The effect of inhibition of CCL2/CCR2 signaling on myeloid lineage cells and osteoclast progenitor subpopulation in collagen induced arthritis

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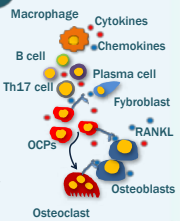
INTRODUCTION

- **Collagen induced arthritis (CIA)** is a mouse model of rheumatoid arthritis
- **Inflammatory mediators** contribute to osteoclast activation and enhanced bone resorption
- **Osteoclast progenitor cells (OCPs)** rise from myeloid lineage and are normally present within bone marrow and circulating monocytes



OBJECTIVES

To investigate effects of CCL2/CCR2 axis blockade on myeloid lineage and myeloid progenitors in mice with collagen-induced arthritis (CIA), especially osteoclast progenitor (OCP) subsets associated to CIA and their osteoclastogenic potential.

METHODS

Treatment protocol

Day 1 — Primary immunisation: CII+CFA
Day 15-20 — Starting treatment
Day 17-22 — Booster dose: CII+IFA
Day 35/45 — Analysis: ELISA, Histology, microCT, Flow cytometry, Cell sorting & cell culture, *In vitro* migration assay

10-week-old male DBA mice

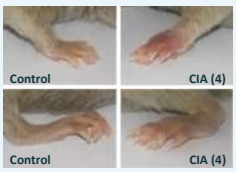
Intradermal injection of chicken type II collagen (CII) emulsified with complete Freund's adjuvant (CFA) at the base of the tail

CII emulsion in incomplete Freund's adjuvant (IFA)

Intraperitoneal injection - 2 day intervals

Groups		Treatment
Control:	ctrl	/
Arthritis:	CIA	PBS
	SMI	molecular inhibitor of CCR2 4mg/kg
	Mtx	methotrexate 2mg/kg
	Mtx+SMI	combined

Visual scoring – 2 day intervals



Sum of grades for each paw:

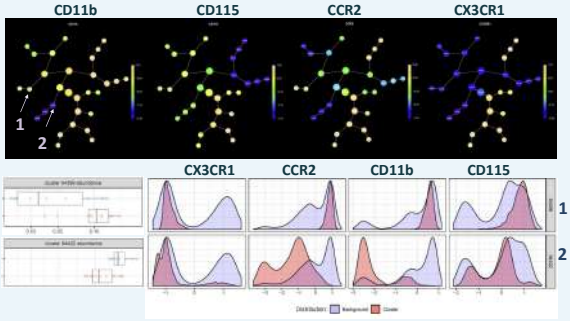
0 = no changes
1 = swelling of one finger/toe
2 = swelling of >1 finger/toe, or slight paw swelling
3 = moderate paw swelling
4 = severe paw swelling

RESULTS

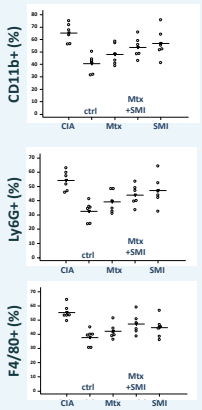
Flow cytometry

Citrus automated flow cytometry analysis

CD3-B220-DX5-Ly6G-CD45+ cells from distal tibia bone marrow (PBM)



Myeloid lineage (PBM)



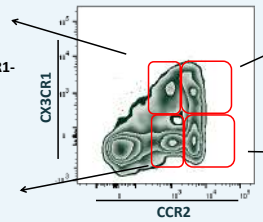
Osteoclast progenitor population

	CCR2lo CX3CR1+	CCR2loCX3CR1+ CD115+
ctrl	12.8±2.4	7.7±1.8
CIA	11.6±1.4	4.6±0.9
SMI	10.8±2.8	3.9±0.8
MTX	13.5±2.7	3.2±2.2
MTX+SMI	12.8±0.2	4.0±0.8

% of CD3-B220-DX5-Ly6G-CD45+ CD11b-/low cells

Distal tibia bone marrow (PBM)

CD3-B220-DX5-Ly6G-CD45+CD11b-/low



	CCR2lo CX3CR1-	CCR2loCX3CR1- CD115+
ctrl	10.7±0.9	1.7±0.7
CIA	13.5±2.2	0.6±0.1
SMI	13.3±2.2	0.7±0.4
MTX	8.3±0.7	0.5±0.3
MTX+SMI	7.4±0.6	0.5±0.2

