OPINION

Alternative pathways of osteoclastogenesis in inflammatory arthritis

Iannis E. Adamopoulos and Elizabeth D. Mellins

Abstract | Osteoclasts are cells of haematopoietic origin that are uniquely specialized to degrade bone. Under physiological conditions, the osteoclastogenesis pathway depends on macrophage colony-stimulating factor 1 (CSF-1, also known as M-CSF) and receptor activator of nuclear factor κB ligand (RANKL). However, an emerging hypothesis is that alternative pathways of osteoclast generation might be active during inflammatory arthritis. In this Perspectives article, we summarize the physiological pathway of osteoclastogenesis and then focus on experimental findings that support the hypothesis that infiltrating inflammatory cells and the cytokine milieu provide multiple routes to bone destruction. The precise identity of osteoclast precursor(s) is not yet known. We propose that myeloid cell differentiation during inflammation could be an important contributor to the differentiation of osteoclast populations and their associated pathologies. Understanding the dynamics of osteoclast differentiation in inflammatory arthritis is crucial for the development of therapeutic strategies for inflammatory joint disease in children and adults.

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Introduction

A number of different forms of chronic arthritis in children and adults can result in erosive disease, causing substantial morbidity. In adults, these potentially destructive arthritides include rheumatoid arthritis (RA), ankylosing spondylitis and psoriatic arthritis (PsA). Bone erosions are observed in more than 45% of patients with early RA and PsA.[1,2] In children, erosive subtypes of arthritis include polyarticular juvenile idiopathic arthritis (JIA), extended oligoarticular arthritis, systemic JIA and PsA. Although bone erosions occur in these childhood diseases, the prevalence of this outcome is not well defined as imaging assessments in children are challenging, owing to the unique features of the growing skeleton.[3]

Epidemiological, immunogenetic and clinical lines of evidence indicate that the various forms of erosive arthritis have distinct aetiologies. Although many factors influence the initiation of musculoskeletal damage, the mechanism of bone and joint destruction seems to represent, in large part, a final common pathway. This pathway relies on the differentiation and activation of osteoclasts, the only specialized cells to resorb bone. Osteoclasts are terminally differentiated cells of the myeloid lineage, and their precursors are mononuclear phagocytes. The regulation of their differentiation under physiological conditions has been extensively studied with genetic experiments in mice.[4] However, emerging evidence, especially from primary human cell cultures, suggests that inflammatory conditions give rise to alternative pathways of osteoclast differentiation and activation. These pathways are less-well-studied than the physiological pathway and involve a variety of cytokines, as mediators, and several cell types, both as targets of these secreted factors and as participants in the cell–cell interactions that lead to differentiation of functional osteoclasts. The contribution of alternative pathways of osteoclast differentiation and activation to erosive potential in inflammatory arthritis is the subject of this Perspectives article.

Competing interests
The authors declare no competing interests.

Physiological bone remodelling

Physiological bone remodelling is orchestrated by two main cell types with opposing functions: osteoblasts, which form new bone, and osteoclasts, which resorb damaged or old bone. Osteoblasts are derived from mesenchymal stem cell (MSC) progenitors, which reside in the bone marrow close to haematopoietic stem cell (HSC) niches. This location enables MSCs to maintain bone marrow homeostasis and to regulate the maturation of both haematopoietic and non-haematopoietic cells. MSCs have broad potential and differentiate into cell types including osteoblasts, osteocytes, adipocytes and chondrocytes (reviewed in detail elsewhere[5]). Osteoclasts, on the other hand, are derived from bone-marrow HSCs, which also have the capacity to differentiate into other cell types, including macrophages and dendritic cells (DCs).[6]

