PhD Day
Zagreb, May 22nd 2015

CHEMOKINE RECEPTOR PROFILE OF OSTEOCLAST PROGENITOR CELLS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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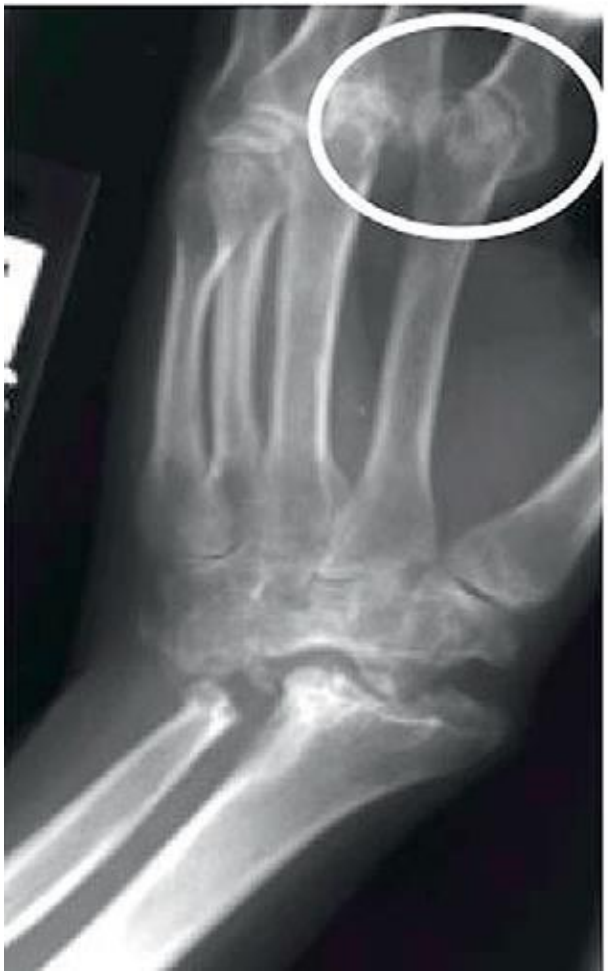
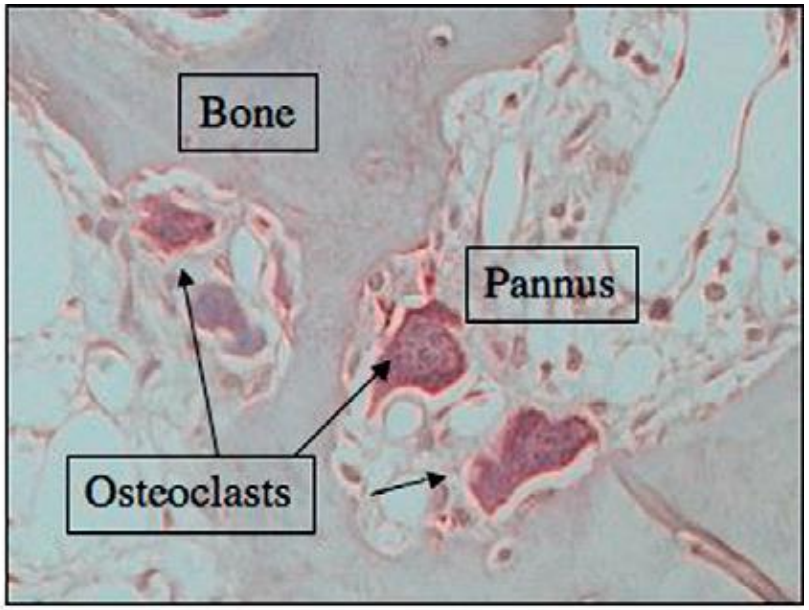
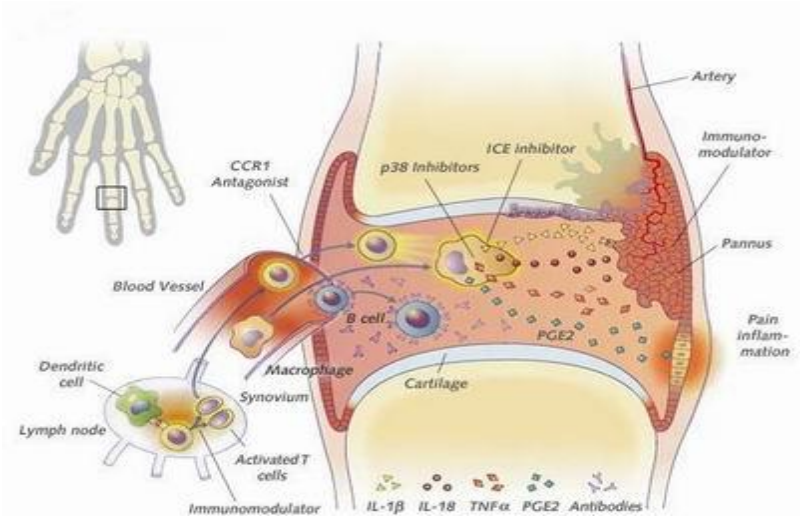
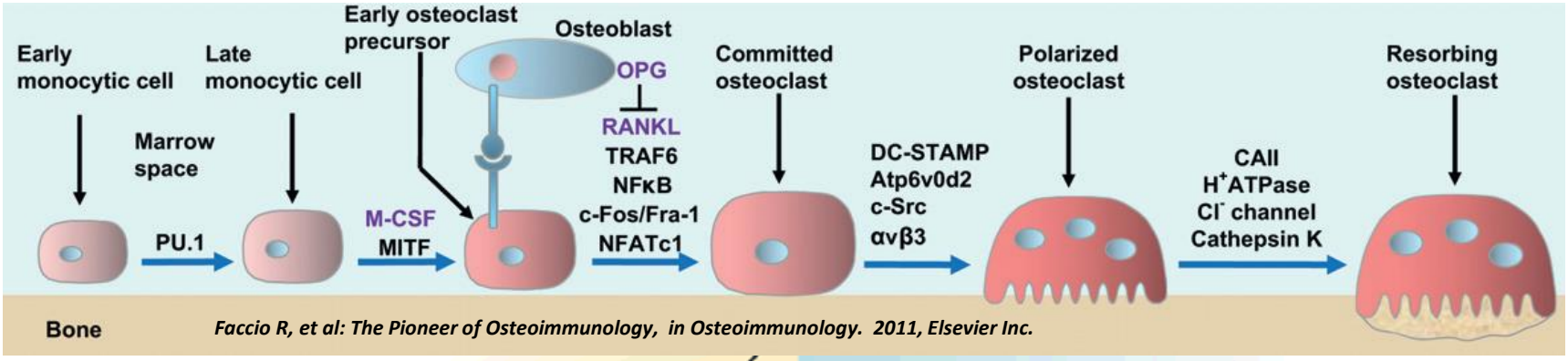
Rheumatoid arthritis



