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CHARACTERIZATION OF OSTEOCLAST PROGENITORS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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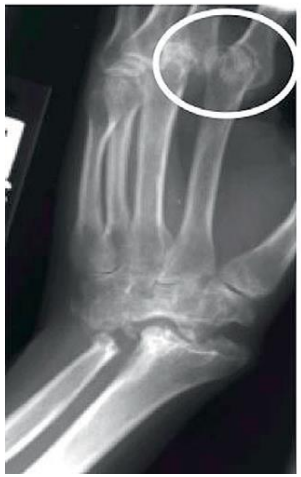
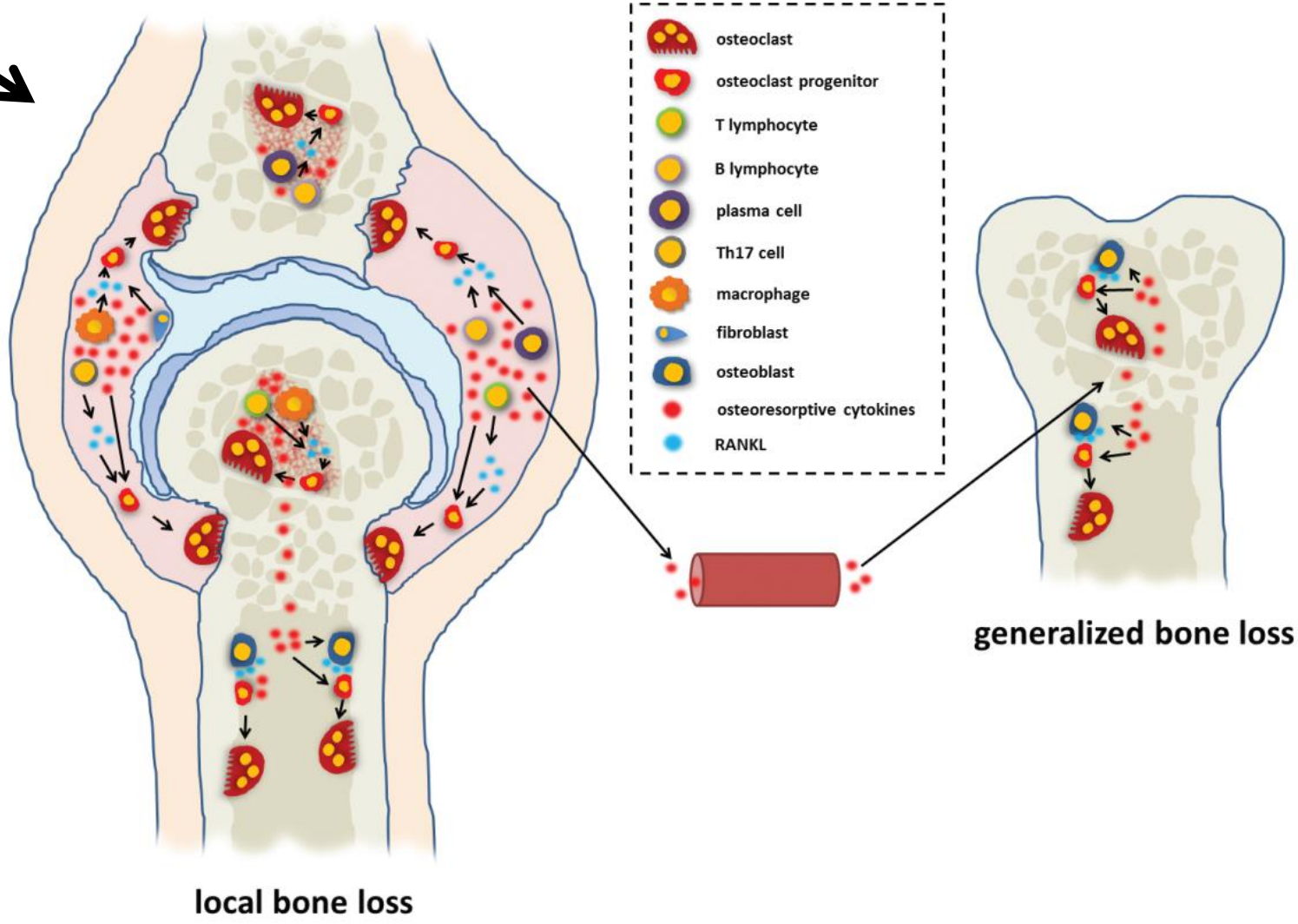
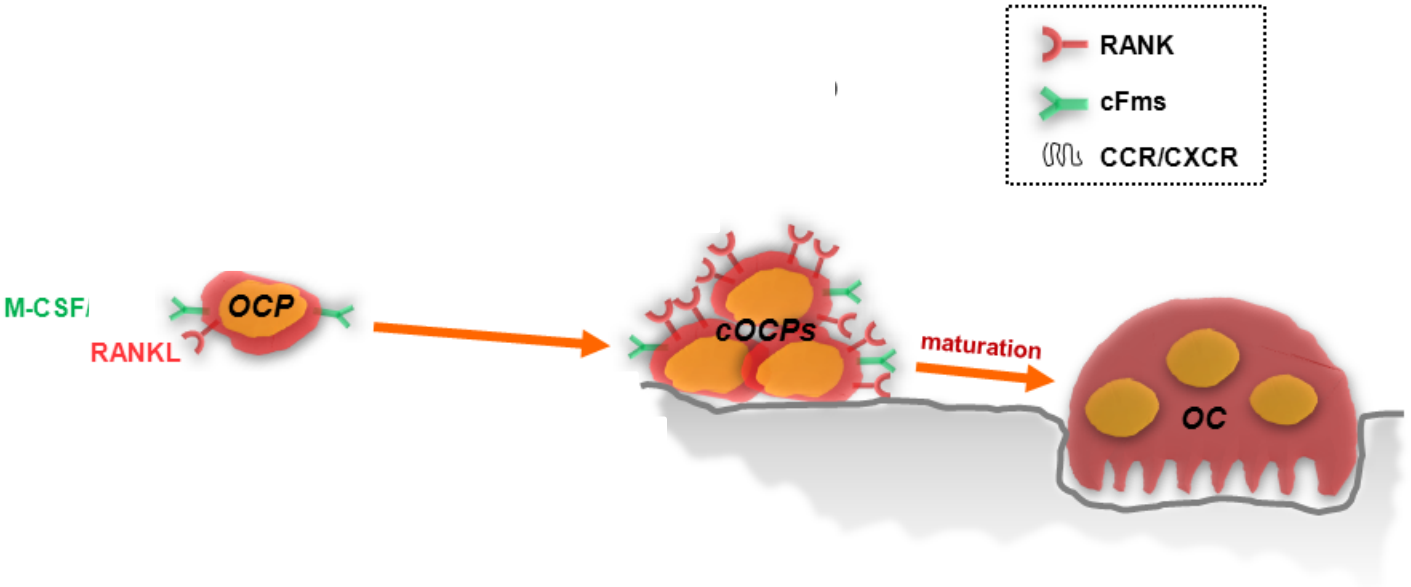
Rheumatoid arthritis





