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Review

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Inflammatory osteolysis: a conspiracy against bone

Gabriel Mbalaviele,¹ Deborah V. Novack,^{1,2} Georg Schett,³ and Steven L. Teitelbaum^{1,2}

¹Department of Medicine, Division of Bone and Mineral Diseases, and ²Department of Pathology and Immunology, Division of Anatomic and Molecular Pathology, Washington University School of Medicine, St. Louis, Missouri, USA. ³Department of Internal Medicine 3, Rheumatology and Immunology, University of Erlangen-Nuremberg, Erlangen, Germany.

There are many causes of inflammatory osteolysis, but regardless of etiology and cellular contexts, the osteoclast is the bone-degrading cell. Thus, the impact of inflammatory cytokines on osteoclast formation and function was among the most important discoveries advancing the treatment of focal osteolysis, leading to development of therapeutic agents that either directly block the bone-resorptive cell or do so indirectly via cytokine arrest. Despite these advances, a substantial number of patients with inflammatory arthritis remain resistant to current therapies, and even effective anti-inflammatory drugs frequently do not repair damaged bone. Thus, insights into events such as those impacted by inflammasomes, which signal through cytokine-dependent and -independent mechanisms, are needed to optimize treatment of inflammatory osteolysis.

Introduction

The skeleton is a dynamic organ that normally maintains its mass by collateral osteoblast activity despite constant removal of effete bone by osteoclasts. Commonly, in states of hormonal deficiency and aging, this balance is compromised as resorption supersedes formation, resulting in global loss of bone. Fortunately, available and effective osteoporotic therapies are able to enhance systemic bone mass and reduce fracture risk in aging individuals and postmenopausal women.

Inflammatory diseases that affect joints, skin and the gut — including rheumatoid arthritis (RA), psoriatic arthritis (PsA), and Crohn's disease — also promote bone loss that is often severe (1–3). Inflammation-driven bone degradation affects the axial and appendicular skeleton early in life and enhances the risk of fracture. Even when not profound, inflammation increases fracture risk, underscoring its skeletal relevance (4, 5).

When inflammation occurs in the vicinity of bone, such as in RA, PsA, orthopedic implant-associated osteolysis, and osteomyelitis, it induces focal erosion that is often devastating. Although major medical achievements have improved the treatment of inflammatory arthritides, limitations remain, such as resistance to therapy in more than one third of RA and PsA patients and failure to restore damaged bone (6). Thus, further understanding of the pathogenesis of various forms of focal osteolysis is necessary to provide a foundation for their prevention and cure.

Immune cells and their products influence the activities of osteoclasts, osteoblasts, and osteocytes to dictate bone mass and strength. This relationship of the immune and skeletal systems was suggested more than 40 years ago by the discovery that stimulated monocytes produce a catabolic, bone resorption-promoting factor that was subsequently proven to be IL-1 β (7, 8). These observations and others, including the discovery of the key osteoclastogenic cytokine, RANKL (9), prompted the discipline of osteoimmunology. In consequence, studies of the means by which cytokines such as

TNF- α , IL-1 β , and IL-17 impact bone cells have provided insights into the mechanisms of the osteolysis of RA and PsA and improved the success of cytokine-inhibiting biological drugs in reducing the crippling peri-articular complications of these diseases. Osteoimmunology has also revealed that postmenopausal osteoporosis reflects an interplay between estrogen deficiency and immune activation (10). Given the excellent reviews on these topics, we will not address the role of inflammation in the pathogenesis of systemic bone loss (11).

Osteoclasts are the principal effectors of inflammatory osteolysis

While resident mesenchymal and immune cells participate in inflammatory bone destruction, the effector cell of focal osteolysis is the osteoclast, the product of myeloid/macrophage precursor fusion (12). In physiological conditions, osteoclast formation is dictated by the interaction of RANKL, a member of the TNF superfamily, with its receptor RANK (9). Interestingly, whereas high-dose RANKL is potently osteoclastogenic, low-dose RANKL may actually increase bone formation (13).

RANK activation by RANKL depends upon cytokine-mediated trimerization of the receptor in a TNF receptor-associated factor 6-dependent (TRAF6-dependent) manner (14–16). RANKL/RANK signaling induces MAP kinases and NF- κ B, eventuating in the activation and expression of NFATc1, the key osteoclastogenic transcription factor (17–20). Elevation of RANKL abundance, which is typically the crucial event regulating bone resorption, is negatively regulated by osteoprotegerin (OPG) (21–23), a decoy receptor with a higher affinity for the osteoclastogenic cytokine than that of RANK; thus, RANKL binding to OPG effectively limits osteoclastogenesis (9, 15). In fact, loss-of-function OPG mutations prompt a severe generalized osteolytic disorder known as juvenile Paget's disease (24). Macrophage colony-stimulating factor (M-CSF) is also essential for osteoclast formation, exerting its effects by signaling via its receptor, C-FMS (25).