Osteoclast differentiation

The development of the mononuclear phagocyte system is controlled primarily by cytokines, with macrophage colony-stimulating factor 1 (CSF-1, also known as M-CSF) as the principal regulator of lineage. CSF-1 signals through the tyrosine kinase receptor CSF-1R, which is ubiquitously expressed during early myeloid lineage commitment, and its expression is maintained by nearly all mononuclear phagocytic cells and by terminally differentiated osteoclasts, highlighting the interdependence of these lineages.[6] The differentiation of osteoclast precursors under physiological conditions is regulated by receptor activator of nuclear factor κB ligand (RANKL, also known as TNF ligand superfamily member 11). RANKL-mediated osteoclast differentiation depends on receptor activator of nuclear factor κB (RANK; TNF receptor superfamily member 11A), the expression of which is induced by CSF-1 stimulation of early-stage osteoclast precursors. CSF-1 activity generates a subset of myeloid cells expressing both CSF-1R and RANK that fuse to become terminally differentiated multinucleated osteoclasts upon further stimulation, on bone surfaces, with CSF-1 and RANKL. Osteoblasts, which control the availability of CSF-1 and RANKL to osteoclast precursors, also regulate the production and secretion of osteoprotegerin (OPG; TNF receptor superfamily member 11B), a soluble decoy receptor for RANKL. In addition, osteocytes are

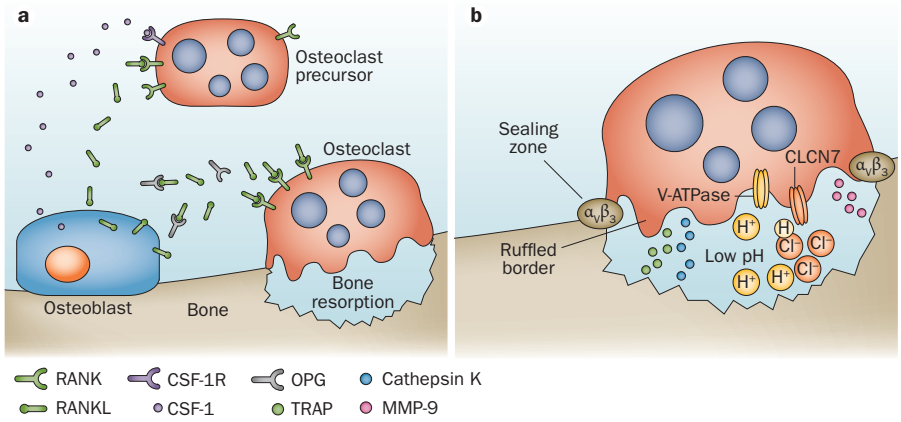

Figure 1 | Bone remodelling and physiological osteoclast differentiation. Schematic representation of **a** | the cellular interactions of the RANKL–RANK–OPG axis amongst osteoblasts, osteoclast precursors and osteoclasts, and **b** | the molecular machinery, acid, lytic enzymes and cytoskeletal organization required to resorb bone. Abbreviations: $av\beta_3$, $av\beta_3$ integrin; CLCN7, chloride channel 7 (H^+Cl^- exchange transporter 7); CSF-1, macrophage colony-stimulating factor 1; CSF-1R, macrophage colony-stimulating factor 1 receptor; MMP-9, matrix metalloproteinase 9; OPG, osteoprotegerin; RANK, receptor activator of nuclear factor κB; RANKL, receptor activator of nuclear factor κB ligand; TRAP, tartrate-resistant acid phosphatase; V-ATPase, vacuolar-type H^+-ATPase.

a major source of RANKL.[7] Thus, the main determinants of osteoclastogenesis are the relative concentrations of CSF-1, RANKL and OPG (Figure 1a).[4,6]

Osteoclast function

Osteoblasts and osteoclasts interact at the bone surface—through the RANKL–RANK–OPG axis—to balance bone formation and resorption and maintain bone homeostasis during skeletal growth in childhood, skeletal remodelling in adolescence, repair after fracture or microfracture, and in response to local biomechanical influences. New bone formation also involves ossification, a well-orchestrated, complex process in which crystals of calcium phosphate are produced by osteoblasts and deposited within the bone's fibrous matrix.[8] The extent of resorption and ossification is, therefore, tightly linked to the number and activity of osteoclasts and osteoblasts, and the regulation of these cells is necessary for bone homeostasis.

