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Feedback inhibition of osteoclastogenesis during inflammation by IL-10, M-CSF receptor shedding, and induction of IRF8

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Abstract

Inflammation plays a key role in excessive bone loss in conditions such as rheumatoid arthritis and periodontitis. An important paradigm in immunology is that inflammatory factors activate feedback inhibition mechanisms to restrain inflammation and limit associated tissue damage. We hypothesized that inflammatory factors would activate similar feedback mechanisms to restrain bone loss in inflammatory settings. We have identified three mechanisms that inhibit osteoclastogenesis and are induced by inflammatory factors, such as toll-like receptor ligands and cytokines: downregulation of expression of costimulatory molecules such as TREM-2; induction of shedding and thereby inactivation of the M-CSF receptor c-Fms, leading to decreased *RANK* transcription; and induction of transcriptional repressors such as interferon regulatory factor 8. It is likely that these mechanisms work in a complementary and cooperative manner to fine tune the extent of osteoclastogenesis in inflammatory settings, and their augmentation may represent an alternative therapeutic approach to suppress bone resorption.

Keywords

inflammation; osteoclasts; toll-like receptors; M-CSF; c-Fms; IRF8

Inflammatory bone resorption

Bone loss is associated with acute and chronic inflammation and is responsible for substantial morbidity associated with inflammatory diseases. This includes local bone loss at sites of inflammation in diseases, such as rheumatoid arthritis (RA), periodontitis, infection, and orthopedic periprosthetic loosening, and systemic bone loss associated with inflammation in these conditions as well as inflammatory diseases such as inflammatory bowel disease that do not occur proximate to bone.^{1–3} Elevations in systemic and circulating inflammatory cytokines also contribute to postmenopausal osteoporosis. Inflammation decreases bone mass by concomitantly suppressing bone formation and increasing osteoclast-mediated bone resorption, thereby disrupting the coupling between these processes that is important for maintaining bone mass under physiological conditions.

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Conflicts of interest

The authors declare no conflicts of interest.

Recently, it has become clear that inflammatory factors suppress bone formation in part by inhibiting osteoblast differentiation, which includes suppression of Wnt signaling.⁴ In addition, inflammatory factors strongly promote bone resorption by inducing the differentiation and bone-resorbing function of osteoclasts.

A large number of inflammatory factors and cytokines, including tumor necrosis factor- α (TNF- α), IL-1, IL-6, and prostaglandins, promote osteoclastogenesis and bone resorption. In addition, microbial products such as bacterial lipopolysaccharides (LPS) and lipopeptides, which are sensed by innate immune receptors such as toll-like receptors (TLRs) 2 and 4, induce inflammatory bone resorption. One mechanism by which inflammatory factors induce osteoclastogenesis is by inducing expression of the most potent osteoclastogenic factor RANKL on mesenchymal and stromal cells or on lymphocytes that infiltrate inflammatory lesions (Fig. 1).^{5–9} Inflammation-induced RANKL that is often ectopically expressed will, in turn, promote osteoclast differentiation by activating the same potent osteogenic signals, that it induces under physiological conditions. In addition, low concentrations of TNF- α and IL-1 synergize with RANKL to augment signaling by its receptor RANK and downstream cellular responses.^{10–13} Mechanisms underlying this synergy are not well understood, but include induction of MITF by IL-1¹¹ and of the master regulator of osteoclastogenesis, NFATc1 by TNF- α .¹³ Finally, inflammatory cytokines have been suggested to induce expression of ligands for ITAM-associated receptors and thereby increase costimulation of RANK signaling and osteoclastogenesis (Fig. 1).¹⁴

The mechanisms illustrated in Figure 1 provide an explanation for the increased osteoclastogenesis and bone resorption which occur in inflammatory settings. However, this model does not take into account that a salient feature of inflammation is the engagement of feedback inhibition that limits the extent of inflammation.^{15,16} This feedback inhibition is required to prevent toxicity, including lethality, associated With excessive inflammatory mediator/cytokine production, and to prevent excessive tissue damage in the setting of inflammation. Indeed, some of the most potent negative feedback mechanisms play a role in guiding the transition from acute inflammation to its resolution, and the concurrent healing of tissue damage and wounds. One interesting possibility is that mechanisms involved in wound healing may also play a role in healing of bone lesions. Feedback mechanisms that suppress and resolve inflammation include production of antiinflammatory cytokines (such as IL-10), expression of inhibitors of inflammatory signaling (such as SOCS proteins and A20), and induction of transcriptional repressors of inflammatory genes (such as ATF-3).

