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## *COMMENTARIES*

# Inhibition of Osteoclast Differentiation by the Interleukin (IL)-1 Family Cytokine IL-33

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**Commentary on:** Schulze J, Bickert T, Beil FT, Zaiss MM, Albers J, Wintges K, Streichert T, Klaetschke K, Keller J, Hissnauer TN, Spiro AS, Gessner A, Schett G, Amling M, McKenzie AN, Horst AK, Schinke T. Interleukin-33 is expressed in differentiated osteoblasts and blocks osteoclast formation from bone marrow precursor cells. *J Bone Miner Res*. 2011 Apr;26(4):704-17.

Zaiss MM, Kurowska-Stolarska M, Böhm C, Gary R, Scholtysek C, Stolarski B, Reilly J, Kerr S, Millar NL, Kamradt T, McInnes IB, Fallon PG, David JP, Liew FY, Schett G. IL-33 shifts the balance from osteoclast to alternatively activated macrophage differentiation and protects from TNF-alpha-mediated bone loss. *J Immunol*. 2011 Jun 1;186(11):6097-105.

**Interleukin (IL)-33 is the most recently described member of the IL-1 family of cytokines. IL-33 mediates its extracellular effects by binding to an IL-1 receptor family member called ST2 and is involved in Th2-type immunity and inflammatory responses. In two recent papers, Schulze *et al*. and Zaiss *et al*. report for the first time *in vivo* data indicating a role for IL-33 in the inhibition of osteoclast differentiation (1;2). The first study, using ST2 knockout (KO) mice, describes decreased bone mass at steady state in the absence of IL-33 signaling, while the second paper shows a protective effect of IL-33 treatment on bone loss in inflammatory conditions in arthritic human TNF-α transgenic (hTNFtg) mice. *In vitro* experiments confirm an inhibitory effect of IL-33 on osteoclast differentiation at an early stage. In fact, IL-33 appears to prevent commitment of bone marrow precursors to the osteoclast lineage, favoring emergence of alternative myeloid cell fates instead. Together, these two studies provide the first *in vivo* context to a series of recently published *in vitro* data concerning the effects of IL-33 on human and mouse bone cells. Finally, based on their observations, Zaiss *et al*. suggest a potential therapeutic use of IL-33 as an inhibitor of bone resorption, although, from this perspective, it must be remembered that IL-33 is also a potent pro-inflammatory cytokine that contributes to increasing bone and cartilage erosion scores in other models of arthritis.**

IL-33 is the most recently discovered member of the IL-1 cytokine family (see (3) for review). IL-33 is constitutively expressed in the nucleus of endothelial and epithelial cells. In addition, IL-33 expression is induced in different types of resident and infiltrated cells in inflamed tissues. IL-33, like IL-1α, is a dual function protein, displaying both nuclear and extracellular effects. The latter are mediated by its binding to an IL-1 receptor family member called ST2. Consistent with expression of ST2 on many cells involved in Th2-type immunity, IL-33 injection induces or amplifies Th2-type responses in various mouse models. In addition, IL-33 also displays pro-inflammatory effects in pathologies that are independent of Th2 immunity, including arthritis.

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Two recent papers now report a role for IL-33 in the inhibition of osteoclast differentiation *in vivo* (1;2). Starting from the observation that treatment with recombinant IL-33 increases RANKL expression in osteoblasts, Schulze *et al*. examined the bone phenotype of ST2 KO mice, anticipating increased bone mass in the absence of IL-33 signaling (1). Unexpectedly, however, ST2 KO mice displayed decreased trabecular mass, resulting from increased bone resorption in the presence of normal bone formation. *In vitro*, addition of IL-33 during the first 4 days of culture abolished generation of $TRAP^+$ multinucleated osteoclasts from bone marrow precursor cells in the presence of M-CSF and RANKL. In fact, IL-33 treatment promoted the emergence of other myeloid cell types, such as alternatively activated M2 macrophages (AAMs), eosinophils or basophils at the expense of osteoclasts. Finally, the authors also reported a direct negative influence of IL-33 on osteoclast differentiation of purified $CD11b^+$ bone marrow cells in the absence of bone marrow stromal cells, and of Raw 264.7 cells.

More recently, Zaiss *et al*. investigated potential effects of IL-33 on bone in an inflammatory context using hTNFtg mice (2). They report that treatment with IL-33, or with an agonistic anti-ST2 antibody, inhibits TNF-$\alpha$-induced bone and cartilage destruction in arthritic mice, although clinical symptoms of arthritis are not improved. Conversely, bone erosion and osteoclast numbers were increased in irradiated hTNFtg mice reconstituted with ST2 KO bone marrow, as compared to control bone marrow. IL-33 treatment or ST2 activation also attenuated TNF-$\alpha$-induced generalized osteopenia and increased serum levels of anti-osteoclastogenic cytokines such as IL-4, IFN-$\gamma$ or GM-CSF. *In vitro*, IL-33 inhibited osteoclast differentiation of $CD11b^+$ bone marrow precursors, in the presence of RANKL or of TNF-$\alpha$, favoring generation of AAMs instead. This shift in cell fate was associated with, and dependent on, production of GM-CSF, and to a lesser extent IL-4, in response to IL-33. Finally, IL-33 also inhibited M-CSF- and RANKL-induced osteoclast differentiation of purified human bone marrow $CD11b^+$ mononuclear cells, but not of $CD14^+$ peripheral blood monocytes, suggesting that also in the human system, IL-33 inhibits osteoclast differentiation by acting on early precursors.

