CHARACTERIZATION OF OSTEOCLAST PROGENITORS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Rheumatoid arthritis

local bone loss

generalized bone loss

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

CXCL12  TNF-α
TNF-α  IL-17
IL-17  IL-21
IL-32  IL-23
IL-33  RANKL independent RANKL
      M-CSF/IL-34

OCP → cOCPs → maturation → OC

RANK

CCR/CXCR

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

CXCL12  TNF-α
TNF-α  IL-17
IL-17  IL-21
IL-32  IL-23
IL-33  RANK independent
M-CSF/IL-34  RANK

OCP

IL-15
IL-17
IL-18
IL-21
IL-22
IL-23
TNF-α
CXCL12

FLS

T Ly

SC/OB

IL-7
IL-18
IL-21
IL-23

↑RANKL

IL-1
IL-6
IL-17
TNF-α

M-CSF

↑RANKL

RANK

cFms

CCR/CXCR

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

Enrolled patients

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

Table 2  Surface marker expression profile of human osteoclast progenitor populations

<table>
<thead>
<tr>
<th>Osteoclast progenitor phenotype</th>
<th>Sourcea</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD14+CD11b+ or CD61+</td>
<td>PBL</td>
</tr>
<tr>
<td>CD3+CD19+CD56+CD14+CD11b+</td>
<td>PBL</td>
</tr>
<tr>
<td>CD14+CD11b+(intB1intB2intB3-)</td>
<td>PBL</td>
</tr>
<tr>
<td>CD14+CD11b+CD51/61+CD16+</td>
<td>PBL</td>
</tr>
<tr>
<td>CD14+RANKhi</td>
<td>PBL (MM)</td>
</tr>
<tr>
<td>CD45+CD14+CD51/61+CD115+RANKhi</td>
<td>PBL</td>
</tr>
<tr>
<td>CD14+CD16+CD33+CD115</td>
<td>GCT</td>
</tr>
<tr>
<td>CD16+(gp-39): CD3+CD4+CD8+CD20+CD56+CD33+MHCIICd4+</td>
<td>PBL, SYN (RA)</td>
</tr>
<tr>
<td>CD3+CD19+CD14+CD16+DC-STEMAP+</td>
<td>PBL</td>
</tr>
<tr>
<td>CD14+MHCIICd4+CD16+</td>
<td>PBL (PsA)</td>
</tr>
</tbody>
</table>

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

- CD11b
- CD14
- SSC
- c-Fms
- RANK

\[ p = 0.582 \]
\[ p = 0.474 \]
\[ p = 0.713 \]

**LYMPH-CD11b+CD14+ (%)**

CTRL: 60, 80, 100
RA: 70, 90, 110

CD115+ (%)

CTRL: 75, 90, 105
RA: 80, 100, 115

RANK+ (%)

CTRL: 65, 85, 100
RA: 70, 90, 110

(n=90 RA, 100 CTRL)
Similar osteoclastogenic potential of OCPs

Control

RA

Number of osteoclasts per well [median (IQR)]

207 (92-514)  p = 0.7970  320 (77-481)

(n=20 RA, 25 CTRL)
Regulation of osteoclast progenitor trafficking

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

CXCL12, TNF-α, IL-17, IL-21, IL-23, IL-33

M-CSF/IL-34, RANK, RANKL

migration, homing, maturation

RANK, cFms, CCR/CXCR
Osteoclast progenitors express chemokine receptors

$n=90$ RA, 100 CTRL
Increased chemokine concentrations and an indication of a blood-joint gradient

