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## **Induction of osteoclast progenitors in inflammatory conditions: Key to bone destruction in arthritis**

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## **Abstract**

The inflammatory milieu favors recruitment and activation of osteoclasts, and leads to bone destruction as a serious complication associated with arthritis and with other inflammatory processes. The frequency and activity of osteoclast progenitors (OCPs) correspond to arthritis severity, and may be used to monitor disease progression and bone resorption, indicating the need for detailed characterization of the discrete OCP subpopulations. Collectively, current studies suggest that the most potent murine bone marrow OCP population can be identified among lymphoid negative population within the immature myeloid lineage cells, as B220<sup>-</sup>CD3<sup>-</sup>CD11b<sup>-/lo</sup>CD115<sup>+</sup>CD117<sup>+</sup>CX3CR1<sup>+</sup> and possibly also Ter119<sup>-</sup>CD11c<sup>-</sup>CD135<sup>lo</sup>Ly6C<sup>+</sup>RANK<sup>-</sup>. In the peripheral blood OCP population bears the monocytoïd phenotype B220<sup>-</sup>CD3<sup>-</sup>NK1.1<sup>-</sup>CD11b<sup>+</sup>Ly-6C<sup>hi</sup>CD115<sup>+</sup>CX3CR1<sup>+</sup>, presumably expressing RANK in committed OCPs. Much less is known about human OCPs and their regulation in arthritis, but the circulating OCP subset is, most probably, comprised among the lymphoid negative population (CD3<sup>-</sup>CD19<sup>-</sup>CD56<sup>-</sup>), within immature monocyte subset (CD11b<sup>+</sup>CD14<sup>+</sup>CD16<sup>-</sup>), expressing receptors for M-CSF and RANKL (CD115<sup>+</sup>RANK<sup>+</sup>). Our preliminary data confirmed positive association between the proportion of peripheral blood OCPs, defined as CD3<sup>-</sup>CD19<sup>-</sup>CD56<sup>-</sup>CD11b<sup>+</sup>CD14<sup>+</sup>, and the disease activity score (DAS28) in the follow-up samples from patient with psoriatic arthritis receiving anti-TNF therapy. In addition, we reviewed cytokines and chemokines which, directly or indirectly, activate OCPs and enhance their differentiation potential, thus mediating osteoresorption. Control of the activity and migratory behavior of OCPs as well as the identification of crucial bone/joint chemotactic mediators represent promising therapeutic targets in arthritis.

**Keyword:** osteoclast progenitors, arthritis, inflammation, cytokines, chemokines, osteoresorption

## **1. Inflammation induced bone loss**

Bone is a highly dynamic tissue important for its mechanical and metabolic functions, and characterized by rapid response to numerous physical, endocrine and paracrine stimuli in physiological and pathological conditions. Among others signals, osteoresorptive mediators (such as interleukin (IL)-1, IL-6, IL-15, IL-17, IL, 21, IL-33, tumor necrosis factor (TNF)- $\alpha$  and receptor activator of nuclear factor- $\kappa$ B ligand (RANKL), CCL2, CXCL12) produced by inflammatory/immune cells create conditions that promote maturation and function of osteoclasts [1-4]. Bone resorption and osteolysis are the prominent features and causes of substantial morbidity in inflammatory processes associated with arthritis as well as with localized bacterial infections of bone and adjacent tissues, peri-prosthetic loosening, vasculitis, connective tissue diseases, chronic viral infections and inflammatory bowel diseases [5-8].

## **2. Bone resorption in arthritis**

Inflammatory arthritides comprise a heterogeneous group of joint disorders that are characterized by chronic inflammatory response as well as periarticular and generalized bone loss due to deregulated bone remodeling [1, 4, 9, 10]. Disruption of joint architecture and bone erosions are the hallmarks of rheumatoid arthritis (RA) but also occur in spondyloarthritis (SpA) and the erosive form of osteoarthritis (OA) [1, 5, 8, 9, 11-13].

Inflamed synovial tissue in RA, with increased vascularity, hyperplasia and accumulation of macrophages, plasma cells, T and B lymphocytes, dendritic cells (DC), natural killer (NK) cells and mast cells, was traditionally postulated as the major pathogenic factor invading adjacent structures (articular cartilage, cortical bone surface and underlying bone marrow) [11, 12]. However, more recent evidence revealed that inflammatory processes in RA spread well beyond the synovial tissue inflammation, particularly affecting subchondral compartment [1, 9].

Most prominent subchondral changes include cortical bone erosions and diffuse bone marrow edema [1, 8, 9, 14]. The bone marrow pathology in the form of bone marrow edema is present at all stages of RA, associated with the disease activity, and also described in OA, SpA and systemic lupus erythematosus. Pathophysiological correlate of bone marrow edema is osteitis, with inflammatory infiltrate (comprised of T and B lymphocytes, plasma cells and macrophages) and active osteoclasts closely juxtaposed to trabecular bone mediating the erosive processes [9, 14].

Moreover, the presence of generalized bone loss, manifested as lower bone mineral density and higher rate of pathological fractures, has been observed in different forms of arthritides and associated with the disease severity. Although generalized osteoporosis in arthritis was usually attributed to immobility and corticosteroid treatment, more recent studies suggest that systemic osteopenia as well as periarticular bone loss and local erosions are caused at least in part by a common pathogenic mechanism that involves inflammation-induced increase in osteoclast number and activity [14-17]) However, the frequency of osteoclast progenitor (OCP) populations, serving as a pool for osteoclast

renewal and maintenance of the expanded osteoclast number, and their migration patterns through peripheral blood and bone sites have not been precisely defined in inflammatory arthritides.