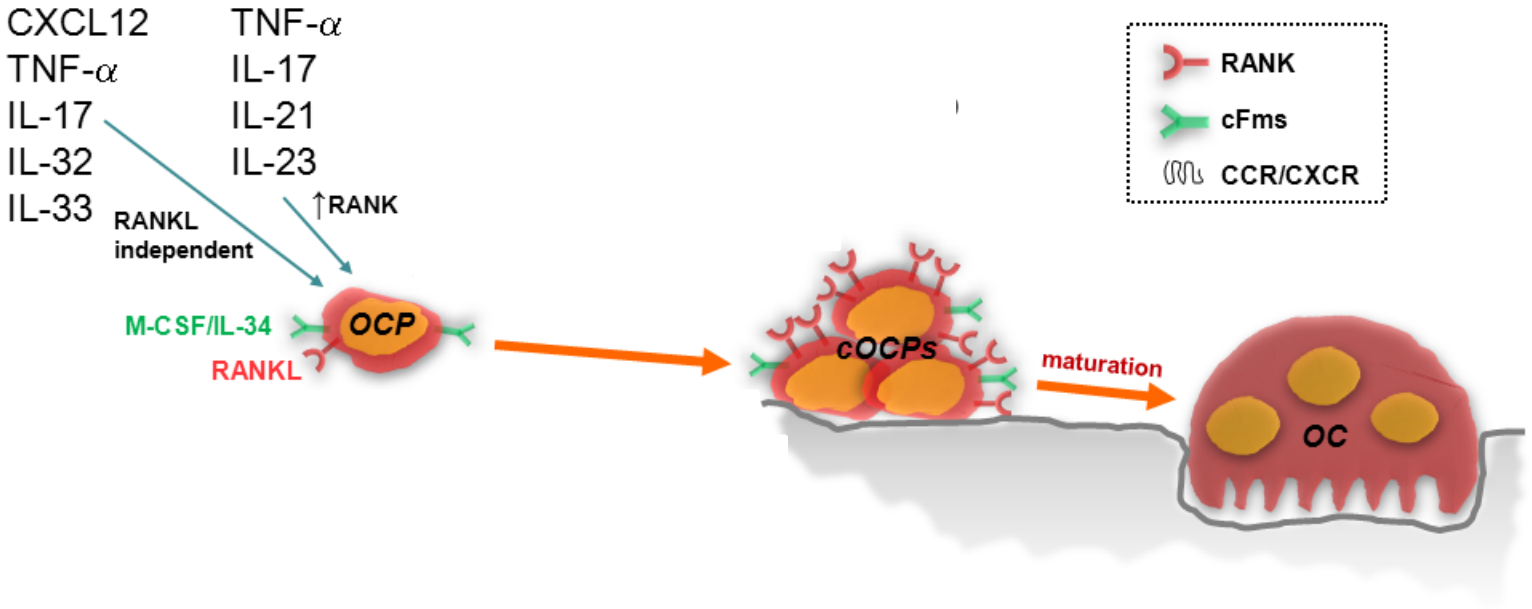
Flegar et al. (2015) Period Biol

Inflammation induced osteoclast activation



OCP – osteoclast progenitor
QOP – circulatory quiescent OCP
cOCP – committed OCP
OC – osteoclast
FLS – fibroblast-like synoviocyte
T ly – T lymphocyte
SC/OB – stromal cell/osteoblast

Inflammation induced osteoclast activation



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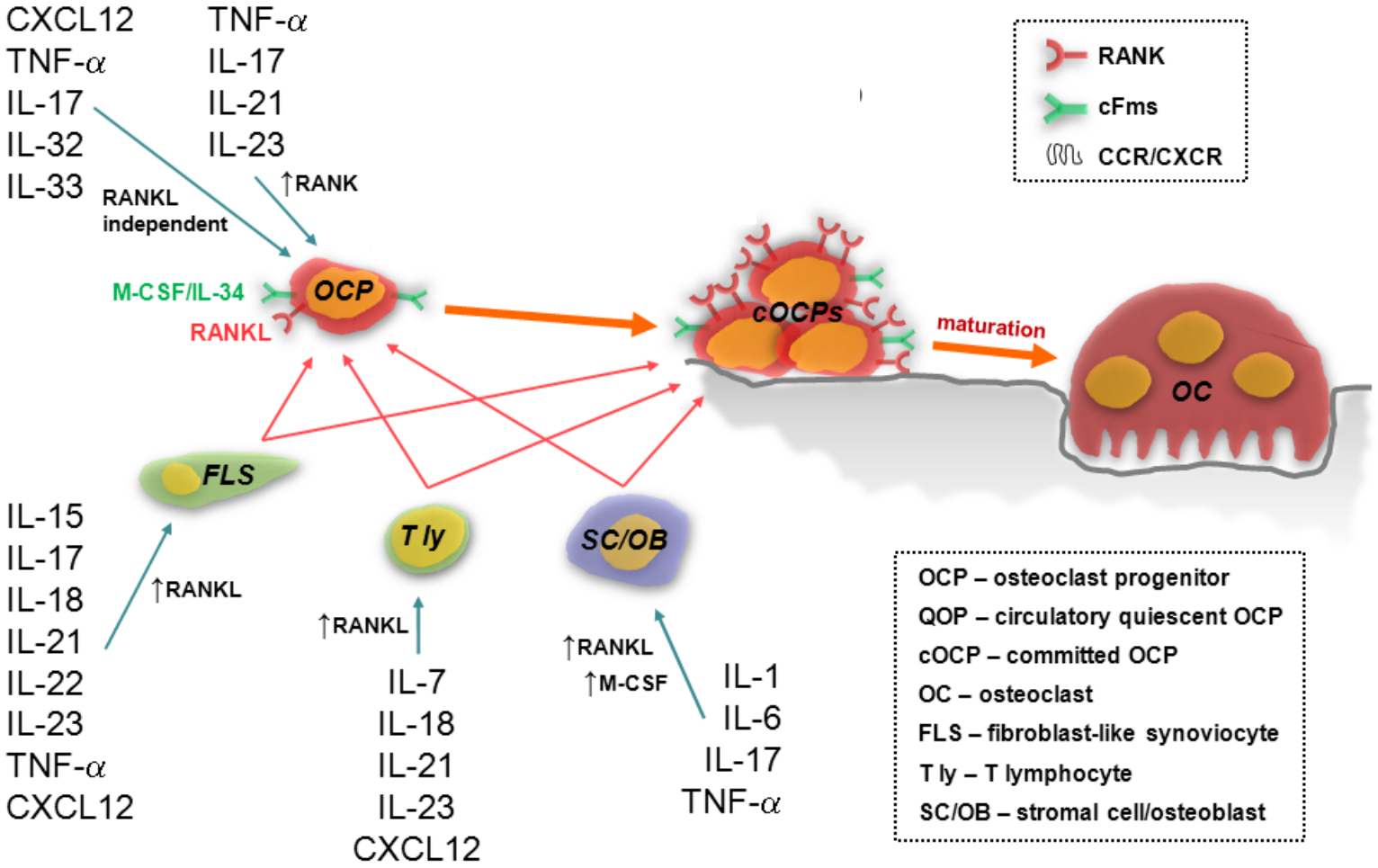
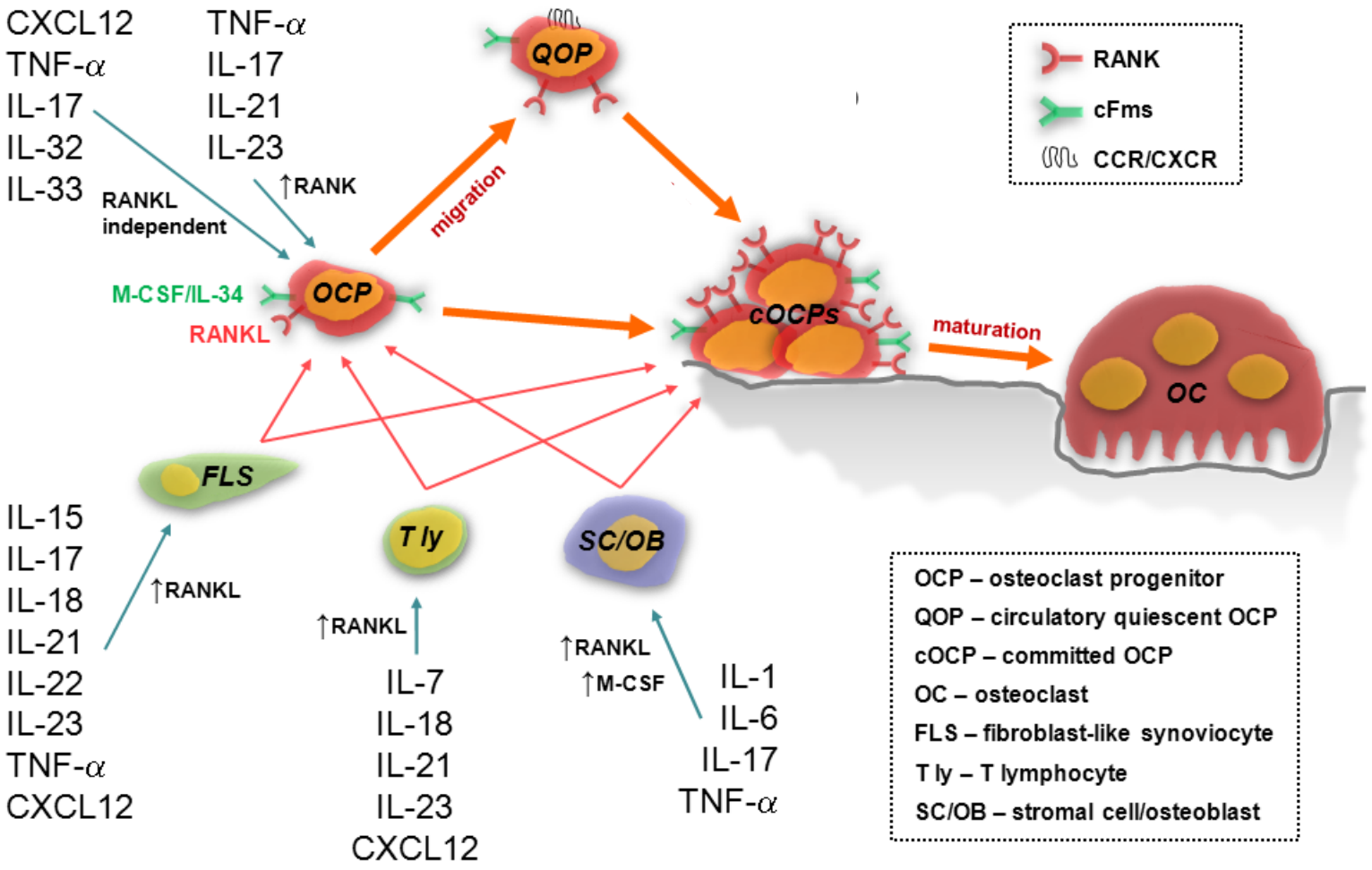