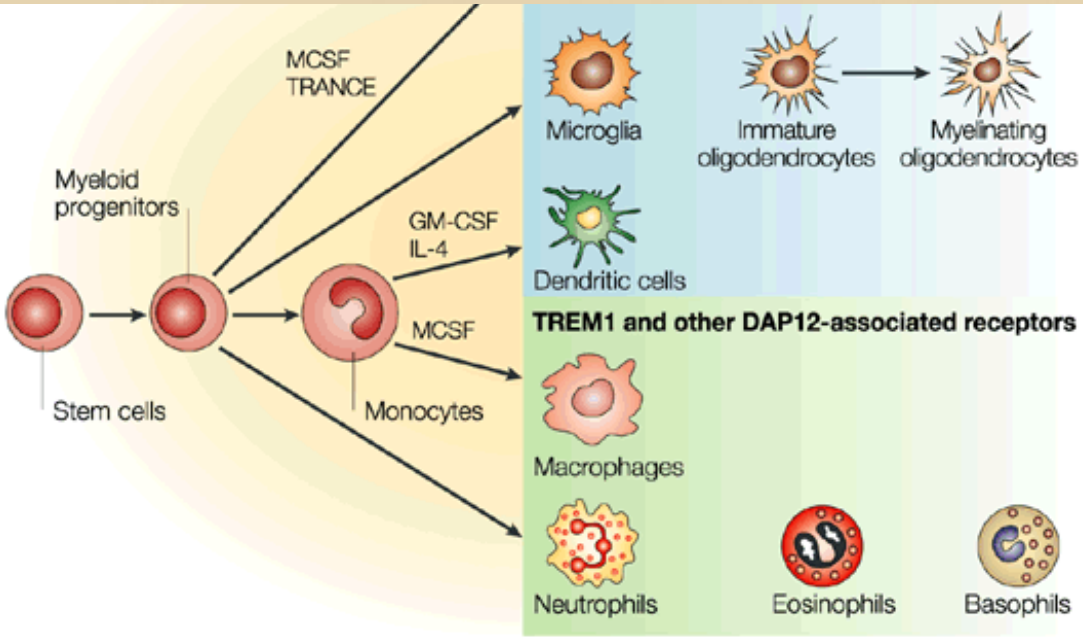




Osteoclast origin and differentiation



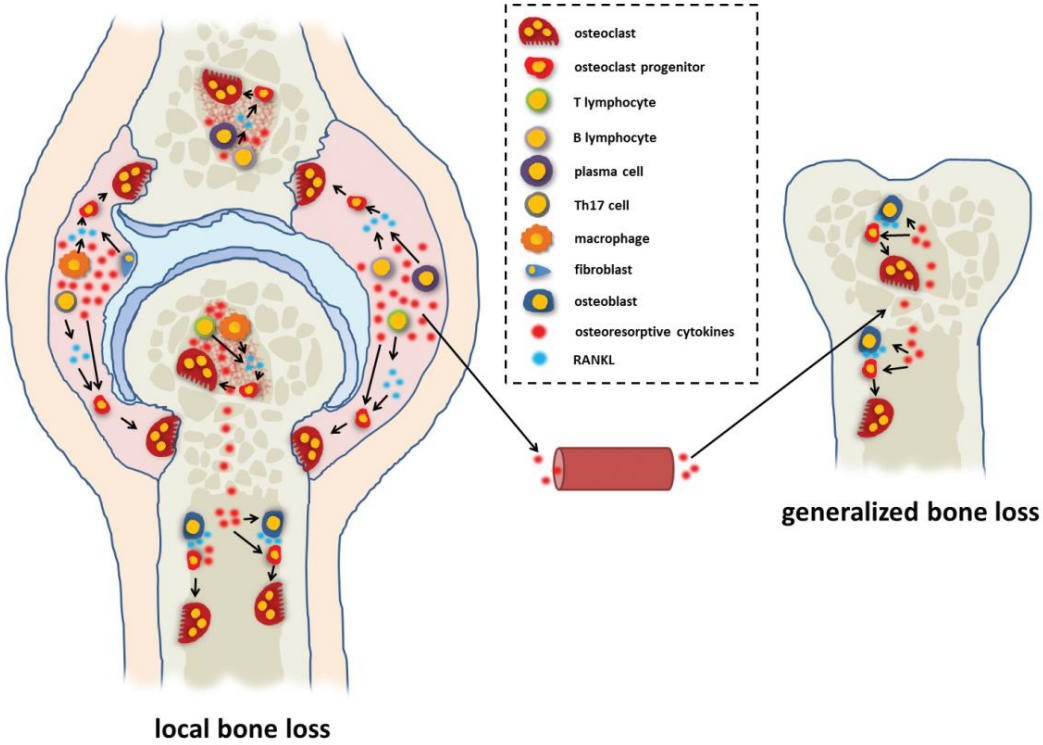
Faccio R, et al: The Pioneer of Osteoimmunology, in Osteoimmunology. 2011, Elsevier Inc.



Marco Colonna (2003)

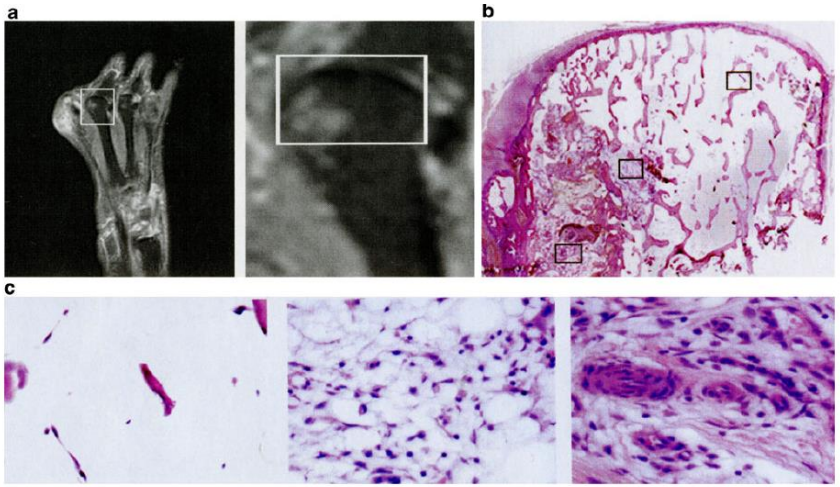
Nature Reviews | Immunology

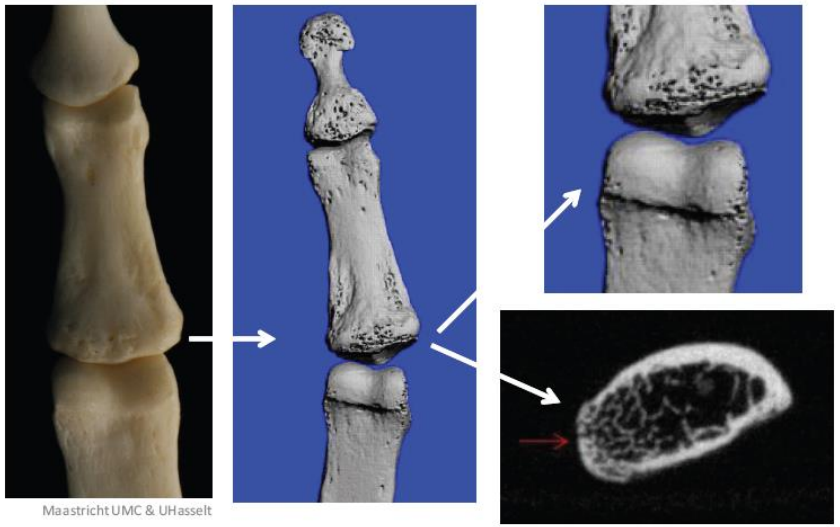
Bone loss in RA: systemic, periarticular and focal



