

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

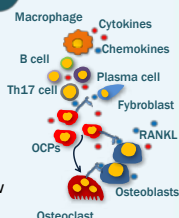
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## INTRODUCTION

- 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

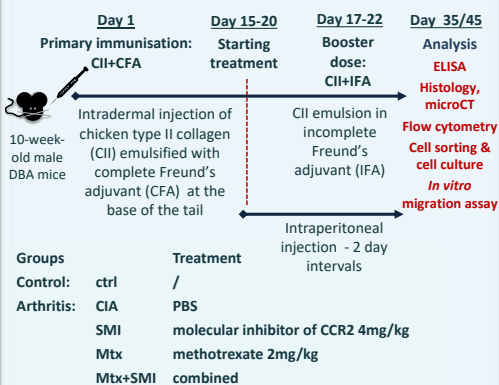


## OBJECTIVES

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



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



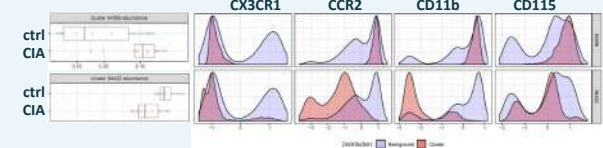
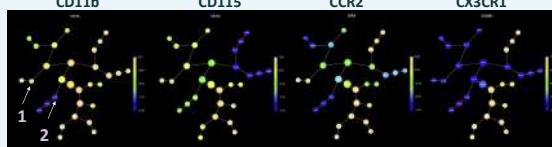
Visual scoring – 2 day intervals  
Sum of grades for each paw:  
0 = no changes  
1 = swelling of one finger/toe  
2 = swelling of >1 finger/toe, or slight paw swelling  
3 = moderate paw swelling  
4 = severe paw swelling

## RESULTS

### Flow cytometry

#### Citrus automated flow cytometry analysis

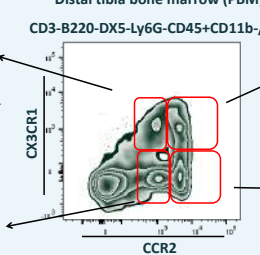
CD3-B220-DX5-Ly6G-CD45+ cells from distal tibia bone marrow (PBM)



#### 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

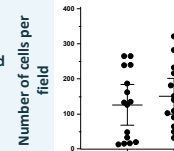
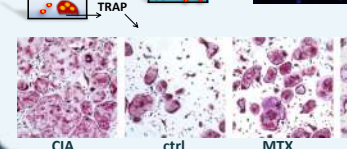
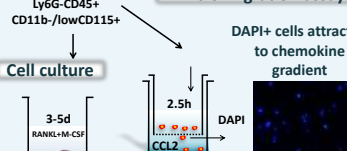


	CCR2+ CX3CR1+	CCR2+CX3CR1+ CD115+
ctrl	10.3±1.1	8.0±2.0
CIA	11.0±2.6	7.7±2.0
SMI	10.3±2.4	5.3±1.0
MTX	15.7±2.8	4.1±2.4
MTX+SMI	17.9±1.7	5.5±1.6

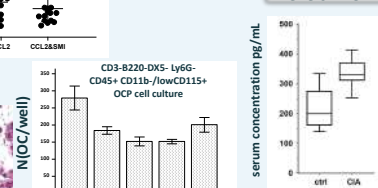
#### FACS sort

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

#### In vitro migration assay

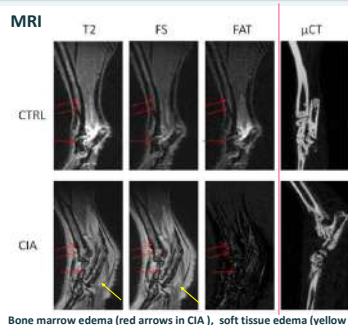
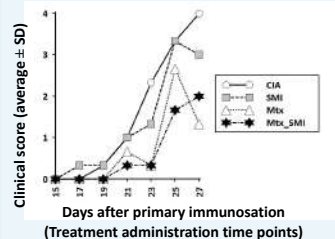


#### CCL2 serum levels in CIA

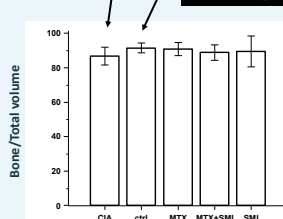
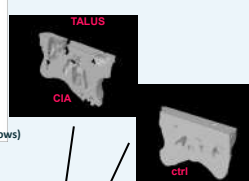


## RESULTS

### Arthritis assessment



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



### 1st tarsometatarsal joint histology



## 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

This work has been fully supported by Croatian Science Foundation under the projects 2414 and 7406.