### 3. Differentiation of osteoclasts

As the exclusive osteoresorptive cells, osteoclasts are directly responsible for physiological bone resorption and pathological bone destruction [18,19]. They arise from myeloid hematopoietic progenitor cells of the monocyte/macrophage lineage through a series of differentiation stages that generate large multinucleated, tartrate-resistant acid phosphatase (TRAP)-positive mature cells [20, 21]. Abnormally enhanced osteoclast formation and activity accelerate pathogenesis of arthritis and other diseases characterized by inflammatory osteolysis, causing bone loss that inevitably results in pain, deformity, osteopenia and structural changes [2, 3, 5, 18, 19].

Osteoclast differentiation is physiologically triggered by RANKL/RANK binding, in the presence of macrophage colony stimulating factor (M-CSF/CSF-1) [22-24], and these essential osteoclastogenic molecules are abundantly expressed in inflammatory conditions such as arthritis and periodontitis [3, 8]. RANKL, expressed on osteoblasts and hypertrophying chondrocytes, as well as on activated T lymphocytes, is up-regulated by pro-resorptive hormones, cytokines and inflammatory mediators, including vitamin D<sub>3</sub>, parathyroid hormone, prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), IL-1, IL-6, IL-7, IL-17 and IL-22 [5, 7, 8]. RANKL pathway is inhibited by osteoprotegerin (OPG), a soluble decoy receptor for RANKL, and RANKL/OPG ratio is a key determinant of the rate of osteoclastogenesis and bone resorption [5, 20]. The increased RANKL/OPG ratio in synovial compartment results in enhanced osteoclast differentiation and formation of bone erosions [2, 3, 7, 8]

M-CSF, through its receptor cFms (CSF-1R/CD115), and the transcription factor PU.1 are involved in the generation of common progenitors for macrophages, DCs and osteoclasts. M-CSF induces the proliferation of OCPs, supports their survival and upregulates RANK expression [23, 24]. IL-34, by binding to cFms, is able to replace M-CSF in RANKL-induced osteoclastogenesis [7, 8, 25].

RANK lacks the intrinsic enzymatic activity in its intracellular domain and transduces signaling by recruiting adaptor molecules from the TRAF (TNF receptor-associated factor) family of proteins, mainly TRAF6 [23, 24]. Upon RANK/TRAF6 complex formation, multiple signaling pathways are activated, including classical and alternative NF- $\kappa$ B pathways, protein tyrosine kinases (Btk/Tec), calcium signaling and mitogen-activated protein kinase (MAPK) pathways (p38 and Erk). These signaling cascades potently induce transcription factor NFATc1 (nuclear factor of activated T cells, cytoplasmic 1), which is a key regulator of osteoclast differentiation *in vitro* and *in vivo*. It positively regulates its own promoter and cooperates with other partners to activate the osteoclast transcriptional machinery [5, 8]. Particularly, activator protein 1 (AP-1) transcription factor complex containing cFos is required for autoamplification of NFATc1 and osteoclast differentiation. AP-1 DNA binding activity has been found to be upregulated in the synovial tissue of RA patients and correlates with the disease activity [5, 23]. The costimulatory receptors OSCAR (osteoclast associated receptor) and

TREM2 (triggering receptor expressed by myeloid cells 2) enhance the osteoclastogenic signal through immunoreceptor tyrosine-based activation motif (ITAM)-containing adaptor proteins (DNAX-activating protein of 12 kDa (DAP12) and Fc-receptor  $\gamma$  chain (FcR $\gamma$ )) and Syk tyrosine kinase activation [5, 7, 8, 23, 24].

#### 4. Characterization of osteoclast progenitors

Given their central role in the osteolytic processes, osteoclast lineage cells (including OCPs) have become a focus of studies investigating inflammatory osteoresorption [1, 4, 5, 8, 9, 13, 18, 19]. Osteoclasts create bone erosions by resorbing periarticular and subchondral bone over a period of months to years, although an individual osteoclast life span is much shorter (2 to 4 weeks) [10, 18, 19, 26]. Thus, mature osteoclasts must be constantly renewed by OCPs in order to sustain pathological bone resorption [8, 9, 24]. Characterization of the discrete OCP subpopulations has become necessary to elucidate the effects of systemic and local stimuli, such as hormones and cytokines/chemokines, on the recruitment and homing of OCPs, on their differentiation to mature osteoclasts, as well as on their activity and lifespan [2, 4, 19, 21]. In this review we have focused on studies defining surface marker expression profile of OCPs, which were further functionally tested in osteoclastogenic *in vitro* assays and osteoresorptive *in vivo* arthritic models/diseases.