Flegar et al. (2015) Period Biol

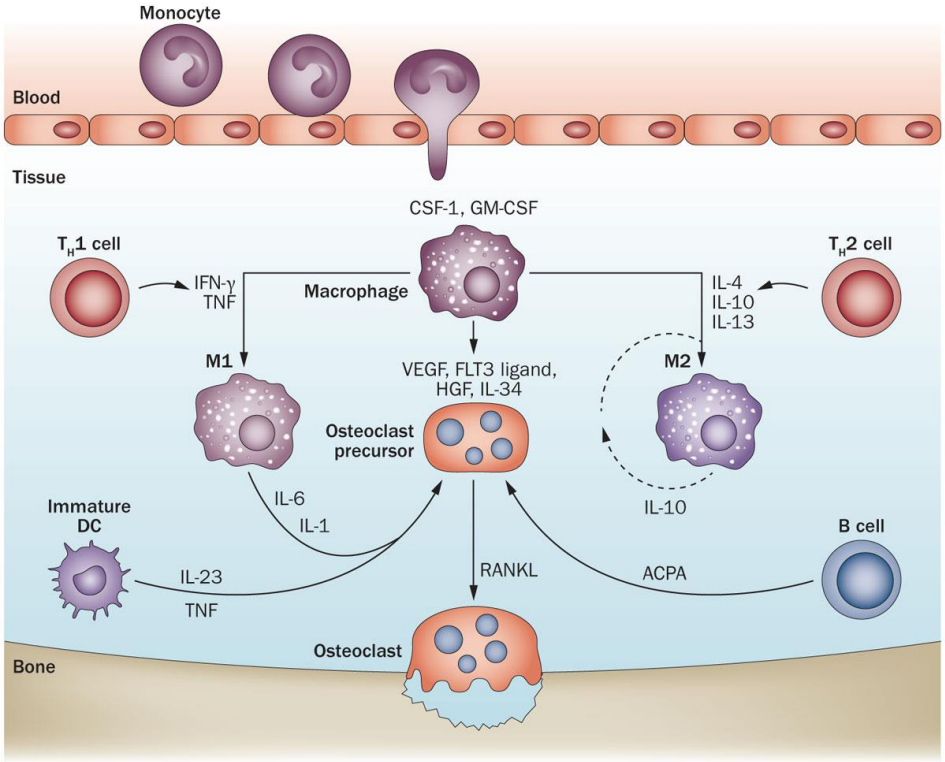
BME (OSTEITIS)





BONE EROSIONS

Inflammation-induced osteoclast activation

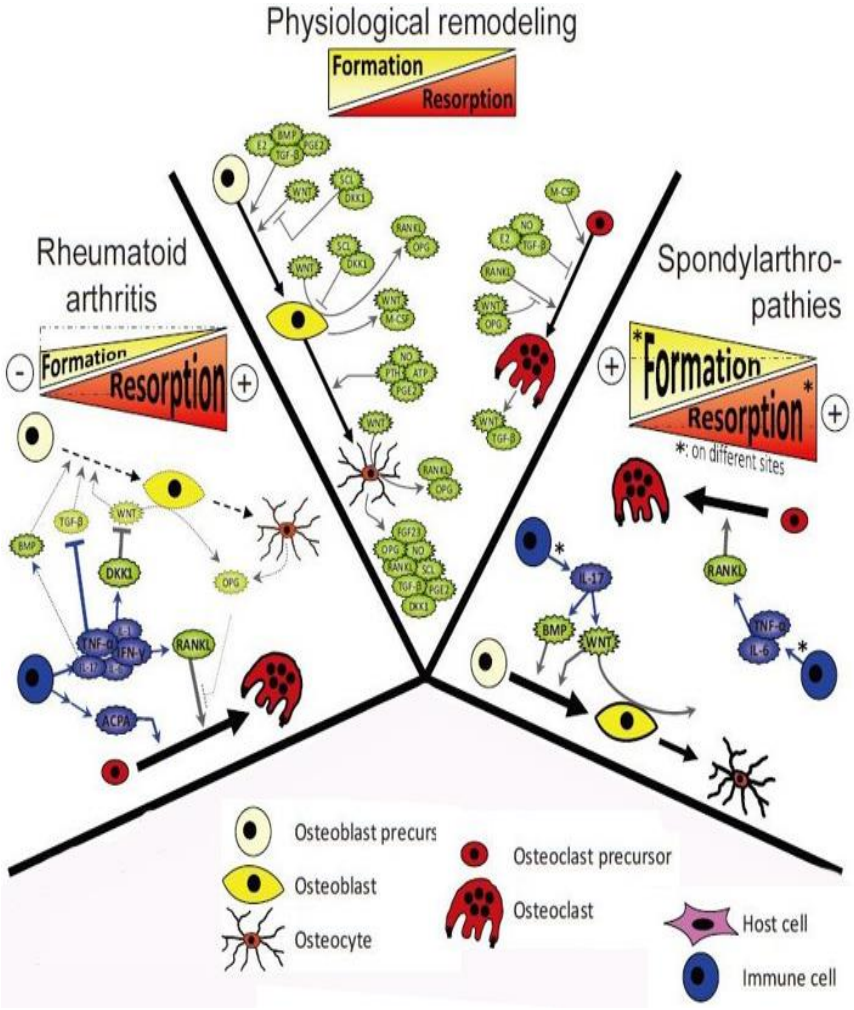


Adamopoulos, I. E. & Mellins, E. D. (2014) Nat. Rev. Rheumatol

Low BMD and osteoporosis – increased fracture risk (mortality!)

- **Rheumatoid arthritis (RA)** *(Lodder M et al. Ann Rheum Dis 2004)*
- **Systemic lupus erythematosus (SLE)** *(Zhu TZ et al. J Rheumatol 2014)*
- **Systemic sclerosis** *(Omair MA et al. Clin Exp Rheumatol 2014)*
- **Dermatomyositis** *(de Andrade DC et al. Rheumatol Int 2012)*
- **Insulin dependent diabetes mellitus** *(Khan TS et al. J Osteoporos 2015)*
- **Multiple sclerosis** *(Kampman MT et al. Acta Neurol Scand Suppl 2011)*
- **Coeliac disease** *(Tau C et al. Eur J Clin Nutr 2006)*
- **Chron's disease** *(Targownik LE et al. Curr Opin Gastroenterol. 2014)*
- **Primary biliary cirrhosis** *(Mounach A et al. J Bone Miner Metab. 2008)*
- **Sjogren's disease** *(Gravani et al. Arthritis Research & Therapy 2015)*

Bone resorption in autoimmune conditions



Modified, according to Gosset M, Int J Orthop 2014;1:124-9.

Prominent osteoresorption and bone erosions

- Rheumatoid arthritis
- Juvenile idiopathic arthritis
  (Malattia C et al. Arthritis Rheum. 2008)