We hypothesized that, similar to feedback inhibition of inflammation, inflammatory factors would induce feedback mechanisms that restrain osteoclastogenesis and prevent excessive bone destruction. In this conceptualization, the extent of inflammatory bone loss is determined by the balance between osteoclastogenic signals and the relative potency of feedback mechanisms that restrain bone resorption and promote healing. During acute and transient inflammation, as occurs in physiological settings, feedback mechanisms effectively limit bone resorption. In contrast, progression of bone erosion during inflammatory diseases is evidence of relatively ineffective feedback inhibition. Based on this hypothesis, we have initiated studies to understand molecular mechanisms that restrain osteoclast formation in an inflammatory setting. We have focused on direct inhibitory effects on osteoclast precursors (OCPs) and on feedback inhibitory mechanisms induced by potent activators of inflammation such as TLR ligands.

IL-10 suppresses human osteoclastogenesis by downregulating TREM-2 expression

Induction of IL-10 and downstream transcription factor STAT3 represents one of the most potent TLR-induced feedback mechanisms that leads to suppression of inflammatory cytokine production. We tested whether IL-10 would similarly inhibit RANKL-induced osteoclast differentiation. We found that IL-10 potently inhibited RANKL-induced expression of the master regulator of osteoclastogenesis NFATc1 and, thus, nearly completely suppressed RANKL-induced gene expression and osteoclast differentiation.¹⁷ The mechanism of IL-10 action was suppression of the expression of TREM-2, a key ITAM-associated receptor required for costimulation of human osteoclast differentiation.^{18,19} Indeed, patients with TREM-2 loss-of-function mutations exhibit defective osteoclastogenesis and bone lesions.^{20–21} IL-10 inhibited TREM-2-dependent calcium signaling downstream of RANK, and blocked RANKL-induced activation of a CaMK-MEK-ERK pathway that is important for osteoclastogenesis. Differences in the role of TREM-2 in human and mouse osteoclastogenesis (where TREM-2 appears to play a lesser role) may be explained by differences in the kinetics and magnitude of TREM-2 expression between human and murine cells. IL-27, a TLR-induced homeostatic cytokine that activates JAK-STAT signaling similar to IL-10, also inhibited osteoclastogenesis by suppressing ERK activation and NFATc1 expression.²² These findings identify the mechanisms by which JAK-STAT signaling by cytokines that are induced by TLRs to feedback and inhibit inflammatory cytokine production have an additional feedback inhibitory function, namely attenuation of osteoclastogenesis.

TLRs inhibit osteoclastogenesis by inducing shedding of the M-CSF receptor c-Fms and suppressing RANK transcription

TLRs are key sensors of microbial infection and mediators of the innate immune and inflammatory response to pathogens. When blood monocytes enter sites of infection/inflammation, they are activated by TLRs, and also by inflammatory cytokines such as TNF- α and IL-1 and ITAM-associated receptors, to assume an inflammatory “M1” macrophage phenotype that is important for host defense (Fig. 2). As the infection clears and inflammation resolves, macrophages transition to an “M2” phenotype that promotes tissue remodeling. In contrast, when OCPs (contained within the circulating monocyte pool) enter noninflamed tissues and are exposed to M-CSF and RANKL adjacent to bone, they develop into osteoclasts (Fig. 2). Given that inflammatory cytokines induce RANKL expression, at inflammatory sites infiltrating myeloid precursors/monocytes will be exposed to a complex microenvironment containing microbial products, inflammatory cytokines, and RANKL (Fig. 2). We reasoned that in this setting, ligation of TLRs by microbial products would suppress osteoclastogenesis to drive myeloid precursors toward an alternative cell fate, namely M1 macrophages necessary for host defense. We confirmed and extended previous work in murine systems^{23–26} to show that engagement of TLR2 or TLR4 on early human OCPs strongly suppressed osteoclastogenesis and investigated underlying mechanisms.²⁷ Strikingly, TLR ligation almost completely abolished RANK expression on human OCPs. A similar, but quantitatively lesser, downregulation of RANK expression after TLR ligation was observed using mouse OCPs, and the TLR4 ligand LPS showed attenuating effects on osteoclastogenesis *in vivo* in mice in two different models.