##### 4.1. Murine osteoclast progenitors

Physiologically, OCPs reside within the bone marrow, spleen and peripheral-blood mononuclear cell (PBMC) pools (Table 1). Investigations of murine bone marrow have identified immature myeloid CD117<sup>+</sup>CD115<sup>+</sup>RANK<sup>-</sup> cells as progenitors with the ability to differentiate into osteoclasts and DCs *in vitro* [27]. In addition, a rare (0.1-0.3%) highly osteoclastogenic population was characterized among lymphoid negative population within immature myeloid subset, expressing a phenotype B220<sup>-</sup>CD3<sup>-</sup>CD11b<sup>-/lo</sup>CD115<sup>+</sup> and the variable level of CD117 (cKit), with CD117<sup>hi</sup> cells being the most potent in generating osteoclasts [28]. Further dissection revealed B220<sup>-</sup>CD3<sup>-</sup>CD11b<sup>-</sup><sup>lo</sup>CD115<sup>+</sup>CD117<sup>+</sup>CX3CR1<sup>+</sup> cells as a common bone marrow progenitors for osteoclasts, macrophages and DCs at the single-cell clonal level, expressing, to a certain degree, other myeloid markers such as Ly6C and F4/80 [29, 30]. By using different combination of surface markers, Xiao et al identified a common bone marrow progenitor for osteoclasts, DCs and macrophages, characterized as B220<sup>-</sup>CD117<sup>+</sup>CD115<sup>+</sup>CD11b<sup>lo</sup>CD27<sup>hi</sup>. In a mouse model of chronic immune activation, sustained CD27/CD70 interaction caused an arrest in osteoclast differentiation and accumulation of OCPs suggesting a skewing toward DC differentiation [31].

Additional two studies attempted to define the OCP subpopulations in murine models of arthritis. Human TNF- $\alpha$  transgenic (hTNF-Tg) mice had a significant increase in bone marrow proliferating CD11b<sup>+</sup>Gr-1(Ly6G)<sup>-/lo</sup> cells as well as circulating CD11b<sup>+</sup>Gr-1(Ly6G)<sup>-/lo</sup> cells [32]. Bone marrow and peripheral blood CD11b<sup>+</sup>Gr-1(Ly6G)<sup>-/lo</sup> cells efficiently differentiated into TRAP<sup>+</sup> osteoclasts upon

stimulation with M-CSF and RANKL. In addition, TNF- $\alpha$  treatment *in vitro* stimulated proliferation and the conversion of CD11b<sup>+</sup>Gr-1<sup>-/lo</sup>CD115<sup>-</sup> to CD11b<sup>+</sup>Gr-1<sup>-/lo</sup>CD115<sup>+</sup> osteoclastogenic cells. In the Sakaguchi (SKG) mouse model of inflammatory arthritis myeloid OCPs, identified as a CD3<sup>-</sup>B220<sup>-</sup>Ter119<sup>-</sup>CD11b<sup>-/lo</sup>Ly6C<sup>hi</sup> population, expanded in bone marrow and had the capacity to differentiate into multinucleated bone-resorbing osteoclasts *in vitro* and *in vivo* [33]. These OCPs were further characterized as being CD135<sup>lo</sup>CD11c<sup>-</sup>CX3CR1<sup>+</sup>CD115<sup>+</sup>RANK<sup>-</sup> and not uniform in the expression of CD117 and osteoclastogenic potential of single-cell clones. Described OCP population was distinct from bone marrow monocyte DC progenitors, but retained the plasticity to differentiate into DCs and macrophages under appropriate culture conditions. In addition, OCPs shared the phenotype with the myeloid-derived suppressor cell (MDSC) population and exerted an immunomodulatory function upon a co-adoptive transfer with SKG CD4<sup>+</sup> T lymphocytes. Sawant et al also observed that bone marrow CD11b<sup>+</sup>Gr-1<sup>+</sup>CD80<sup>lo</sup>CD115<sup>+</sup>F4/80<sup>-</sup> MDSCs of breast tumor-bearing mice, found among the immature myeloid population, can be differentiated into functional osteoclasts [34].

Cells with osteoclastogenic potential also exist in blood and peripheral hematopoietic organs. Both splenic and peripheral blood monocyte progenitors have been identified to share the B220<sup>-</sup>CD3<sup>-</sup>NK1.1<sup>-</sup>CD11b<sup>+</sup>Ly-6C<sup>hi</sup>CD115<sup>+</sup>CX3CR1<sup>+</sup> phenotype, with the capacity to differentiate into osteoclasts, macrophages and DCs. This OCP population was negative for the granulocyte marker Ly6G and expressed intermediate level of CD117 [30]. Circulating long-lived quiescent lineage-committed OCPs (QOPs) were characterized as CD115<sup>lo</sup>RANK<sup>hi</sup> cells, with almost no expression of myeloid markers CD11b, F4/80 and Gr-1, and were also present in very low numbers in bone marrow [35]. In response to different osteoclastogenic stimuli QOPs had the potential to differentiate into osteoclasts *in vitro* and *in vivo* [35, 36].

In models of arthritis *in vivo*, great expansion of CD11b<sup>+</sup>RANK<sup>+</sup> splenocytes was observed, possibly serving as a source of osteoclasts responsible for bone destruction in collagen induced arthritis (CIA) established in the IFN- $\gamma$ R knock-out mice [37]. Another study using the CIA model revealed that *in vivo* depletion of CD11b<sup>+</sup>Gr-1<sup>+</sup>CCR2<sup>+</sup> monocytes by anti-CCR2 antibody significantly attenuated the severity of arthritis [38]. Systemic increase in TNF- $\alpha$  has been shown to mediate an enlargement of peripheral CD11b<sup>hi</sup>CD115<sup>+</sup> OCP population in the hTNF-Tg mouse model. Expanded CD11b<sup>hi</sup> splenocyte population was negative for CD3, B220, F4/80 and CD117, and inhomogeneous for Gr-1 and RANK (<20% cells) [39].