The magnitude of bone degradation depends upon both the number of osteoclasts and their individual resorptive capacity. As stated above, physiological osteoclast abundance is dictated principally by RANKL, M-CSF, and OPG, which are modulated by cytokines

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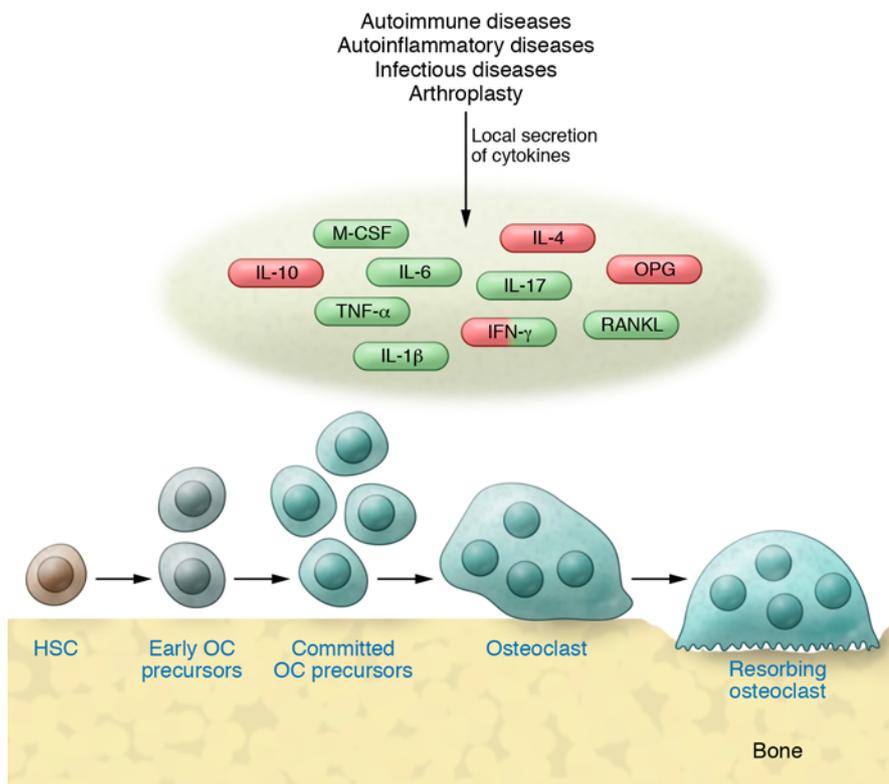


Figure 1. Cytokines are key regulators of inflammatory osteolysis. Despite differences in the pathogenesis of various inflammatory diseases, proximal regulators of osteoclastogenesis are the same, although the “cocktail” of these factors may differ. These factors may act at one or more aspects of the osteoclastogenic pathway from osteoclast generation to activation of resorption. While the homeostatic actions of pro-osteoclast factors (green) are balanced by those that are anti-osteoclast (red), pro-osteoclast factors dominate in inflammatory conditions, thereby increasing osteoclast number and enhancing the cell’s capacity to resorb bone. Some factors, such as IFN- γ , have context-dependent effects on osteoclasts (see text). HSC, hematopoietic stem cell; OC, osteoclast.

in states of inflammatory bone disease. Whereas RANKL increases osteoclast number by promoting precursor differentiation, M-CSF does so by enhancing proliferation of progenitors and restricting apoptosis. The capacity of the individual osteoclast to resorb bone, on the other hand, is largely a product of cytoskeletal organization. The resorptive machinery polarizes toward the cell-bone interface, enabling its transport into an isolated resorptive microenvironment in which degradation of the mineralized and organic components of the skeleton occurs sequentially (26). This process involves fusion of cytoplasmic vesicles containing matrix-degrading molecules to the bone-apposed plasma membrane. Fusion of these lysosome-derived vesicles to the plasma membrane generates the ruffled border, the unique resorptive organelle of the osteoclast, which transports mineral-mobilizing HCl and organic matrix-degrading cathepsin K into the resorptive space (27–31). Polarization of resorptive molecule-containing vesicles is initiated by signals derived from mineralized matrix via cell surface integrins, particularly $\alpha v \beta 3$ (32). In addition to their role in osteoclast formation, both RANKL and M-CSF profoundly enhance bone resorption by the mature osteoclast via canonical organization of its cytoskeleton (33). Given the abundance of RANKL and M-CSF in states of inflammatory osteolysis, it is likely that the robust bone destruction reflects both increased numbers of osteoclasts and enhanced resorptive capacity of the mature cell. Thus, understanding the means by which inflammation destroys bone must be viewed in the context of interplay between cytokines that regulate these two critical osteoclastogenic factors.

