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COMMENTARIES

RANKing Bone Resorption Versus Inflammation: Infection Makes the Decision

Sylvie Ferrari-Lacraz[1] and Danielle Burger[2]

[1]Division of Immunology and Allergy, Transplantation Immunology Unit, Department of Internal Medicine, and Department of Genetic and Laboratory Medicine, University Hospital and Faculty of Medicine, University of Geneva, Geneva, Switzerland
[2]Division of Immunology and Allergy, Inflammation and Allergy Research Group, Hans Wilsdorf Laboratory, Department of Internal Medicine, University Hospital and Faculty of Medicine, University of Geneva, Geneva, Switzerland

Commentary on: Ji JD, Park-Min KH, Shen Z, Fajardo RJ, Goldring SR, McHugh KP, Ivashkiv LB. Inhibition of RANK expression and osteoclastogenesis by TLRs and IFN-gamma in human osteoclast precursors. *J Immunol*. 2009 Dec 1;183(11):7223-33.

Osteoclasts are primary actors in physiologic and pathologic bone resorption whose differentiation and maturation are triggered by inflammatory cytokines including receptor activator of NF-κB ligand (RANKL). That Toll-like receptor (TLR) occupancy by bacterial products inhibits osteoclast differentiation was demonstrated in the past mainly in a mouse system (1). Ji *et al*. (2) report the mechanisms underlying the inhibition of osteoclast precursor differentiation into noninflammatory mature osteoclasts during microbial infection in a human system. The results show that TLRs and IFNγ (*i.e.*, a potent macrophage differentiation/maturation factor) synergize to inhibit human osteoclastogenesis by triggering complementary mechanisms that work in tandem to downregulate the expression of RANK and triggering receptor expressed by myeloid cells (TREM)-2 expression. Indeed, TLR ligation triggers the diminution of c-Fms (the colony-stimulating factor-1 (M-CSF) receptor) that is shed out by metalloproteinases and whose expression is further diminished by IFNγ. The study by Ji *et al*. (2) demonstrates the coordinated down-regulation of RANK and TREM-2 expression and sheds new light on mechanisms underlying the potent downregulation of human osteoclastogenesis by TLR ligation. Along with a recent report showing the activation of TLRs by new synthetic, non-toxic compounds (3), this study opens the way toward a clinical setting of new anti-osteoclastogenic therapeutic agents.

Originating from bone marrow stem cells, osteoclasts derive from the mononuclear/phagocytic lineage (4). The regulation of osteoclastogenesis by immuno-modulatory receptors suggests that osteoclasts, similar to their related myeloid cells, are tightly controlled by an array of receptors that allows them to sense and respond to their local microenvironment like other innate and adaptive immune cells (5). In the bone marrow environment, mesenchymal stromal cells have an important role in the differentiation of osteoclasts, and a close interaction between hematopoietic precursors and mesenchymal cells is needed for the proper differentiation of osteoclasts (6). Stromal cells and osteoblasts, as well as surrounding bone marrow cells, express cytokines and growth factors to initiate and support osteoclast differentiation; monocytes and T cells being

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the major cytokine-producing cells (7). Osteoclasts derive from a subset of monocytes/macrophages expressing the receptor RANK. Osteoclast development requires the activation of specific cell surface receptors on pre-osteoclasts. On one side, the two most important receptors for the differentiation of osteoclasts are RANK and c-Fms (7). On another side, two factors are critical and sufficient to induce the differentiation of hematopoietic precursors into multinucleated, bone-resorbing cells: M-CSF and RANKL (8-10). Originally identified as a regulator of macrophage formation, M-CSF is an essential survival factor for all cells of the monocyte/macrophage lineage including osteoclast precursors, pre-osteoclasts and multinucleated mature osteoclasts. Although M-CSF does not induce osteoclast formation in the absence of RANKL, it upregulates RANK on a subset of monocytes enabling them to respond to RANKL and differentiate into osteoclasts (5). The proof-of-concept is that, both in humans and mice, deletion or inactivating mutations of the RANKL and RANK genes result in the absence of osteoclasts, resulting in osteopetrosis (8;11;12).

TLRs are a family of innate immune receptors responsible for initiating inflammation. TLRs are the most well-studied pattern recognition receptors (PRRs) (13). These receptors are expressed by various cell types in the immune system, and recognize molecular structures present on microbes that are not found in the host (14). TLRs exist as cell surface receptors (*e.g.,* TLR2, TLR4) or intracellular receptors (*e.g.,* TLR3, TLR7, TLR8, TLR9). That TLR occupancy by bacterial products inhibits osteoclast differentiation was previously demonstrated in a mouse system (1). Ji *et al*. (2) report the mechanism underlying the inhibition of osteoclast precursor differentiation into noninflammatory mature osteoclasts during microbial infection in a human system. By using ligands of TLR4 (LPS) and TLR2 (Pam_3Cys), they demonstrate how, when an organism is in danger and has to fight infection and/or microbial, toxic products, the fate of pre-osteoclasts can swerve to that of monocyte-macrophages, key cells involved in the innate immune response. They demonstrate that with danger signals, the organism will be devoted to its defense and will cause precursor cells to develop into macrophages in order to clear the infection rather than to become committed to bone homeostasis. However, it should also be emphasized that TLR agonists such as LPS were also described as pro-osteoclastogenic since they mediate *in vivo* and *in vitro* bone resorption and induce osteoclast formation by directly stimulating RANKL expression in osteoblasts, the production of pro-inflammatory cytokines in macrophages, and the survival of osteoclasts (15;16). These contrasting actions compared to the current anti-osteoclastogenic data (2) show that according to the dose, the timing and the cell culture microenvironment (concomitant use of RANKL), LPS may display opposite effects.