Chemokine receptor expression, attributed to OCPs, is crucial for their selective attraction to bone and subsequent sustained pathological bone resorption in arthritis [5, 8, 40, 41]. Besides the already described expression of CX3CR1 (fractalkine (CX3CL1) receptor) and CCR2 (receptor for monocyte chemotactic protein 1 (MCP-1)/CCL2) [29, 33, 42], OCPs also express CXCR4, a receptor for stromal cell-derived factor 1 (SDF-1/CXCL12), secreted by bone marrow stromal cells, vascular endothelium and immature osteoblasts [40, 41, 43].



#### 4.2. Human osteoclast progenitors

Peripheral blood monocyte pool, originating from myeloid bone marrow progenitors, contains DC and macrophage progenitors as well as, at low frequency, OCPs (Table 2). It was estimated that 1-2% of peripheral blood monocytes are capable of differentiating into osteoclasts under physiological conditions [44-46]. Circulatory OCPs, found in higher frequency in arthritic patients, migrate to bone surfaces attracted by osteoclastogenic signals and subsequently differentiate into osteoclasts creating bone erosions [46, 47].

Several monocyte markers were used, such as CD11b, CD14, CD16 or CD51/61, to purify human peripheral OCPs and test their osteoclastogenic potential *in vitro*, upon treatment with cytokines (including RANKL, M-CSF, TNF- $\alpha$ , IL-6, IL-17 and IL-32), synovial fibroblasts or synovial fluid from arthritic patients [47-57]. The osteoclast forming capacity of CD14<sup>+</sup> cells has been observed to be higher compared with CD11b<sup>+</sup> or CD61<sup>+</sup> cells [53], and to correspond to the expression level of CD16 and DC-STAMP (dendritic cell-specific transmembrane protein) [54]. Under *in vitro* osteoclastogenic conditions CD14<sup>+</sup> monocytes acquired CD51/61 (integrin  $\alpha$ v $\beta$ 3/vitronectin receptor) and CD16 (FcR $\gamma$ III) markers. Among the circulatory CD14<sup>+</sup>CD11b<sup>+</sup>CD51/61<sup>+</sup> monocyte population, CD14<sup>hi</sup>CD16<sup>+</sup> subset was significantly increased in multiple myeloma patients [55]. Peripheral blood and bone marrow CD14<sup>+</sup>RANK<sup>hi</sup> monocytes differentiated more efficiently into osteoclasts compared with CD14<sup>+</sup>RANK<sup>int</sup> and CD14<sup>+</sup>RANK<sup>lo</sup> cells. Moreover, CD45<sup>+</sup>CD14<sup>+</sup>CD51/61<sup>+</sup>CD115<sup>+</sup>RANK<sup>+</sup> cells were identified as committed OCPs in a giant cell tumor of bone [56]. Although OCPs express  $\beta$ 2 integrins such as CD11b/CD18, CD11b expression seems to be a negative regulator of the earliest stages of osteoclast differentiation by transiently suppressing RANK expression. The RANKL signal induced a switch in the integrin expression from  $\beta$ 2 to  $\beta$ 3 on CD14<sup>+</sup> OCPs, attenuating the  $\beta$ 2 integrin negative role in osteoclastogenesis [47].

Monocytes can be dissected based on the expression of CD16, into CD16<sup>+</sup> and CD16<sup>-</sup> subsets that differ in their migratory pattern, cytokine profile and lineage commitment [44, 46, 57]. In physiological conditions, OCPs have been found among CD14<sup>+</sup>CD16<sup>-</sup> monocyte pool, possibly within the subset of immature (proliferating) monocytes (defined as CD14<sup>+</sup>CD16<sup>-</sup>CD64<sup>+</sup>CD33<sup>+</sup>CD13<sup>lo</sup>CD115<sup>+</sup>) [58]. Komano et al described a highly osteoclastogenic subpopulation of peripheral blood monocytes, expressing the CD14<sup>+</sup>CD16<sup>-</sup>(CD33<sup>hi</sup>)CD115<sup>lo</sup> phenotype [46]. However, the CD16<sup>+</sup> subset increases in inflammatory conditions and migrates into the sites of inflammation [44, 46]. Peripheral blood samples from patients with PsA, particularly those with bone erosions, showed significantly expanded OCP population that resides within the CD14<sup>+</sup>CD16<sup>+</sup> monocyte pool [57]. A marked expansion of CD16<sup>+</sup> monocytes in peripheral blood and their accumulation in the synovial tissue was also reported in RA. This monocyte subset overlapped with human cartilage gp-39 expression (a possible autoantigen in RA), did not express lymphoid markers (CD3<sup>-</sup>CD4<sup>-</sup>CD8<sup>-</sup>CD20<sup>-</sup>CD56<sup>-</sup>), but weakly expressed some other myeloid markers (CD33<sup>lo</sup>MHCII<sup>lo</sup>CD14<sup>lo</sup>) [59]. However, CD16<sup>-</sup> cells also accumulate in RA synovium, and differ from CD16<sup>+</sup> cells by their expression of

CD51/61, which is important for committed OCP migration and associated with osteoclast maturation [46, 47]. Described results indicate that CD14<sup>+</sup>CD16<sup>+</sup> monocytes represent a more mature (mostly quiescent) stage in osteoclast differentiation sequence compared with CD14<sup>+</sup>CD16<sup>-</sup> and CD14<sup>+</sup>CD16<sup>int</sup> subsets [57, 58]

