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CROATIAN IMMUNOLOGICAL SOCIETY FOUNDED 1968.

Hrvatsko imunološko društvo

Vas poziva na predavanje

"The life and death of eosinophils"

koje će održati

Nives Zimmermann
M.D., Associate Professor

Division of Allergy and Immunology
Cincinnati Children's Hospital Medical Center
University of Cincinnati
Ohio, SAD

u četvrtak, 13. srpnja 2017., u 12.00 sati

seminarska dvorana, I. kat
Hrvatski institut za istraživanje mozga
Medicinski fakultet Sveučilišta u Zagrebu
Šalata 12, Zagreb

Ovu suradnju je potpomogla Hrvatska zaklada za znanost pod projektom broj 7406 – "Molekularni posrednici koštane resorpcije uvjetovane receptorom Fas".

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Curriculum Vitae: NIVES ZIMMERMANN, M.D.
Associate professor of Pediatrics, University of Cincinnati College of Medicine, Cincinnati Children's Hospital Medical Center

RESEARCH FIELD: eosinophils, myeloid lineage cells, chemokines, cell death, asthma, allergy

TOTAL NUMBER OF RESEARCH PAPERS (NUMBER OF CITATIONS): 59 (2466)

EDUCATION

1995 M.D.	University of Zagreb School of Medicine

EMPLOYMENT HISTORY

2008-present	Associate Professor of Pediatrics, University of Cincinnati College of Medicine, Cincinnati Children's Hospital Medical Center
2002-2008	Assistant Professor of Pediatrics, University of Cincinnati College of Medicine, Children's Hospital Medical Center
1999-2002	Instructor of Pediatrics, University of Cincinnati College of Medicine, Children's Hospital Medical Center, Cincinnati, Ohio

PROFESSIONAL MEMBERSHIPS
American Academy of Allergy, Asthma and Immunology
American Association of Immunologists
American Heart Association, Cardiovascular Sciences
National Academy of Inventors: Cincinnati Chapter, charter member

AWARDS AND HONORS

1992	Dean's Award, University of Zagreb
1994	Summer student program, Karyn Kupcinet International Science School; Dr. Avner Yayon, Department of Chemical Immunology, Weizmann Institute of Science, Rehovot, Israel
1999	Symposia funded scholarship, Keystone Symposia
2004	Woman physician in allergy junior faculty development award (special recognition)
2014	ARTrust™ and Donald Y. M. Leung, MD PhD FAAAAI-JACI Lecture: Investing Together in Our Future award

RECENT RESEARCH SUPPORT

2013-2015	NIH/NIAID "Molecular mechanism of eosinophil cell death", Role: Principal Investigator
2010-2012	NIH/NIAID "Role for acidity and GPR65 in food allergy", Role: Principal Investigator
2010-2012	NIH/NIAID "ALA/AAAAI Allergic Respiratory Diseases Award Mechanisms of airway acidification in asthma", Role: Principal Investigator
2010-2012	Department of Defense (PI: Rothenberg, Marc) "Candidate Gene Approach for Eosinophilic Esophagitis" Role:Co-Investigator
2009-2011	NIH/NIAID"Role of Acidic Environment in Eosinophilic Inflammation", Role: Principal Investigator
2007-2010	Dana Foundation (Bochner, Bruce) "Novel genetic and therapeutic approaches focusing on Siglec-8 for the diagnosis and treatment of human idiopathic eosinophilic disorders", Role:Co-Investigator

RECENT RESEARCH PAPERS

1. Kano G, Bochner BS, Zimmermann N.Regulation of Siglec-8-induced intracellular reactive oxygen species production and eosinophil cell death by Src family kinases. Immunobiology. 2017 Feb;222(2):343-349. doi: 10.1016/j.imbio.2016.09.006. Epub 2016 Sep 20.
2. Zhu X, Hogan SP, Molkentin JD, Zimmermann N. Cyclophilin D regulates necrosis, but not apoptosis, of murine eosinophils. Am J Physiol Gastrointest Liver Physiol. 2016 Apr 15;310(8):G609-17. doi: 10.1152/ajpgi.00389.2015. Epub 2016 Feb 18.
3. Zimmermann N, Rothenberg ME. Mechanism of enhanced eosinophil survival in inflammation. Blood 2015 Jun 18;125(25):3831-2.
4. Wen T, Mingler MK, Wahl B, Khorki ME, Pabst O, Zimmermann N, Rothenberg ME. Carbonic anhydrase IV is expressed on IL-5-activated murine eosinophils. J Immunol. 2014 Jun 15;192(12):5481-9
5. Zhu X, Mose E, Hogan SP, Zimmermann N. Differential eosinophil and mast cell regulation: mast cell viability and accumulation in inflammatory tissue are independent of proton-sensing receptor GPR65. Am J Physiol Gastrointest Liver Physiol. 2014 Jun 1;306(11):G974-82.
6. Mao H, Kano G, Hudson SA, Brummet M, Zimmermann N, Zhu Z, Bochner BS. Mechanisms of Siglec-F-Induced Eosinophil Apoptosis: A Role for Caspases but Not for SHP-1, Src Kinases, NADPH Oxidase or Reactive Oxygen. PLoS One. 2013 Jun 28;8(6)
7. Kano G, Almanan M, Bochner B, Zimmermann N. Mechanism of Siglec-8-mediated Cell Death in IL-5-activated Eosinophils: Role for ROS-enhanced MEK/ERK Activation. J Allergy Clin Immunol. 2013; 132: 437-45.
8. Collison A, Hatchwell L, Verrills N, Wark PA, de Siqueira AP, Tooze M, Carpenter H, Don AS, Morris JC, Zimmermann N, Bartlett NW, Rothenberg ME, Johnston SL, Foster PS, Mattes J. The E3 ubiquitin ligase midline 1 promotes allergen and rhinovirus-induced asthma by inhibiting protein phosphatase 2A activity. Nat Med. 2013 Feb;19(2):232-7.
9. Herbert DR, Orekov T, Roloson A, Ilies M, Perkins C, O'Brien W, Cederbaum S, Christianson DW, Zimmermann N, Rothenberg ME, Finkelman FD. Arginase I suppresses IL-12/IL-23p40-driven intestinal inflammation during acute schistosomiasis. J Immunol. 2010;184(11):6438-46.
10. Kottyan LC, Collier AR, Cao KH, Niese KA, Hedgebeth M, Radu CG, Witte ON, Khurana Hershey G, Rothenberg ME, Zimmermann N. Eosinophil viability is increased by acidic pH in a cAMP and GPR65-dependent manner. Blood. 2009; 114(13):2774-82.

The life and death of eosinophils

Nives Zimmermann, M.D.

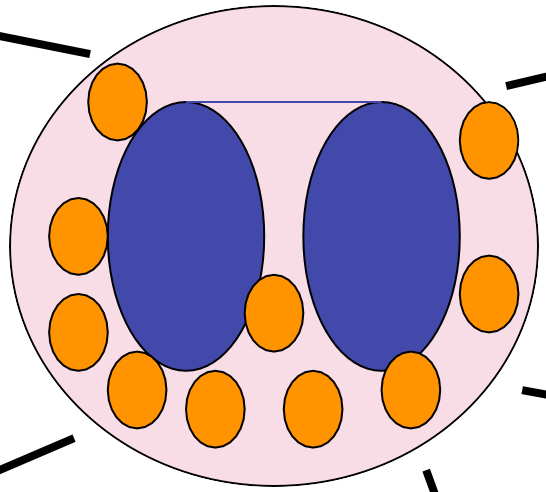
Division of Allergy and Immunology, Cincinnati Children's Hospital, and

Department of Pathology, University of Cincinnati

This collaboration is supported by Croatian science foundation (project MEFRA, nr. 7406)

Photo courtesy of Mitch Grayson

Eosinophils



Antigen presentation
- B7.1 (CD80)
- MHC II

Chemokines
- eotaxin
- MIP-1
- RANTES

Neuro-mediators
- Substance P
- VIP

Lipid mediators
- PAF
- leukotrienes

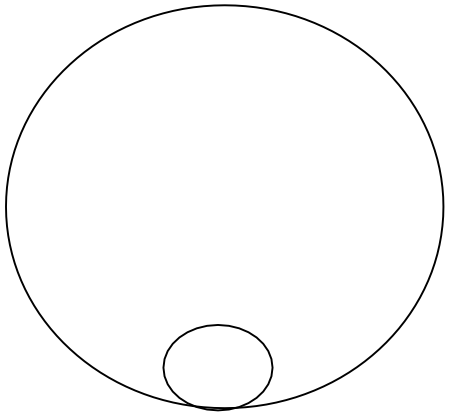
Cytotoxic secretory products
- ECP
- EDN
- EPO
- MBP

Cytokines

IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-12, IL-13, IFN-g, GM-CSF, TGF-a/b, TNF-a

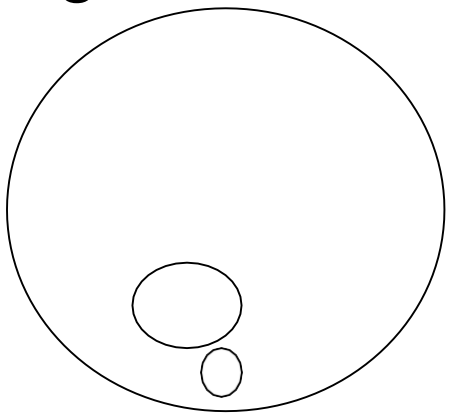
Eosinophil degranulation

Exocytosis (incl. compound exocytosis)



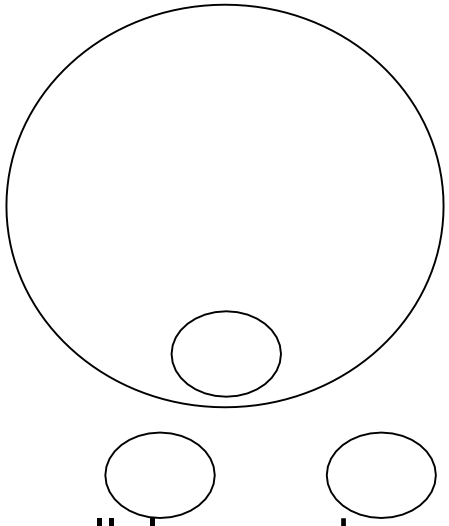
Granule fuses with membrane and content gets released LAMPs 1,2,3 (CD107a and b, CD63) are found on cell surface

Piecemeal degranulation



Secretory vesicles transport content from granule and release it; can be selective release of content

Cytolysis



Extracellular membrane-coated granules; presumably associated with plasma membrane rupture (a.k.a. cell death); some evidence of ability of granules to function independently

Eosinophil-associated diseases

- Parasites
- Allergic diseases
- Asthma
- Eosinophilic esophagitis (EoE), and other EGID
- Hypereosinophilic syndrome/chronic eosinophilic leukemia (HES/CEL)