Osteoclast expression of bone-degrading enzymes, such as tartrate-resistant acid phosphatase (TRAP), cathepsin K and matrix metalloproteinase 9, regulates the bone resorption process. The activity of these enzymes is amplified by osteoclast expression of chloride channel 7 (H^+Cl^- exchange transporter 7) and vacuolar-type H^+-ATPase, which together acidify the extracellular space, optimizing the pH for the function of the bone-degrading enzymes during bone resorption.[4] Formation of a late-endosome-like, bone-facing osteoclast membrane region (ruffled border) facilitates directed secretion of enzymes within a defined extracellular space; this space is maintained by a sealing zone regulated by $a_v\beta_3$ integrins.[4] Dynamic structures, consisting of condensed arrays of podosomes interconnected by filamentous actin, concentrate the enzymes and H^+ ions in the bone-resorbing area (Figure 1b). A fully mature osteoclast is identified as a multinucleated giant cell that has all the aforementioned functional characteristics, which are best characterized *in vitro* by bone resorption assays.

Genetically, there is no single regulator of osteoclast action. Rather, the genes that mediate osteoclast functions are regulated by a large number of transcription factors, including microphthalmia-associated transcription factor (MITF), PU.1, nuclear factor κB (NFκB) and nuclear factor of activated T cells cytoplasmic 1 (NFATc1).[4] Many of the gene targets of these factors overlap, suggesting that the extent of concurrent upstream signalling could regulate the intensity of osteoclastogenesis and bone resorption. For instance, osteoclast-related target genes of MITF *CTSK* and *ACP5* (encoding cathepsin K and TRAP, respectively) are also transcriptional targets of PU.1 and NFATc1.[9,10] Simultaneous activation of these transcription factors can enhance osteoclastogenesis and lead to bone pathology.

Pathological osteoclastogenesis

The diversity and plasticity of haematopoietic precursors, in combination with an array of mediators that activate signalling cascades during inflammation, probably contribute to the heterogeneity of the various forms of inflammatory arthritis. Thus, the specific erosive phenotype of the various inflammatory arthritides might reflect differences in the availability of osteoclast precursors within the inflammatory infiltrate and in the nature of the cytokine milieu. In the remainder of this article, we summarize findings that support an emerging paradigm that, under inflammatory conditions, additional pathways are activated to generate osteoclasts.

Osteoclast precursors

Macrophages

Although it is widely appreciated that myeloid cells are crucial in synovial inflammation and the abundance of synovial macrophages correlates with progression of joint erosion,[11] understanding of the specific role of this cell type in the molecular pathogenesis of arthritic damage has been hindered, at least in part, by macrophage heterogeneity. Data from the Immunological Genome Project reveal that murine macrophages from various organs are transcriptionally diverse, with minimal overlap.[12] Similarly, high-dimensional cytometric analysis of human bone marrow shows a large number of distinct monocyte/macrophage phenotypes.[13]

An important contributor to macrophage diversity is the ability of these cells to adapt to stimuli in the microenvironment, including the cytokine milieu and cell–cell interactions with other innate immune cells. These polarizing factors lead to different activation states and can affect the capacity of macrophages to become osteoclasts. The initial paradigm for classification of macrophage activation states was the M1–M2 model. M1 refers to 'classical' activation of macrophages by IFN-γ from, for example, type 1 T helper (T_H1) cells, whereas M2 refers to 'alternative' activation by IL-4 and IL-13 from, for example, type 2 T helper (T_H2) cells.[14] However, macrophages are also activated during infection and tissue injury, through Toll-like receptors (TLRs), nucleotide-binding and oligomerization domain-like receptors, retinoid acid-inducible gene-I-like receptors, C-type lectin receptors and immunoreceptor tyrosine-based activation motif (ITAM)-associated receptors. Activation of macrophage TLRs can lead to NFκB activation and inhibition of RANK expression, attenuating the RANKL and CSF-1 signalling pathways and thus osteoclastogenesis.[15] On the other hand, C-type lectin receptors and ITAM-associated

receptors expressed by osteoclast precursors, such as osteoclast-associated receptor (OSCAR), provide co-stimulatory signals that synergize with RANK signalling to promote osteoclastogenesis.[16–18]