Inflammation induced osteoclast activation



Sucur A, Katavic V, Kelava T, Jajic Z, Kovacic N, Grcevic D. Induction of osteoclast progenitors in inflammatory conditions: key to bone destruction in arthritis. Int Orthop 2014

Enrolled patients

	Control Group	Rheumatoid Arthritis
Sample No & Type	100 (peripheral blood)	106 (106 peripheral blood, 9 synovial fluid)
Age (years)	62.38±14.72	65.83±11.84
Male/female	12/88	10/96
DAS28	-	5.76±1.45
SE (mm/h)	-	33.44±23.99
CRP (mg/L)	-	19.00±22.40
RF (IU/L, n=58)	-	84.75 [13.9-264.37]
aCCP (EU/L, n=33)	-	68.7 [1.85-281.5]

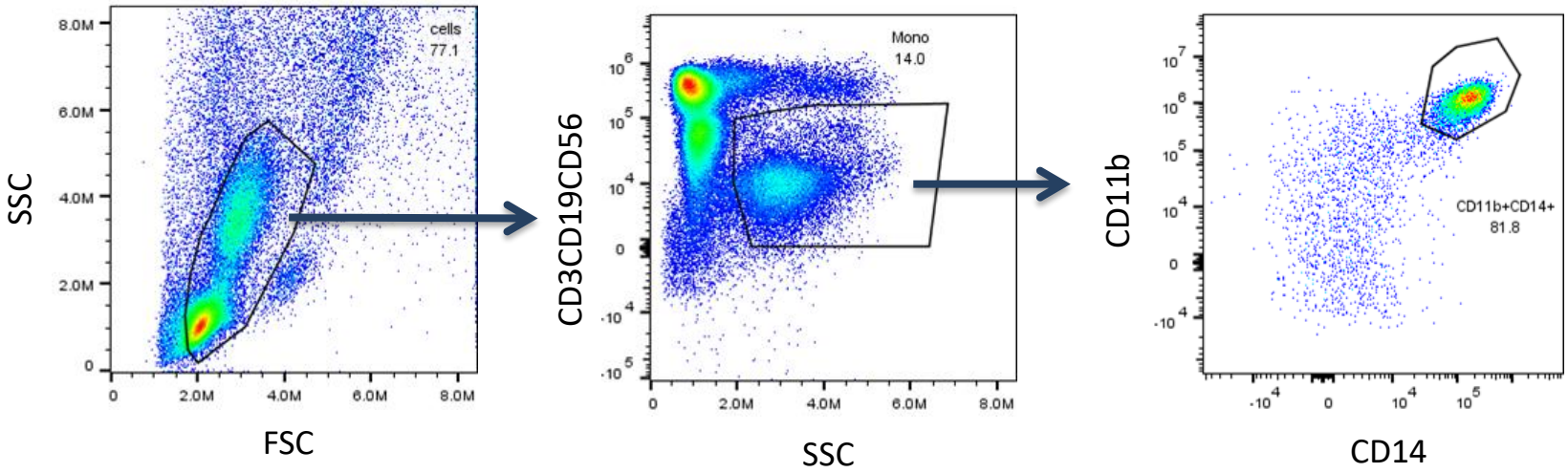
Osteoclast progenitor phenotype

Table 2 Surface marker expression profile of human osteoclast progenitor populations

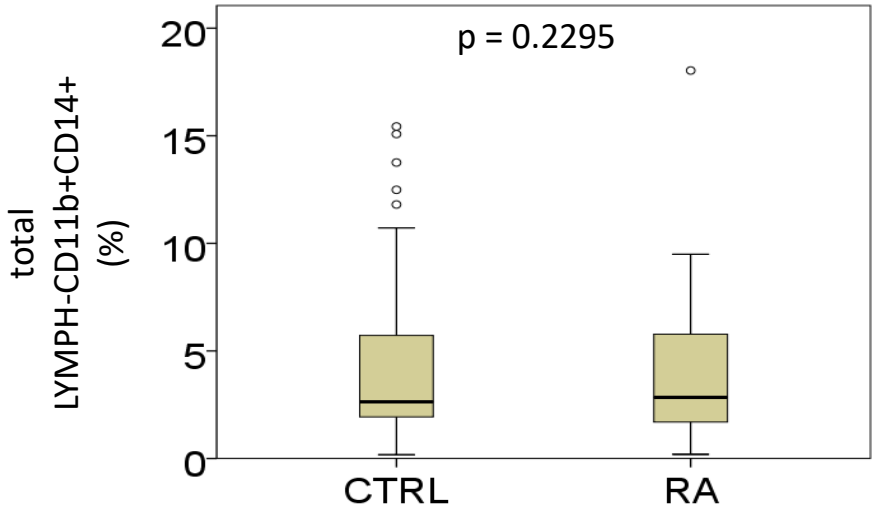
Osteoclast progenitor phenotype	Source[a]
$CD14^+$; $CD11b^+$; or $CD61^+$	PBL
$CD3^-CD19^-CD56^-CD14^+CD11b^+$	PBL
$CD14^+CD11b^+$ ($int\beta1^+int\beta2^+int\beta3^-$)	PBL
$CD14^{hi}CD11b^+CD51/61^+CD16^+$	PBL (MM)
$CD14^+RANK^{hi}$	PBL, BM
$CD45^+CD14^+CD51/61^+CD115^+RANK^+$	GCT
$CD14^+CD16^-(CD33^{hi})CD115^{lo}$	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)