Cytokine-induced focal osteolysis in RA

In many circumstances, osteolysis occurs adjacent to inflammation, such as in arthritic periarticular tissue. The secreted products of infiltrating immune cells perpetuate the inflammatory response

that recruits osteoclasts, the direct mediators of the osteolytic component of the disease (Figure 1). Thus, anti-osteolytic strategies include inflammatory cytokine inhibition, such as TNF- α and IL-6 receptor blockade, both of which indirectly diminish osteoclastogenesis, as well as direct inhibition of osteoclast formation by agents such as denosumab, a humanized anti-RANKL antibody (34). The fact that NF- κ B mediates both inflammation and osteoclast formation suggests its candidacy as a therapeutic target in inflammatory osteolysis (35–38). While an NF- κ B-focused strategy may be compromised by the broad biological necessity of the transcriptional complex, systemic administration of peptide-siRNA nanocomplexes targeting NF- κ B subunit p65 dramatically suppresses the inflammatory and bone-erosive components of experimental arthritis without apparent off-target effects (39).

A number of inflammatory cytokines may contribute to the periarticular bone loss of RA, but the most significant is TNF- α . Macrophages and T cells express TNF- α in the arthritic synovial tissue as a membrane-residing protein that does not promote osteoclast formation until it is cleaved to its soluble form (40). Like RANKL, TNF- α exerts its biological effects by clustering and trimerizing three monomeric receptors (41). In fact, TNF- α activates two known receptor complexes, namely TNF receptor type 1 (TNFR1) and type 2 (TNFR2), and each prompts different intracellular events. TNFR1, which is principally stimulated by soluble TNF- α , transmits proinflammatory signals and synergizes with RANK to promote osteoclastogenesis (42). Signals induced by TNFR2, which largely recognizes membrane-residing TNF- α , are pro-immunogenic and anti-inflammatory (43). Thus, TNFR1 is responsible for the crippling effects of RA, PsA, and other forms of focal osteolysis, such as that complicating orthopedic implantation (44, 45). In fact, there is compelling evidence that complica-

tions of anti-TNF- α therapy substantially reflect a failure to distinguish TNFR1 and TNFR2 (46, 47). Recently developed strategies, including manipulation of affinities for the two receptors to promote selective binding and thus inhibition of TNFR1 while sparing TNFR2, may attenuate the problematic properties of TNF- α while maintaining those that are salutary (48).

TNF- α potently stimulates osteoclast formation, but whether it does so independently of RANKL is controversial. Administration of TNF- α to RANKL-deficient mice fails to induce meaningful formation of the bone-resorptive cell (49), but TGF- β may substitute for RANKL in certain circumstances (50). Challenging the concept that RANKL-independent osteoclast formation may participate in inflammatory bone loss is the finding that *Rankl*-knock-out mice are protected from arthritic bone erosions (51). On the other hand, deletion of RBP-J, a transcription factor that inhibits osteoclast formation in the context of inflammation, enables RANKL-independent, TNF- α -induced, but not physiological, osteoclastogenesis (52). Similarly, absence of TRAF3 or NF- κ B2/p100 alleviates inhibition of osteoclast formation in the context of TNF stimulation (53). Furthermore, combined TNF- α and IL-6 promote osteoclast formation and bone erosions in RANK-depleted myeloid precursors. Osteoclasts are also present, though diminished, in arthritic mice with inducible RANK deficiency (54). It therefore appears that in certain circumstances TNF- α may promote osteoclast formation in the absence of RANKL, but the biological significance of this event is yet to be defined. It is also clear that TNF- α and RANKL are synergistic, and the inflammatory cytokine needs only minimal amounts of RANKL to directly stimulate macrophages to differentiate into osteoclasts (55, 56). The osteoclast-inducing properties of relatively low quantities of TNF- α are largely the product of enhanced RANKL expression by mesenchymal cells, such as those residing in pannus, the inflamed and hypertrophied synovium of arthritic joints (57). As TNF- α abundance increases, its osteoclastogenic effects progressively reflect direct stimulation of myeloid precursors, while the contribution made by increasing RANKL diminishes (55, 57, 58). Thus, osteolysis accompanying relatively modest elevations of ambient TNF- α depends upon responsive stromal cells. Alternatively, in states of severe periarticular inflammation, TNF- α may fully exert its bone-erosive effects by directly promoting differentiation of osteoclast precursors and their expression of RANK receptor independent of cytokine-responsive stromal cells and T lymphocytes (57). The capacity of TNF- α to prompt RANK expression and osteoclast formation is dependent upon its induction of M-CSF by stromal cells (59), which increases osteoclast precursor abundance (60). Importantly, antibody blockade of the M-CSF receptor C-FMS completely arrests TNF- α -induced osteolysis, with minimal effects on macrophage number. C-FMS blockade also limited local and systemic resorption in two mouse models of inflammatory arthritis (61). Thus, M-CSF and its receptor are candidate therapeutic targets in inflammatory osteolysis.