Dendritic cells

Some evidence suggests that immature murine DCs can differentiate into functional osteoclasts *in vitro* and *in vivo* through interactions with $CD4^+$ T cells and various unknown factors present in the bone marrow microenvironment.[19,20] Immature human DCs exposed to unidentified factors in RA synovial fluid are also induced to differentiate into osteoclasts.[21] Similarly, in multiple myeloma, IL-17A stimulates immature DCs to differentiate into osteoclast-like cells.[22] Evidence to date suggests that maturation of DCs might attenuate their differentiation into osteoclasts, but this area requires further investigation.[23] DCs are a heterogeneous cell lineage that includes various subtypes, such as $CD11c^+CD4^+$ cells, $CD11c^+CD8^+$ cells and $CD11c^+CD4^-CD8^-$ cells, and it remains to be determined whether a unique subset of immature DCs function as osteoclast precursors.[24]

Osteoclast differentiation

Engagement of CSF-1R is required for the differentiation and functional maturation of macrophages and DCs from their myeloid precursors. In the naturally occurring CSF-1-deficient *Csf1*$^{op/op}$ mouse strain, a 50–70% reduction (in comparison with wild-type mice) in all $CD11c^+$ DC subsets and a severe deficiency in mature macrophages are detected, along with impaired osteoclastogenesis and resulting osteopetrosis.[25,26] Notably, *Csf1*$^{op/op}$ mice recover with age, suggesting that CSF-1-independent osteoclastogenesis pathways exist.[25] However, neither the osteoclast precursor subtypes nor the particular (cytokine) stimuli involved in this model are yet defined.

Alternatives to CSF-1

Despite the knowledge gap related to the *Csf1*$^{op/op}$ model, other cytokines and growth factors, including granulocyte colony-stimulating factor (GM-CSF), have been reported to stimulate osteoclast differentiation in the absence of CSF-1 signalling.[27–30] GM-CSF induces expression of DC-specific transmembrane protein (DC-STAMP) and NFATc1, which leads to fusion of osteoclast precursors in cultured bone marrow-derived cells *in vitro*, resulting in the formation of multinucleated giant cells.[31] Importantly, expression of DC-STAMP is not exclusive to DCs; this protein is expressed at the surface of $CD14^+CD16^-$ and $CD14^+CD16^+$ osteoclast precursors.[32] Transcriptome analysis of human-derived and mouse-derived bone marrow cells treated with GM-CSF suggests that these osteoclast precursors are more like macrophages than they are like DCs.[33,34]

Although GM-CSF increases the number of immature DCs, which include osteoclast precursors, it also induces shedding of CSF-1R, resulting in disruption of its phosphorylation by CSF-1 and the induction of osteoclastogenesis by CSF-1 and RANKL produced by monocytes.[24,35] Therefore, GM-CSF has dual roles in osteoclastogenesis as it can both promote and inhibit osteoclast differentiation depending on the type of precursors and the osteoclastogenesis pathway utilized. In inflammatory arthritis, a different set of osteoclast precursors is available, which might respond to a variety of pro-inflammatory cytokines and compensate for the loss of CSF-1 signalling.[21,36]

Other factors that can substitute for CSF-1 include vascular endothelial growth factor (VEGF), which is produced by many cells including endothelial cells, macrophages, neutrophils, fibroblasts and T cells. VEGF prevents osteopetrosis in *Csf1*$^{op/op}$ mice *in vivo,* and the combination of VEGF and RANKL is sufficient to induce osteoclast formation *in vitro*.[27] However, terminally differentiated osteoclasts are smaller in size when cultured with VEGF plus RANKL than when cultured with CSF-1 plus RANKL, suggesting that fusion might be less intense, and osteoclast activity is possibly less efficient, in the former condition. In another study, Fms-related tyrosine kinase 3 (FLT3) ligand, which is produced by bone marrow stromal cells, partly compensated for loss of CSF-1 by inducing RANK expression on osteoclast precursors from *Csf1*$^{op/op}$ mice and promoting survival of terminally differentiated osteoclasts.[28] In these experiments, FLT3 ligand was ≥10-fold more effective for osteoclast generation than VEGF. Similar results have been obtained with hepatocyte growth factor (HGF), which signals through the c-Met tyrosine kinase receptor and engages downstream effectors such as proto-oncogene tyrosine-protein kinase Src, phosphatidylinositol-3 kinase, Sos a guanine nucleotide exchange factor for Ras, and the adaptor molecules Grb2 and Shc. HGF plus RANKL also results in osteoclast formation in cultures of human cells, although, as with VEGF plus RANKL, osteoclast activity was considerably lower than in cultures treated with CSF-1 plus RANKL.[29] These observations with VEGF, FLT3 ligand and HGF have been corroborated in human osteoclastogenesis assays.[37]