Sucur A Int Orthop 2014

Frequency and phenotype of OCPs

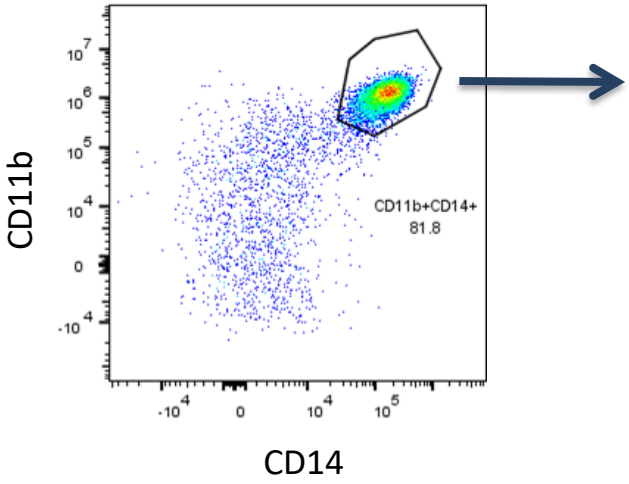


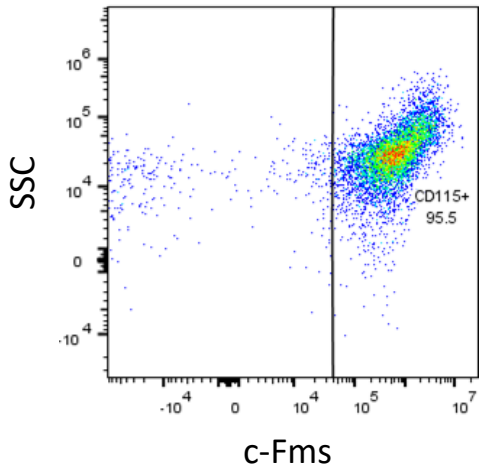
Similar frequency of OCPs in the PBMC

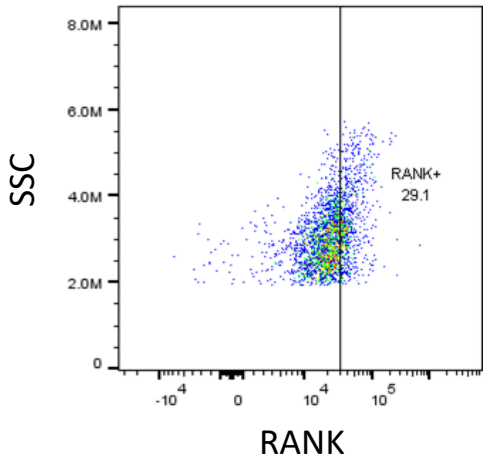


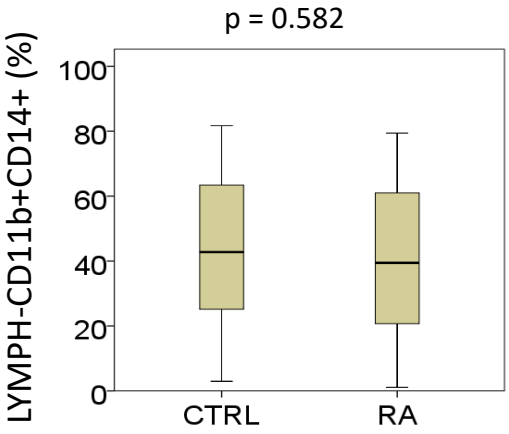
(n=90 RA, 100 CTRL)

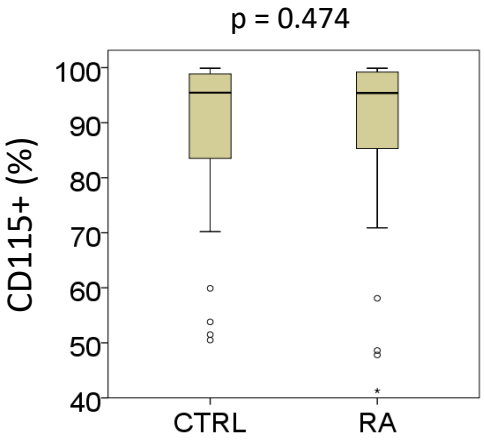
Subpopulation similarly expresses crucial receptors for OC differentiation

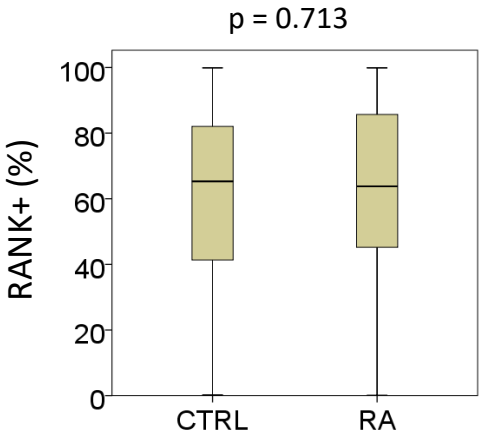






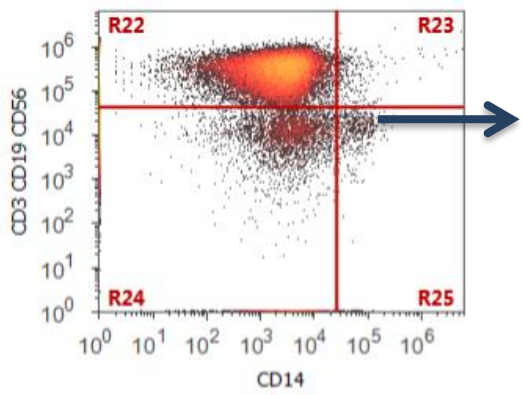


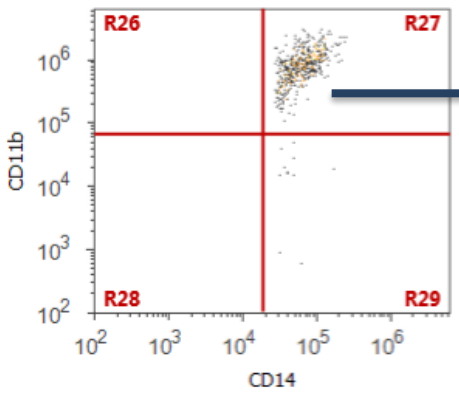




(n=90 RA, 100 CTRL)

Similar osteoclastogenic potential of OCPs





12 day cell culture

OCL count by TRAP enzyme stain

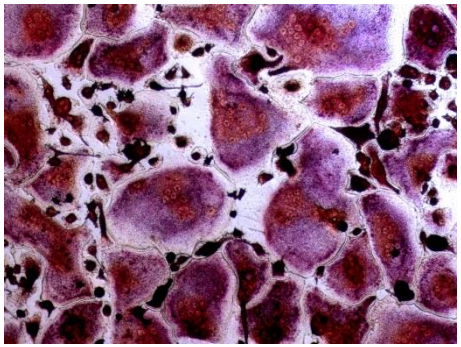
4 days

60 ng/mL M-CSF

8 days

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

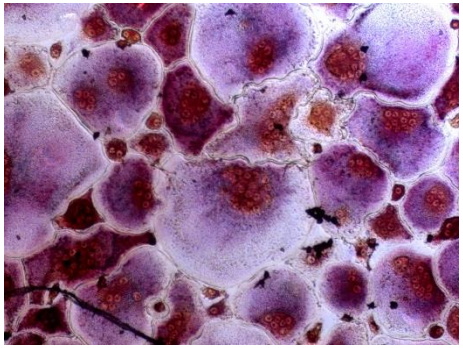
control



207 (92-514)