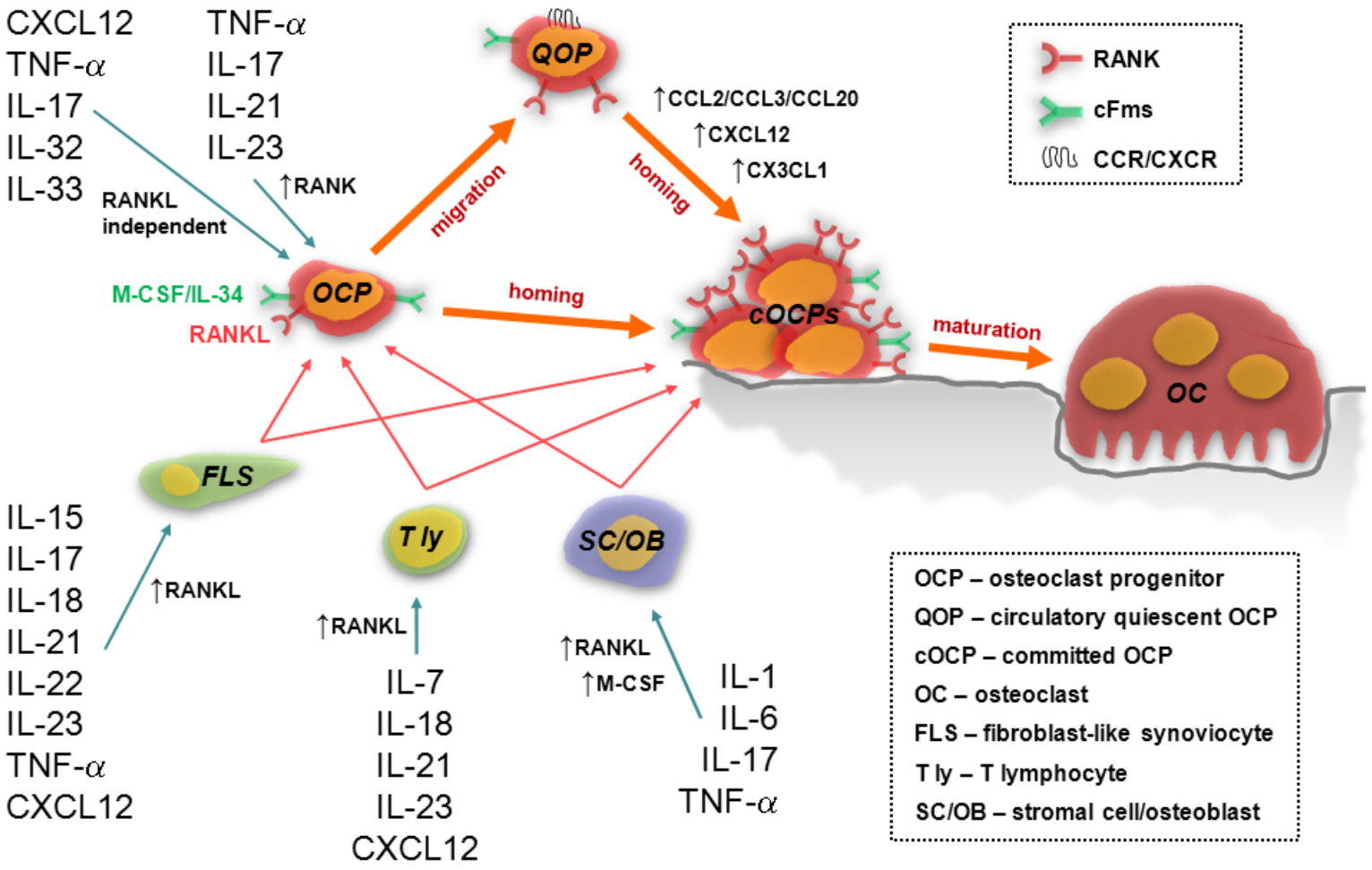
Excessive bone formation with or without osteolysis

- Ankylosing spondylitis
- Reactive arthritis
- Psoriatic arthritis

Autoimmunity affecting joints without alterations in bone

- Entheropatic arthritis, Systemic lupus erythematosus, Sjogren‘s syndrome, Familial mediterannean fever

Regulation of OCP trafficking



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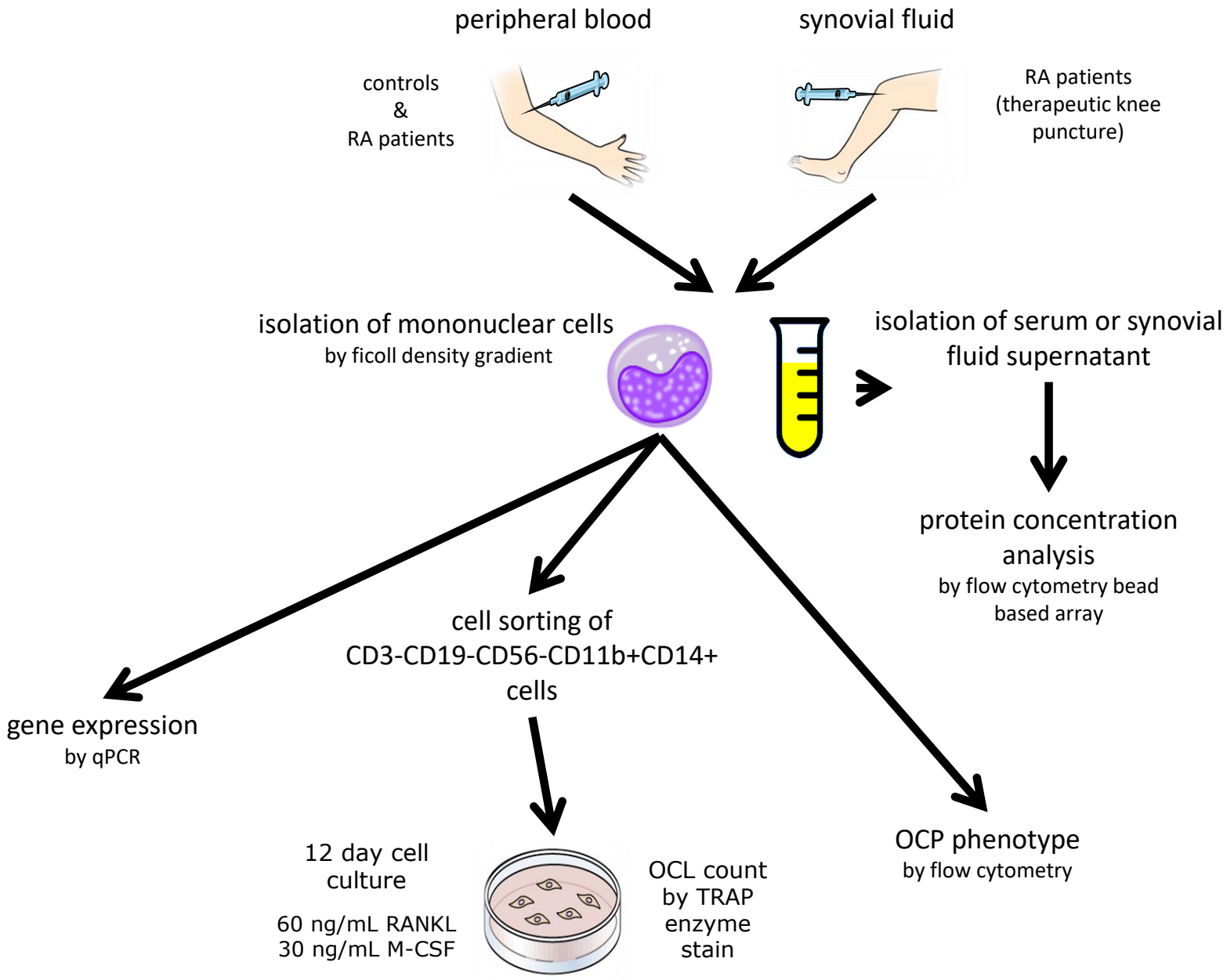
Materials and methods



Osteoclast progenitor phenotype

MOUSE **lymphoid negative, CD11b^{low}, Ly6C^{hi}, CD115^{+}, CCR2^{+}**

Table 1 Surface marker expression profile of mouse osteoclast progenitor populations

Osteoclast progenitor phenotype	Source[a]
CD117^{+}CD115^{+}RANK^{-}	BM
B220^{-}CD3^{-}CD11b$^{-/lo}$CD115^{+}CD117^{+}CX3CR1^{+}	BM
B220^{-}CD3^{-}NK1.1^{-}CD11b^{+}Ly6C^{hi}CD115^{+}CX3CR1^{+}	PBL, SPL
B220^{-}CD117^{+}CD115^{+}CD11b^{lo}CD27^{+}	BM
CD115^{lo}RANK^{hi} (mostly CD11b^{-}F4/80^{-}Gr-1^{-})	BM, PBL
CD11b^{+}Gr-1^{+}CD80^{lo}CD115^{+}F4/80^{-}	BM (TM)
CD11b^{+}Gr-1^{+}CCR2^{+}	PBL, SYN (CIA)
CD3^{-}B220^{-}Ter119^{-}CD11b$^{-/lo}$Ly6C^{hi}	BM (SKG)
CD135^{lo}CD11c^{-}CD115^{+}CD117^{+}CX3CR1^{+}RANK^{-} CD11b^{+}Gr-1(Ly6G)$^{-/lo}$	BM, PBL (hTNF-Tg)
B220^{-}CD3^{-}F4/80^{-}CD117^{-}CD11b^{hi}CD115^{+}	SPL (hTNF-Tg)
CD11b^{+}RANK^{+}	SPL (IFN-γR KO CIA)