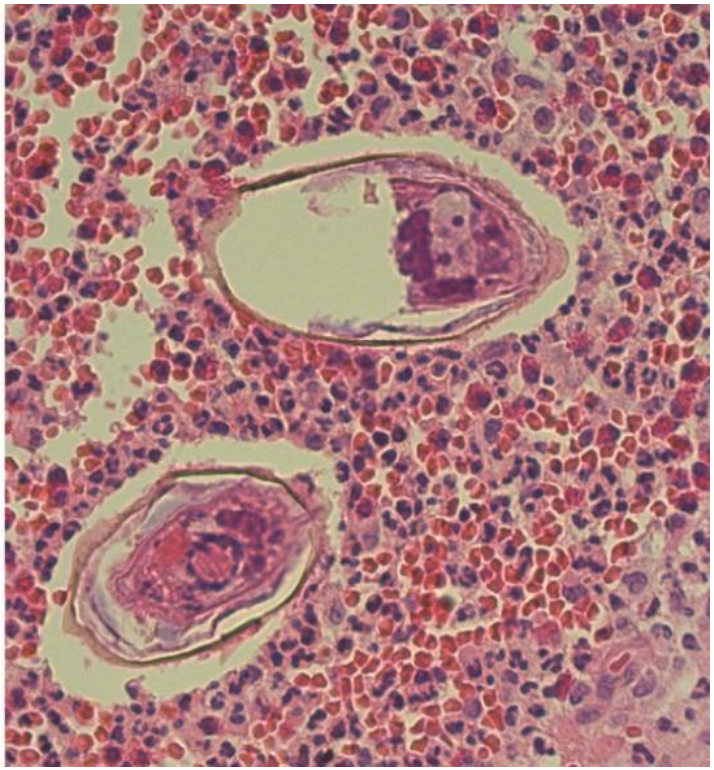


Image from Becker SL et al. Euro Surveill. 2015

Factors that influence eosinophil accumulation

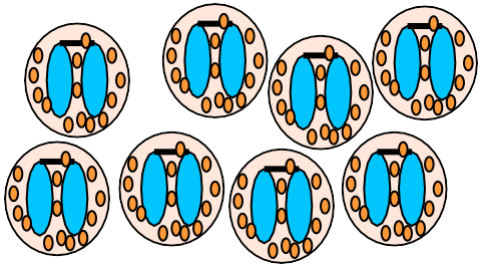
IN

OUT

Recruitment

Development *in situ* from progenitors

Steady state # of eosinophils



Egress

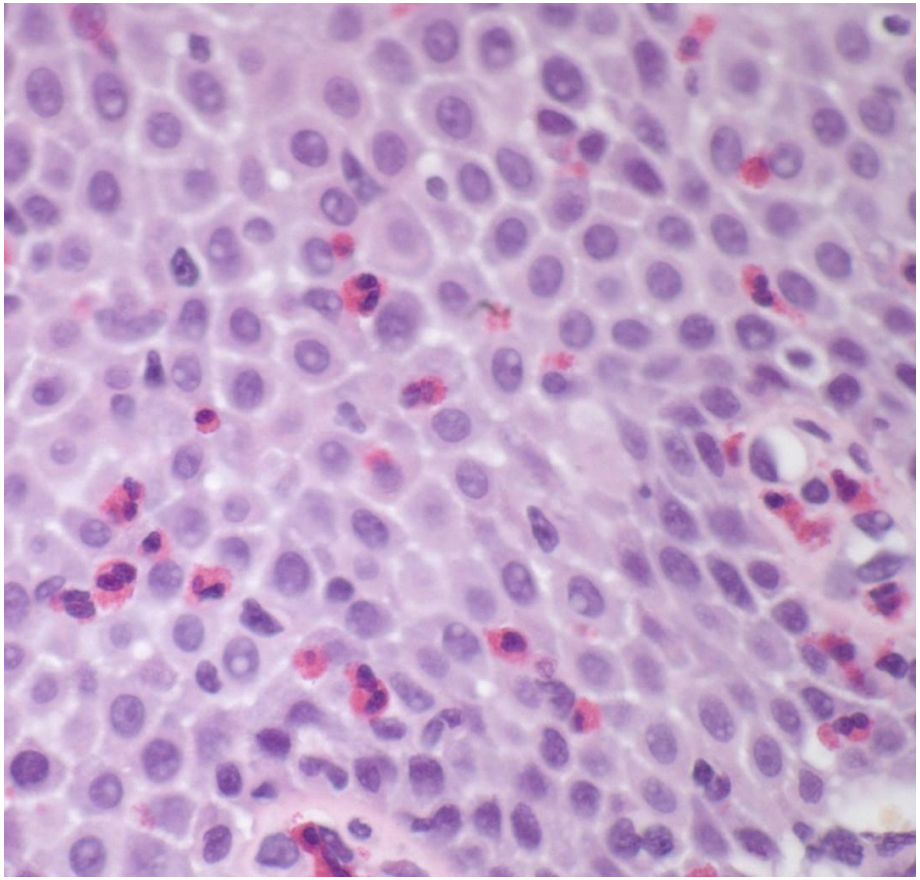
Death

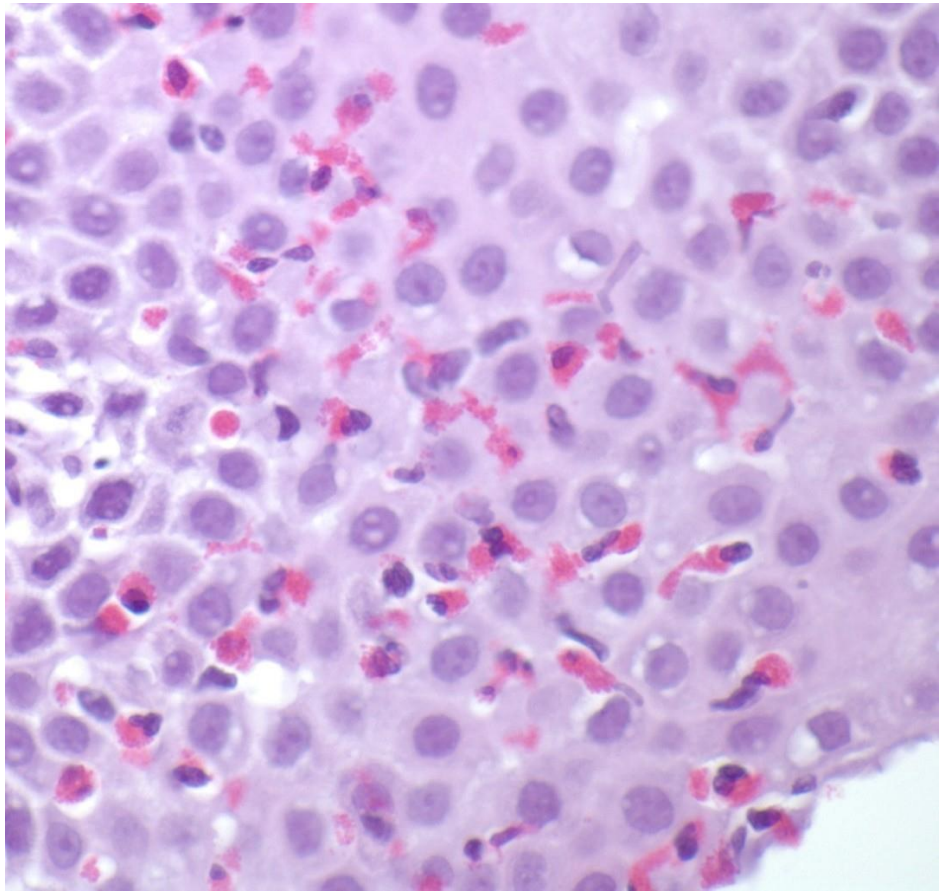
Role of eosinophil cell death in disease

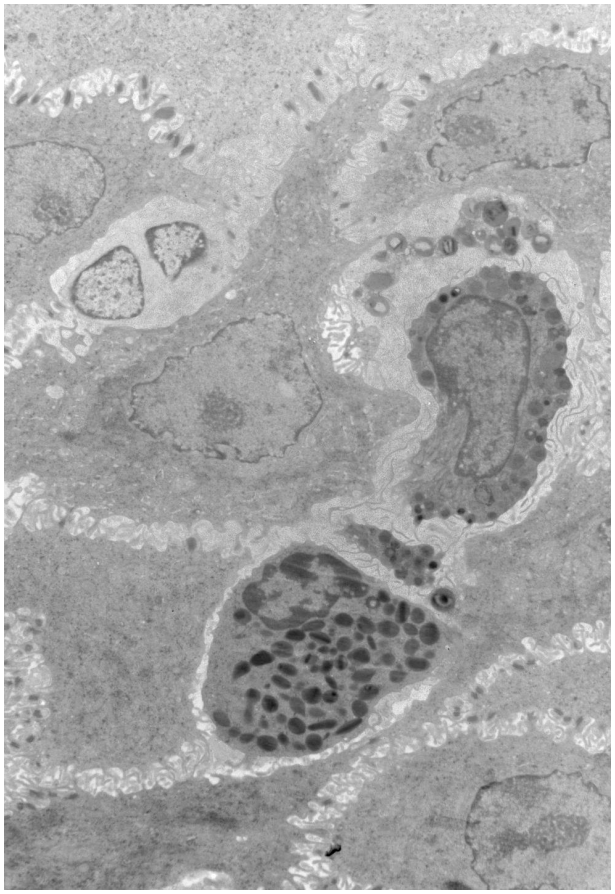
- Ultrastructural and immunohistochemical studies in human tissue from patients with eosinophilic diseases demonstrate significant eosinophil cytolysis/necrosis
- even though the number of eosinophils was decreased in the bronchial tissue of patients with asthma by ~50% after anti-IL-5 treatment, the deposition of extracellular eosinophil granule protein MBP did not differ from before treatment or with placebo, correlating with lack of treatment effect in some cases
- intact extracellular eosinophil granules have the ability to function as secretory organelles extracellularly after eosinophil cytolysis

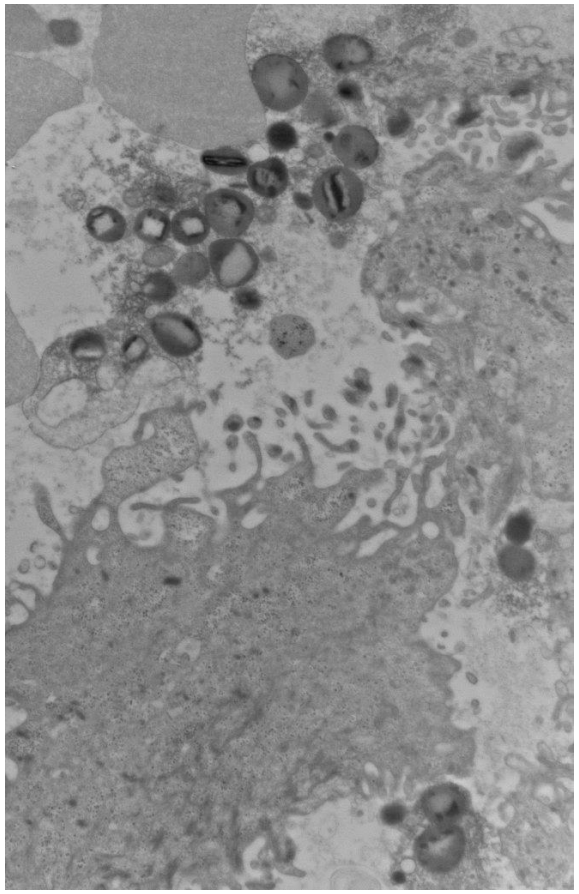
Uller L, et al: *Am J Respir Crit Care Med* 2004; Armengot M, et al: *Am J Rhinol Allergy* 2009; Colombel JF, et al:**.** *Gut* 1992; Tai PC, et al: *Lancet* 1987; Wright BL, et al: *N Engl J Med* 2011; Flood-Page PT, et al: *Am J Respir Crit Care Med* 2003; Neves JS, et : *Proc Natl Acad Sci U S A* 200

EoE with intact eosinophils and Cfegs (clusters of free eosinophil granules)





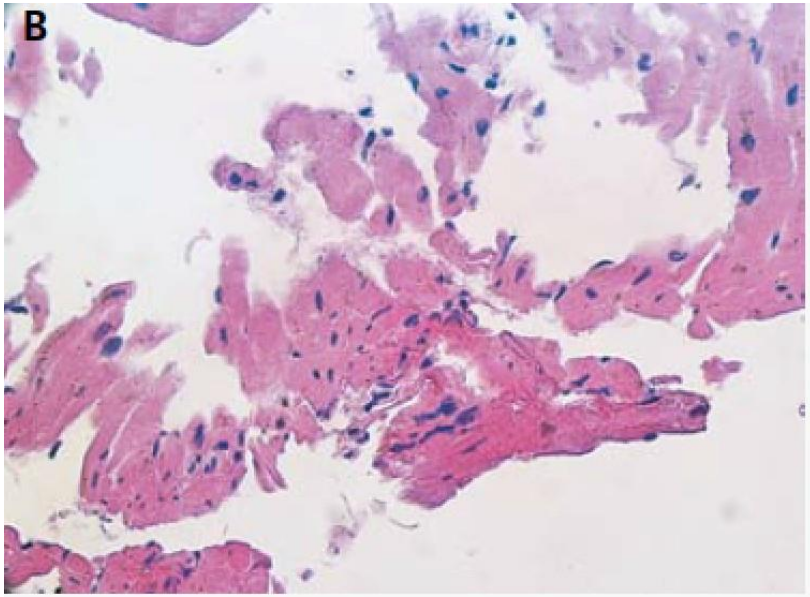


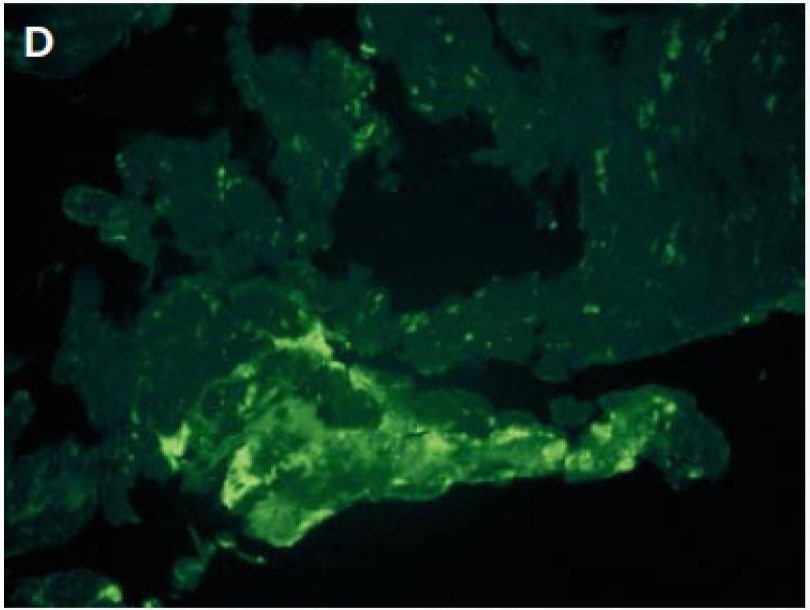


Margaret Collins, MD

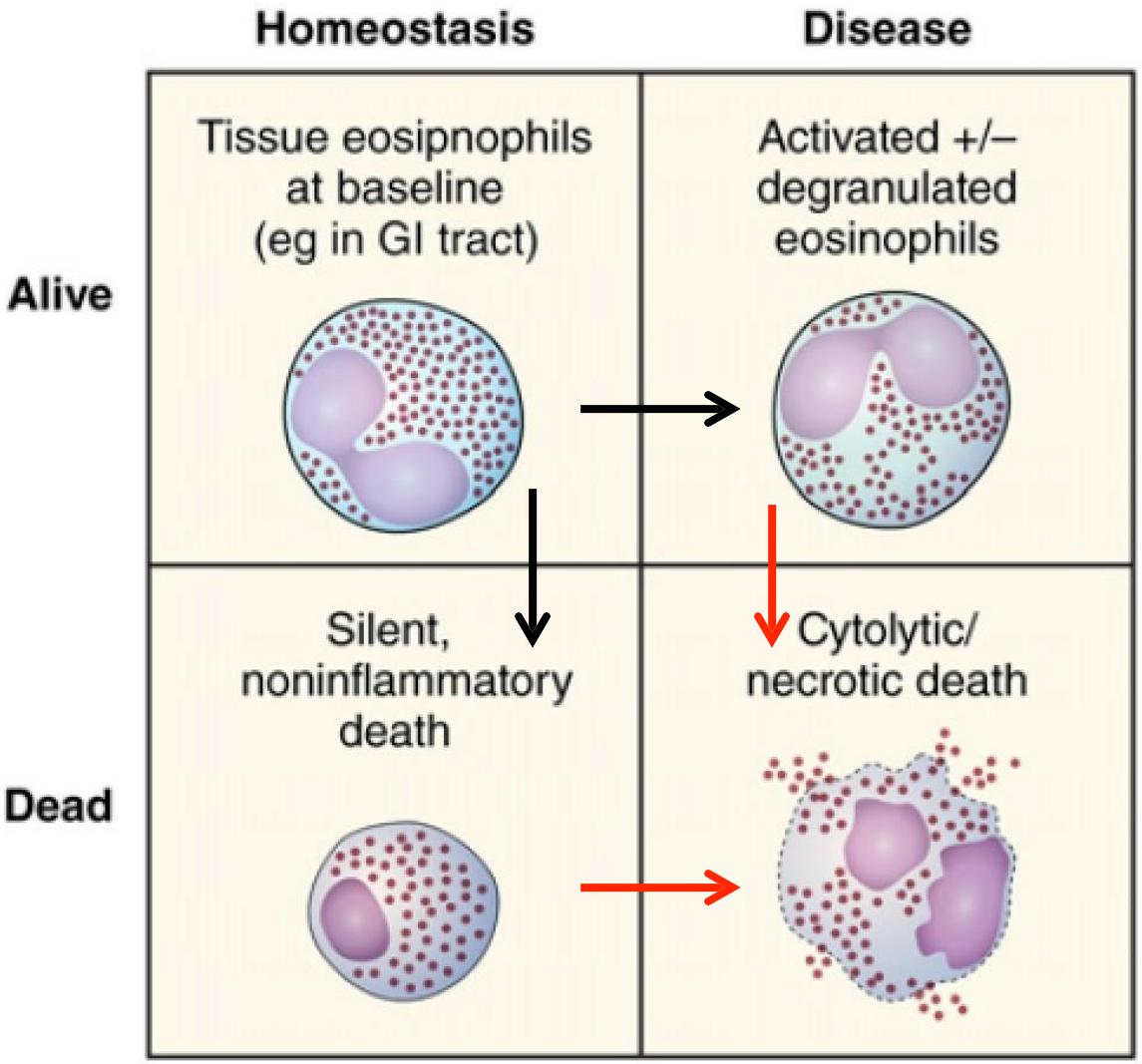
- Patient with hypereosinophilia and brain microemboli; endomyocardial disease was suspected but no eosinophils were seen by H&E

- Staining for anti-MBP



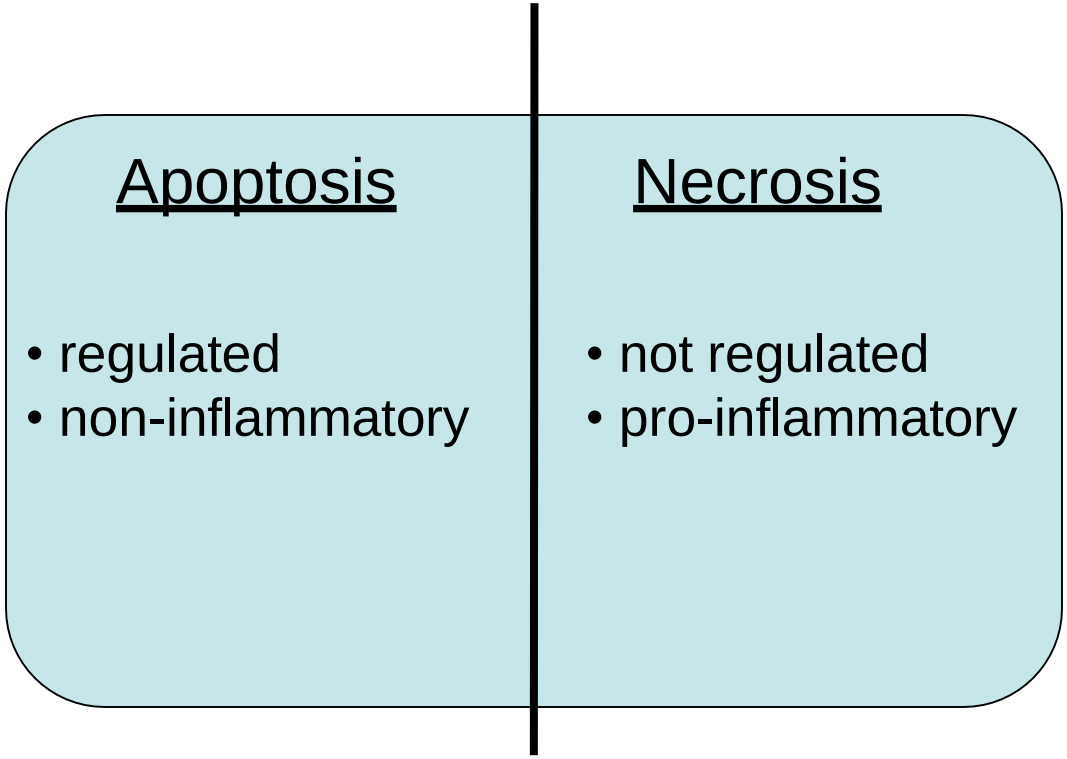


- Wright BL, Leiferman KM and Gleich GJ. Eosinophil Granule Protein Localization in Eosinophilic Endomyocardial Disease. New Engl J Med 2011;365:2.