CSF-1R is also the receptor for IL-34. IL-34 is expressed in various tissues and promotes the differentiation and viability of monocytes and macrophages. However, targeted ablation of IL-34 does not lead to substantial alterations in the development of bone marrow, splenic or liver macrophages; only the development of Langerhans cells and microglia are inhibited.[38] Nevertheless, IL-34 has been shown to affect osteoclast formation, both *in vivo* and *in vitro*, and has been proposed as an important factor in osteoclastogenesis.[39]

Overall, the various substitutes for CSF-1 and their partial rescue of both macrophage and DC development suggest that the cytokine milieu and inflammatory infiltrate in the arthritic joint could determine the specific origin(s) of osteoclasts in particular types of arthrtitis.

Alternatives to RANKL

In inflammatory arthritis, an increase in RANKL secretion by synovial fibroblasts and type 17 T helper (T_H17) cells has been documented.[40,41] Moreover, RANKL knockout mice are protected against bone erosion in the K/B×N serum transfer model of inflammatory arthritis and accordingly, in RA clinical trials a humanized anti-RANKL antibody reduced bone destruction.[42,43] Although RANKL undoubtedly has a role in both physiological and pathological bone destruction, there is also evidence for RANKL-independent osteoclastogenesis pathways. A compelling example is the presence of TRAP-positive and cathepsin K-positive multinucleated osteoclasts in RANK-deficient osteopetrotic mice after the administration of TNF.[25,44] In separate studies, mouse bone marrow macrophages differentiated into functional osteoclasts in the presence of CSF-1, TNF and IL-1α when the RANKL pathway was neutralized by OPG or anti-RANK antibody.[45] Further support for alternatives to the RANK–RANKL axis in osteoclast differentiation comes from follow-up experiments in which haematopoietic precursors from RANKL, RANK and TNF receptor-associated factor 6 (TRAF6)-null mice differentiated into osteoclasts upon stimulation with TNF in the presence of cofactors such as TGF-β.[46]

TRAF6-deficient precursors from spleen fail to differentiate into mature osteoclasts *in vitro* in the presence of both CSF-1

Table 1 | Summary of the effects of various cytokines and growth factors on osteoclastogenesis*

Effect(s) on osteoclastogenesis	Cytokines and growth factors	References
Promotion	TNF, IL-1, IL-17,IL-23	21,36,44,46,48,50–53,55
Inhibition	IL-4, IL-12, IL-27, IL-33	35,55–57
Promotion and inhibition	GM-CSF, IL-6	21,31,33–36,54,55
Support	IL-34, VEGF, HGF, PIGF, FLT3 ligand	27–29,37,39

*This table serves as a summary aid and does not intend to capture the complexity of cytokine networks described in the text. Abbreviations: FLT3, Fms-related tyrosine kinase 3; GM-CSF, granulocyte colony-stimulating factor; HGF, hepatocyte growth factor; PIGF, placenta growth factor; VEGF, vascular endothelial growth factor.