HUMAN **lymphoid negative, CD11b^{+}, CD14^{+}, CD16^{+}, CD115^{low}**

Table 2 Surface marker expression profile of human osteoclast progenitor populations

Osteoclast progenitor phenotype	Source[a]
CD14^{+}; CD11b^{+}; or CD61^{+}	PBL
CD3^{-}CD19^{-}CD56^{-}CD14^{+}CD11b^{+}	PBL
CD14^{+}CD11b^{+} (intß1^{+}intß2^{+}intß3^{-})	PBL
CD14^{hi}CD11b^{+}CD51/61^{+}CD16^{+}	PBL (MM)
CD14^{+}RANK^{hi}	PBL, BM
CD45^{+}CD14^{+}CD51/61^{+}CD115^{+}RANK^{+}	GCT
CD14^{+}CD16^{-}(CD33^{hi})CD115^{lo}	PBL, SYN (RA)
CD16^{+}(gp-39): CD3^{-}CD4^{-}CD8^{-}CD20^{-}CD56^{-}CD33^{lo}MHCII^{lo}CD14^{lo}	PBL, SYN (RA)
CD3^{-}CD19^{-}CD14^{+}CD16^{+}DC-STAMP^{+}	PBL (PsA)
CD14^{+}(MHCII^{+})CD16^{+}	PBL (PsA)

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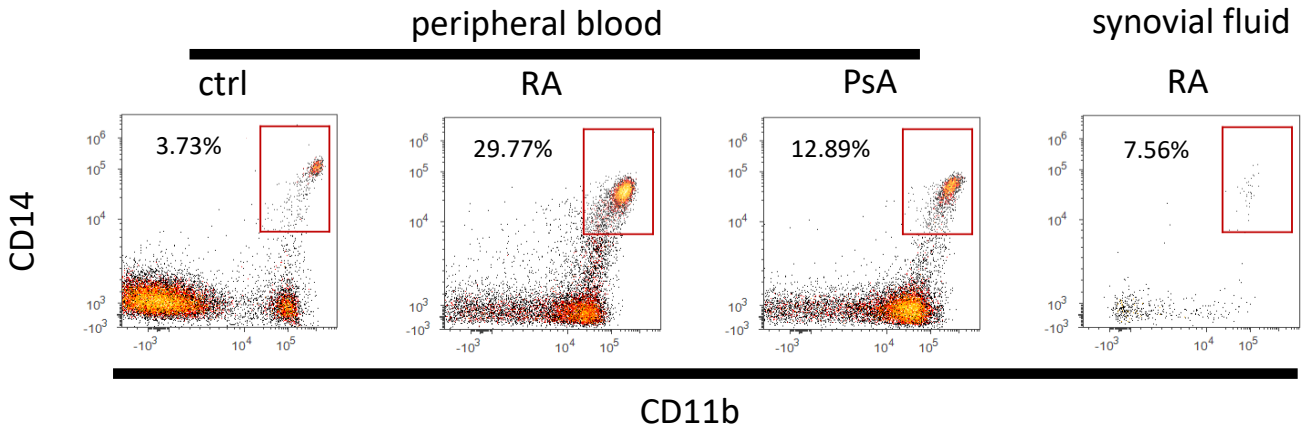
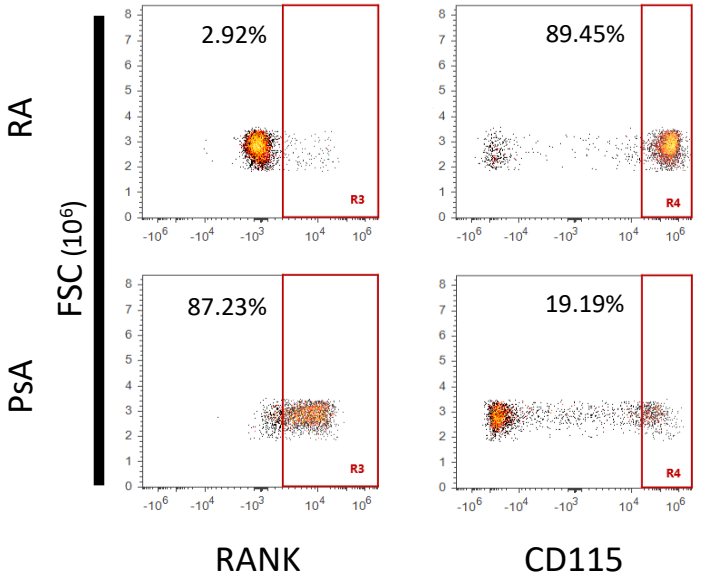
Frequency and phenotype of OCPs

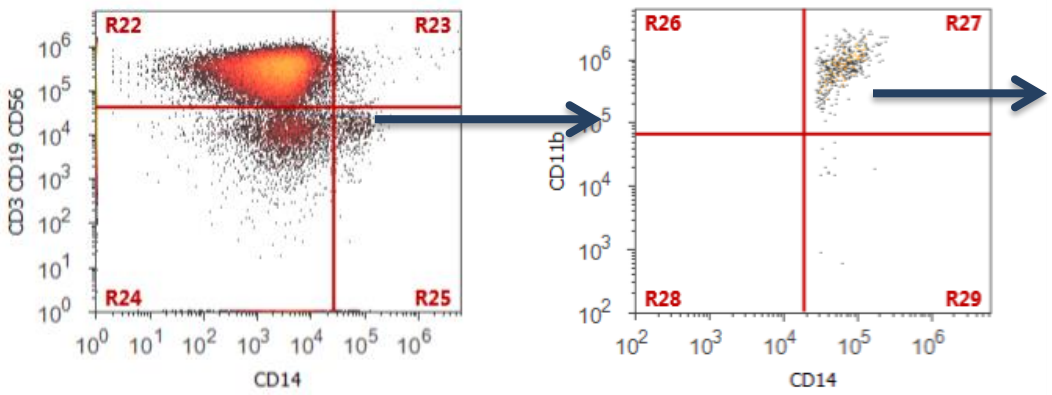
Phenotype of $CD3^{-}CD19^{-}CD56^{-}$ mononuclear cells



Phenotype of $CD11b^{+}CD14^{+}$ lymphoid marker negative cells



Osteoclastogenic potential of OCPs



12 day cell culture

OCL count by TRAP enzyme stain

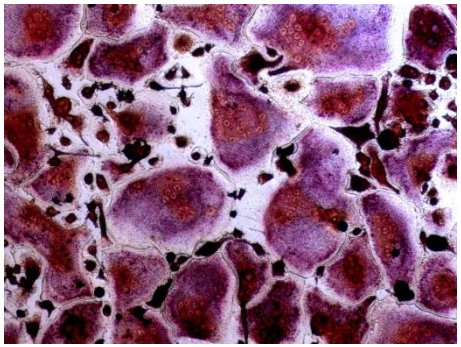
4 days

60 ng/mL M-CSF

8 days

60 ng/mL RANKL
30 ng/mL M-CSF

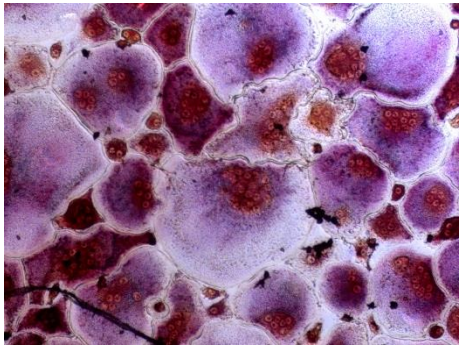
control



207 (92-514)