Table 1. Reported *in vitro* effects of IL-33 on bone cells.

| Species | Cell type | Effect | Reference |
|---|---|---|---|
| Mouse | $CD11b^+$ BM cells | Inhibition of RANKL-induced OC differentiation | 1;2 |
| Mouse | Total BM or spleen cells | Inhibition of RANKL-induced OC differentiation | 6 |
| Mouse | BMM precursors | No direct effect on differentiation of immature OC progenitors | 6 |
| Mouse | Calvarial osteoblasts | No effect on mineralization | 1 |
| Mouse | Calvarial osteoblasts | Enhanced mineralization | 6 |
| Human | $CD11b^+$ BM cells | Inhibition of RANKL-induced OC differentiation | 2 |
| Human | $CD14^+$ blood monocytes | No effect on OC differentiation | 2;5;6 |
| Human | $CD14^+$ blood monocytes | Induction of OC differentiation | 4 |
| Human | BMSC-derived osteoblasts | No effect | 5 |

BM: bone marrow; BMM: bone marrow macrophage; BMSC: bone marrow stromal cell; OC: osteoclast.

The picture emerging from these studies, as well as from a series of somewhat controversial *in vitro* data concerning the effects of IL-33 on human and mouse bone cells (Table 1) (4-6), is thus that IL-33 decreases osteoclast differentiation by acting at early stages on bone marrow precursors to prevent commitment to the osteoclast lineage, and to favor emergence of alternative myeloid cell fates instead, such as AAMs in particular (Fig. 1). In agreement with this hypothesis, several studies have failed to detect any effect, negative or positive, of IL-33 on osteoclast

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differentiation of purified $CD14^+$ peripheral blood monocytes, which represent a later stage of monocyte differentiation (2;5;6) (Fig. 1). At odds with these observations, however, Mun and colleagues (4) reported a strong RANKL-independent stimulatory effect of IL-33 on differentiation of $CD14^+$ monocytes into osteoclasts. While the reasons for this discrepancy remain unclear, they might relate to differences in purity of cell preparations or in culture and assay conditions used, as discussed in (6).