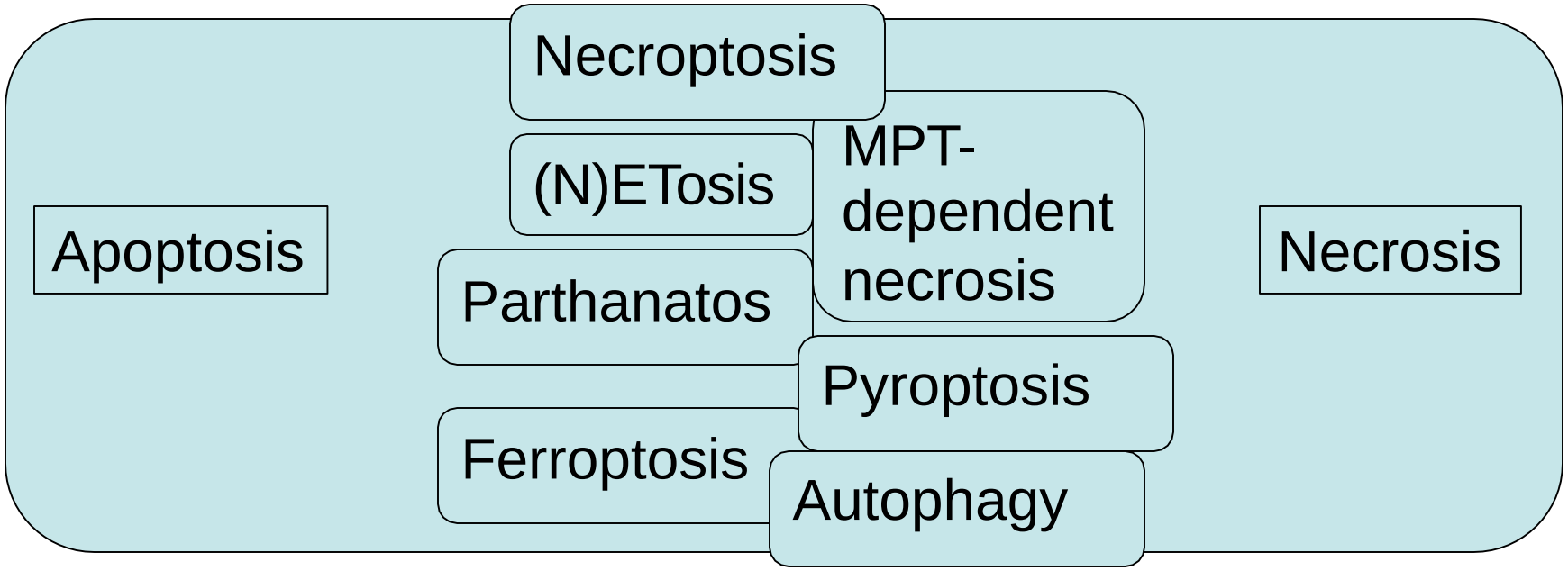


From Zimmermann N and Rothenberg ME. Blood 2015; 125:3831

Cell death


Apoptosis
• regulated
• non-inflammatory
Necrosis
• not regulated
• pro-inflammatory

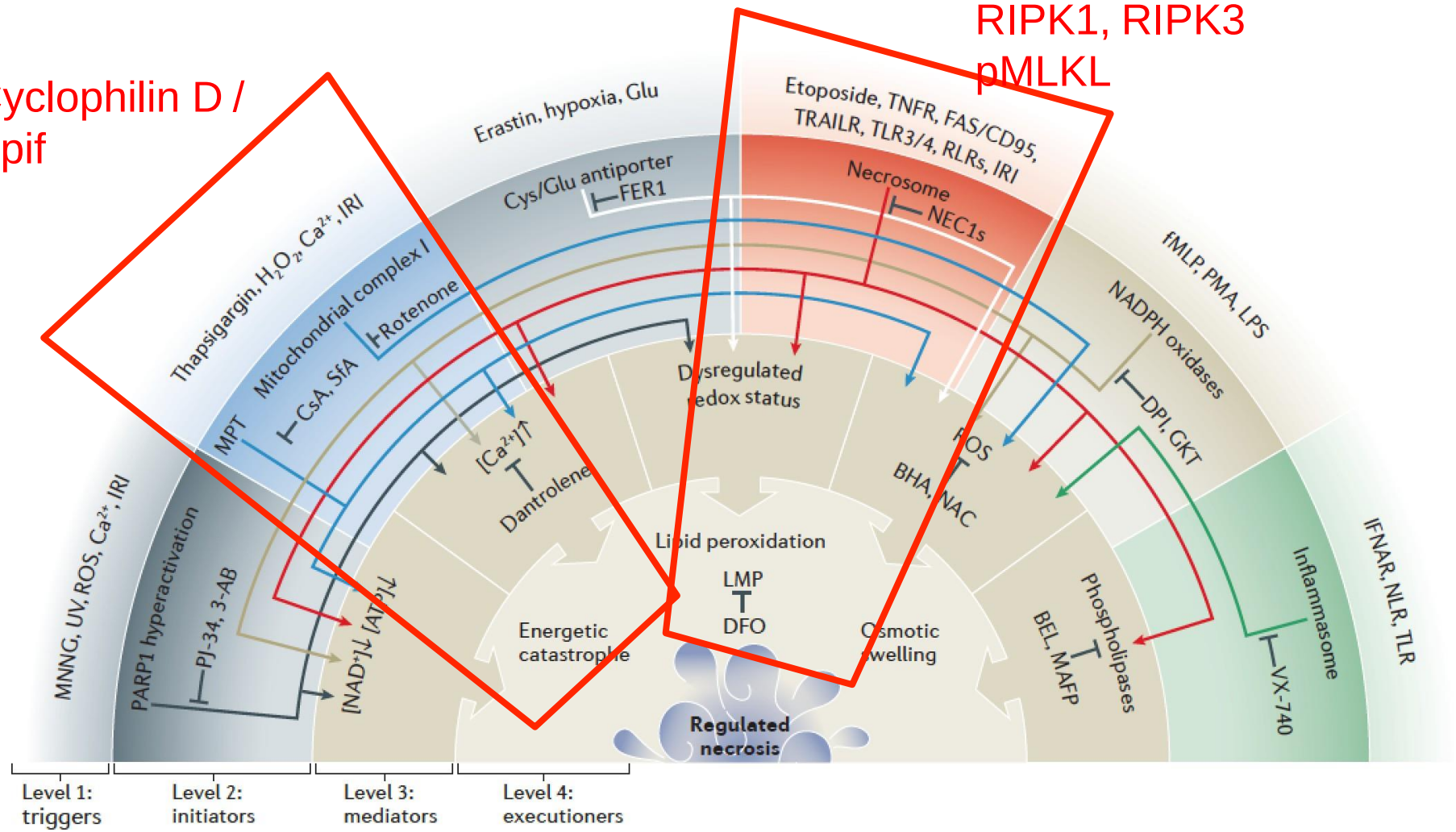
Cell death



Caspases etc

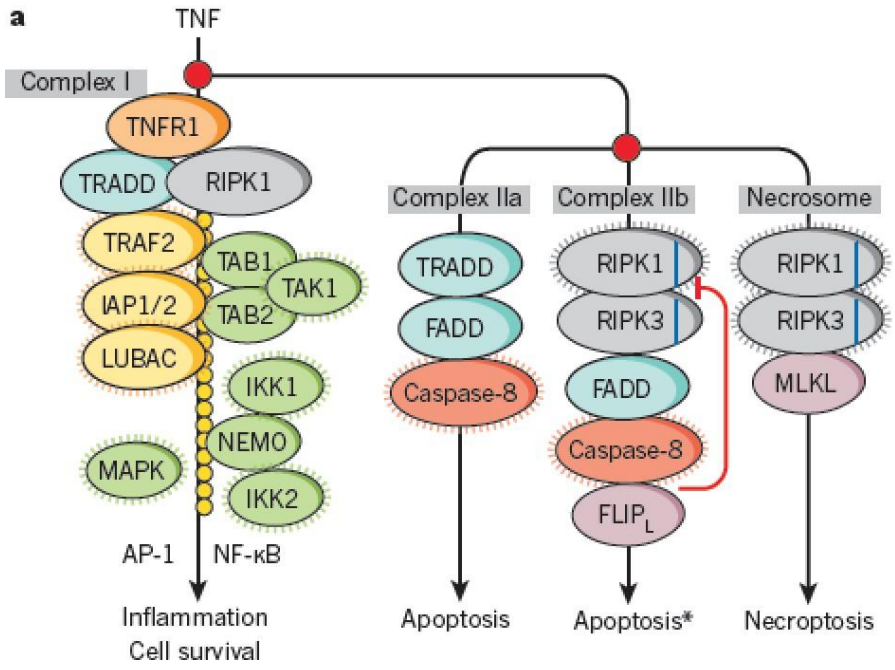
RIP kinases etc

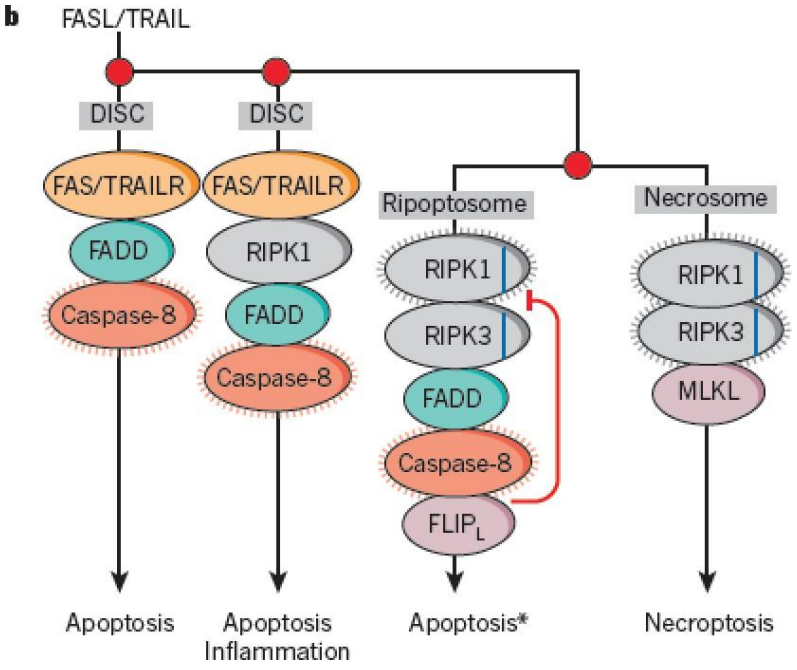
Regulated cell death pathways



From Vanden Berghe T et al. Nature Reviews 2014;15:135

Necroptosis





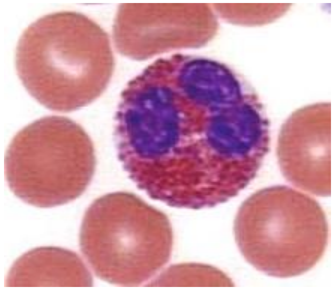
Pasparakis and Vandenabeele. Nature 2015; 517:311

Regulation of eosinophil viability

- Survival factors: IL-5, IL-3, GM-CSF, TNFα, IFNs, acidic microenvironment, β-agonists etc.
- Death-inducing signals: ligation of Fas, glycans that crosslink Siglec-8, corticosteroids, theophylline (cAMP-independent), TGF-β etc.

Survival factors

\+ + + + +

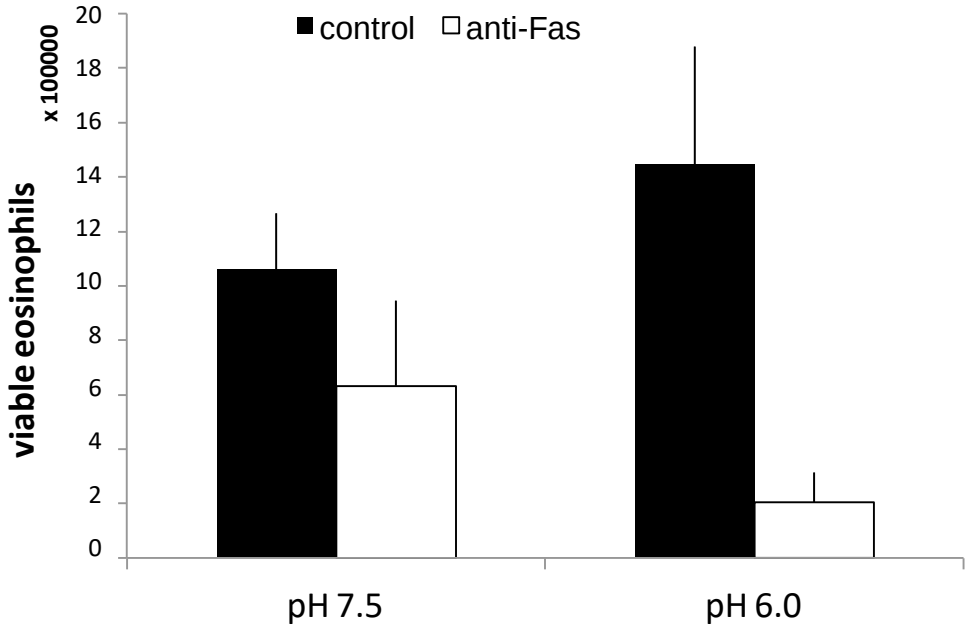


Grand total → Cell fate

\- - - - - - -

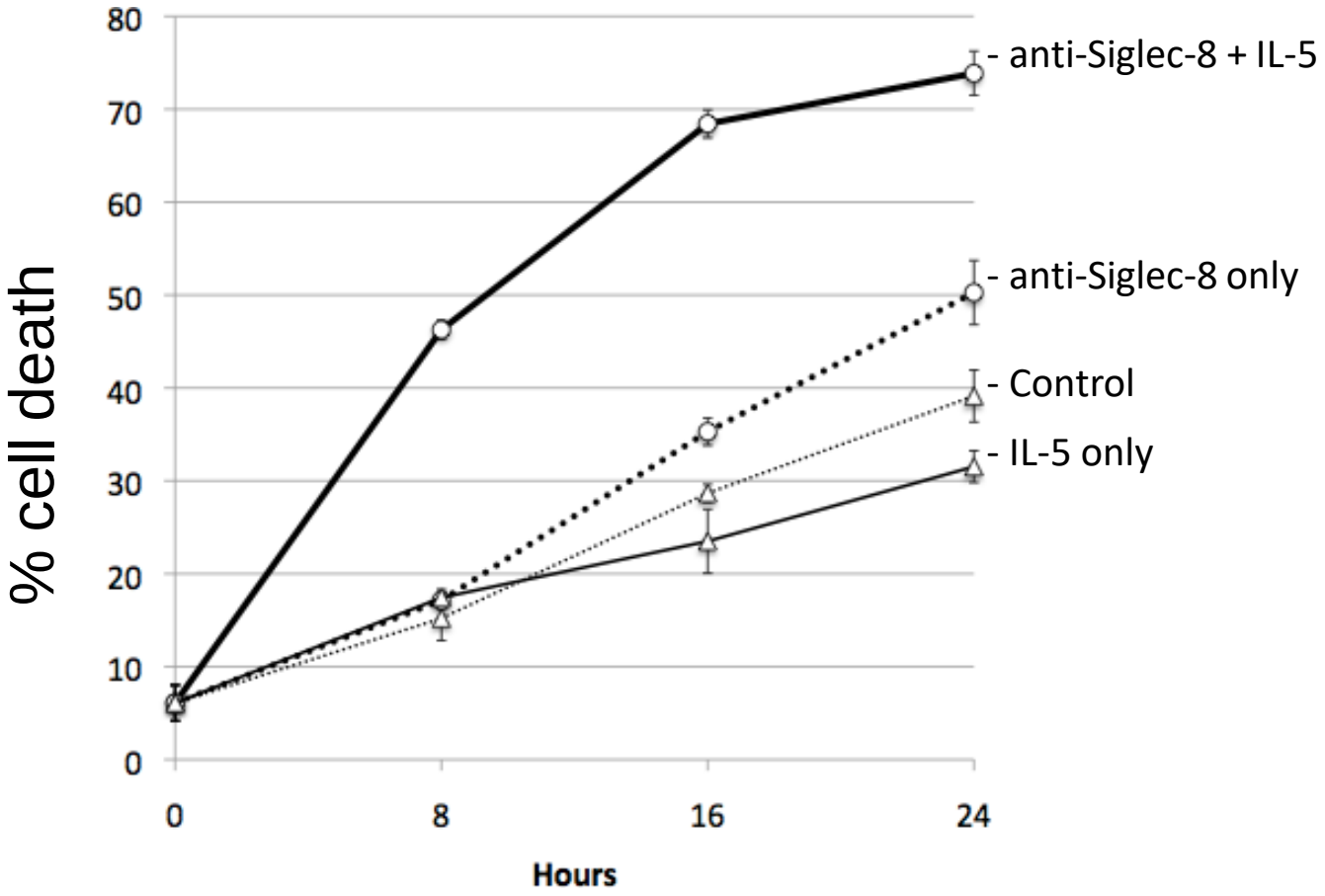
Death factors

Mild acidity enhances eosinophil survival, but exacerbates anti-Fas-induced eosinophil cell death