Although the cell-surface phenotype of peripheral OCPs has been extensively studied in arthritis, the functional relations of circulating OCPs to bone marrow OCPs and their bone-directed homing, mostly governed by chemokine signals, have not been clearly established. CXCR4, unique CXCL12 receptor, is highly expressed in human OCPs and mature osteoclasts, indicating that CXCL12 may be important for chemotaxis, differentiation and survival of human osteoclast lineage cells [60]. CCR1 and CCR5, which interact with inflammatory protein 1 $\alpha$  (MIP-1 $\alpha$ /CCL3), have been detected in bone marrow derived OCPs, able to generate osteoclasts *in vitro* when stimulated with MIP-1 $\alpha$  [61].

Osteoclastogenic potential of peripheral blood was identified as biomarker of erosive disease in RA, psoriatic arthritis (PsA) and other forms of SpA [5, 46, 52, 53, 56, 57, 59]. Based on our previous study, which showed the osteoclastogenic potential of CD3<sup>-</sup>CD19<sup>-</sup>CD56<sup>-</sup>CD14<sup>+</sup>CD11b<sup>+</sup> PBMCs [52], we assessed the association in the frequency of such OCPs and clinical disease activity score (DAS28) in the follow-up samples of PsA patients during anti-TNF treatment (Enbrel, 50 mg/week) (Figure 1). Our results revealed positive correlation between the proportion of peripheral OCP and DAS28 score (in 3 out of 4 patients) as well as reduction of both parameters with anti-TNF treatment (in 2 out of 4 patients), suggesting favorable therapeutic response (Figure 1).

#### 4.3. Effect of arthritic mediators on osteoclast progenitors

Under pathological arthritic conditions, the process of osteoclastogenesis is markedly enhanced by various innate and acquired immune mediators, particularly proinflammatory cytokines, which act on OCPs directly or indirectly, through RANK/RANKL/OPG system [3, 17, 20]. It has been shown that even a small rise in the level of systemic inflammation can precipitate osteodestruction [2, 21, 62]. Thus, investigation of factors that promote osteoclast activity may provide insight into the events responsible for the pathological bone loss seen in arthritis. Due to the length constrain, we are discussing only the positive regulators of OCPs and their described roles in arthritis (Figure 2). Additional positive and negative regulators of osteoclast differentiation and activity associated with inflammatory conditions have been identified and extensively reviewed by several recent publications [5-8].

The altered cytokine profile created by inflammatory cells is a crucial part of arthritis etiology, with proinflammatory cytokines (elevated systemically and produced within synovial compartment) playing a key role in arthritic bone destruction [5-8, 40, 41, 63]. Particularly, TNF- $\alpha$  potently induces osteoclastogenesis through several mechanisms: by upregulating RANK expression in OCPs and RANKL expression in osteoblasts and synovial fibroblasts; by affecting OCPs directly and independently of RANKL; and by inducing M-CSF expression in bone marrow stromal cells [32, 39,

64, 65]. TNF-induced bone loss is highly dependent on IL-1, and IL-1-deficient hTNF-Tg mice show no bone loss and cartilage destruction. IL-1 stimulates osteoclastogenesis by increasing production of RANKL, M-CSF and PGE<sub>2</sub> in osteoblast lineage cells, while decreasing OPG [64, 66]. IL-6, induced by TNF- $\alpha$ , contributes to bone destruction by stimulating RANKL expression and by regulating development of Th17 lymphocytes [67]. TNF- $\alpha$  upregulates IL-34, which mediates chemotactic migration of OCPs and supports the RANKL-induced osteoclastogenesis in the absence of M-CSF [25].

Besides the activation of proinflammatory loop by innate immune cells, Th17 lymphocytes and associated cytokine profile have also been proven to mediate development of inflammatory arthritis [5, 7]. IL-17, a hallmark Th17 cytokine, stimulates bone destruction and is present in the synovium of RA patients. It is also produced by mast cells, a major source of IL-17 in inflammatory arthritis. *In vitro*, IL-17 stimulates osteoclastogenesis mostly indirectly by inducing RANKL expression (in osteoblasts and synovial fibroblasts) and production of proinflammatory cytokines (IL-6, IL-8, TNF- $\alpha$  and IL-1), but may also upregulate RANK in human OCPs and act in a RANKL-independent manner [68]. Some potent arthritic mediators exert their effects in synergy with IL-17, such as IL-23, which is able to elicit severe arthritis with a profound bone-resorptive phenotype. It enhances osteoclast differentiation by upregulating RANK expression in peripheral blood OCPs [69]. IL-32, induced by IL-17, stimulates TNF- $\alpha$  and IL-1 $\beta$  production, and promotes maturation of OCPs independently of RANKL [70]

Other cytokines contribute to the pathology of arthritis mainly by increasing RANKL expression in CD4<sup>+</sup> T lymphocytes or synovial fibroblasts. IL-7 induces overexpression of RANKL in T lymphocytes and its blockade significantly reduces monocyte attraction, osteoclast differentiation and bone erosion in CIA [71]. IL-21 stimulates osteoclastogenesis through an induction of RANKL in CD4<sup>+</sup> T lymphocytes, and is upregulated in the synovia and sera of RA patients [72]. Although its role in osteoclastogenesis is still controversial, IL-18 is known to induce M-CSF, GM-CSF, RANKL and OPG in synovial fibroblasts and/or T lymphocytes [73]. IL-22 promotes osteoclastogenesis in RA by upregulating RANKL expression in synovial fibroblasts, through the p38 MAPK/NF- $\kappa$ B and JAK-2/STAT-3 pathways [74]. In contrast, SOFAT (secreted osteoclastogenic factor of activated T-cells), produced by activated T lymphocytes, induces osteoclastogenesis independently of RANKL. As chronic T lymphocyte activation is involved in RA etiology, SOFAT can present additional link in the cross-talk between bone and immune cells in arthritis [75].