p = 0.7970

RA

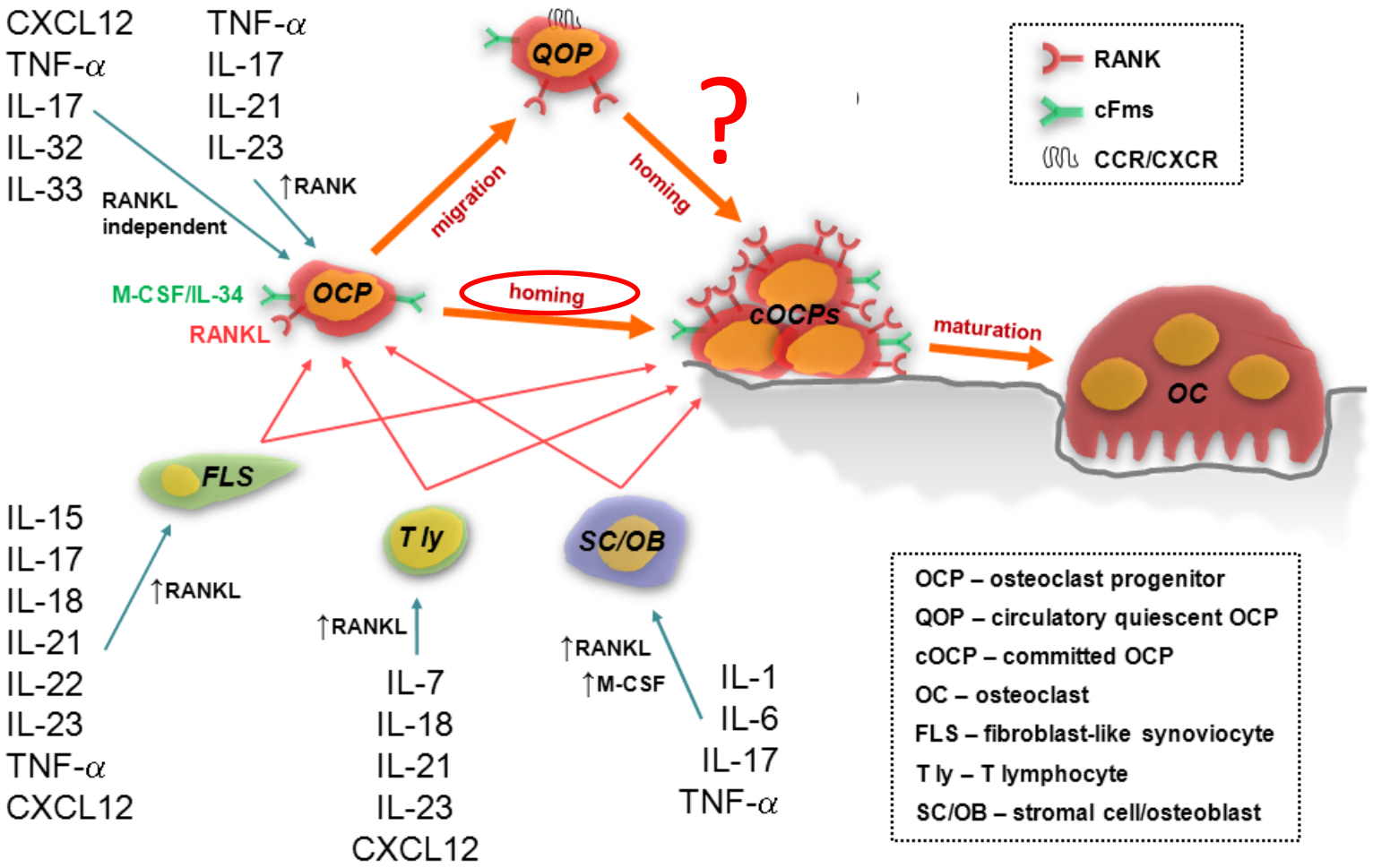


320 (77-481)

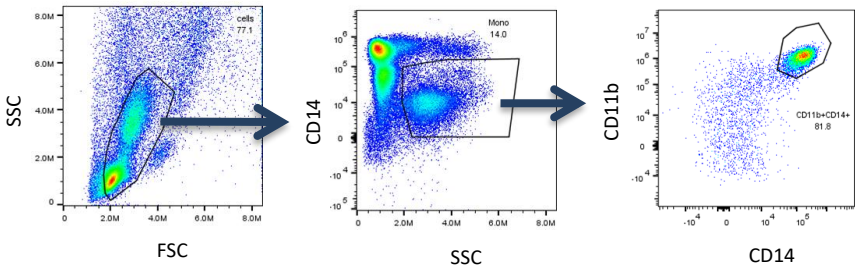
number of osteoclasts per well [median (IQR)]

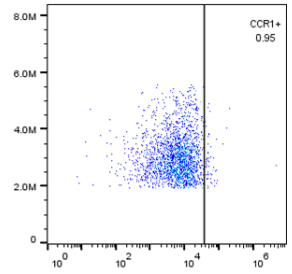
(n=20 RA, 25 CTRL)

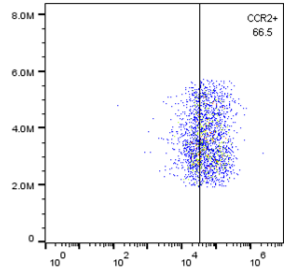
Regulation of osteoclast progenitor trafficking

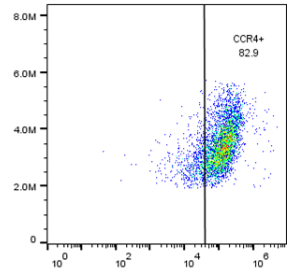


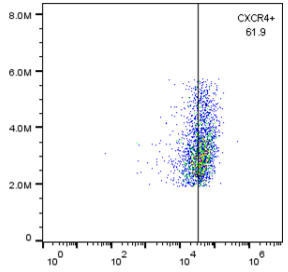
Osteoclast progenitors express chemokine receptors

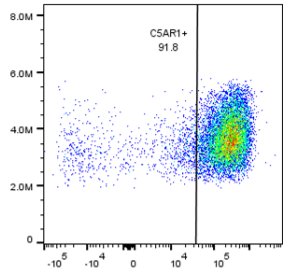




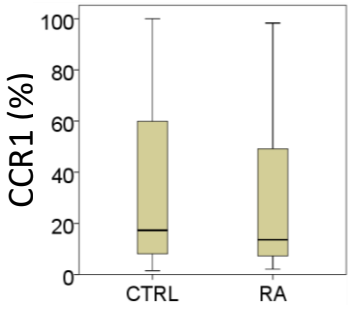




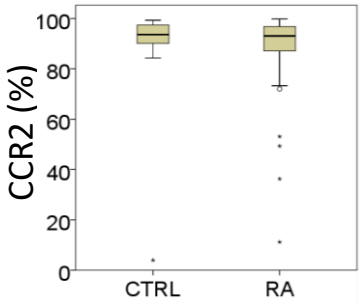




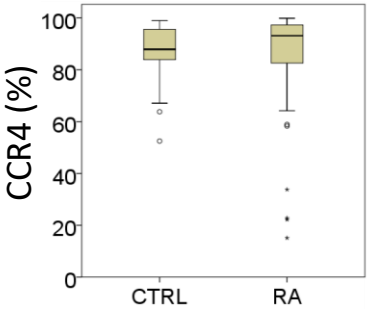
p = 0.810



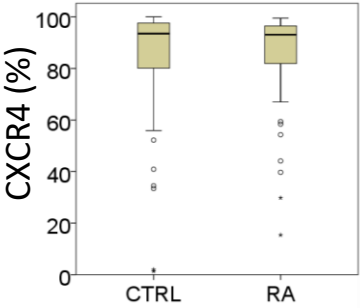
p = 0.691



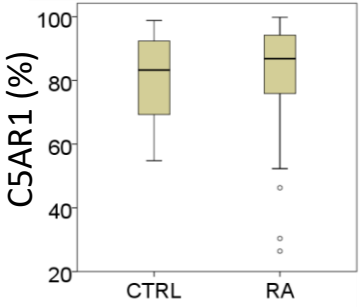
p = 0.327



p = 0.860

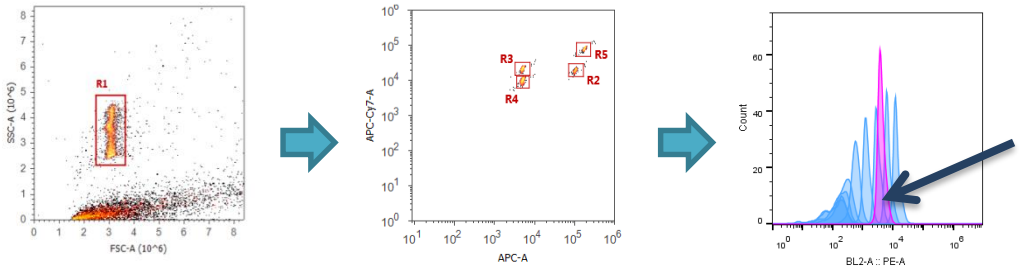


p = 0.235



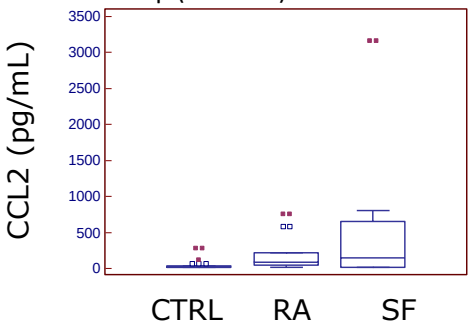
(n=90 RA, 100 CTRL)