p = 0,7970

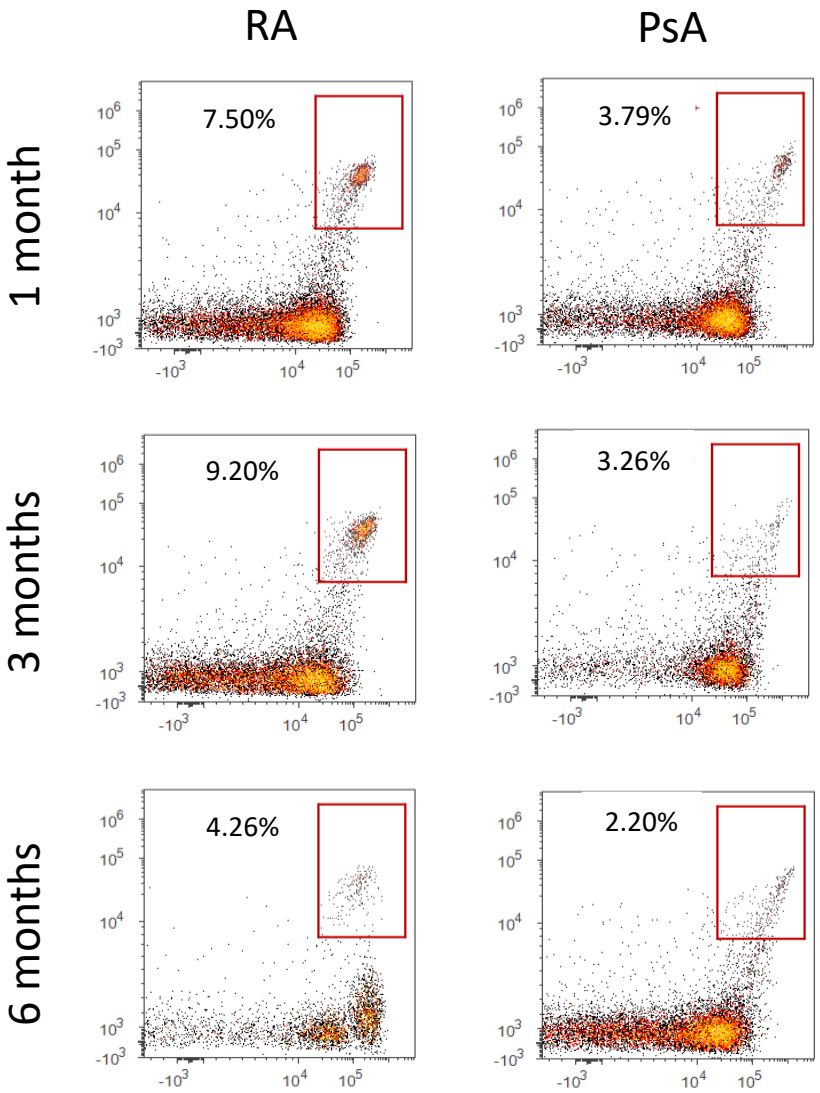
RA



320 (77-481)

number of osteoclasts per well [median (IQR)]

Frequency of OCPs during anti-TNF therapy

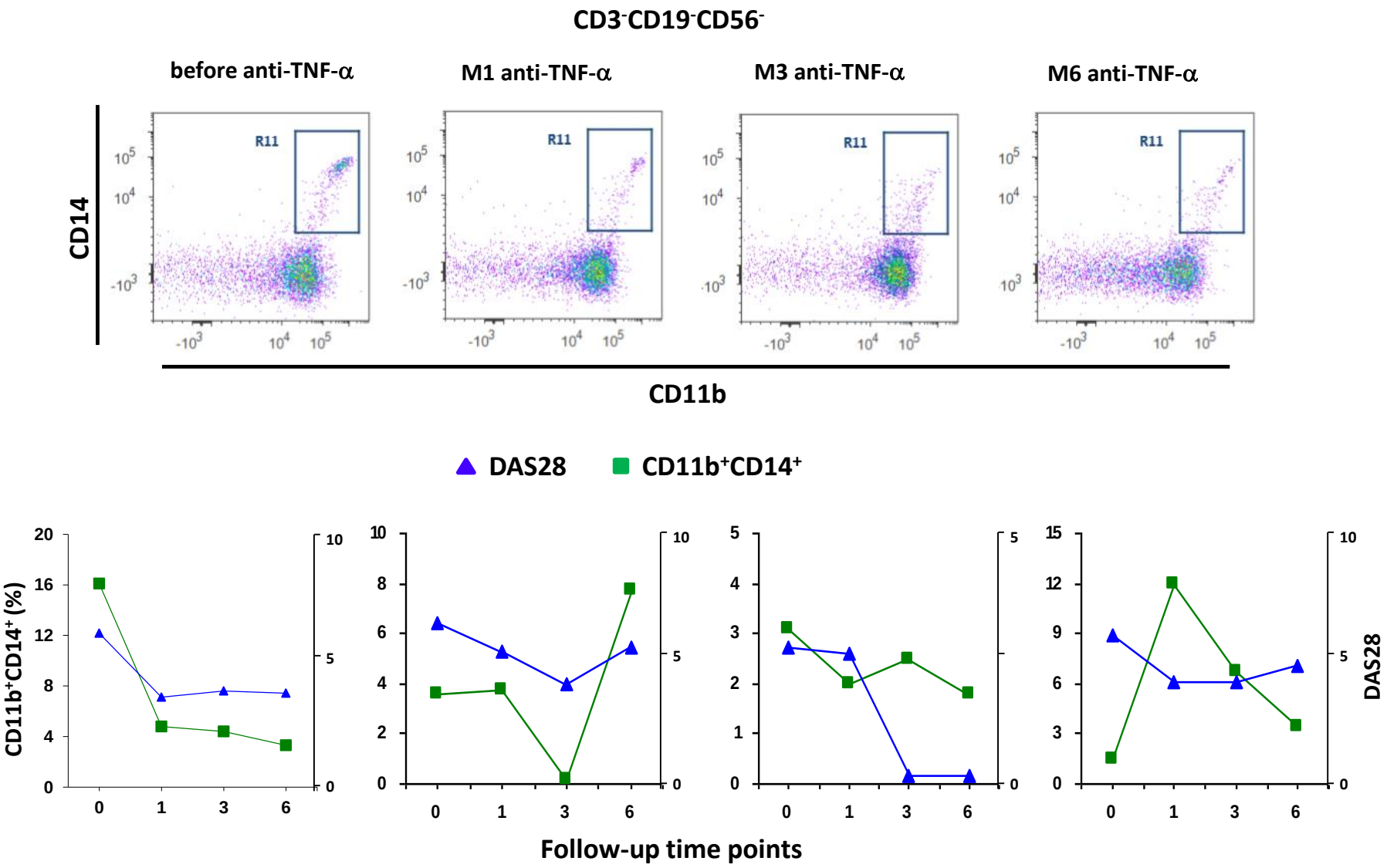


Osteoclastogenic culture in relation to anti-TNF therapy

1st month	3rd month	6th month
49±8	275±47	384±56

number of osteoclasts per well

Correlation of DAS28 with OCP frequency



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Conclusions #1

- OCPs , found among the CD3−CD19−CD56−CD11b+CD14+ subpopulation of peripheral blood mononuclear cells, are able to differentiate into mature OCs in vitro, and appear to be specifically induced in RA and PsA