Through inflammation and its mediators, immune cells not only influence immune responses but also the metabolism of each organ in the body, including bone. In contrast to the mechanisms described by Ji *et al*. (2), who studied the innate immune response triggered by danger signals, in chronic inflammation the adaptive immune system, through immune and bone cell interactions, plays a major role in bone remodeling. Indeed, bone resorption is often observed in inflammatory diseases, as in rheumatoid arthritis (RA) with subchondral bone erosion, as well as in cancer with osteolytic bone metastasis. These local and systemic bone losses are caused by the bone-resorbing effects of osteoclasts. In many pathological and inflammatory conditions, the maintenance and amplification of inflammatory reactions lead to osteoclastogenesis and increased bone turnover; this is due to the premise that during inflammation, activated immune cells produce many pro-inflammatory cytokines, which modulate osteoblast and osteoclast activity leading to a high rate of bone remodeling. The inducer cells in this process are immune cells such as activated macrophages, dendritic cells and T cells, which produce cytokines and soluble mediators able to stimulate osteoclast

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differentiation and activation. Moreover, activated T cells induce osteoclastogenesis by stimulating synovial macrophages to secrete pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6 (17), and as demonstrated more recently, IL-15 and IL-17, which in turn activate osteoclastogenesis either directly or indirectly, via RANKL production in synovial fibroblasts (18-21). Thus, the imbalance of the stimulatory and inhibitory factors/activities of T cells may cause the aberrant osteoclast formation observed in RA.

The influence of IFN-γ on osteoclast function is controversial, and depending on the culture conditions it has been described as inhibiting (22;23) or facilitating osteoclastogenesis (24). *In vivo*, administration of IFN-γ produces a pro-osteoclastogenic effect. Indeed, IFN-γ functions indirectly to upregulate antigen presentation activity, through the induction of class II transactivator (CIITA), causing activation of T cells and secretion of osteoclastogenic cytokines (24). This indirect pro-osteoclastogenic effect may be moderated by direct counteracting anti-osteoclastogenesis effects of IFN-γ that inhibit NFκB through TRAF6 degradation (23), and by the mechanisms described by Ji *et al*. (2) (*i.e*., downregulation of RANK, TREM2 and c-Fms). A negative feedback regulatory mechanism via type I IFN has been described previously (25). Activation by RANKL leads to up-regulation of IFN-β, which mediates a feedback mechanism blocking further c-Fos-dependent activity. As such, it was shown that mice deficient in the IFN-α/β receptor (IFNAR1) suffer from an osteoporotic phenotype that is characterized by an increase in osteoclasts. In contrast, the results of Ji *et al*. (2) demonstrate that in the human system IFN-β is poorly induced upon TLR ligation and that type II (IFNγ), rather than type I IFNs, is involved in the inhibition of osteoclast formation.

The results of Ji *et al*. (2) further demonstrate that TLR ligation induces a bias towards the pro-inflammatory differentiation of cells by diminishing the expression of TREM-2. TREM-1 and -2 are members of a family of single immunoglobulin-like domain-comprising receptors expressed on a variety of innate immune cells of the myeloid lineage (26). Currently TREM-1 is thought of as an amplifier of the immune response, while TREM-2 is believed to be a negative regulator (27). Thus diminution of TREM-2 expression is also indicative of a bias towards a more inflammatory phenotype. It was recently reported that a modified TLR3 ligand (CpG-KSK13) displays an inhibitory effect on osteoclast formation in both mouse and human systems (3). This inhibitory effect was due to the downregulation of TREM-2 expression in osteoclast precursors, thus providing the possibility of utilizing TLR modified ligands as anti-osteoclastogenic therapeutic agents (3).

In conclusion, the findings of Ji *et al*. (2) reveal molecular processes by which TLR ligands and IFNγ synergize to suppress osteoclastogenesis. Indeed, myeloid precursor responses to M-CSF are coordinately regulated by inflammatory stimuli. Reversion of osteoclast precursor differentiation towards macrophage differentiation/maturation requires cooperation between microbial products provided by infectious agents (*e.g.,* TLR ligands) and stimuli produced during the immune response (*e.g.,* IFN-γ). By elucidating mechanisms underlying the inhibition of osteoclast formation upon infection, the work of Ji *et al*. (2) opens the way toward a clinical setting of new anti-osteoclastogenic therapeutic agents.

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