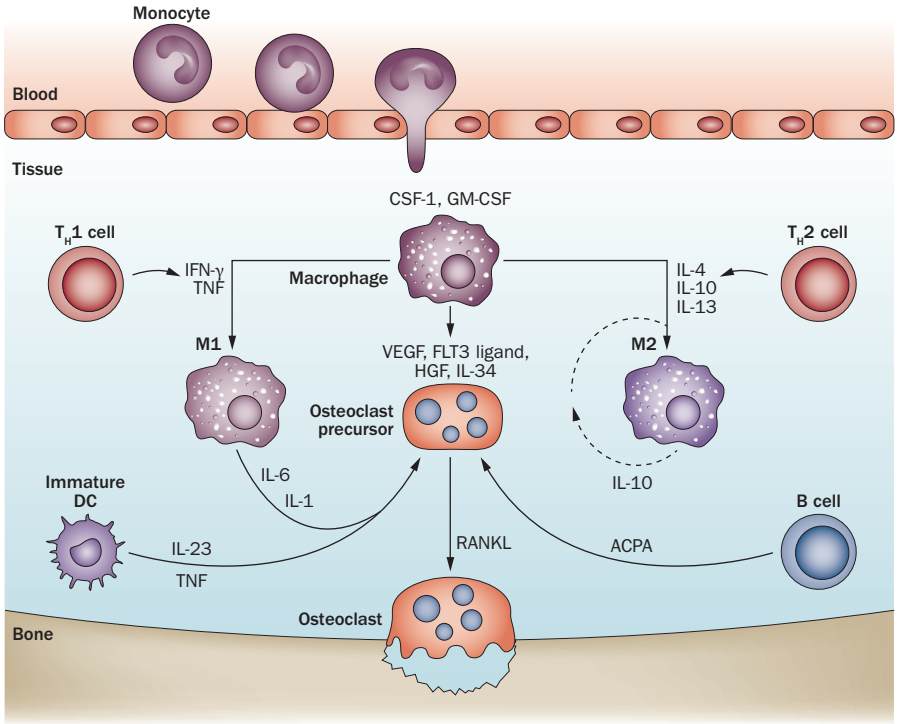

Figure 2 | Activation of macrophages and pathological osteoclast differentiation. Graphical representation of the cellular and molecular interactions between T_H1 and T_H2 cytokines and the polarization of M1 and M2 macrophages, with potential pathways leading to osteoclast differentiation. An undefined osteoclast precursor cell differentiates into an osteoclast via the RANKL pathway. Other cytokine-mediated and growth-factor-mediated pathways can substitute for CSF-1 and RANKL and interact with this precursor (or possibly other precursors) to generate osteoclasts. ACPA might also be able to induce the differentiation of myeloid precursors, highlighting the diversity of signals that can promote osteoclastogenesis. Abbreviations: ACPA, anti-citrullinated protein antibodies; CSF-1, macrophage colony-stimulating factor; DC, dendritic cell; FLT3, Fms-related tyrosine kinase 3; GM-CSF, granulocyte colony-stimulating factor; HGF, hepatocyte growth factor; RANKL, receptor activator of nuclear factor κB ligand; T_H1 cell, type 1 T helper cell; T_H2 cell, type 2 T helper cell; VEGF, vascular endothelial growth factor.

and RANKL, indicating that RANKL-dependent osteoclastogenesis is mediated predominantly by TRAF6.[6] TRAF6 also mediates signalling from other TNF receptor superfamily members, including CD40 (TNF receptor superfamily member 5), and from IL-1 receptor (IL-1R) and TLR family members. Interestingly, bone marrow macrophages overexpressing CD40 formed osteoclasts after stimulation with either anti-CD40 antibodies or TGF-β in the presence of RANK-neutralizing antibodies.[47] Similarly, overexpression of IL-1R type 1 in bone marrow macrophages is sufficient to drive osteoclastogenesis in the presence of RANKL blockade with a RANK–F_c fusion protein, suggesting that IL-1, which mediates TNF-induced osteoclastogenesis, can also act independently of RANKL.[48,49] TNF has been shown to induce NFATc1 activity in human macrophages and prime them for enhanced osteoclastogenesis in response to RANKL.[50] Therefore, TNF can act both independently of, and synergistically with, RANKL.