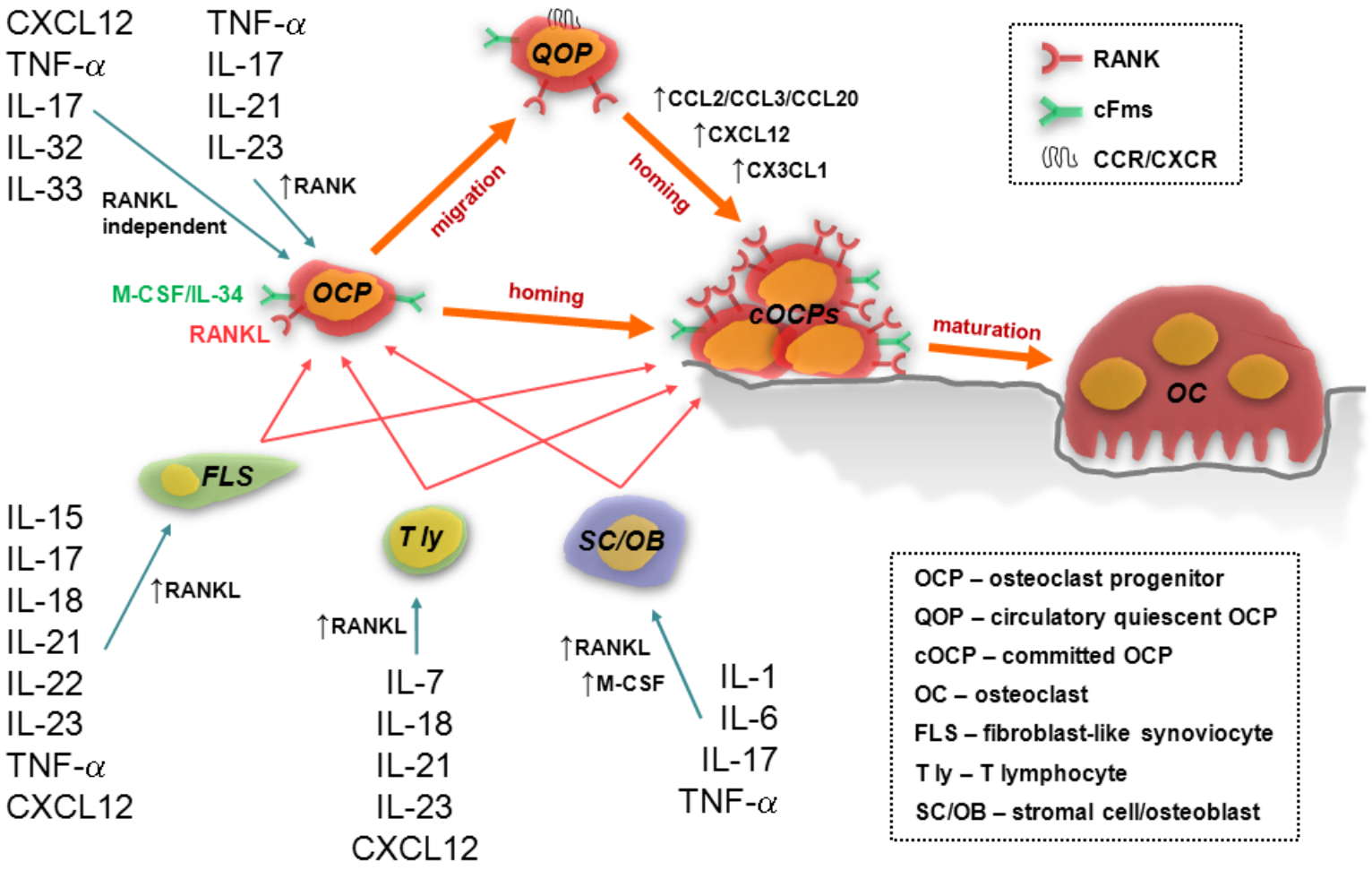
- differentiation potential of sorted OCPs did not significantly differ in RA

- OCPs differ by surface marker expression in RA and PsA:
    - RA OCPs are RANK negative, highly express CD115
    - PsA OCPs highly express RANK, ~1/5 express CD115

- anti-TNF treatment lowered the frequency of peripheral OCPs, which correlated with a lower DAS28, and could be used to monitor the reponse to therapy

- anti-TNF treatment only transiently suppressed osteoclastogenic potential of peripheral OCPs, indicating that additional therapeutic modalities, besides TNF-blocking agents, could be considered for sustained antiresorptive effect

Regulation of OCP trafficking



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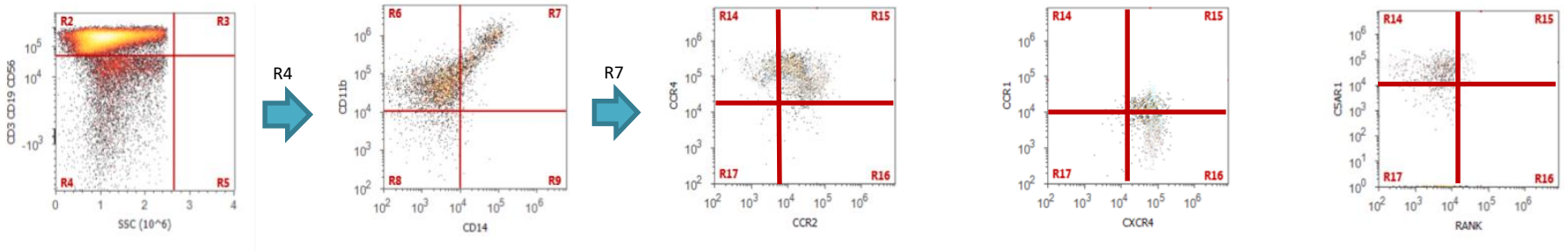
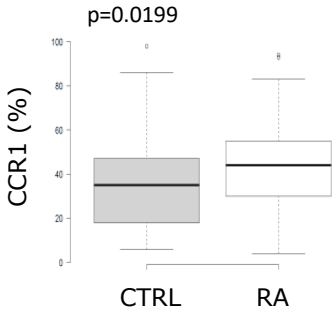
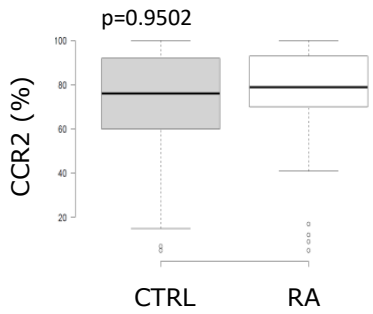
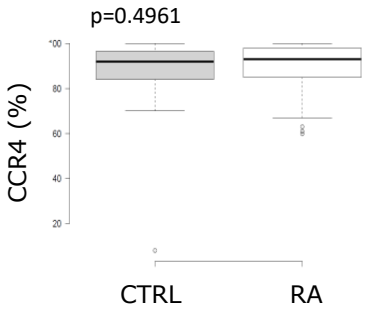
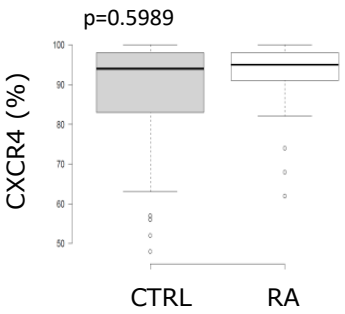
Chemokine receptor phenotype of OCPs