Inhibition of RANK expression by TLRs occurred at the level of *RANK* gene transcription. As *RANK* transcription is induced by M-CSF (CSF-1) acting through its receptor c-Fms (also termed CSF-1R), we investigated the effects of TLR ligation on c-Fms expression.²⁷ Strikingly, TLR stimulation induced rapid (within 15 minutes) and near complete

downregulation of cell surface expression of c-Fms, without affecting expression of intracellular c-Fms precursor protein or mRNA. Concurrently, levels of soluble c-Fms extracellular domain accumulated in culture supernatants. These results indicated that ILRs induce ectodomain shedding of c-Fms, thereby making OCPs refractory to M-CSF and diverting them from the osteoclast lineage (Fig. 3). Downregulation of cell surface c-Fms was sensitive to MMP inhibitors and was dependent on activation of p38 and ERK MAPKs. These results support a model whereby canonical TLR signaling important for inflammatory responses also induces c-Fms shedding to inhibit osteoclastogenesis (Fig. 3). Strong activation of MAPKs by additional inflammatory factors such as IL-1 or orthopedic wear debris, similarly induced c-Fms shedding,^{28,29} thereby suggesting a more general role for c-Fms shedding in inflammatory feedback inhibition of osteoclast differentiation.

Feedback inhibition by IRF8 mediates the effects of TLRs and TNF- α on osteoclastogenesis

Interferon regulatory factor 8 (IRF8), a transcription factor specifically expressed in immune cells,³⁰ is a key negative regulator for osteoclastogenesis and bone metabolism in humans and mice, and its downregulation by RANKL is essential for osteoclastogenesis.³¹ IRF-8 expression is induced by IFN- γ or by costimulation with IFN- γ and TLRs,^{32,33} and augmented IRF-8 expression may contribute to the inhibitory effects of IFN- γ on osteoclastogenesis, and also to the well-documented suppressive effects of TLRs on OCP cells. We found that RANKL-induced downregulation of IRF8 is abrogated by TLR activation or IFN- γ treatment (Fig. 4). The inhibitory effect of TLRs on osteoclastogenesis at early stages is compromised by IRF8 deficiency. In an LPS-induced inflammatory bone resorption model, IRF-8 deficient mice exhibit enhanced osteoclast formation and more dramatic bone destruction than WT littermates.³¹ These data suggest that enhanced or sustained IRF8 expression contributes to the mechanisms by which TLRs and IFN- γ inhibit osteoclastogenesis, and IRF8 plays an important feedback inhibitory role in inflammatory osteoclastogenesis and bone resorption.

TNF- α is a key inflammatory cytokine important for the pathogenesis of autoimmune/inflammatory diseases such as RA, psoriatic arthritis, and periodontitis. In addition to triggering chronic inflammation, TNF- α plays a critical role in driving pathologic osteoclastogenesis and bone resorption (osteolysis) in these inflammatory diseases that are associated with bone destruction.^{34–36} Pathologic bone destruction is a major cause of morbidity and disability in inflammatory arthritis patients. The mechanisms by which TNF- α promotes inflammatory bone resorption include activation of osteoblastic/stromal cells to express M-CSF and RANKL, increasing cellular responses by augmentation of expression of M-CSF receptor c-Fms and RANK in OCPs, and increasing OCP numbers in inflammatory settings. TNF- α also induces additional inflammatory cytokines, such as IL-1 and IL-6, to augment osteoclastogenesis and bone resorption.^{8,36–38} Interestingly, the effects of these mechanisms on osteoclastogenesis are eventually mediated by RANK and dependent on RANK signaling. Although TNF- α alone can act directly on OCPs, its osteoclastogenic capacity is markedly lower than that of RANKL in both mouse and human systems.^{8,13,39–42} Genetic evidence obtained using RANK or RANKL knock-out mice demonstrates that TNF- α cannot induce osteoclast formation *in vivo* independently of RANK signaling.^{8,40} Despite eliciting similar activating pathways as does RANKL, TNF- α does not effectively induce osteoclastogenesis. One explanation for this difference is that RANK induces a unique (as yet unknown) signal required for osteoclast differentiation. An alternative nonmutually exclusive explanation is that stronger feedback inhibitory mechanisms may be induced in response to TNF- α than to RANKL, and these inhibitory mechanisms prevent TNF- α from inducing osteoclast differentiation as effectively as does RANKL.