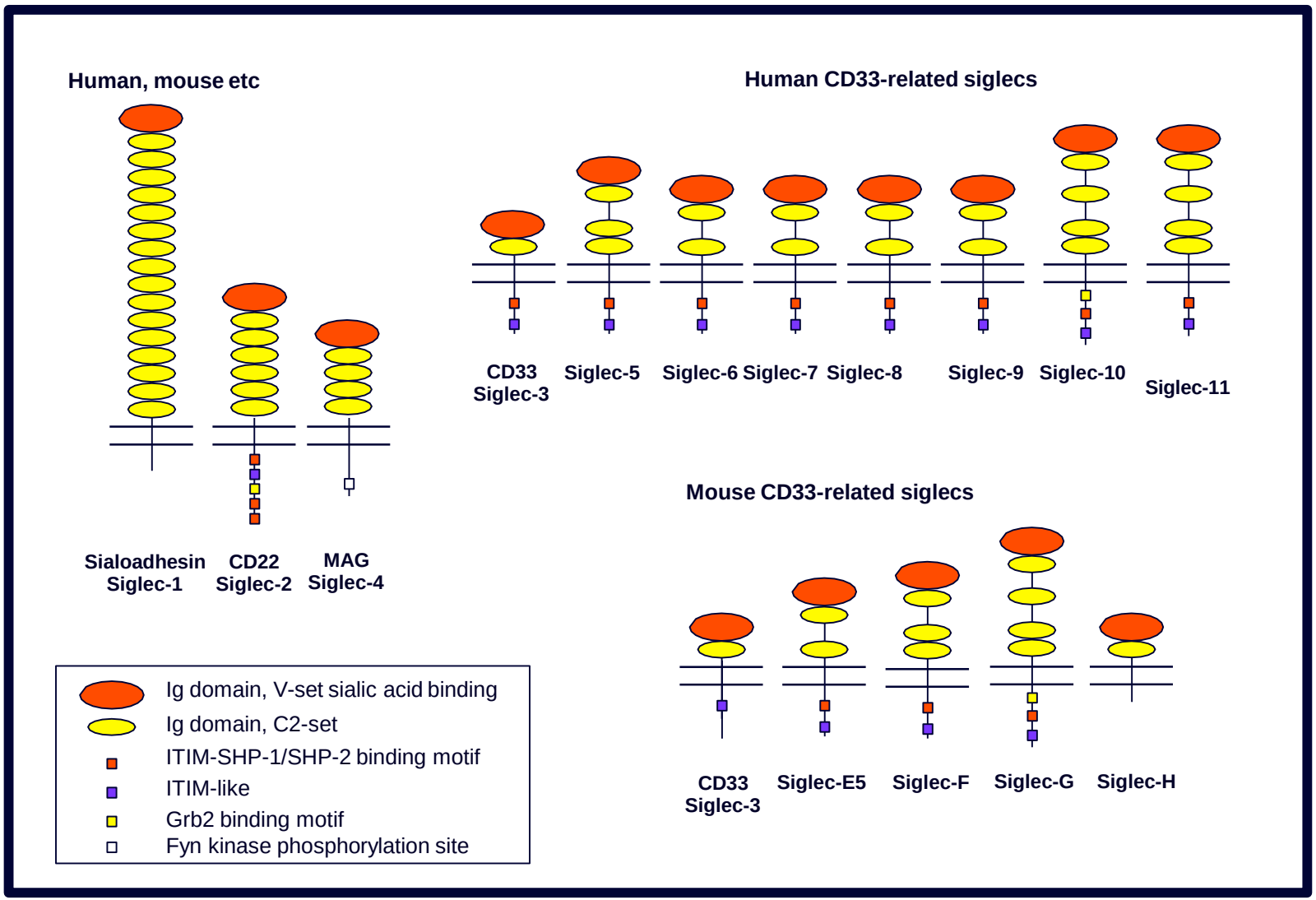


Kottyan, L et al. Blood 2009

Siglec-8 engagement induces eosinophil cell death

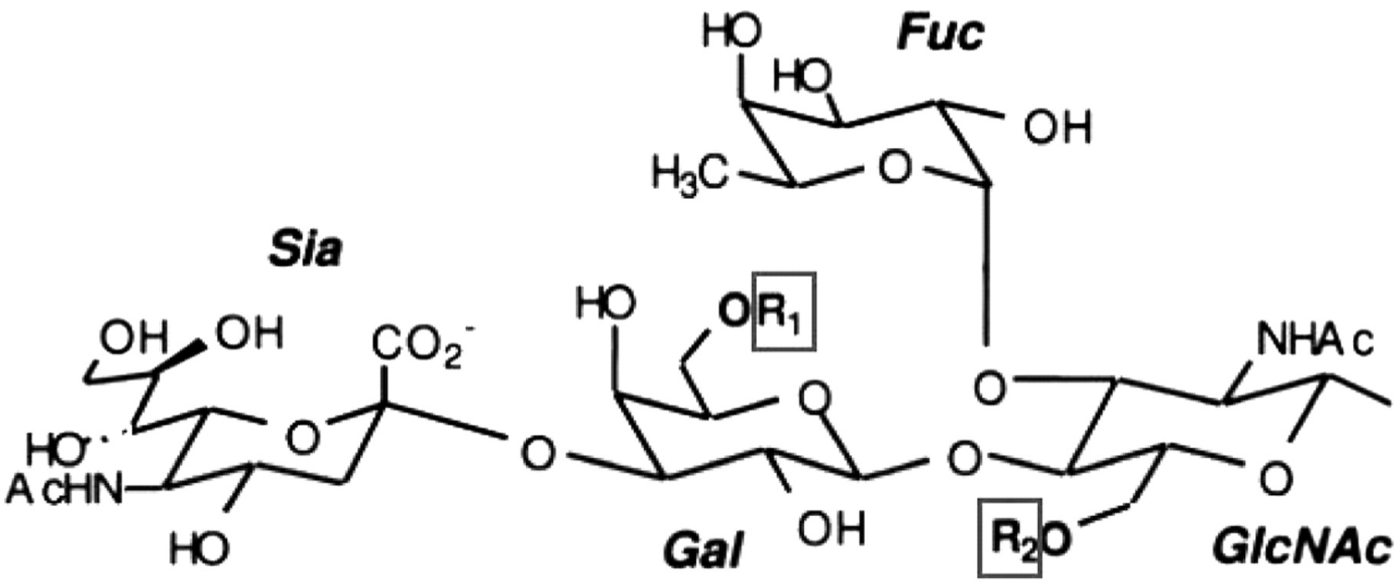


Nutku E et al *Blood* 2003



Crocker, Curr Opin Pharmacol 5:431, 2005

Structures of glycans used to verify specificity of Siglec-8-Ig binding



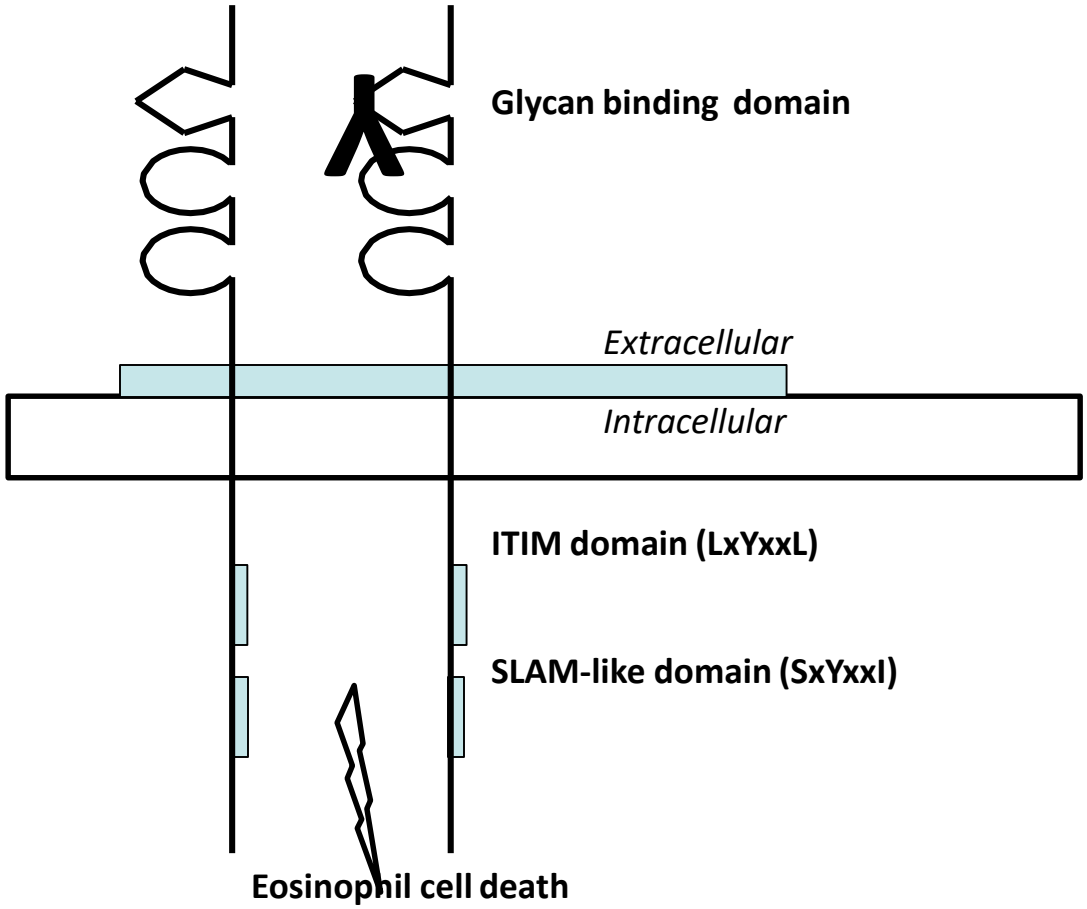
R1=R2=H; SLe^x , NO BINDING

R1=SO_3, R2=H; 6'-sulfo-SLe^x, BINDING

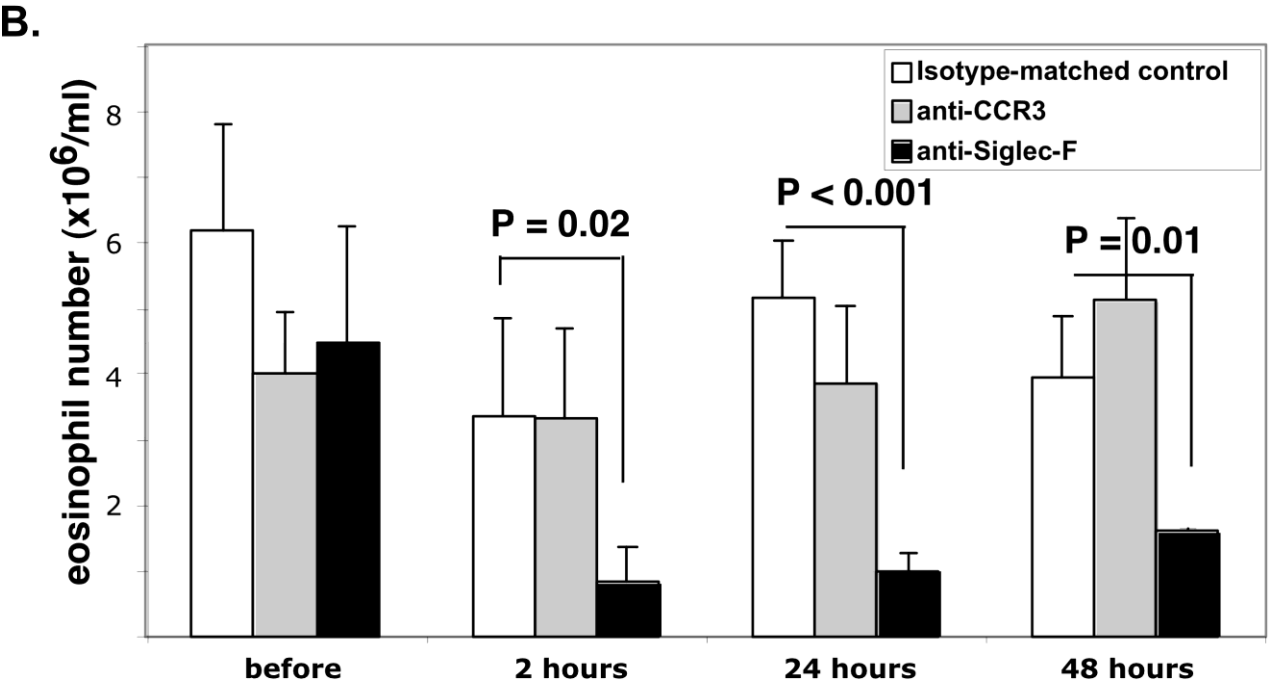
R1=H, R2=SO_3; 6-sulfo-SLe^x , NO BINDING

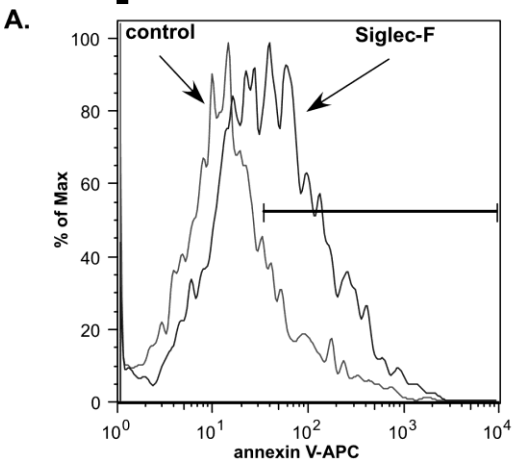
Bochner, B. S. et al. J. Biol. Chem. 2005;280:4307-4312