Physiological bone remodeling is characterized by a “coupling” of osteoclast and osteoblast activity, and the same holds true in pathological conditions such as PsA (45). In contrast, the robust osteoclastogenesis of RA is accompanied by suppressed bone formation, and thus its erosive lesions exhibit limited osteoblast activity even in face of therapeutic intervention (62). TNF- α

potently activates the canonical RelA pathway, which inhibits osteoblast differentiation, and therefore could contribute to the uncoupling of RA bone resorption from formation (63). However, this conclusion is controversial, as others report that NF- κ B positively affects osteogenesis (64). Given the central role of Wnt signaling in osteoblast differentiation, the failure of RA focal osteolysis to heal is perhaps more likely due to the osteogenic-suppressive properties of TNF- α mediated through its induction of dickkopf-1 (DKK-1), a potent inhibitor of Wnt activation (65–67). Interestingly, in contrast to TNF- α arrest, which suppresses osteoclastogenesis but does not increase bone formation, blockade of DKK-1 does both, thus repairing erosive lesions (66).

IL-1 β , which is abundant in RA pannus and other inflamed tissues, induces and partners with TNF- α to promote inflammatory osteolysis (58). In this circumstance, TNF- α enhances the synthesis of IL-1 β by synovial fibroblasts (68) and macrophages. IL-1 β , in turn, stimulates expression of RANKL and RANK as well as its own receptor. In fact, IL-1 β mediates approximately 50% of TNF- α -induced osteoclastogenesis. The interdependency of TNF- α , RANKL, and IL-1 β lends credence to the concept that combined blockade more effectively prevents inflammatory bone loss than single cytokine inhibition (69).

Autoantibody-induced focal osteolysis in RA

While molecular effectors of innate immunity have been long recognized as mediators of bone loss, until recently little was known about the role of B cell-produced immunoglobulins as the key molecular effectors of adaptive immunity in skeletal degradation. Clinical observations suggested that rheumatoid factor, an immune complex induced by an IgM autoantibody against the Fc portion of IgG, and anti-citrullinated protein autoantibodies are potent risk factors for focal osteolysis in RA. Anti-citrullinated vimentin antibodies directly stimulate myeloid lineage cells to produce TNF- α and IL-8, which in turn induce osteoclastogenesis (70, 71). Furthermore, individuals with such antibodies but no signs of RA develop skeletal abnormalities, indicating a direct effect on bone (72). Immune complexes such as rheumatoid factors also stimulate osteoclast formation and function by cross-linking Fc receptors and activating SYK (73, 74). Importantly, anti-citrullinated protein autoantibodies and rheumatoid factors additively trigger bone loss (75).

IL-17 and focal osteolysis in PsA

In contrast to RA, in which TNF- α predominates, PsA is characterized by robust activation of the IL-23/IL-17 axis (76). IL-17 is produced by Th17 and innate lymphoid (ILC3) cells exposed to IL-23. IL-17-expressing T cells infiltrate the skin and the joints of psoriatic patients and likely drive their clinical manifestations. Th17 cells synthesize IL-17 and express RANKL, both of which directly induce osteoclast differentiation (77, 78). Additionally, IL-17 potentiates RANKL's osteoclastogenic capacity by upregulating RANK on osteoclast precursor cells (79). IL-17 also indirectly promotes bone destruction by inducing synovial fibroblasts and macrophages, respectively, to express TNF- α , IL-1, and IL-6 (80, 81). In fact, inducing osteolytic cytokines, especially TNF- α , in these cells, may be the principal means by which IL-17 causes arthritic osteolysis. In contrast to IL-17, IL-23,

the essential inducer of Th17 differentiation, does not directly promote osteoclast formation. The deleterious effects of IL-17 on bone also reflect suppressed osteogenesis via downregulation of Wnt and BMP pathways (12, 82).

Approximately 90% of PsA patients develop bone erosions that correlate with the magnitude of joint destruction, establishing a pathogenic role of the osteoclast. In contrast to RA, whose bone destructive complications are exclusively resorptive, PsA is characterized by not only robust bone resorption but also exuberant osteogenesis, particularly in regions of attachment of ligaments to bone (entheses), which can eventuate in ankyloses (83). While RA is exclusively characterized by synovial inflammation, patients with PsA also develop inflamed entheses that may reflect IL-23 and IL-17 activation. Although inflammation is a prerequisite for new bone formation at enthesal sites, IL-17 is unlikely the responsible factor, as it suppresses rather than promotes osteogenesis.