We considered the possibility that IRF8 may mediate feedback inhibition of TNF- α -induced osteoclastogenesis, thereby preventing TNF- α from inducing osteoclastogenesis as effectively as RANKL. We found that IRF8, which functions as a transcriptional repressor, is a potent suppressor of TNF- α induced osteoclastogenesis; IRF8 was the first transcriptional repressor identified among the newly described negative regulators of osteoclast differentiation.^{31,43} We found that IRF8 deficiency dramatically enhanced the osteoclastogenic capacity of TNF- α , resulting in significantly enhanced NFATc1 induction, elevated expression of NFATc1 target genes, and increased osteoclast differentiation. Mechanistically, IRF8 binds to NFATc1 and suppresses its DNA binding ability and transcriptional activity, thereby inhibiting NFATc1 autoamplification and expression of NFATc1 target osteoclast marker genes. Downregulation of IRF8 is required for osteoclast differentiation.³¹ However, IRF8 expression decreases more slowly and weakly after TNF- α than after RANKL stimulation, indicating that TNF- α signaling has a weaker ability to overcome the transcriptional repression of inhibitory regulators, such as IRF8, than does RANKL (Zhao *et al.*, unpublished data). The partially maintained expression of IRF8 after TNF- α stimulation may be secondary to a feedback mechanism that promotes IRF8 expression, and thus attenuates the downregulation of IRF8 that occurs as part of osteoclast differentiation. Differential regulation of IRF8 expression after RANKL versus TNF- α stimulation provides one explanation of the different osteoclastogenic properties of RANKL and TNF- α .

Consistent with the importance of TNF- α -induced feedback inhibition in preventing osteoclast differentiation in response to this cytokine, TNF- α has been shown to induce the p100 inhibitor of the noncanonical NF- κ B pathway that also feeds back to restrain osteoclastogenesis.^{44–45} These results, together with our findings with IRF8, support the notion that feedback inhibitory molecules, such as IRF8 and p100, serve as nonredundant key negative regulators that not only suppress physiological osteoclastogenesis but also block osteoclast differentiation in response to inflammatory cytokines such as TNF- α , and thus serve an important homeostatic function to prevent excessive bone resorption in both physiological and inflammatory settings. Factors that induce or maintain IRF8 expression in inflammatory settings would function to restrain pathologic osteoclast differentiation. Identification of signaling pathways, additional factors, and mechanisms that regulate IRF8 expression and function presents a promising approach to restrain inflammatory bone resorption.

Conclusions

A role for inflammation in bone loss is well appreciated, and several mechanisms by which inflammatory factors suppress bone formation and promote bone resorption have been described. In contrast, activation of feedback inhibition by inflammatory factors, a concept that is well established in the immunology field, has not been fully appreciated in the osteoimmunology and bone biology fields. We have initiated investigation of such feedback inhibition, and have identified three mechanisms that inhibit osteoclastogenesis and are induced by inflammation: suppression of costimulatory molecules such as TREM-2; suppression of *RANK* transcription by induction of ectodomain shedding and thereby inactivation of c-Fms; and induction of transcriptional repressors such as IRF8. It is likely that these mechanisms work in a complementary and cooperative manner to fine tune the extent of osteoclast generation in inflammatory settings. Interestingly, these inhibitory mechanisms are operative and effective at the level of OCPs, suggesting that inhibition of osteoclast differentiation is linked with myeloid cell fate decisions. It is notable that inflammatory stimuli regulate the network of transcriptional repressors that restrain osteoclastogenesis, which should spur future research in this area. Identification and augmentation of novel feedback inhibitory mechanisms induced by inflammation represents

an attractive therapeutic alternative to current approaches that aim to suppress activating pathways.

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Figure 1.

Induction of bone resorption by inflammatory factors. Inflammatory factors and cytokines such as TLR ligands, TNF- α , and IL-1 promote bone resorption by inducing RANKL expression, synergizing with RANK signaling, and inducing ligands of costimulatory ITAM-associated receptors.

Figure 2.

Regulation of differentiation of myeloid precursors that enter inflammatory sites. Monocytes and osteoclast precursors that enter inflammatory sites from the circulation are exposed to microbial products, inflammatory factors and cytokines, and RANKL. These factors induce both activating and feedback inhibitory signals that regulate cell fate decisions and determine the balance between inflammatory M1 macrophage versus osteoclast differentiation. Feedback inhibitory mechanisms also will determine the quantitative extent of osteoclastogenesis.

Figure 3.

TLRs induce c-Fms ectodomain shedding to suppress osteoclastogenesis. TLRs activate MAPK pathways that induce matrix metalloprotease (MMP)-mediated ectodomain shedding of c-Fms. This results in a loss of responsiveness of cells to M-CSF, including loss of M-CSF-induced expression of RANK.

Figure 4.

Regulation of IRF8 expression by RANKL, IFN- γ , and LPS. Murine bone marrow derived macrophages were treated with RANKL (80 ng/mL) and M-CSF (20 ng/mL) in the absence or presence of IFN- γ (10 ng/mL) or LPS (10 ng/mL) for the indicated times. IRF8 mRNA expression was determined by RT-PCR.