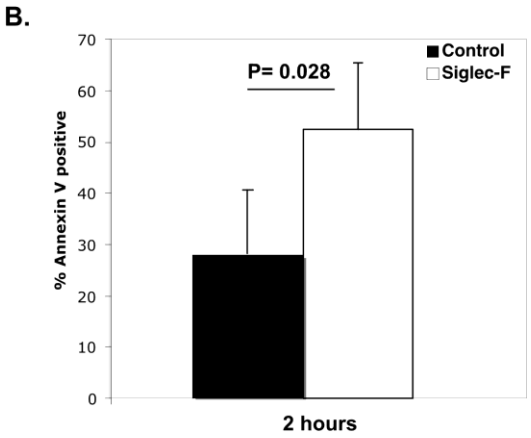
Siglec-8 crosslinking



Anti-Siglec-F treatment of mice decreases number of eosinophils

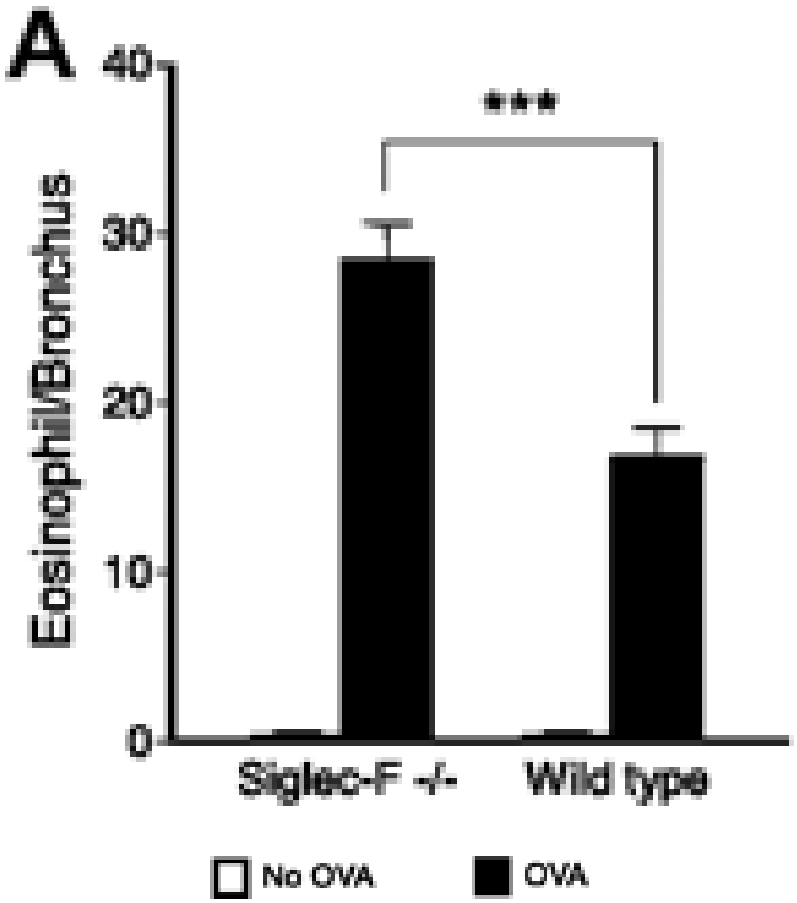






From Zimmermann N et al Allergy 2008

Siglec-F-deficient mice have enhanced eosinophilia following allergen challenge

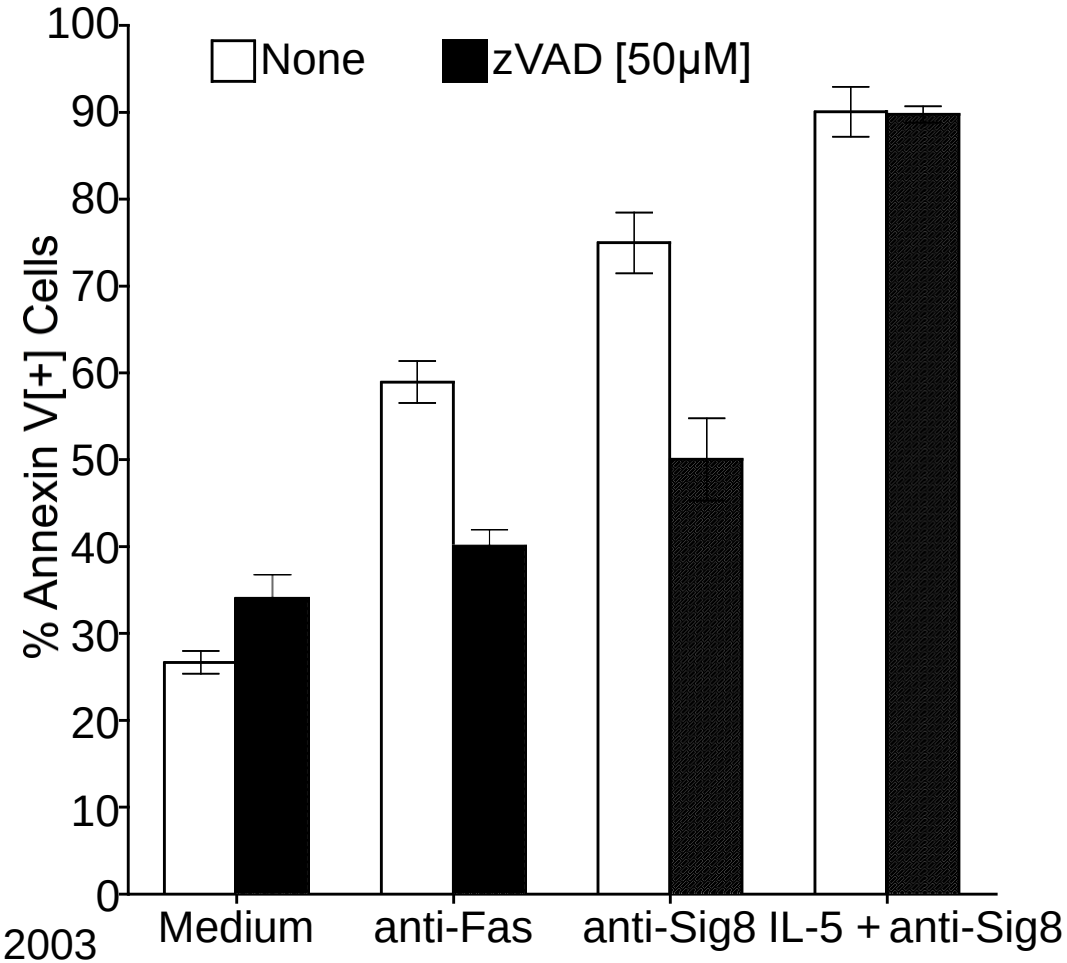


From Zhang M et al Blood 2007

Summary I

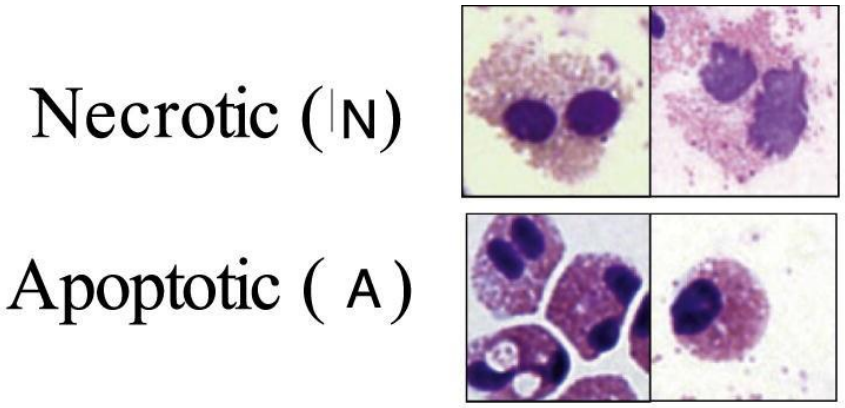
- Siglec-8 is a novel target: relatively specific for eosinophils, more effective on activated (tissue?) eosinophils
- Targeting Siglec-F in mouse models shows improvement of disease outcomes
- Paradoxical aspect of Siglec-8-induced eosinophil cell death is that this effect is significantly enhanced by IL-5, an eosinophil survival factor
- goal is to understand what are the consequences of this different type of cell death, and how this enhancement occurs on a molecular level (as this could be exploited to enhance anti-Siglec-8 therapeutics as well as provide new conceptual model for Siglec-8 signaling)

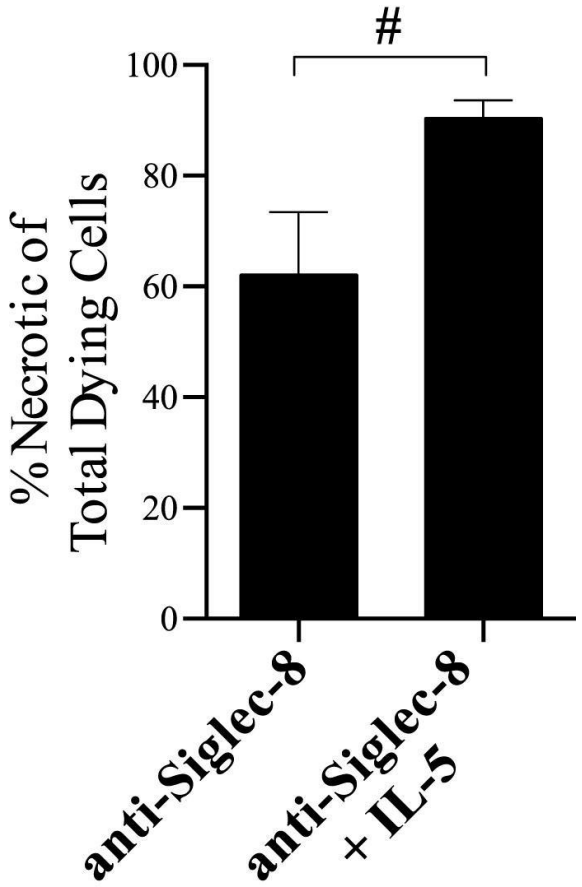
Anti-Siglec-8 induced cell death is <u>caspase-independent</u> in IL-5-stimulated eosinophils



Nutku E et al *Blood* 2003

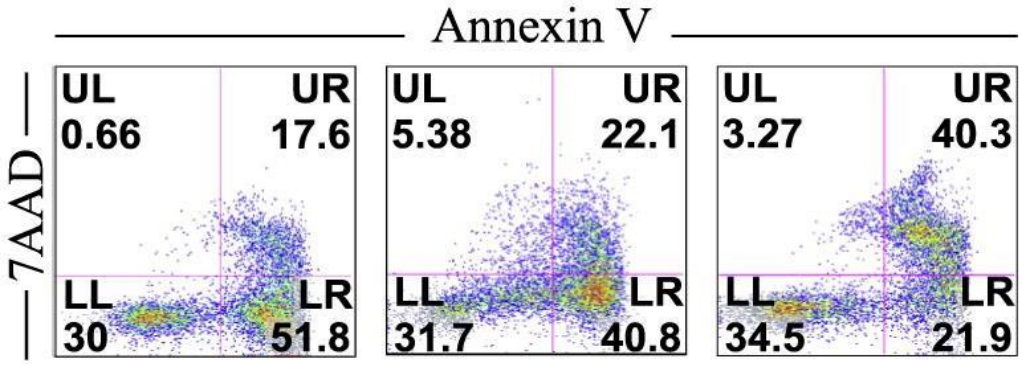
Different mode of anti-Siglec-8-induced cell death in IL-5-activated eosinophils





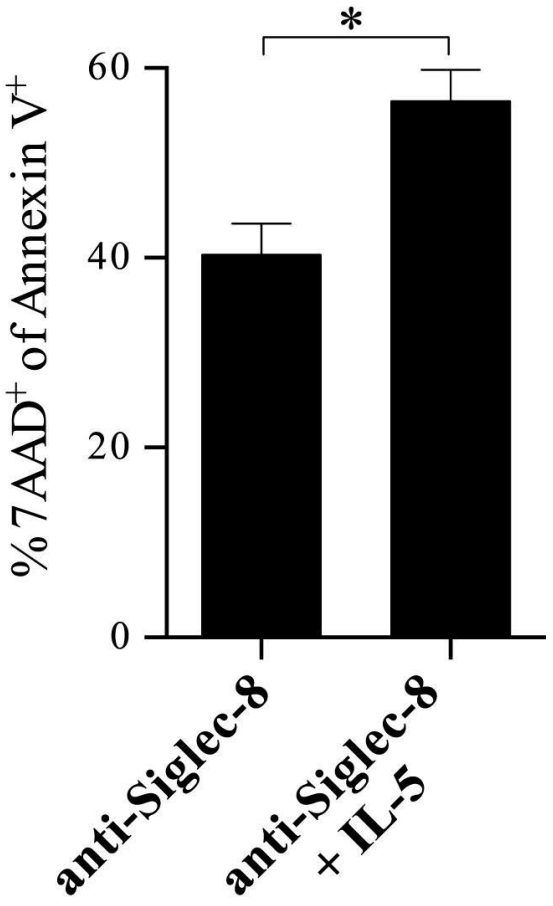
J Allergy Clin Immunol 2013; 132:437-445