Increased chemokine concentrations and an indication of a blood-joint gradient

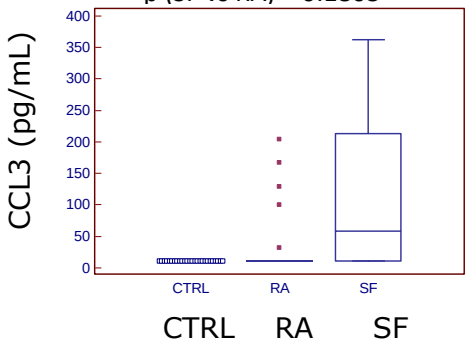


calculate concentrations of unknown samples based on MFI of standard set

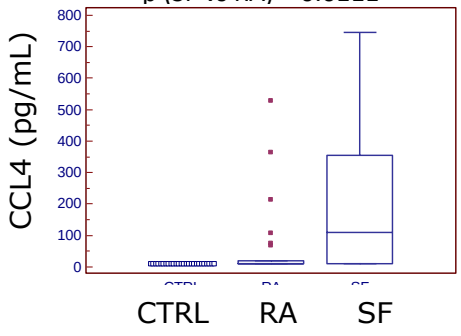
p (RA vs CTRL) < 0.001
p (SF vs RA) = 0.0875



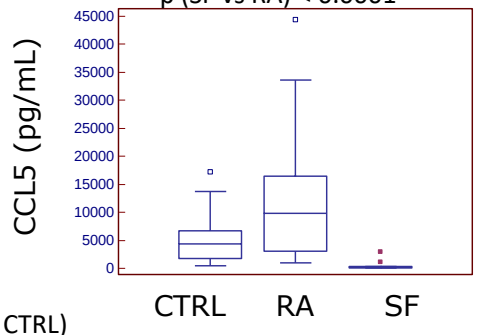
p (RA vs CTRL) = 0.004
p (SF vs RA) = 0.1803



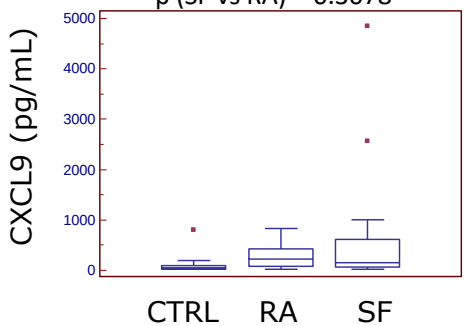
p (RA vs CTRL) < 0.001
p (SF vs RA) = 0.0111



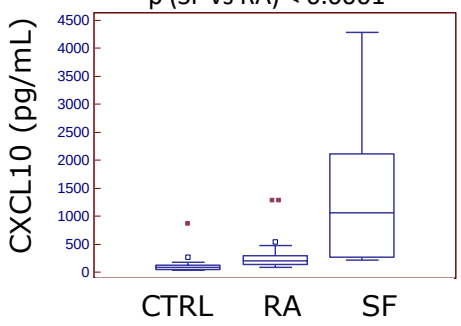
p (RA vs CTRL) = 0.043
p (SF vs RA) < 0.0001



p (RA vs CTRL) < 0.001
p (SF vs RA) = 0.3078

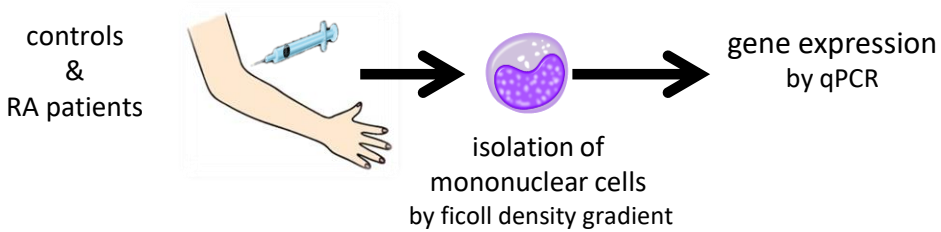


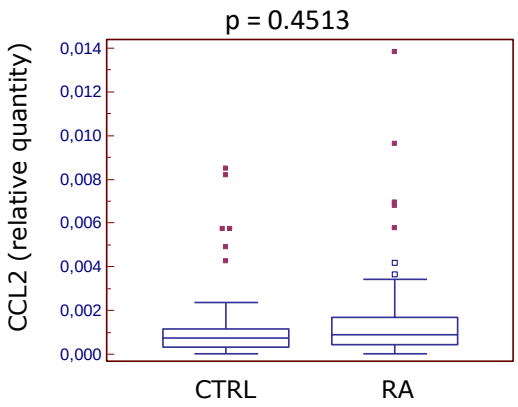
p (RA vs CTRL) < 0.0001
p (SF vs RA) < 0.0001

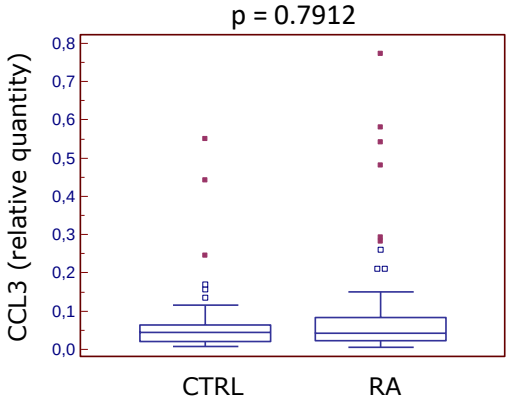


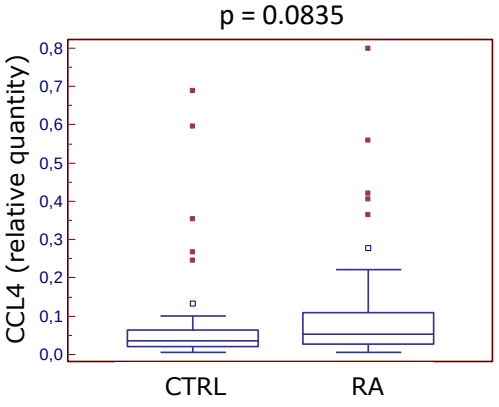
(n=28+9 RA, 28 CTRL)

Chemokine gene expression in PBMC





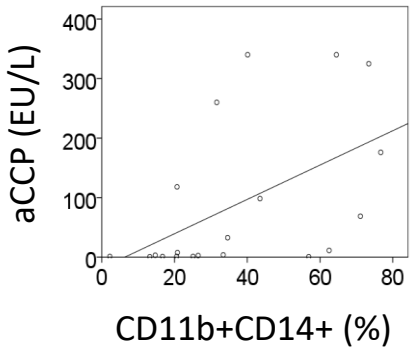




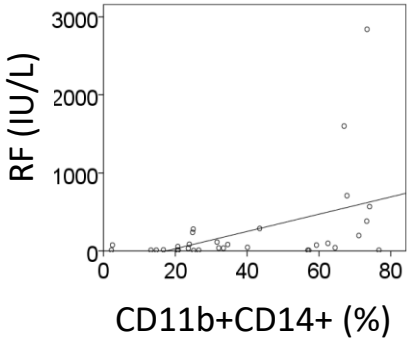
(n=81 RA, 72 CTRL)

Association of population frequencies with clinical parameters and chemokine levels