Immunoregulatory mechanisms inhibiting focal arthritic osteolysis

Macrophages are central to the pathogenesis of focal osteolysis, as they are a principal source of inflammatory cytokines and can differentiate into osteoclasts. In fact, the abundance of macrophages in pannus parallels disease severity (84). While the osteoclast is a macrophage lineage cell, its precise origin remains controversial and it is likely influenced by specific environmental circumstances. This is particularly true regarding the relationship of osteoclasts to classically activated, proinflammatory M1 and alternatively activated, immunoregulatory M2 macrophages. Assumption of the M2 phenotype typically requires IL-4 and IL-13, which inhibit osteoclast formation and induce myeloid progenitors to develop into non-bone-resorbing polykaryons (85). IL-4 and IL-13 are the products of Th2 lymphocytes and eosinophils, respectively. These cells suppress inflammatory arthritis and hence its osteolytic consequences (86). TNF- α , which promotes osteoclast formation, blunts polarization to the M2 phenotype directly and by arresting production of IL-13 by eosinophils that are present in arthritic joints (86, 87). While these observations suggest that osteoclasts are not derived from alternatively activated M2 macrophages, inflammatory arthritis in mice recruits osteoclast precursors with features of both M1 and M2 cells (88). Hence, differentiation of a monocytic precursor into osteoclasts may essentially depend on the cytokine micro-milieu to which the cell is exposed, thus allowing a modicum of plasticity in monocyte differentiation.

In addition to stimulating resorption, immune cells may retard osteoclast formation and bone loss. For example, CD4⁺ FoxP3⁺ Tregs are potent inhibitors of osteoclast differentiation, exerting their effects by cell contact (89). The anti-resorptive capacity of Tregs reflects their anti-inflammatory properties. They also directly block osteoclast formation by liganding CD80/CD80 surface receptors with CTLA4, thereby inducing indoleamine 2,3-deoxygenase (IDO) (90). In inflammatory conditions, however, the immune-suppressive capacity of Tregs is compromised by proinflammatory cytokines, which promote targeted dephosphorylation of FoxP3 (91). A second population of Tregs that express CD8, known as Tregs, is directly induced via interaction with osteoclasts (92). These Tregs inhibit the resorptive activity of osteoclasts, rather than inhibiting their differentiation in vitro,

and limit bone loss in mice following RANKL injection or ovariectomy (92–94). Whether the osteoclast-suppressive properties of Tregs or Tregs are compromised in inflamed joints is unknown.

Understanding the role of Th1 cells in the skeleton has been more complex than exploring Tregs. Activated Th1 cells were originally considered pro-osteoclastogenic, as they express RANKL (95). Th1 cells, however, actually suppress osteoclast formation in vitro, postulated to be via co-production of IFN- γ , challenging their role in RA-associated focal osteolysis (18, 78). Surprisingly, however, human CD4⁺IFN- γ ⁺ T but not CD4⁺IFN- γ ⁻ T cells express RANKL and thus promote osteoclastogenesis (96). Importantly, CD4⁺IFN- γ ⁺ T cells are selectively increased in the circulation of RA patients and IFN- γ stimulates osteoclast formation and bone loss in vivo (96, 97). Thus, Th1-produced IFN- γ appears to directly inhibit and indirectly stimulate osteoclast formation, with the latter predominating in vivo.

Skeletal changes associated with excessive inflammasome activation

Inflammasomes are intracellular protein complexes expressed mainly by myeloid cells, including osteoclasts (98, 99), but also develop in osteoblasts and chondrocytes (98, 100, 101). They protect against infections upon recognition of microbial structures known as pathogen-associated molecular patterns (PAMPs) (102). Inflammasomes also restore tissue integrity after injury by sensing danger-associated molecular patterns (DAMPs) from damaged cells (103–105). They function primarily by converting pro-IL-1 β and pro-IL-18 to their biologically active forms (103).

Inflammasomes are assembled by nucleotide-binding oligomerization domain, leucine-rich repeat-containing proteins (NLRP1, NLRP3, NLRC4, NLRP6, and NLRP12), absent in melanoma 2-like (AIM 2-like) receptors (known as ALRs), or pyrin (ref. 103 and Figure 2). These proteins act as receptors for PAMP- and DAMP-associated molecular patterns. Ligand recognition enables the recruitment and oligomerization of apoptosis-associated speck-like protein containing a caspase activation recruitment domain (CARD). Complex assembly activates caspase 1, which proteolytically processes pro-IL-1 β and pro-IL-18. The NLRP3 inflammasome signaling cascade cleaves poly(ADP-ribose) polymerase 1 (PARP1) (106–108).

Owing to its robust activation by monosodium urate crystals, the NLRP3 inflammasome plays a key role in gout (109). The NLRP3 inflammasome is also activated in RA synovium, consistent with increased IL-1 β secretion (110, 111). TNF- α stimulates IL-1 β expression in vitro, suggesting the latter cytokine may drive pro-IL-1 β transcription in RA. Although TNF- α and IL-1 β regulate each other's expression and osteoclastogenic properties (58), the superior efficacy of TNF- α inhibitors in RA relative to those that block IL-1 is consistent with the apical role of TNF- α in generating focal osteolysis in this disease.