Several cytokines overexpressed in synovial tissue may act directly on OCPs, mediating arthritic processes. Besides the already described TNF- $\alpha$  and IL-32, IL-33 induces osteoclastogenesis from human monocytes, by activating MAPKs, NF- $\kappa$ B and Syk/PLC $\gamma$  pathways independently of RANKL [76]. IL-15 directly promotes differentiation of rodent OCPs to preosteoclasts *in vitro*, through the MAPKs and NF- $\kappa$ B pathways, but also upregulates RANKL expression in synovial fibroblasts [77]. Although some of the proinflammatory cytokines (ie IL-6, IL-18, IL-33) may exhibit the anti-

osteoclastogenic effects, it mostly applies to the early stages of osteoclastogenesis, whereas in chronic inflammatory conditions these cytokines predominately work as pro-osteoclastogenic mediators [5, 6] Chemoattractants directing the homing of OCPs from circulation to bone sites are not fully characterized [7, 8, 40, 41]. Among the best defined is CXCL12, which directly promotes early stages of osteoclast development. It also attracts OCPs to bone surfaces, and activates T lymphocytes and synovial fibroblasts in RA to secrete RANKL [43, 60, 78]. CCL20 is over-expressed in subchondral bone in RA, and is able to induce OCP expansion and osteoclast differentiation [79] Although treatment of RA patients with anti-CCL2 antibody failed to produce clinical improvement, recent studies revealed that serum CCL2 level is associated with osteoclastogenic potential of peripheral blood OCPs and clinical markers of arthritis activity [52, 80].

## **5. Conclusions**

*In vivo* plasticity, as a hallmark of the myeloid lineage, enables the flexible lineage commitment of monocyte progenitors toward osteoclasts, macrophages or DCs, depending on the cytokine and chemokine milieu in the bone marrow, circulation and synovium. Within the pool of immature monocytes, OCPs are found in higher frequency in the peripheral blood and synovial tissue of patients with RA and SpA, mediating bone loss locally, in the form of bone erosions and joint osteolysis, and systemically, with loss of skeletal bone density. Moreover, the size and activity of OCP population corresponds to arthritis severity, and may be used to monitor disease progression and bone resorption. Thus, osteoclasts represent a functional link between joint inflammation and structural damage in arthritis. However, the human OCP subpopulations are not yet precisely defined and it is, still, unclear by which exact mechanisms the cytokine/chemokine network induces activation and egress of OCPs from hematopoietic organs and attracts them to joint and bone lesions. A number of immunomodulatory treatments aimed to inhibit osteoclast differentiation and activity are in use or under investigation, including biological therapies aimed to block IL-1, TNF- $\alpha$ , IL-6 and RANKL, but with only partial effect in reducing bone resorption. Control of the migratory behavior of OCPs and identification of crucial bone/joint chemotactic mediators represent promising therapeutic targets in arthritis, with the fine dissection of the discrete OCP populations being the necessary prerequisite for such approaches.

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## **Conflict of Interest**

The authors declare that they have no conflict of interest.

## Refereces

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**Table 1.** Surface marker expression profile of mouse osteoclast progenitor populations

Osteoclast progenitor phenotype	Source <sup>a</sup>	Reference
CD117 <sup>+</sup> CD115 <sup>+</sup> RANK <sup>-</sup>	BM	27
B220 <sup>-</sup> CD3 <sup>-</sup> CD11b <sup>-lo</sup> CD115 <sup>+</sup> CD117 <sup>+</sup> CX3CR1 <sup>+</sup> B220 <sup>-</sup> CD3 <sup>-</sup> NK1.1 <sup>-</sup> CD11b <sup>+</sup> Ly-6C <sup>hi</sup> CD115 <sup>+</sup> CX3CR1 <sup>+</sup>	BM PBL, SPL	28, 29, 30
B220 <sup>-</sup> CD117 <sup>+</sup> CD115 <sup>+</sup> CD11b <sup>lo</sup> CD27 <sup>hi</sup>	BM	31
CD115 <sup>lo</sup> RANK <sup>hi</sup> (mostly CD11b <sup>-</sup> F4/80 <sup>-</sup> Gr-1 <sup>-</sup> )	BM, PBL	35
CD11b <sup>+</sup> Gr-1 <sup>+</sup> CD80 <sup>lo</sup> CD115 <sup>+</sup> F4/80 <sup>-</sup>	BM (TM)	34
CD11b <sup>+</sup> Gr-1 <sup>+</sup> CCR2 <sup>+</sup>	PBL, SYN (CIA)	38
CD3 <sup>-</sup> B220 <sup>-</sup> Ter119 <sup>-</sup> CD11b <sup>-lo</sup> Ly6C <sup>hi</sup> CD135 <sup>lo</sup> CD11c <sup>-</sup> CD115 <sup>+</sup> CD117 <sup>+</sup> CX3CR1 <sup>+</sup> RANK <sup>-</sup>	BM (SKG)	33
CD11b <sup>+</sup> Gr-1(Ly6G) <sup>-lo</sup>	BM, PBL (hTNF-Tg)	32
B220 <sup>-</sup> CD3 <sup>-</sup> F4/80 <sup>-</sup> CD117 <sup>-</sup> CD11b <sup>hi</sup> CD115 <sup>+</sup>	SPL (hTNF-Tg)	39
CD11b <sup>+</sup> RANK <sup>+</sup>	SPL (IFN- $\gamma$ R KO CIA)	37