Different mode of anti-Siglec-8-induced cell death in IL-5-activated eosinophils



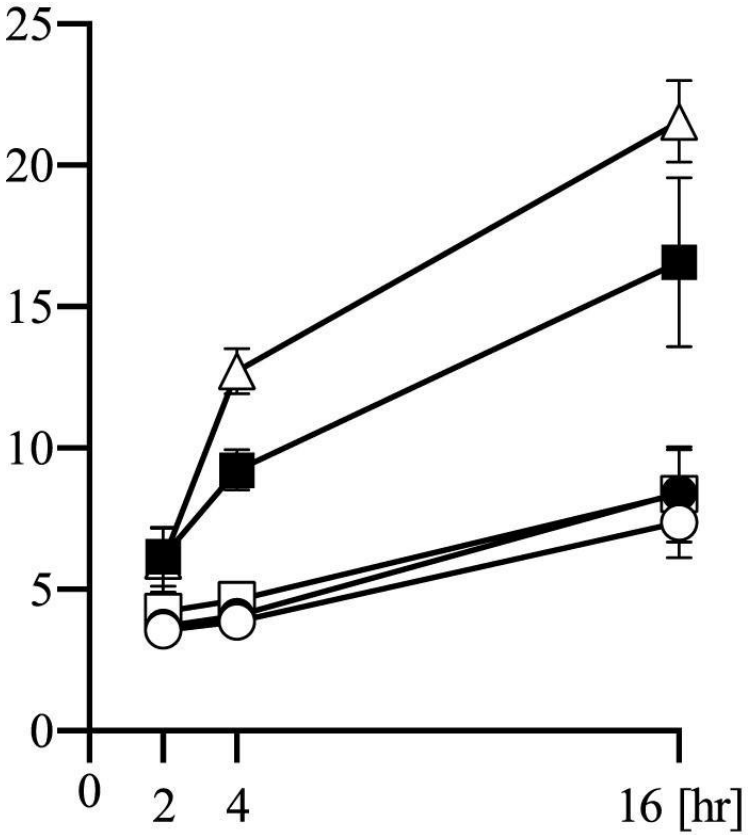
Anti-Siglec-8

Anti-Siglec-8 + IL-5



J Allergy Clin Immunol 2013; 132:437-445

EPO release from anti-Siglec-8/IL-5-treated eosinophils



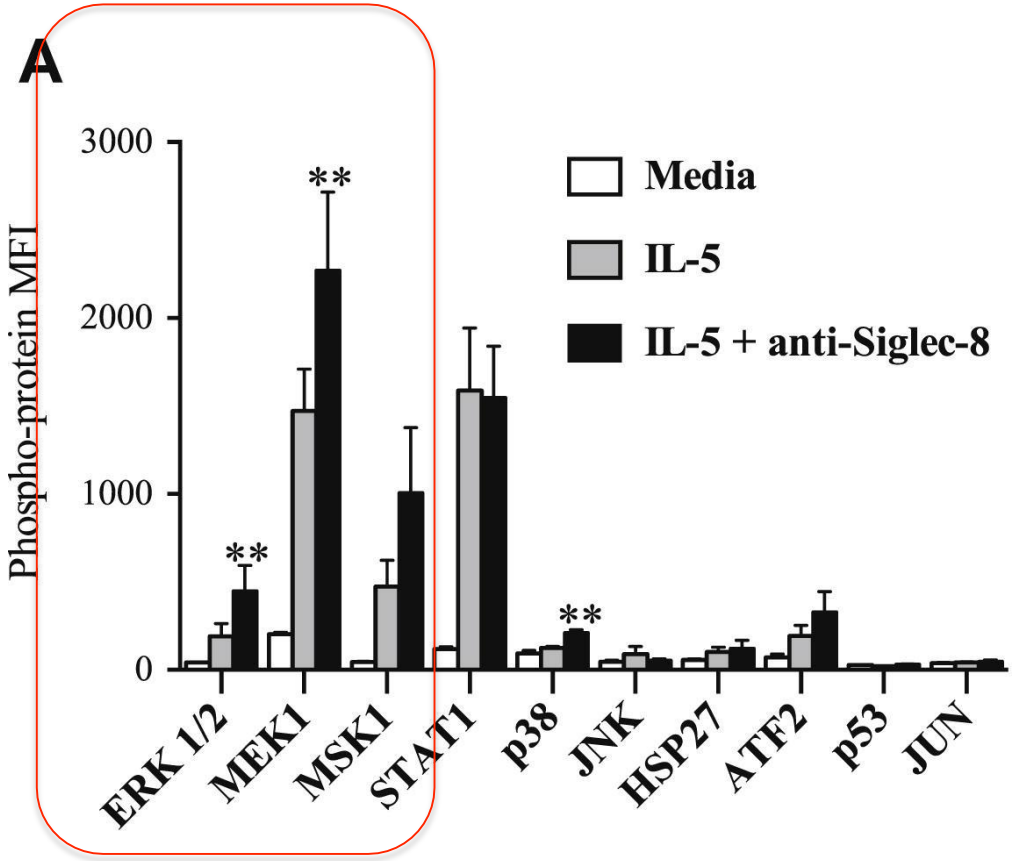
PMA

Anti-Siglec-8/IL-5

Anti-Siglec-8 alone
Ctrl atb/IL-5
Ctrl atb alone

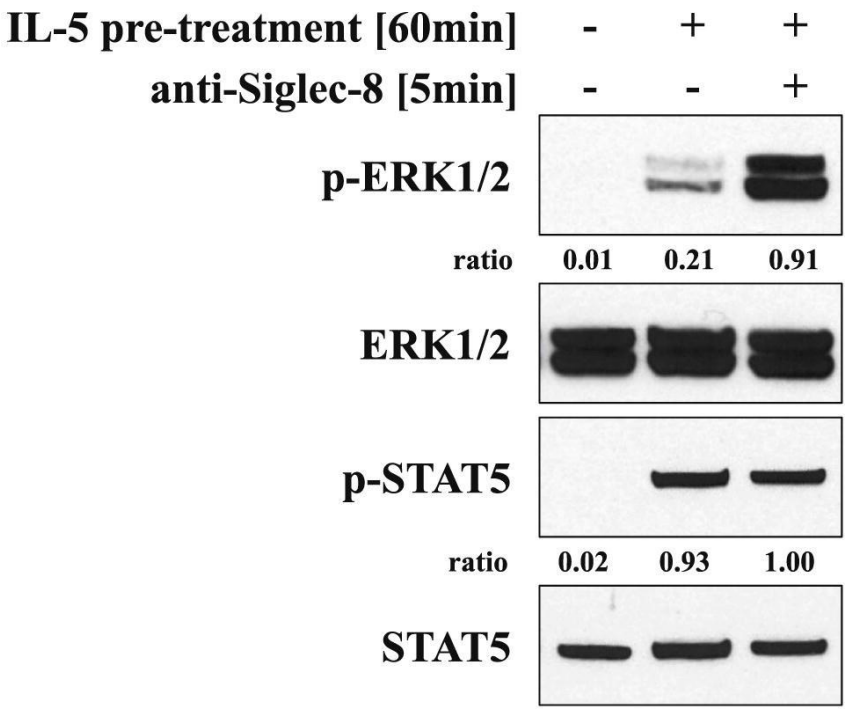
J Allergy Clin Immunol 2013; 132:437-445

ERK pathway phosphorylation is enhanced by anti-Siglec-8



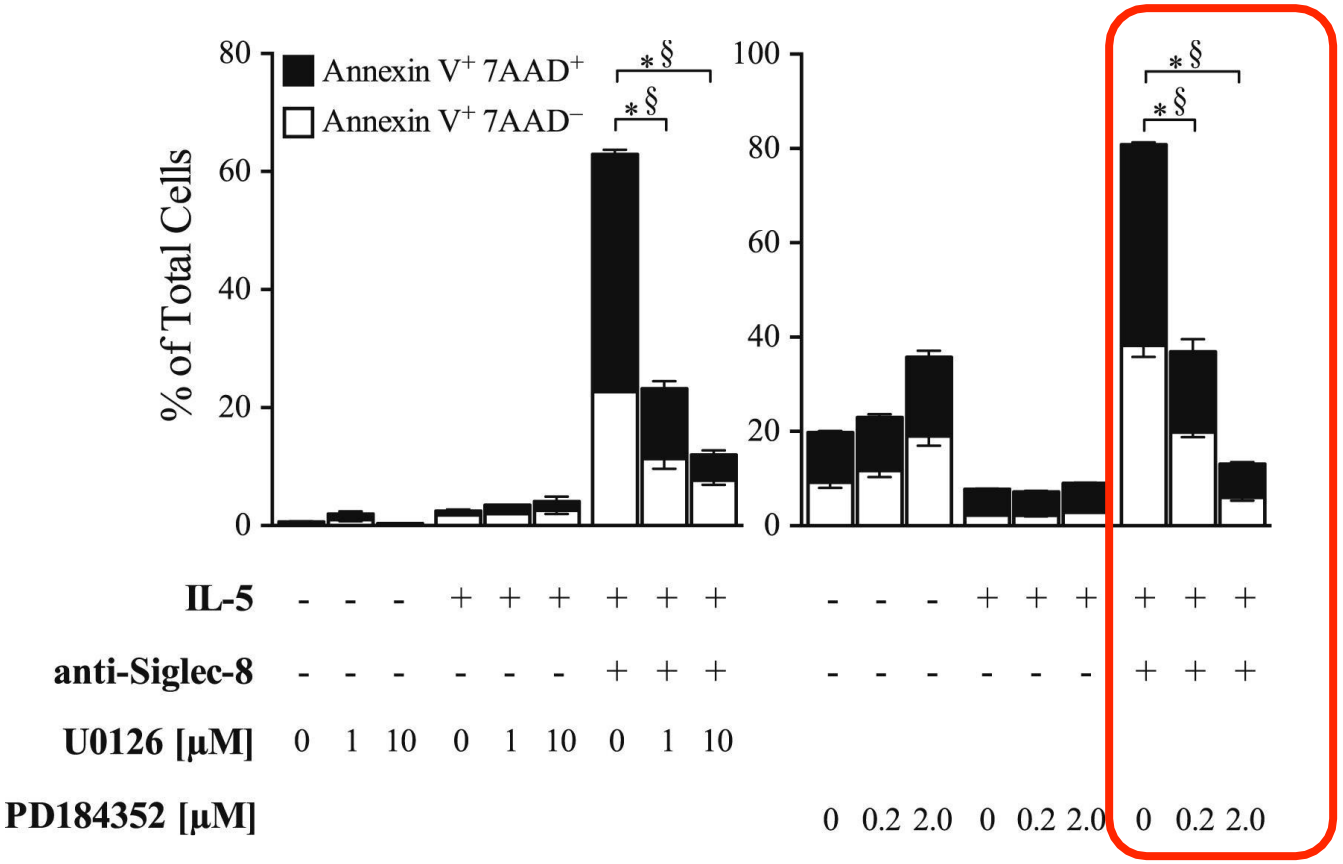
J Allergy Clin Immunol 2013; 132:437-445

ERK pathway phosphorylation is enhanced by anti-Siglec-8



J Allergy Clin Immunol 2013; 132:437-445

ERK activation is required for IL-5 + anti-Siglec-8 induced eosinophil cell death

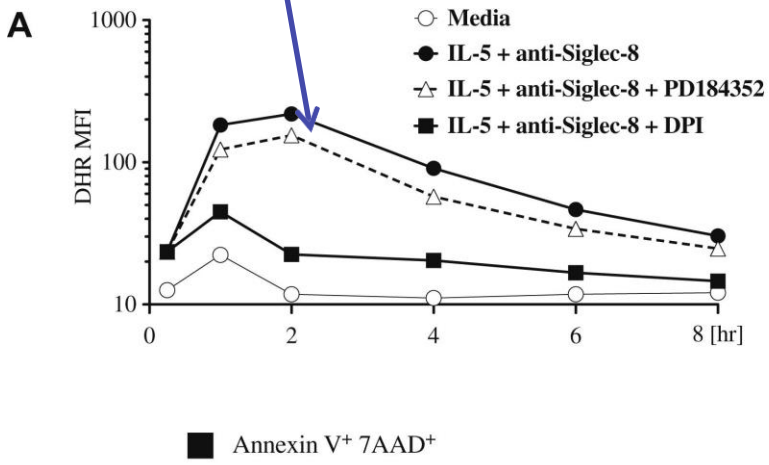


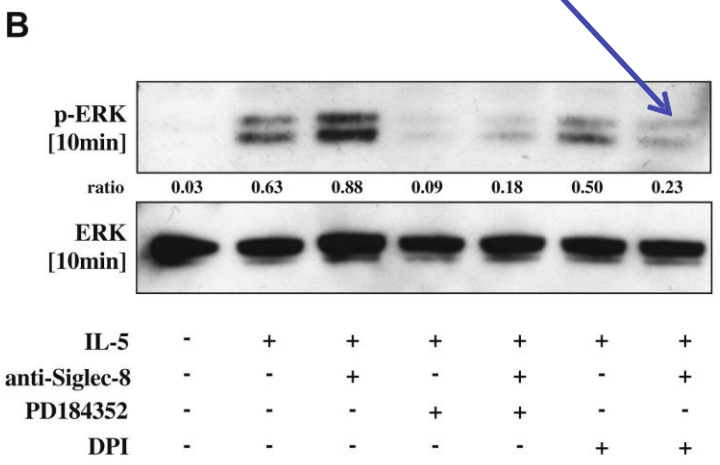
J Allergy Clin Immunol 2013; 132:437-445

Enhanced ERK phosphorylation is dependent on ROS

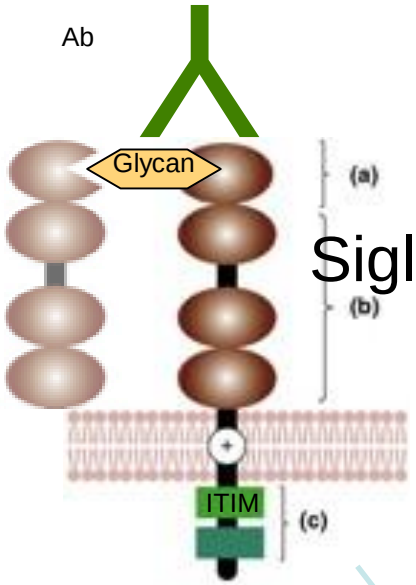
Inhibiting ROS does inhibit ERK enhancement

Inhibiting MAPK does not inhibit ROS

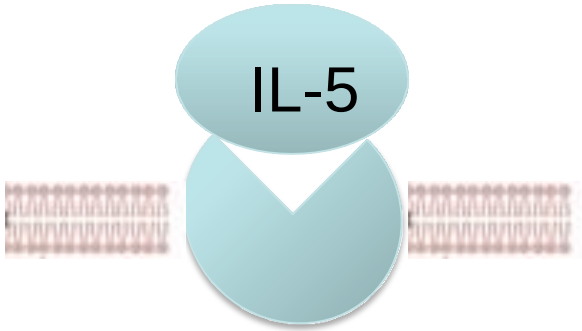




J Allergy Clin Immunol 2013; 132:437-445



Siglec-8



SFK

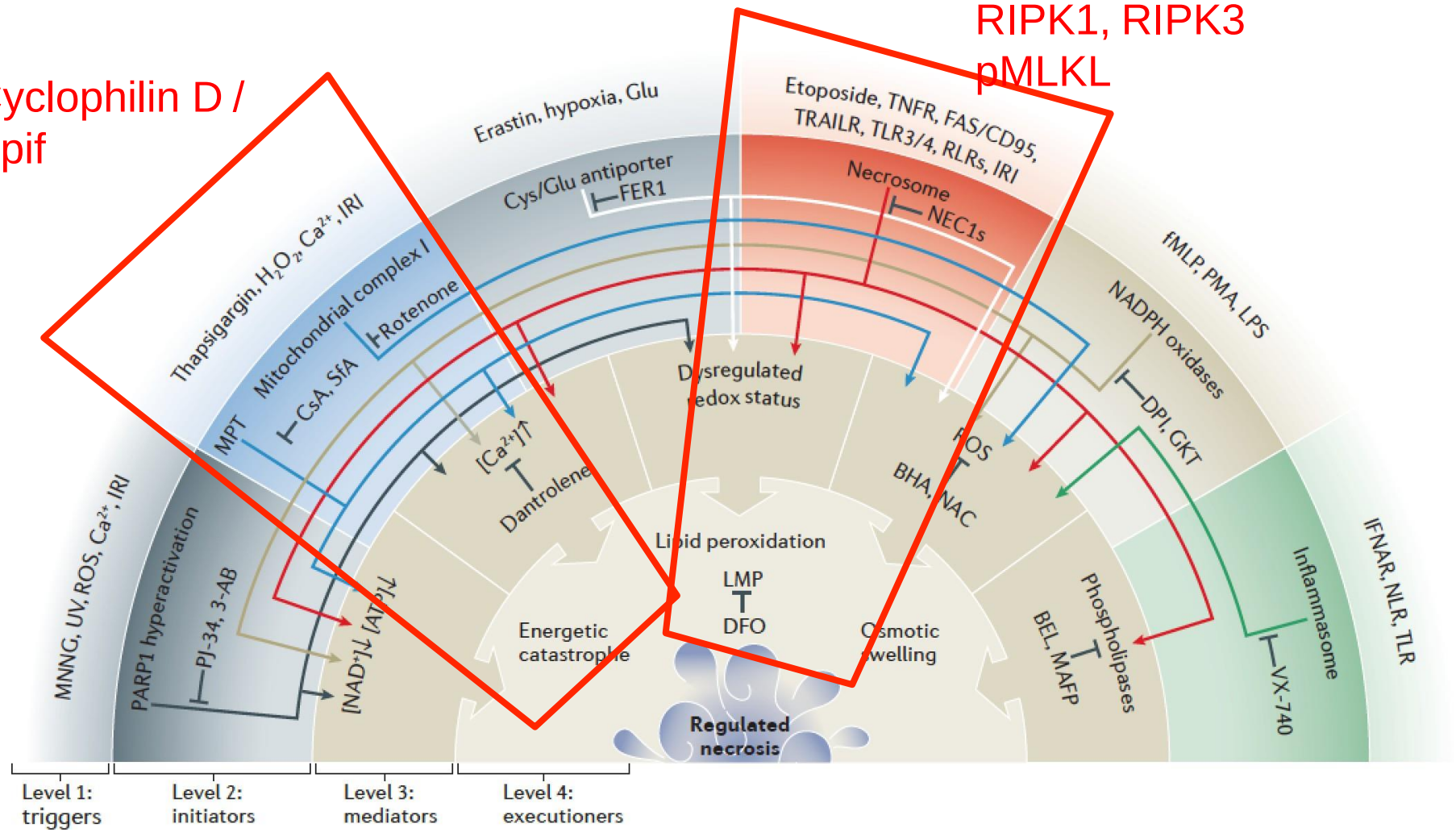
ROS → Erk → Akt STAT5

Cell death

Differentiation
Activation
Priming
Survival

Kano G, Bochner BS, Zimmermann N. Immunobiology. 2017;222:343.