As stated above, inflammasomes also promote cleavage of PARP1 (106–108). While it has yet to be studied in the context of RA, PARP1 proteolysis occurs during osteoclastogenesis (98, 99, 112, 113). A proteolytically stable PARP1 mutant antagonizes osteoclastogenesis, whereas PARP1 deficiency enhances this process (107, 114), indicating that PARP1 negatively regulates osteoclast differentiation.

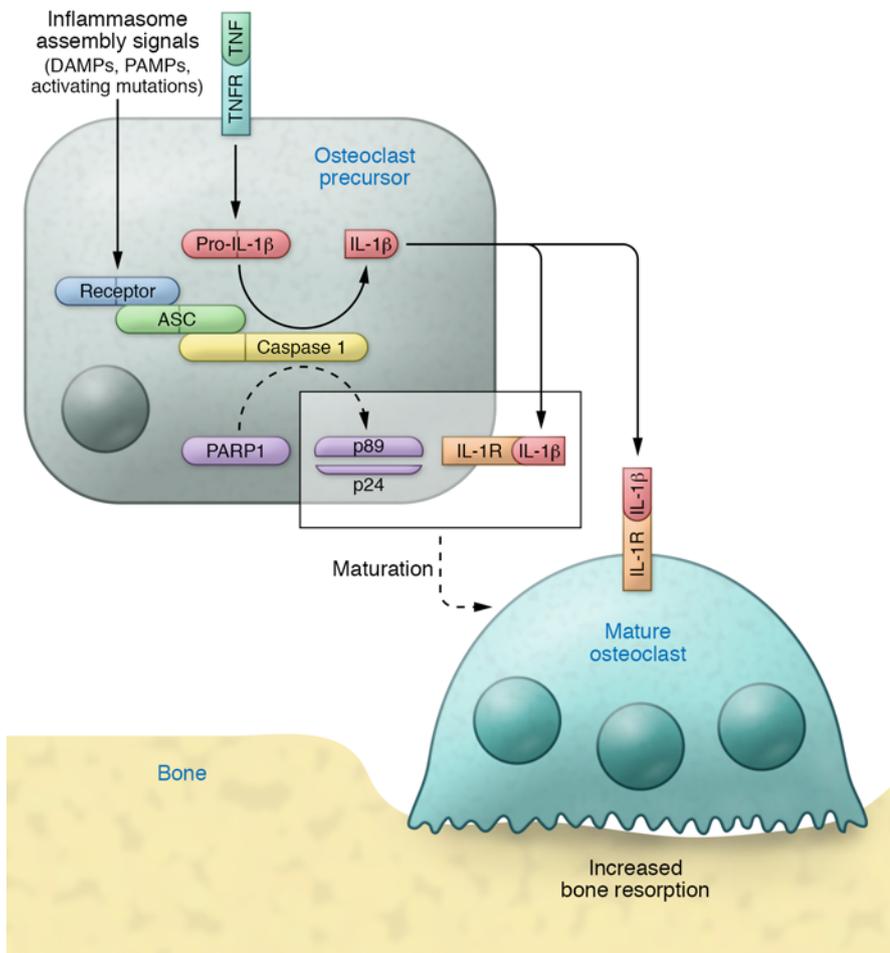


Figure 2. The inflammasomes in osteolysis. Cytokines such as TNF induce the expression of pro-IL-1β, which lacks the signal peptide for secretion. Recognition of damage- or pathogen-associated molecular patterns (DAMPs or PAMPs) by the receptor/sensor component of the inflammasome (e.g., NLRP3, NLRC4), or activating mutations of some of these receptors, leads to the assembly of the inflammasome, a protein complex containing the receptor, apoptosis-associated speck-like protein containing a CARD (ASC) and caspase 1. Once assembled, this active complex cleaves pro-IL-1β into active IL-1β and triggers cascades that lead to PARP1 cleavage into p89 and p24 fragments. In precursors sensing DAMPs or PAMPs, cleaved PARP1 and autocrine effects of IL-1β, acting through IL-1R, promote maturation of osteoclasts into bone-resorbing cells. Secreted IL-1β can also act in a paracrine manner to increase bone resorption by nearby osteoclasts. Dashed arrows indicate multiple steps.

Inflammasome mutations may prompt stimulus-independent activation and are the cause of cryopyrin-associated periodic syndromes (CAPS). Neonatal-onset multisystem inflammatory disease (NOMID), the most severe CAPS, is attended by bone loss and skeletal deformation (101, 115–118). IL-1-blocking agents have limited efficacy against NOMID skeletal lesions, while other symptoms related to systemic inflammation are rapidly resolved (119–122). While the human disease is phenocopied in mouse models with global NOMID mutation (98, 123), mice bearing the mutation only in osteoclasts lack systemic inflammation but have severe osteoporosis (99), indicating a cell-autonomous role in the bone-resorptive cell.