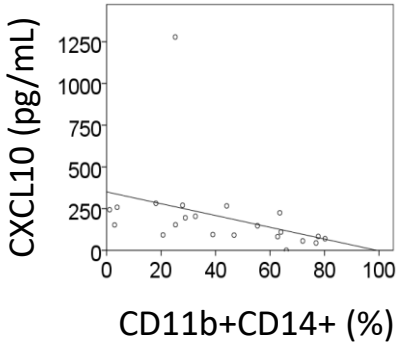
ρ = 0.626
p = 0.003



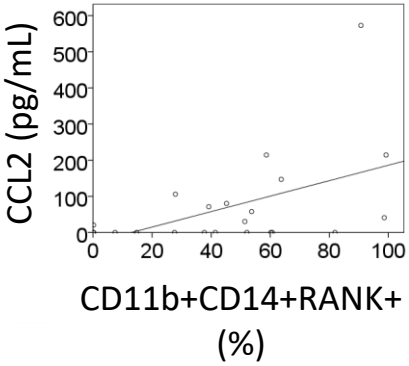
ρ = 0.468
p = 0.007



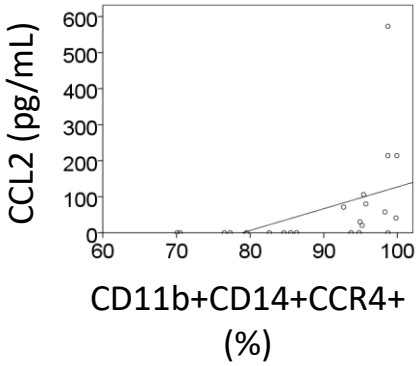
ρ = -0.711
p = 0.003



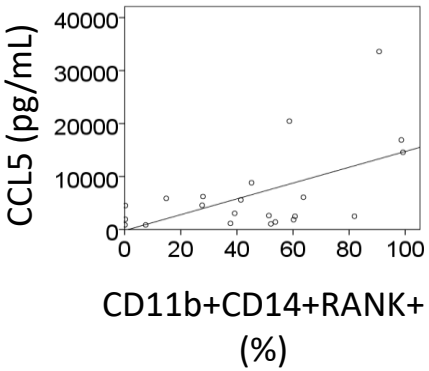
ρ = 0.468
p = 0.028



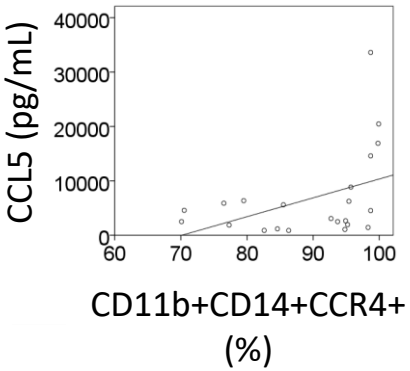
ρ = 0.749
p = 0.0001



ρ = 0.452
p = 0.035

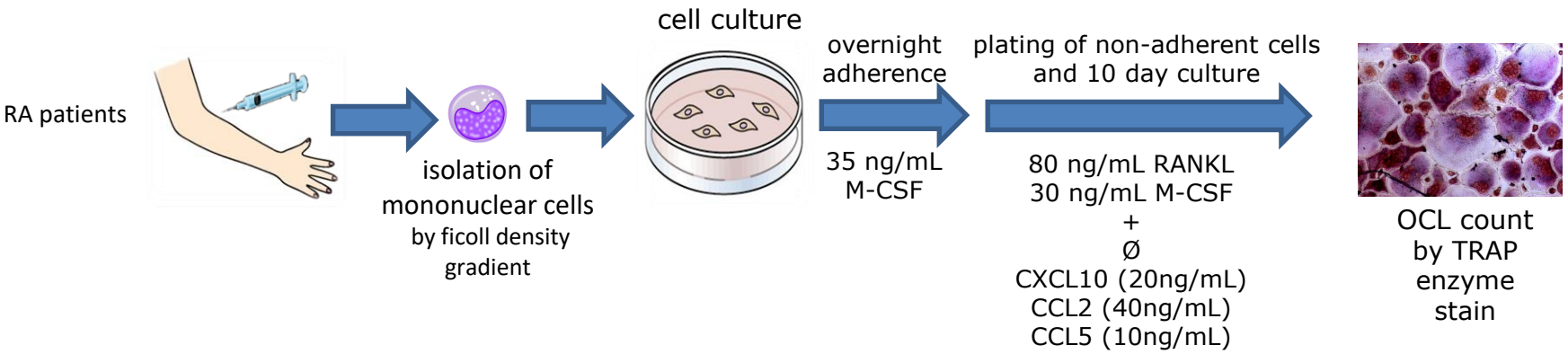


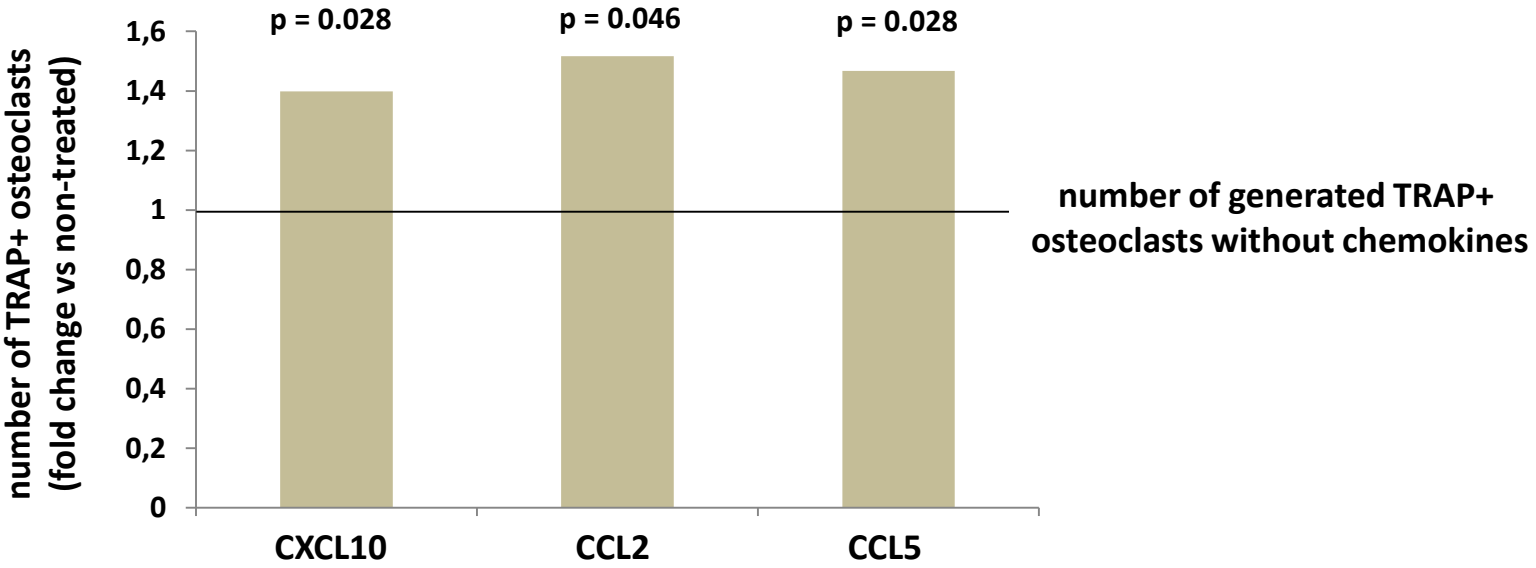
ρ = 0.469
p = 0.028



(n=22 RA + CTRL)