<sup>a</sup> Osteoclast progenitors, capable of differentiating into functional osteoclasts, were characterized using multiple surface markers by flow-cytometry, in bone marrow (BM), peripheral blood (PBL), spleen (SPL) and synovium (SYN), in wild type and genetically modified mice under physiological and pathological conditions. RANK, receptor activator of nuclear factor- $\kappa$ B; TM, breast-tumor bearing mice; SKG, Sakaguchi model of arthritis; hTNF-Tg, human tumor necrosis factor  $\alpha$  transgenic model of arthritis; IFN- $\gamma$ R KO, interferon  $\gamma$  receptor knock-out mice; CIA, collagen induced arthritis.

**Table 2** Surface marker expression profile of human osteoclast progenitor populations

Osteoclast progenitor phenotype	Source <sup>a</sup>	Reference
CD14 <sup>+</sup> ; CD11b <sup>+</sup> ; or CD61 <sup>+</sup>	PBL	53
CD3 <sup>-</sup> CD19 <sup>-</sup> CD56 <sup>-</sup> CD14 <sup>+</sup> CD11b <sup>+</sup>	PBL	52
CD14 <sup>+</sup> CD11b <sup>+</sup> (intβ1 <sup>+</sup> intβ2 <sup>+</sup> intβ3 <sup>-</sup> )	PBL	47
CD14 <sup>hi</sup> CD11b <sup>+</sup> CD51/61 <sup>+</sup> CD16 <sup>+</sup>	PBL (MM)	55
CD14 <sup>+</sup> RANK <sup>hi</sup> CD45 <sup>+</sup> CD14 <sup>+</sup> CD51/61 <sup>+</sup> CD115 <sup>+</sup> RANK <sup>+</sup>	PBL, BM GCT	56
CD14 <sup>+</sup> CD16 <sup>-</sup> (CD33 <sup>hi</sup> )CD115 <sup>lo</sup>	PBL, SYN (RA)	46
CD16 <sup>+</sup> (gp-39): CD3 <sup>-</sup> CD4 <sup>-</sup> CD8 <sup>-</sup> CD20 <sup>-</sup> CD56 <sup>-</sup> CD33 <sup>lo</sup> MHCII <sup>lo</sup> CD14 <sup>lo</sup>	PBL, SYN (RA)	59
CD14 <sup>+</sup> CD16 <sup>+</sup> DC-STAMP <sup>+</sup>	PBL (PsA)	54
CD14 <sup>+</sup> (MHCII <sup>+</sup> )CD16 <sup>+</sup>	PBL (PsA)	57