In humans, gain-of-function mutations in NLRC4 cause a disorder reminiscent of macrophage activation syndrome (MAS) (124–127), a life-threatening complication of systemic juvenile idiopathic arthritis (JIA). JIA responds to IL-1 blockers (128, 129), indicating the inflammasome-activated cytokine participates in this osteolytic disorder. Consistent with the human phenotype, transgenic mice expressing constitutively active NLRC4 develop inflammatory arthritis (130). Similarly, activating mutations of *MEFV*, encoding pyrin, cause familial Mediterranean fever (FMF), which is characterized by excessive IL-1β that drives autoinflammation associated with arthritis and bone loss (131, 132). The efficacy of IL-1 blockers underscores the pathogenic action of this cytokine in FMF (133).

Additionally, mice harboring mutant *MEFV* establish that the pyrin inflammasome is proinflammatory and causes massive cartilage and bone erosion (134).

Chronic recurrent multifocal osteomyelitis (CRMO) is an auto-inflammatory osteolytic disorder. Anti-inflammatory cytokines (e.g., IL-10) are diminished, whereas their proinflammatory counterparts (e.g., IL-1β, IL-6, and TNF-α) are increased. This imbalance presumably reflects NLRP3 inflammasome hyperactivation and causes exaggerated osteoclastogenesis (135). Immunohistochemical staining of bone of CRMO patients shows that the NLRP3 inflammasome is increased in osteoclasts (136). Supporting the role of cytokines in bone loss during CRMO, TNF-α or IL-1 blockers are efficacious (135). Mice harboring a spontaneous missense mutation in the proline-serine-threonine phosphatase-interacting protein 2 (*Pstpip2*) gene, which abolishes protein expression, develop a phenotype reminiscent of CRMO, with enhanced osteoclastogenesis and bone resorption (137). These mutant mice overproduce IL-1β due to combined and redundant actions of the NLRP3 inflammasome and caspase 8 (138–140), suggesting that inflammasome-independent IL-1β release may occur in some situations.

Pathogenesis of periprosthetic osteolysis

Periprosthetic osteolysis is the most significant long-term complication of total hip replacement. Prosthetic-wear particles activate innate immune responses, causing excessive bone resorption and,

ultimately, implant failure (141, 142). The prevalence of myeloid cell responses indicates that the NLRP3 inflammasome likely participates in prosthetic loosening, implicating crystalline particles in the pathogenesis of NLRP3-mediated diseases. Furthermore, calvarial bone resorption that is induced by polymethylmethacrylate (PMMA) particles is reduced in the absence of NLRP3 (143). The size and shape of metal alloys affect the amplitude of the inflammasome response (144). Plastic components of the prostheses also activate the inflammasome. Phagocytosis-enhanced production of reactive oxygen species and rupture of phagosomes, which release cathepsins in the cytoplasm, are the presumed mechanisms of NLRP3 inflammasome activation by particles. On the other hand, prosthetic particles generate priming signals through TLRs that induce expression of pro-IL-1 β and NLRP3 (145, 146). The NLRP3 inflammasome, however, appears to supersede TLR4 signaling in prosthetic-wear particle-induced osteolysis (147). On the other hand, the non-NLR inflammasome AIM2 and not NLRP3 may mediate the acute phase of PMMA-induced foreign body responses (148).

Infection-associated osteolysis

Immune activation during infection causes profound local bone loss. Although the term may refer to any instance of bone inflammation, osteomyelitis (OM) is primarily used to describe the infection-induced form of disease whose progression is the result of interplay among pathogens, the cellular and matrix constituents of bone, and the immune system. Bacteria may cause bone necrosis through direct killing of osteoblasts, but the inflammatory response is likely the major event by which infection induces osteoclastogenesis and bone degradation. The most frequently identified microorganism in OM is *Staphylococcus aureus*, which is seeded into bone through both hematogenous and direct (e.g., trauma, implant) routes in children and adults (149).

Bacterial products of *Staphylococcus aureus*, which grows primarily in the extracellular bone microenvironment and forms biofilms, induce an inflammatory response. Factors released by cells adjacent to bacteria include TNF- α , IL-1 β , IL-6, IFN- γ , and IL-12 as well as the chemokines CCL3 (also known as MIP1 α), CXCL1, CXCL2 (also known as MIP2 α), and CXCL8 (150–154). Further linking mechanisms of bone loss in infectious and non-infectious inflammatory states, the NLRP3 inflammasome is implicated in the response of innate immune cells to *Staphylococcus aureus* infection. Hemolysins, bacterial lipoproteins, and Pantone-Valentine leukocidin from *Staphylococcus aureus* are all activators of NLRP3 capable of inducing IL-1 β release (155–157). In addition to the caspase 1-mediated IL-1 β processing event, NLRP3 activation induces expression of a variety of cytokines in an NF- κ B-dependent, caspase 1-independent fashion (158). As discussed above, although individual inflammasome factors may inhibit osteoclast formation or function, combined release is highly osteolytic (151, 159, 160).