Regulated cell death pathways



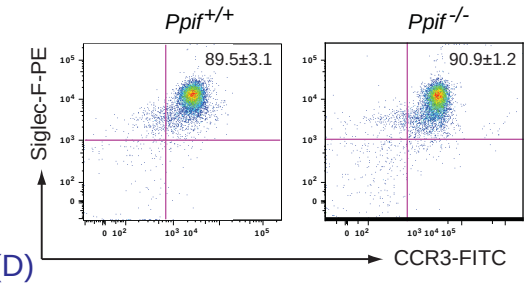
From Vanden Berghe T et al. Nature Reviews 2014;15:135

Mouse eosinophils methods

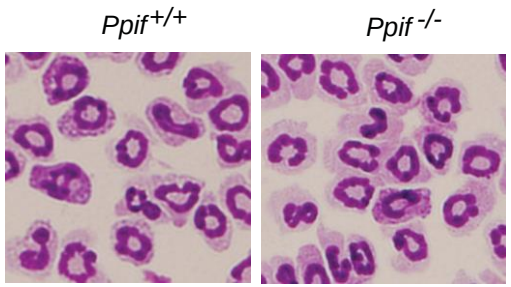
SCF
FLT3L

IL-5

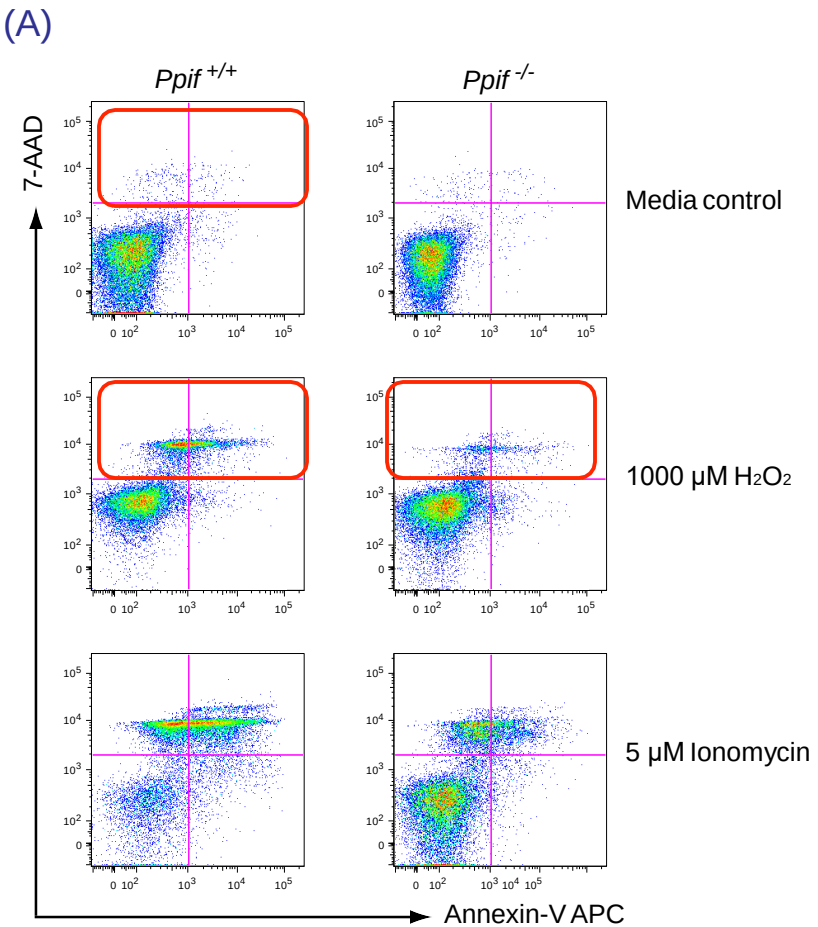
(C)

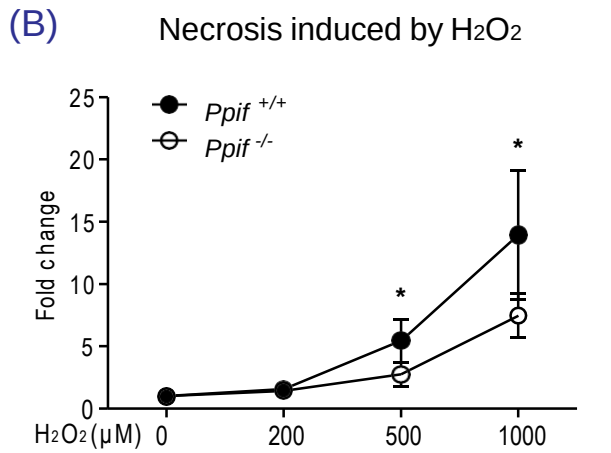


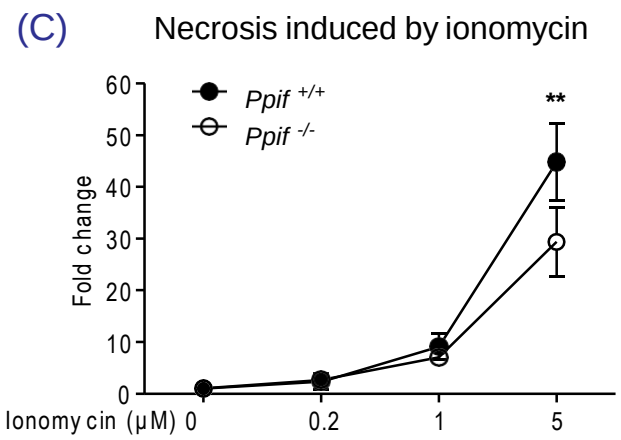
(D)



Cyclophilin D-deficient eosinophils have decreased regulated necrosis

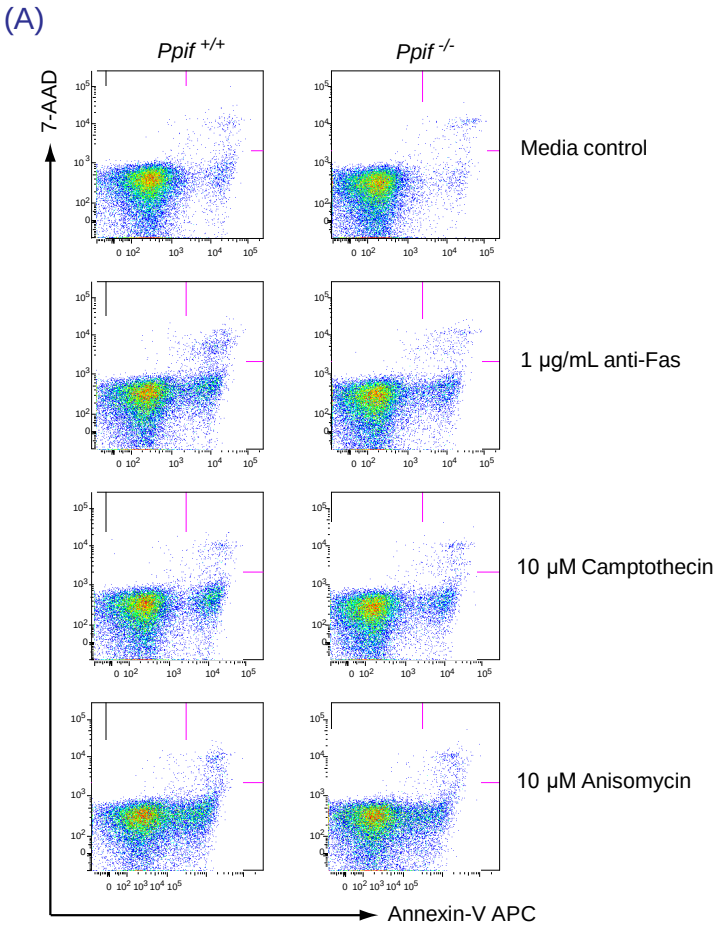


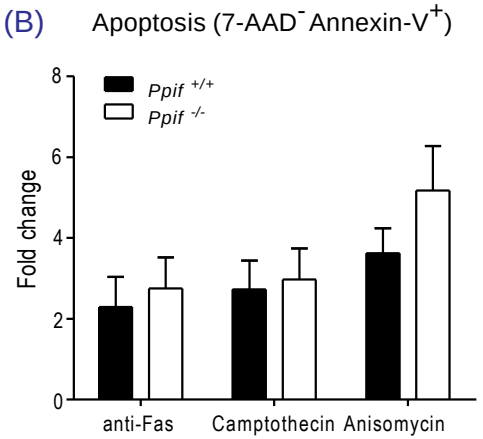


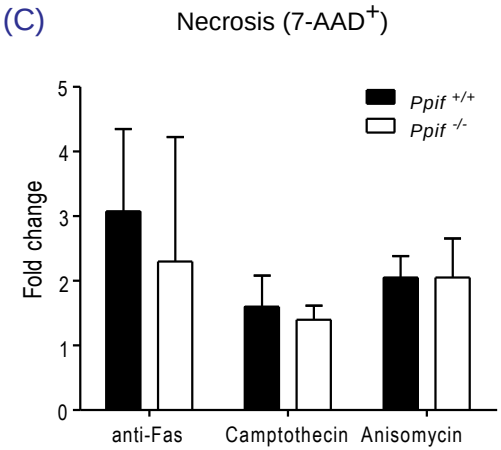


Zhu X et al. AJP: gastro 2016

Cyclophilin D does not have an effect on eosinophil apoptosis

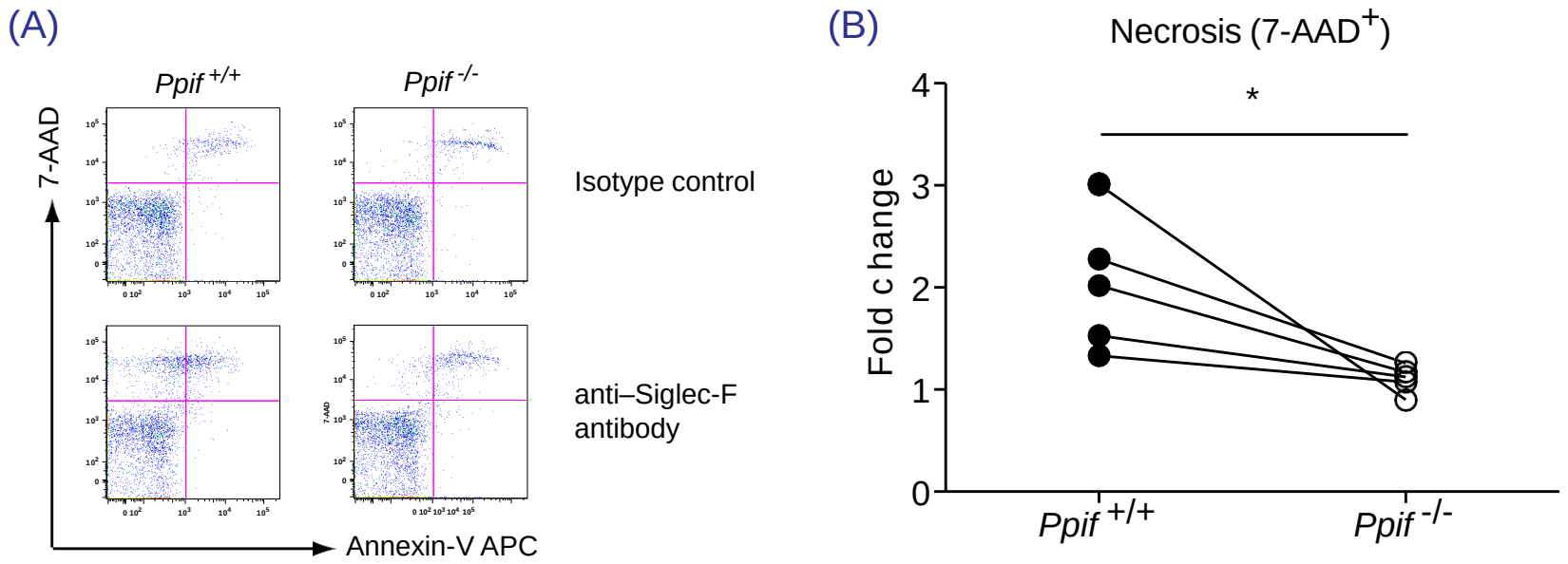






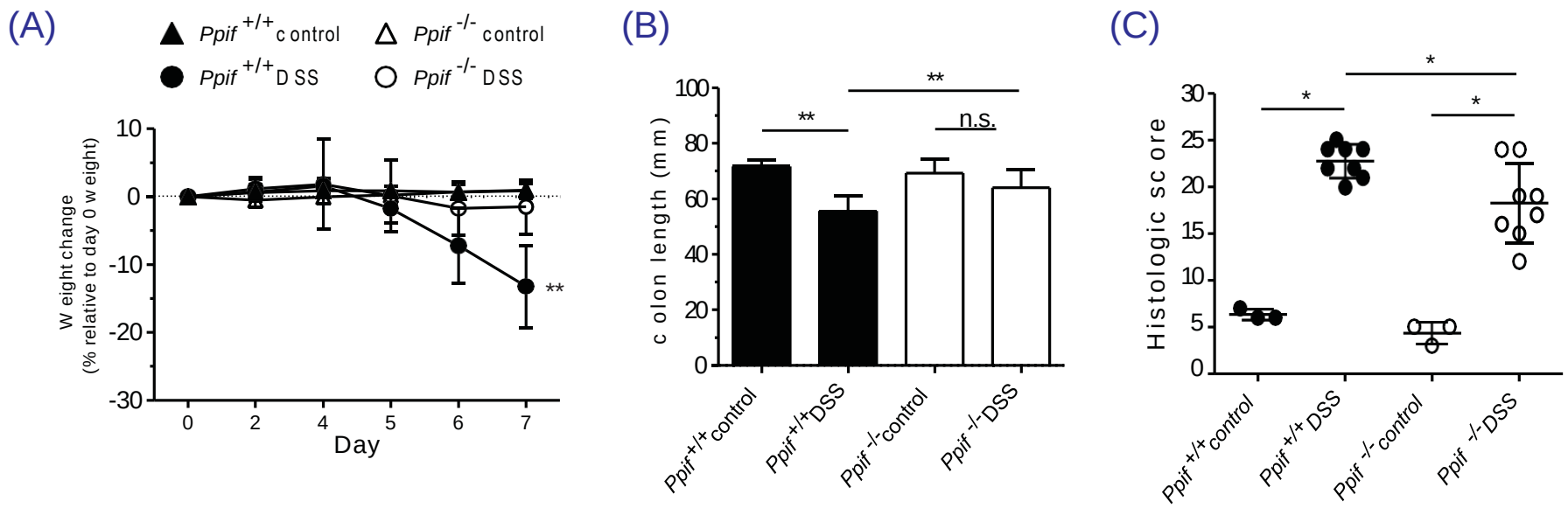
Zhu X et al. AJP: gastro 2016

Cyclophilin D-deficient eosinophils have decreased Siglec-F-induced cell death



Zhu X et al. AJP: gastro 2016

Cyclophilin D-deficient mice are protected in DSS-induced colitis model



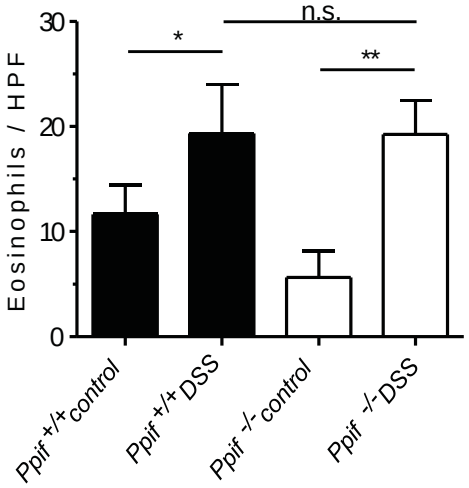
Zhu X et al. AJP: gastro 2016

Decreased cytolysis of eosinophils in the colon of cyclophilin D-deficient DSS-treated mice