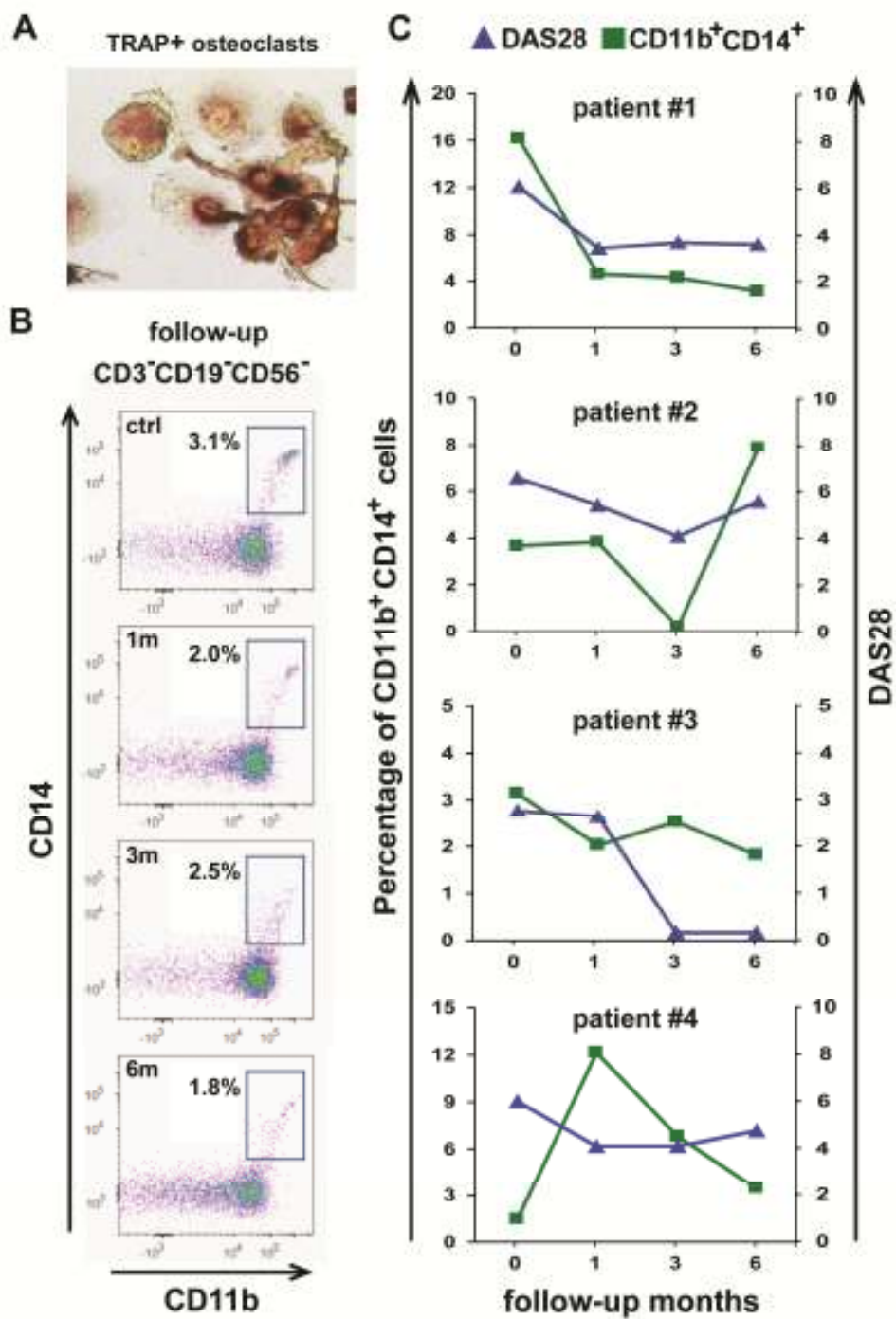
<sup>a</sup> Osteoclast progenitors, capable of differentiating into functional osteoclasts, were characterized using multiple surface markers by flow-cytometry in bone marrow (BM), peripheral blood (PBL) and synovium (SYN), in control samples and patients with malignant (multiple myeloma (MM), giant cell tumor of bone (GCT)) and chronic joint diseases (psoriatic arthritis (PsA), rheumatoid arthritis RA). RANK, receptor activator of nuclear factor-κB; int, integrin; DC-STAMP, dendritic cell-specific transmembrane protein; gp-39, human cartilage gp-39, a possible autoantigen in RA; MHC, major histocompatibility complex.

## Figure legends

**Figure 1.** Effect of anti-TNF- $\alpha$  therapy on osteoclast progenitors in psoriatic arthritis. Peripheral blood samples of patients with psoriatic arthritis (PsA; 2 females/2 males, age range 47-67 years, arthritis duration range 8-14 years, with the clinical form of spondylitis with peripheral arthritis; with the approval of the Ethics Committee and informed consent) were collected in a time-course of anti-TNF- $\alpha$  therapy (Enbrel, 50 mg/week): immediately prior to therapy introduction (ctrl), and at month 1 (1m), month 3 (3m) and month 6 (6m) of the treatment. Peripheral-blood mononuclear cells were analyzed for the frequency of osteoclast progenitors (OCPs), bearing phenotype CD3<sup>-</sup>CD19<sup>-</sup>CD56<sup>-</sup>CD14<sup>+</sup>CD11b<sup>+</sup>, by flow-cytometry. A) Sorted OCPs were differentiated by the addition of RANKL (60 ng/mL) and M-CSF (40 ng/mL), and analyzed for the presence of TRAP<sup>+</sup> osteoclast-like cells at day 12 of culture; representative image. B) Dynamic changes in OCP frequency during the follow-up period of TNF- $\alpha$  therapy. Density plots represent the percentages of lymphoid negative cells (CD3<sup>-</sup>CD19<sup>-</sup>CD56<sup>-</sup>) in the context of monocytoid CD14 and CD11b markers for the representative patient. C) Association of OCP frequency and clinical disease activity score (DAS28) in a time-course of anti-TNF- $\alpha$  therapy (patients #1 to #4).



Figure 1



**Figure 2.** Perpetuation of osteoclast progenitor activity by pro-resorptive cytokines and chemokines in arthritis. The possible scenario of exaggerated and sustained osteoresorption is initiated by overstimulation of osteoclast progenitor (OCP) activity by inflammatory/immune mediators. These mediators (mostly cytokines and chemokines), overexpressed in arthritis, can potentially promote osteoclastogenesis by having direct (on OCPs) or indirect (through other cells in the microenvironment, such as stromal cells, osteoblasts, T lymphocytes and fibroblast-like synoviocytes) effects on RANK/RANKL expression. OCPs, generated in hematopoietic tissues in response to the essential osteoclastogenic factors RANKL and M-CSF, migrate either directly to the sites of bone resorption or are first released into circulation as quiescent osteoclast progenitors (QOPs) and then home to bone attracted by chemotactic mediators. Once attached to the bone surface, committed OCPs, highly expressing RANK, differentiate into mature bone-resorbing osteoclasts in response to osteoclastogenic stimuli. For clarity, only the most important positive regulators of osteoclast differentiation in arthritis are shown. IL, interleukin; TNF- $\alpha$ , tumor necrosis factor  $\alpha$ ; RANK, receptor activator of nuclear factor- $\kappa$ B; RANKL, RANK ligand; M-CSF, macrophage colony-stimulating factor; cFms, M-CSF receptor; CXCR/CCR, chemokine receptors.

**Figure 2**