Other inflammatory cytokines also affect osteoclast development. IL-23 overexpression expands a myeloid-lineage osteoclast precursor *in vivo* and induces bone loss in mice.[51] IL-17 enhances osteoclastogenesis by inducing expression of RANK on human $CD14^+$ osteoclast precursors.[52] Whether IL-17 can also contribute to osteoclastogenesis independent of the RANK–RANKL axis is unknown. The pleiotropic cytokine IL-6 synergizes with TNF and induces osteoclastogenesis and bone resorption *in vitro*.[53] Interestingly, inhibition of osteoclast formation by an antibody to IL-6 receptor (IL-6R) was rescued by the addition of soluble gp130 (the β subunit of IL-6R), suggesting that IL-6R–gp130 interaction is required for the osteoclastogenic effect of IL-6.[54] Reports on the effects of IL-6R signalling on bone destruction are contradictory, as reviewed elsewhere.[55] Similarly conflicting actions have been described for IL-27 (reviewed elsewhere[56]), as the actions of both IL-6 and IL-27 depend on dosage, timing, the nature and availability of precursors and the synergy, or lack thereof, with other proinflammatory cytokines. Taken together, the data currently support the idea that various inflammatory cytokines can modulate osteoclastogenesis, as summarized in Table 1. Defining the osteoclast precursor(s) in settings where these cytokines are expressed, and identifying those that are $CSF\text{-}1R^-RANK^-$ myeloid cells, is of great interest.

Osteoclastogenesis in inflammation

Intriguingly, osteoclastogenic cytokines that might substitute for RANKL are commonly released by M1 macrophages (Figure 2). By contrast, M2 macrophages are generally anti-inflammatory and produce high levels of IL-10 and IL-1R antagonist.[21] Indeed, M2 polarization seems to inhibit the osteoclastogenic signal. For example, IL-33, which has been described to amplify polarization of M2 macrophages, inhibits TNF-mediated bone destruction *in vivo* and directly inhibits its early RANKL-induced osteoclastogenesis *in vitro*.[57] However, the simple model of inflammation-induced osteoclastogenesis being regulated by M1 or M2 polarization is questionable. Inflammatory arthritis reportedly expands populations of mouse osteoclast precursors with a mixed M1–M2 surface phenotype and myeloid suppressor function.[58] Therefore, just as the T_H1–T_H2 paradigm has been replaced by a more complex picture of T-cell polarization that includes T_H17, T_H9 and T_H22 cells, it is

tempting to speculate that a more nuanced understanding of macrophage heterogeneity lies ahead.[59] This knowledge might be required to reveal and characterize the specific cell subsets involved in osteoclast differentiation in inflammatory arthritis.

Conclusions

In RA, joint-associated B cells are skewed toward production of anti-citrullinated protein antibodies (ACPA),[60] and some evidence directly implicates ACPA in the activation of osteoclasts and induction of bone resorption.[60,61] Other contributors to bone turnover in inflammatory arthritis include oxidative stress and an uncoupling of osteoblast and osteoclast activities. As we have discussed here, RANKL-independent pathways of osteoclast differentiation are suggested to contribute to bone destruction both independently of and in synergy with the RANK–RANKL axis during inflammatory arthritis. The relevant myeloid subpopulations might differ among the various subtypes of erosive arthritis, and the phenotype of the resultant bone-resorbing cells could be more heterogeneous than currently appreciated. Indeed, if osteoclasts generated by alternative pathways have unique markers, it might be possible to detect osteoclast precursor subpopulations in the various forms of arthritis, and define the molecular mechanisms underlying their activation. Detailed understanding of osteoclast activation could lead to new therapeutic strategies for inflammatory arthritis.

*Division of Rheumatology, Allergy and Clinical Immunology, University of California, Shriners Hospitals for Children Northern California, 2425 Stockton Boulevard, Room 653A, Sacramento, CA 95817, USA (**I.E.A.**). Division of Pediatric Rheumatology, Stanford University School of Medicine, 300 Pasteur Drive, Stanford, CA 94305, USA (**E.D.M.**).*
Correspondence to: I.E.A.
iannis@ucdavis.edu

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Author contributions

Both authors researched the data for the article, provided substantial contributions to discussions of content, wrote the article and undertook review and/or editing of the manuscript before submission.