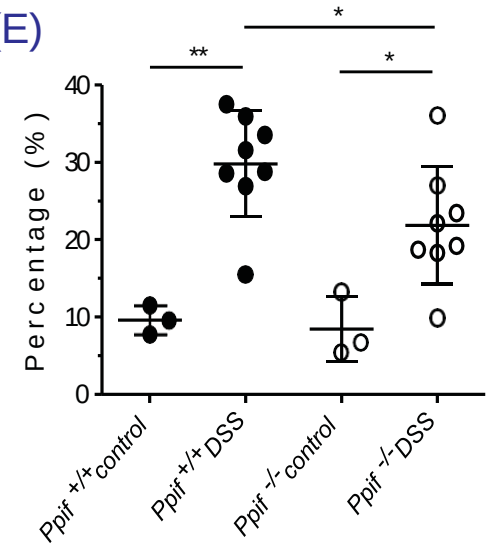
TOTAL

CYTOLYTIC

(D)



(E)



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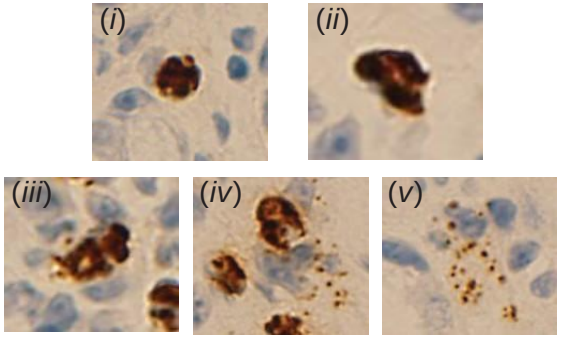
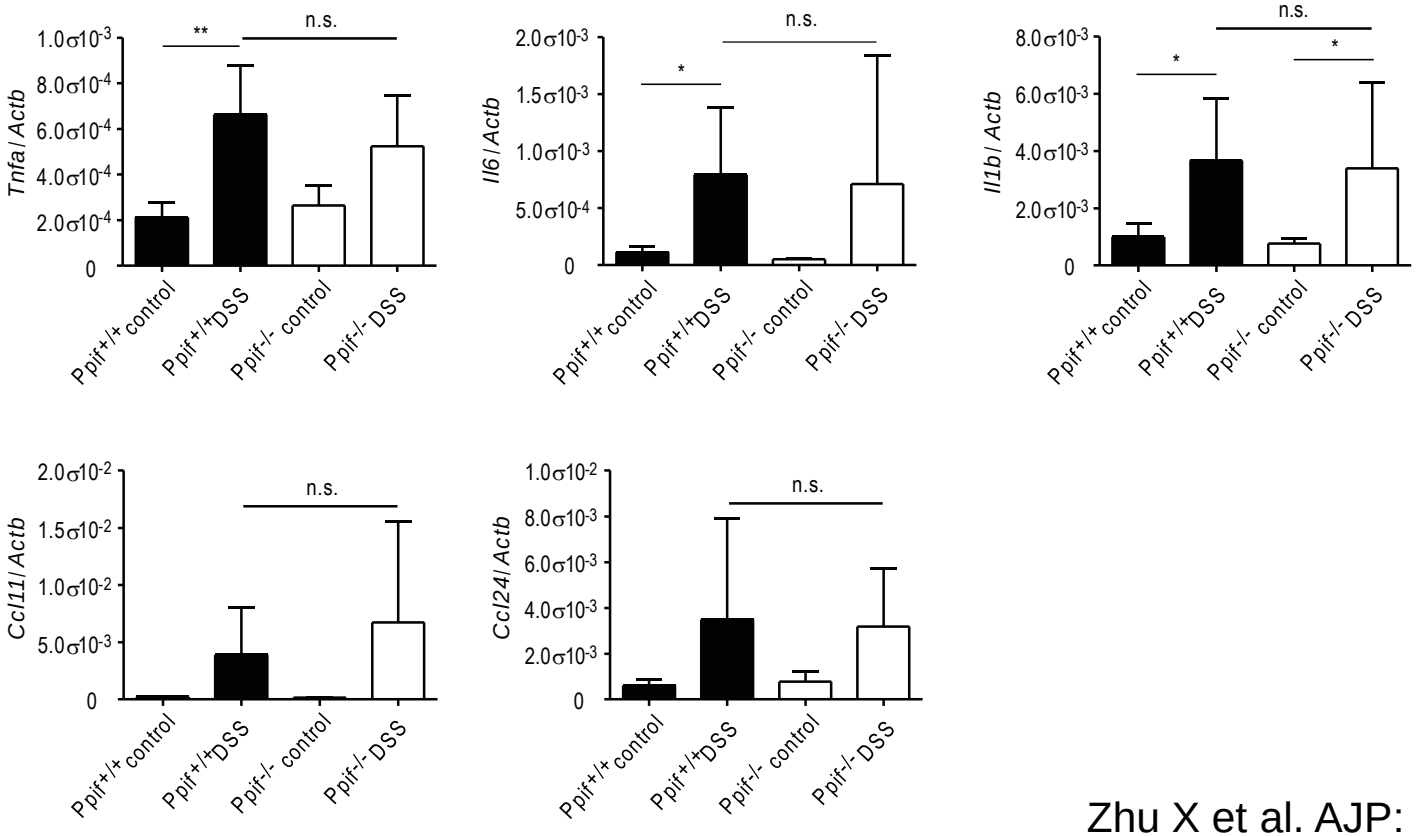


Zhu X et al. AJP: gastro 2016

Inflammatory milieu is not altered by cyclophilin-D deficiency

(G)



Zhu X et al. AJP: gastro 2016

Summary

- Cyclophilin D-mediated regulated necrosis pathway is active in eosinophils
- Siglec-F-induced cell death is mediated by cyclophilin D, at least in part
- Eosinophil cytolysis and disease outcomes in DSS colitis are inhibited by inhibition of cyclophilin D-regulated necrosis

Current/future goals

- Define spectrum of cell death pathways in eosinophils *in vivo*
- Determine pathophysiological consequences of these various cell death modalities

Acknowledgement

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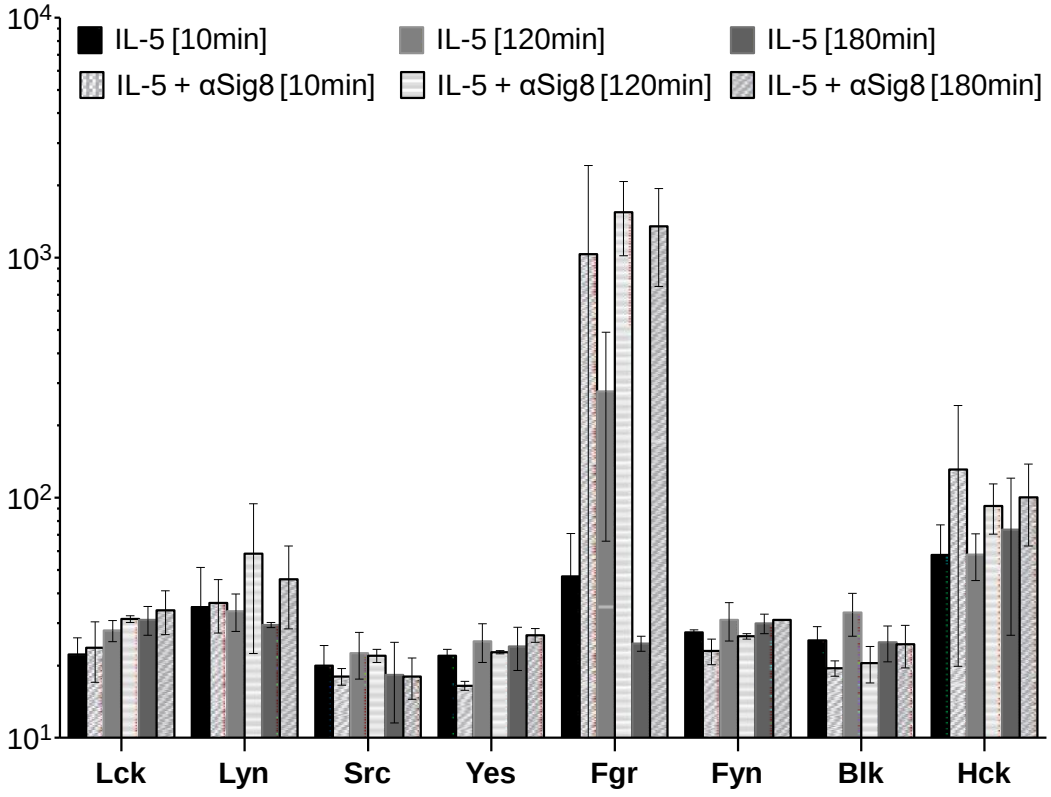
- Blood donors, patients, and CCED RAs and CRCs

Is there a role for Src family kinases (SFK)?

- SFK have been shown to be activated by ITIM-bearing receptors, including Siglecs (SHP dephosphorylates inhibitory pY)
- SFK have been shown to induce ROS accumulation

Src kinase Fgr is phosphorylated by Siglec-8 engagement

C

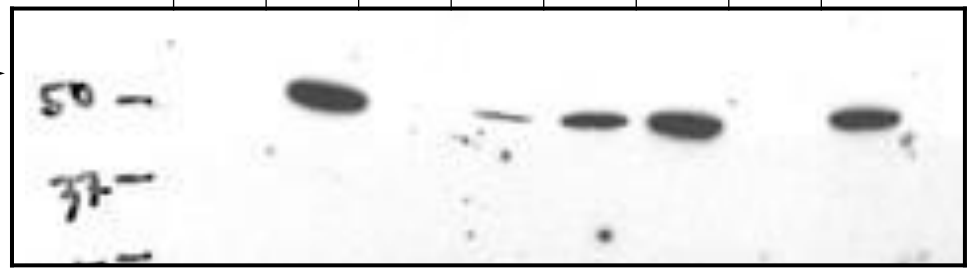


Src kinase is phosphorylated by Siglec-8 engagement

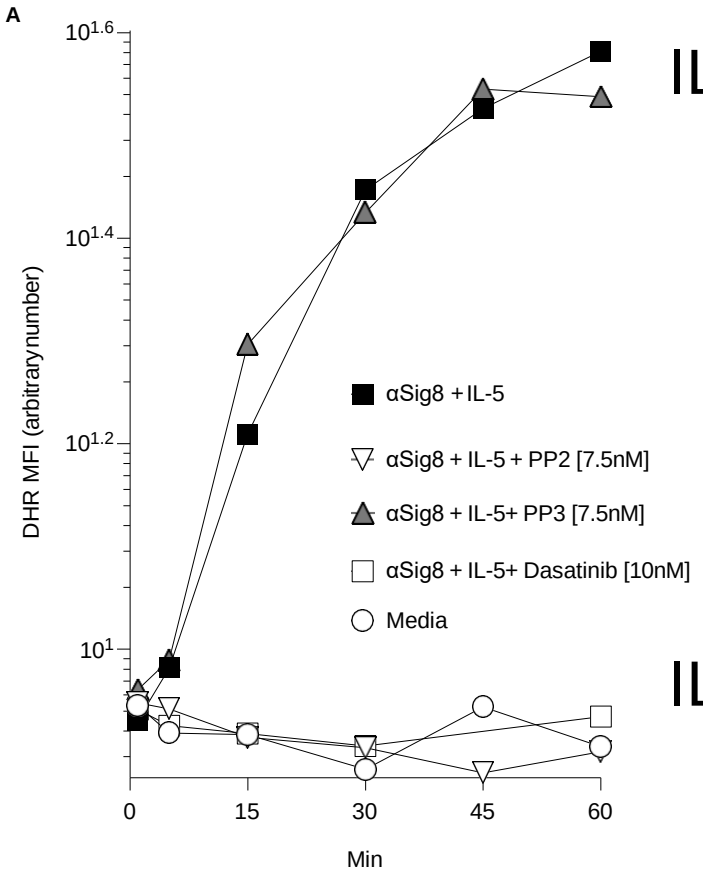
B

	Cytoplasmic Fraction							
Min	10		60		120		180	
IL-5	+	+	+	+	+	+	+	+
7C9	–	+	–	+	–	+	–	+

Phospho-SFK (Tyr537)



SFK are required for Siglec-8-induced ROS production

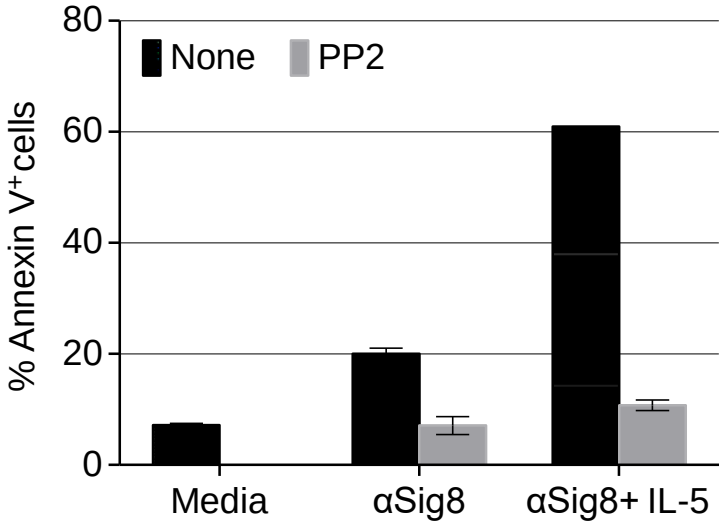


IL-5/anti-Siglec-8
+/- PP3

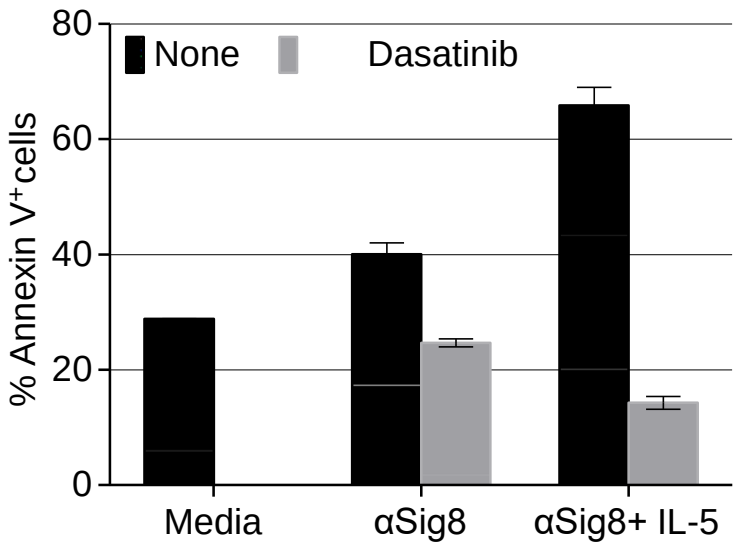
IL-5/anti-Siglec-8
+/- PP2, dasatinib

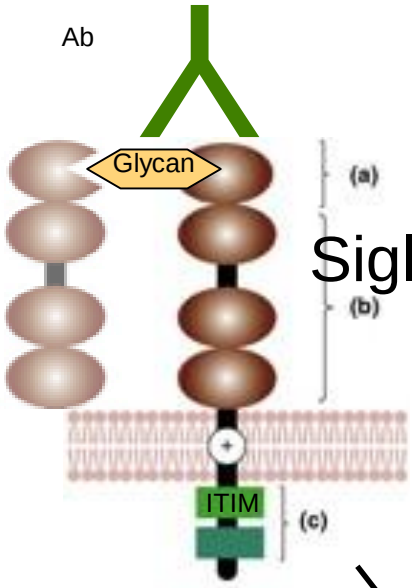
SFK are required for Siglec-8-induced cell death

B

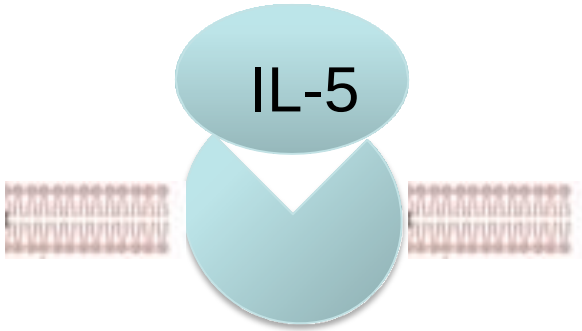


C





Siglec-8



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