- CCL2 (pg/mL)
  - \( p \) (RA vs CTRL) < 0.001
  - \( p \) (SF vs RA) = 0.0875

- CCL3 (pg/mL)
  - \( p \) (RA vs CTRL) = 0.004
  - \( p \) (SF vs RA) = 0.1803

- CCL4 (pg/mL)
  - \( p \) (RA vs CTRL) < 0.001
  - \( p \) (SF vs RA) = 0.0111

- CCL5 (pg/mL)
  - \( p \) (RA vs CTRL) = 0.043
  - \( p \) (SF vs RA) < 0.0001

- CXCL9 (pg/mL)
  - \( p \) (RA vs CTRL) < 0.001
  - \( p \) (SF vs RA) = 0.3078

- CXCL10 (pg/mL)
  - \( p \) (RA vs CTRL) < 0.0001
  - \( p \) (SF vs RA) < 0.0001

(\( n = 28 + 9 \) RA, 28 CTRL)
Chemokine gene expression in PBMC

controls & RA patients

isolation of mononuclear cells by ficoll density gradient

gene expression by qPCR

CTRL               RA
CTRL               RA
CTRL               RA

p = 0.4513
p = 0.7912
p = 0.0835

(n=81 RA, 72 CTRL)
Association of population frequencies with clinical parameters and chemokine levels

\[
\rho = 0.468 \\
p = 0.028
\]

CCL2 (pg/mL)

\[
\rho = 0.749 \\
p = 0.0001
\]

CD11b+CD14+CCR4+ (%)

\[
\rho = 0.452 \\
p = 0.035
\]

CD11b+CD14+RANK+ (%)

\[
\rho = 0.469 \\
p = 0.028
\]

CD11b+CD14+CCR4+ (%)

\[
\rho = -0.711 \\
p = 0.003
\]

CXCL10 (pg/mL)

\[
\rho = 0.626 \\
p = 0.003
\]

aCCP (EU/L)

\[
\rho = 0.468 \\
p = 0.007
\]

RF (IU/L)

\[
\rho = 0.452 \\
p = 0.035
\]

CD11b+CD14+ (%)
Osteoclastogenic effect of chemokines

RA patients

- Isolation of mononuclear cells by ficoll density gradient

Cell culture

- Overnight adherence
- Plating of non-adherent cells and 10 day culture

- 35 ng/mL M-CSF
- 80 ng/mL RANKL
- 30 ng/mL M-CSF + Ø
- CXCL10 (20 ng/mL)
- CCL2 (40 ng/mL)
- CCL5 (10 ng/mL)

OCL count by TRAP enzyme stain

Number of generated TRAP+ osteoclasts without chemokines

Number of TRAP+ osteoclasts (fold change vs non-treated)

CXCL10: p = 0.028
CCL2: p = 0.046
CCL5: p = 0.028

(n=10 RA)
Osteoclast progenitor migration assay

RA patients

isolation of mononuclear cells by ficoll density gradient

cell culture

overnight adherence

35 ng/mL M-CSF

80 ng/mL RANKL

30 ng/mL M-CSF

plating of non-adherent cells and 2 day culture

adherent cells plated on transwell

3 hrs

Ø

CXCL10 (20ng/mL)

CCL2 (40ng/mL)

CCL5 (10ng/mL)

1

3 hrs

remove cells from upper membrane and fixate cells on lower membrane

cell count by DAPI stain

number of migrated cells without chemokines

number of migrated cells (fold change vs non-treated)

CXCL10

CCL2

CCL5

p = 0.182

p = 0.213

p = 0.008

(n=10 RA)
Peripheral blood OCPs exhibit chemotaxis

RA patients

isolation of mononuclear cells by ficoll density gradient

cell culture

overnight adherence

35 ng/mL M-CSF

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

plating of non-adherent cells and 2 day culture

Ø

CXCL10 (20ng/mL)

CCL2 (40ng/mL)

CCL5 (10ng/mL)

adherent cells plated on transwell

3 hrs

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

8-9 day cell culture

OCL count by TRAP enzyme stain

number of generated TRAP+ osteoclasts without chemokines

number of TRAP+ osteoclasts (fold change vs non-treated)

p = 0.109

p = 0.180

p = 0.042

CXCL10

CCL2

CCL5

(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 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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