In addition to mediating osteolysis via activation of immune cells, *Staphylococcus aureus* directly affects osteoblasts. Staphylococcal protein A binds to and activates TNFR1 on osteoblasts, thereby stimulating NF- κ B and promoting IL-6 secretion (161). The cell wall component peptidoglycan stimulates TLR2 in a NOD2-dependent manner, thereby inducing RANKL expression

by osteoblasts. Concordant OPG remains unchanged (159, 162, 163) or decreases (164), contributing to osteoclast recruitment and osteolysis. Bone loss likely also occurs through reduced osteoblast viability as well as reduced activity induced by substances released from staphylococcal biofilms (163, 164).

Alveolar bone loss due to periodontitis is another frequent form of infection-mediated inflammatory osteolysis. In this context, the most common pathogen is *Porphyromonas gingivalis*, although the human disease is likely polymicrobial, as the oral cavity is not a sterile site (165). Alveolar bone loss elicited by *Porphyromonas gingivalis* shares many features of lysis attending OM. Cytokine release (166) as well as induction of RANKL downstream of TLR2 and NOD2 are the prominent pathways leading to osteoclast activation in periodontitis (167, 168). IL-17 is also markedly increased in periodontitis and appears to be an important factor in both inflammation and osteolysis of the periodontal tissue (169). *Porphyromonas gingivalis* also releases cysteine proteases called gingipains, which do not directly induce osteoclastogenesis (170) but may act via differential degradation of osteoclast-modulatory cytokines, including OPG (171).

Therapeutic perspective

In the not-too-distant past, many patients with RA or PsA were incapacitated because of focal osteolysis, which destroyed periarticular bone. Insights gained into the pathogenesis of inflammatory osteolysis, and in particular the interplay of the immune and skeletal systems, has led to substantial therapeutic progress. RANKL inhibition by denosumab, for example, arrests progression of arthritic osteolysis, supporting the concept that stimulated osteoclastogenesis is the essential event in inflammatory bone loss (172). However, RANKL inhibition, per se, has no intrinsic anti-inflammatory properties and therefore must be accompanied by anti-inflammatory agents in these disorders (172). All current cytokine inhibitors approved for the treatment of inflammatory diseases, which include compounds specifically targeting IL-1, IL-6R, IL-17, IL-12/23, and in particular TNF- α , limit osteolysis (173–175). The efficacy of TNF- α inhibitors appears to reflect combined anti-inflammatory properties and direct suppression of bone-resorbing osteoclasts. IL-6R inhibitors, which are used exclusively to treat RA, and IL-12/23 and IL-17 blocking agents, predominantly employed in psoriatic arthritis, exert similar bone-protective effects. The impressive skeletal-sparing properties of IL-1 blockers are surprising, as these drugs minimally diminish the articular inflammation of RA, supporting the concept that the cytokine's primary effect, in the disease, is direct osteoclast recruitment and activation (176). Clinically, IL-1 inhibitors may have their primary bone-protective role in crystal arthropathies such as gout, where IL-1 β plays a dominant role as an inflammatory cytokine (177). A small molecule inhibitor of the NLRP3 inflammasome has shown efficacy in rodent disease models (178), thus providing proof of concept that pharmacologic inhibition of this inflammasome is a viable therapeutic strategy. However, there is no report on NLRP3 inhibitors in clinical development. Drugs interfering with adaptive immune response, particularly T cell activation, also regulate bone resorption. CTLA4, for instance, which retards T cell activation and is effective in RA, binds osteoclast precursors and arrests their differentiation (179). The anti-osteoclastogenic properties of Tregs are mediated by CTLA4, which these cells constitutively express (179).

Since infection-induced osteolysis is primarily the result of the host response rather than the direct action of pathogens, blockade of cytokines and/or RANKL could be a useful adjunct to debridement and antibiotic therapy. RANKL blockade has been successful in animal models of periodontitis (180–182). Patients treated with anti-TNF- α therapy for other disorders have also shown some improvement in alveolar bone loss (183).

In summary, while the clinical consequences of skeletal inflammation have long been appreciated, recent discoveries of immune pathways controlling bone homeostasis have added greatly to our understanding as to how these pathological events occur. Inflammatory cytokines, autoantibodies, and DAMPs/PAMPs induce osteoclast differentiation and osteolysis in auto-inflammatory, autoimmune, and infectious diseases. These discoveries prompted therapeutic interventions, such as cytokine blockers, which selectively disrupt the detrimental skeletal effects

of chronic immune activation and inflammation, thereby limiting their associated bone loss. Further examination of immune pathways will likely lead to additional approaches to the treatment of inflammatory osteolysis.

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Address correspondence to: Steven L. Teitelbaum, Washington University School of Medicine, Department of Pathology and Immunology, Campus Box 8118, 660 South Euclid Avenue, St. Louis, Missouri 63110, USA. Phone: 314.454.8463; E-mail: teitelbs@wustl.edu.

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