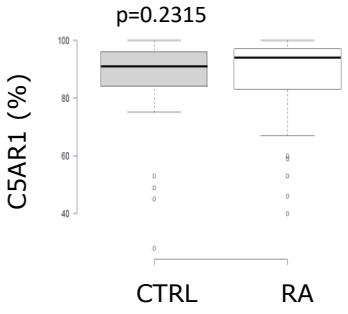




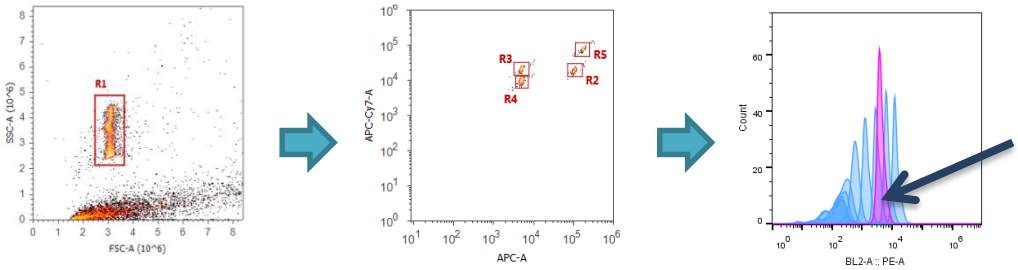




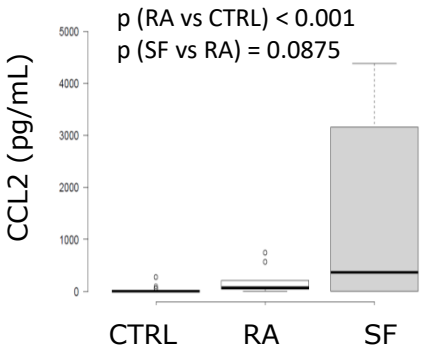


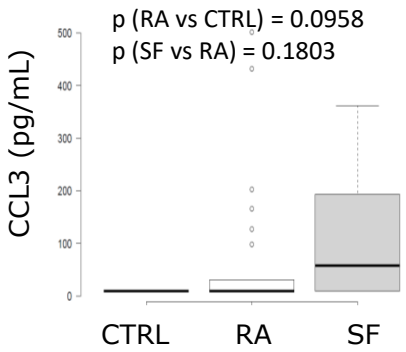


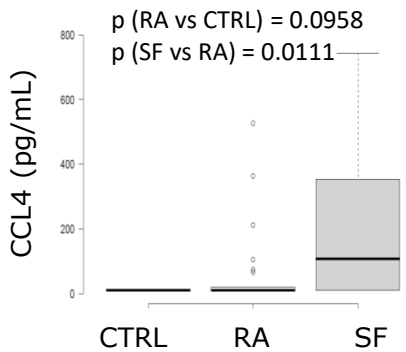
Chemokine concentrations in control serum vs serum and SF of RA patients

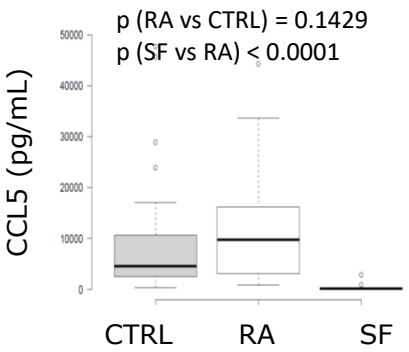


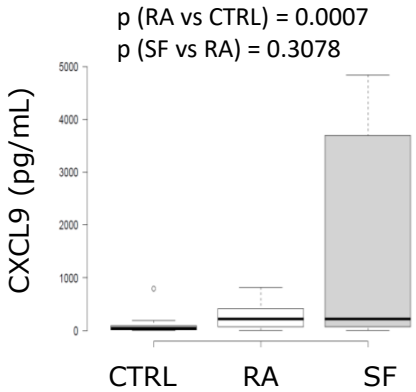
calculate concentrations of unknown samples based on MFI of standard set

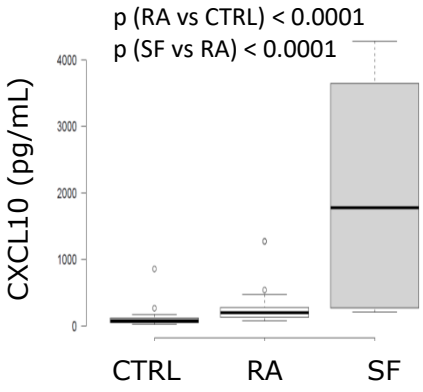




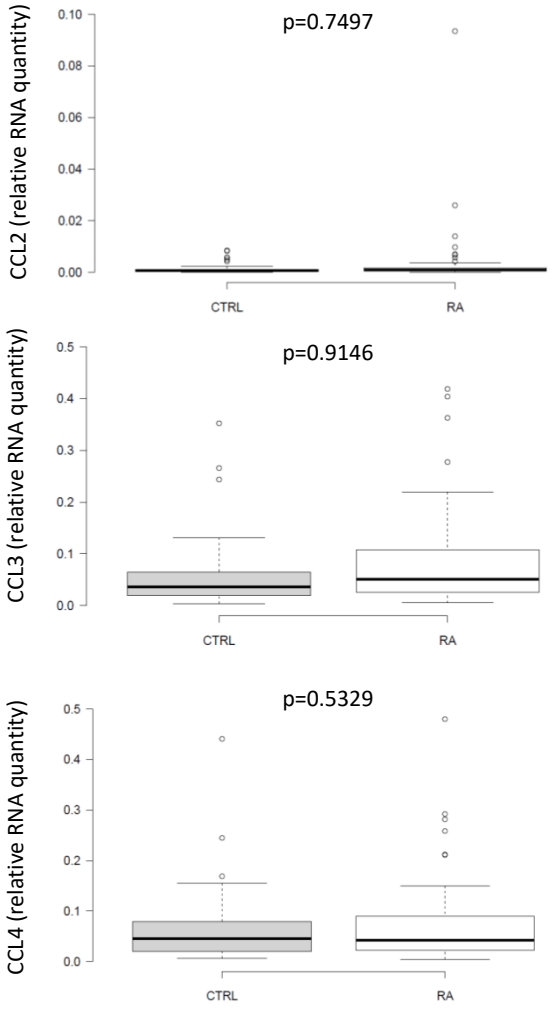


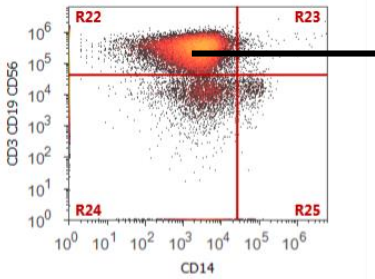


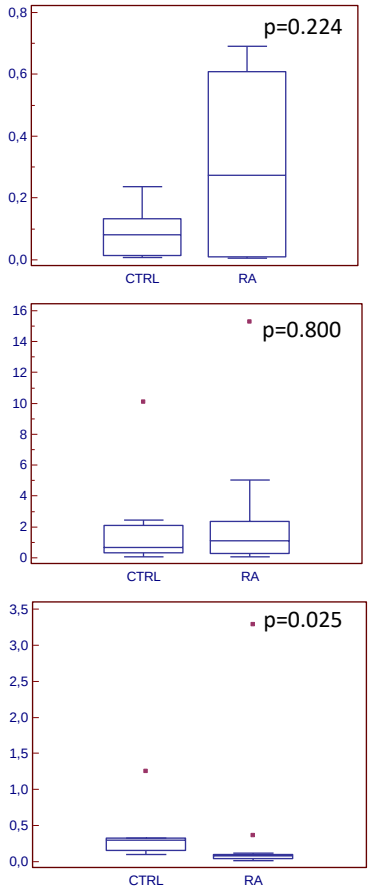




Chemokine gene expression in PBMC and PB lymphocytes







Conclusions #2

- human peripheral blood OCPs similarly expressed CCR1, CCR2, CCR4 and CXCR4 in RA and healthy subjects

- CCL2, CXCL9 and CXCL10 serum levels were significantly higher in RA

- CCL4 and CXCL10 levels in synovial fluid were significantly higher compared to serum

- the source of chemokines appears to be other than PBMC/ PB lymphocytes

- elevated chemokine concentrations and a possible blood-joint chemokine gradient in RA suggest a chemotactic mechanism of OCP migration to affected joints

Acknowledgements

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