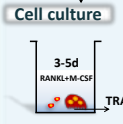
	CCR2+ CX3CR1+	CCR2+CX3CR1+ CD115+
ctrl	10.3±1.1	8.0±2.0
CIA	11.0±2.6	7.7±2.0
SMI	10.3±2.4	5.3±1.0
MTX	15.7±2.8	4.1±2.4
MTX+SMI	17.9±1.7	5.5±1.6

	CCR2+ CX3CR1-	CCR2+CX3CR1- CD115+
ctrl	10.7±4.2	6.9±4.4
CIA	15.0±2.9	7.2±2.2
SMI	18.0±1.1	7.1±2.5
MTX	9.1±1.6	1.6±0.8
MTX+SMI	9.6±3.0	2.1±0.8

FACS sort

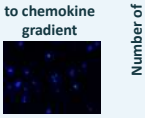
CD3-B220-DX5-Ly6G-CD45+ CD11b-/lowCD115+

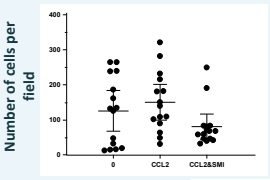
Cell culture

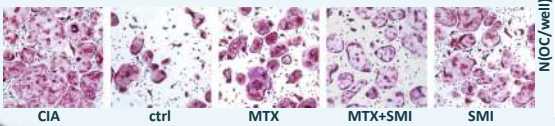


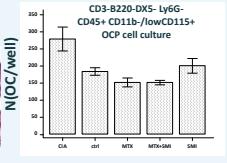
In vitro migration assay

DAPI+ cells attracted to chemokine gradient

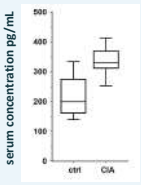






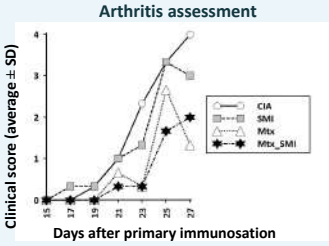


CCL2 serum levels in CIA

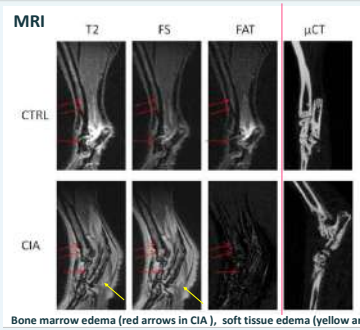


RESULTS

Arthritis assessment

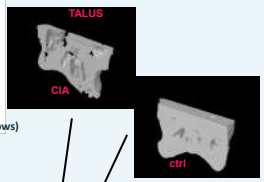


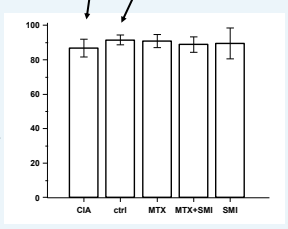
MRI



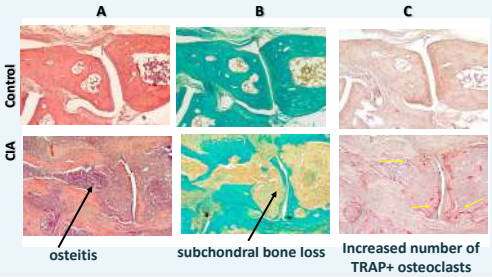
Bone marrow edema (red arrows in CIA), soft tissue edema (yellow arrows)

Hind paw microcomputerised tomography scans with bone erosions and increased bone volume loss in CIA





1st tarsometatarsal joint histology



A. Haematoxylin and eosin
B. Goldner trichrome
C. TRAP stain

osteitis
subchondral bone loss
Increased number of TRAP+ osteoclasts

CONCLUSIONS

- Osteoclast progenitors (OCP) are induced in CIA
- OCPs express CCR2+ at the substantial level and are susceptible to chemotactic signals
- OCP subset expressing CCR2 may contribute to bone resorption in arthritis
- Therapeutic blocking of CCL2/CCR2 chemokine signaling may be a promising approach to antagonize enhanced osteoresorption in inflammatory diseases

DISCLOSURE

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