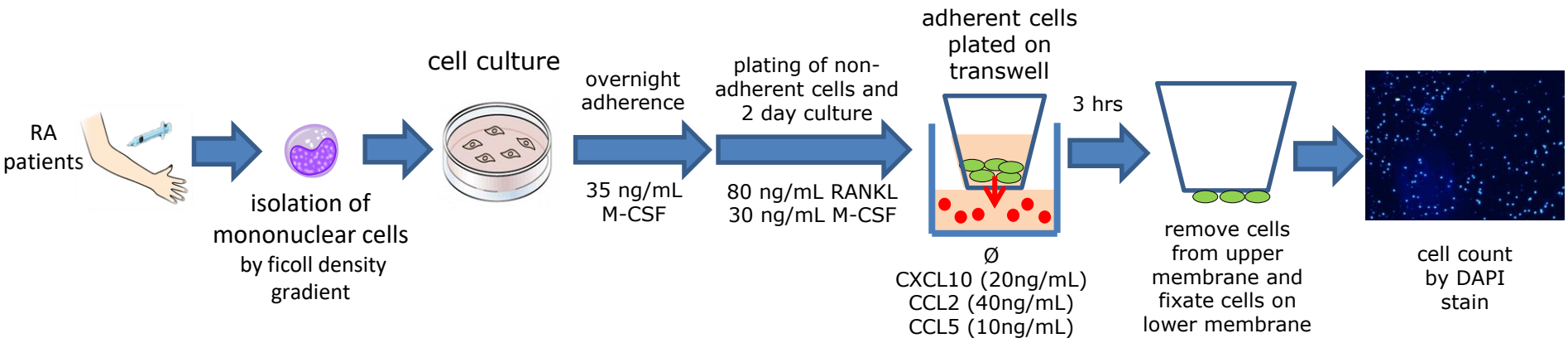
Osteoclastogenic effect of chemokines

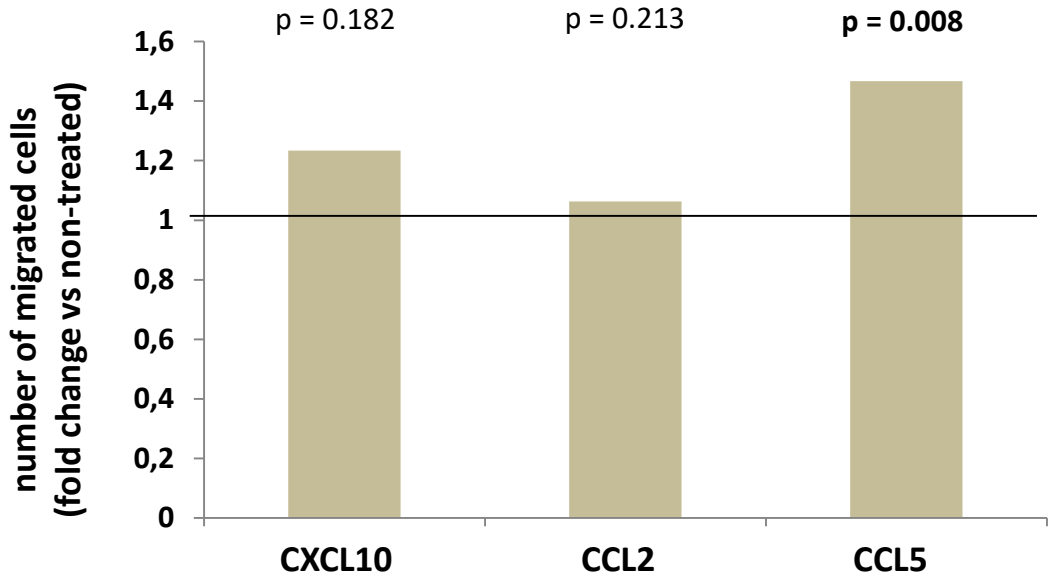




(n=10 RA)

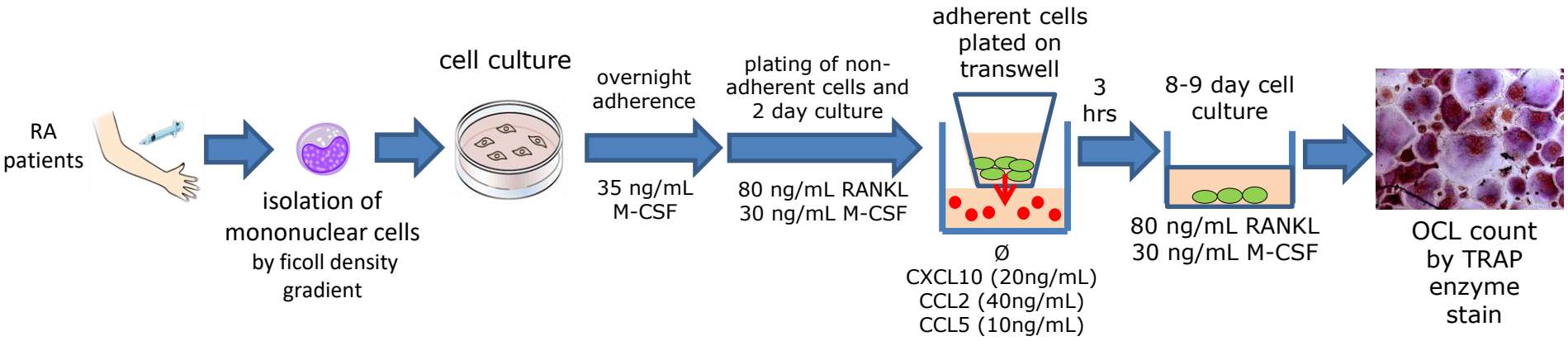
Osteoclast progenitor migration assay

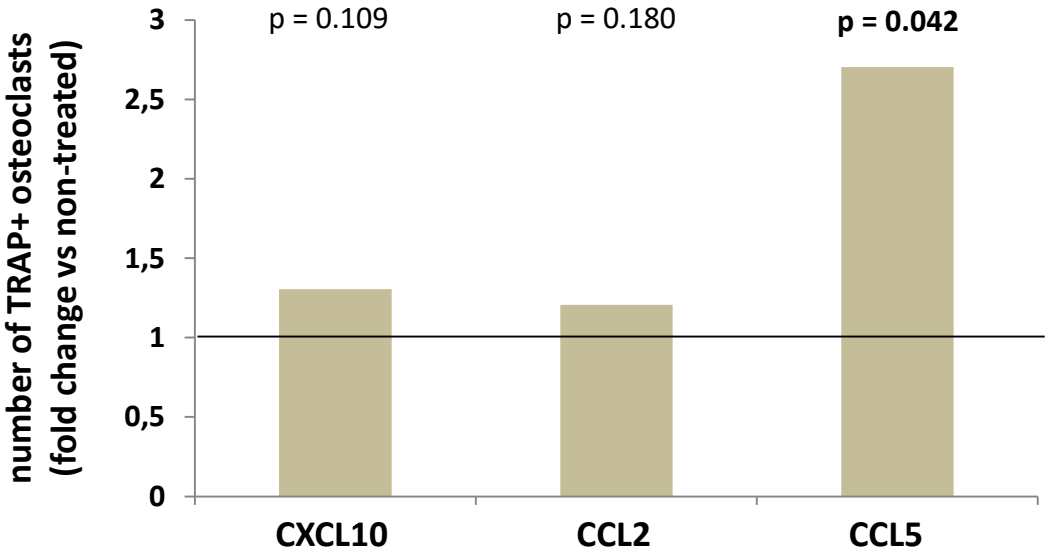




(n=10 RA)

Peripheral blood OCPs exhibit chemotaxis





number of generated TRAP+ osteoclasts without chemokines

(n=10 RA)

Conclusions

- OCPs , found among the CD3−CD19−CD56−CD11b+CD14+ subpopulation of peripheral blood mononuclear cells, express crucial receptors for OC differentiation and are able to differentiate into mature OCs *in vitro* – with similar phenotype and differentiation potential in RA and control samples

- human peripheral blood OCPs express CCR1, CCR2, CCR4 and CXCR4, and at similar levels in in RA and control samples

- CCL2, CCL3, CCL4, CCL5, CXCL9 and CXCL10 serum levels were significantly higher in RA, while CCL4 and CXCL10 levels in synovial fluid were significantly higher compared to serum

- CCL2, CCL5 and CXCL10 exhibit a marked osteoclastogenic effect

- OCPs exhibit strong chemotaxis towards CCL5

- **elevated chemokine concentrations, a possible blood-joint/bone chemokine gradient in RA and chemotactic ability of peripheral blood OCPs suggest a possible mechanism of OCP migration to affected joints**

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