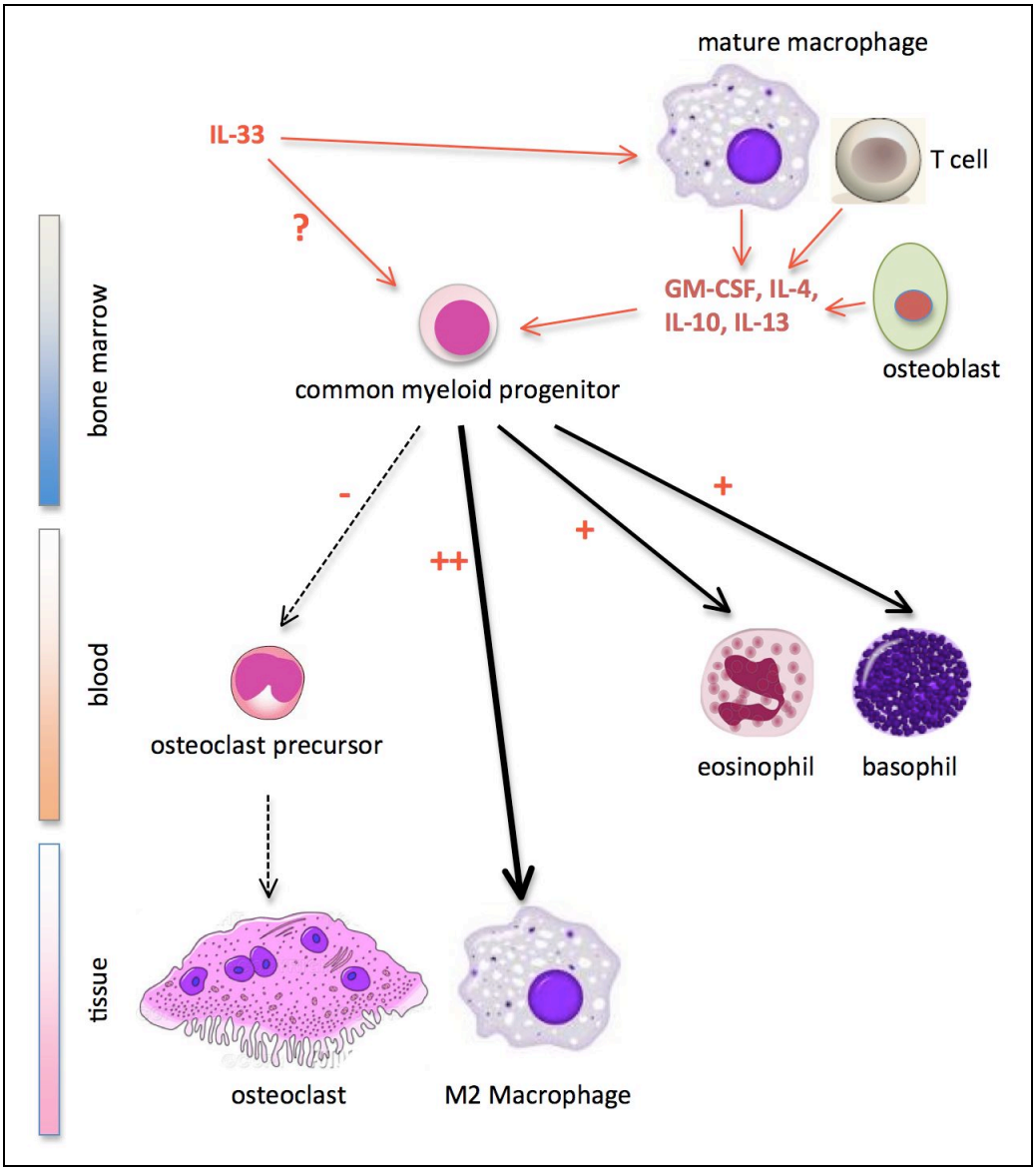


Fig. 1. Inhibitory effect of IL-33 on osteoclast differentiation. IL-33 acts at early stages on bone marrow myeloid progenitors to prevent commitment to the osteoclast lineage, and to favor emergence of alternative cell fates, such as M2 macrophages, eosinophils or basophils. Recent data suggest that this effect is indirect and mediated by the production of anti-osteoclastogenic cytokines, such as GM-CSF, IL-4, IL-10 and IL-13, by various cell types, which include mature macrophages, T cells or osteoblasts.

Another twist to this interesting story is that the effect of IL-33 on bone marrow macrophage precursors to limit commitment to the osteoclast lineage is probably indirect,

and mediated by the production of a combination of anti-osteoclastogenic factors such as GM-CSF, IL-4, IL-13 and IL-10, which together optimally inhibit osteoclast differentiation (6). While production of such factors has been observed in cultures of purified mouse $CD11b^+$ bone marrow cells, and thus in absence of bone marrow stromal cells (1;2), a recent study suggests that it requires the presence of cell types other than the osteoclast precursors themselves, including, for instance, mature macrophages, T cells or osteoblasts (6) (Fig. 1).

Another open question lies with the influence of IL-33 on bone formation. Reported *in vitro* findings disagree concerning effects of IL-33 on human and mouse osteoblast cultures, and in particular concerning a potential stimulation of matrix mineralization (1;5;6). Furthermore, *in vivo* data indicate normal bone formation in ST2 KO mice (1), such that the relevance of reported *in vitro* effects of IL-33 on osteoblasts remains to be established.

Finally, while Zaiss *et al*. report a protective effect of IL-33 on bone loss in the hTNFtg model, IL-33 was previously shown to be a potent pro-inflammatory mediator in other models of arthritis, including collagen-, antigen- and K/BxN serum transfer-induced arthritis (7-10). In these models, IL-33 contributes to increasing bone and cartilage erosion scores, although this effect may be at least in part indirect and mediated by pro-inflammatory effects of IL-33 and induction of RANKL expression in the joint (8). This dual role of IL-33 in the control of bone turnover is reminiscent of similar effects reported for IL-1. Indeed, exposure to IL-1β prior to, or together with, RANKL, was reported to suppress osteoclastogenesis in human $CD14^+$ monocytes and rheumatoid arthritis synovial fluid macrophages (11). To the contrary, there is substantial evidence to indicate that, *in vivo*, IL-1 acts primarily as a pro-resorptive cytokine in many physiological and pathological situations. As for IL-1, suppressive functions of IL-33 on bone resorption may thus be context-dependent and biologically relevant only in specific circumstances. This important issue clearly warrants further study.

In summary, two recent papers demonstrate an inhibitory effect of IL-33 on bone resorption by a unique mechanism. While controversial findings have been reported concerning the effects of IL-33 on osteoclast and osteoblast differentiation and activity *in vitro*, which to some extent may reflect differences in experimental systems and cell populations used, these studies now provide an *in vivo* context for further definition of the role of IL-33 in bone biology and of potential therapeutic opportunities involving this cytokine.

**Conflict of Interest:** None reported.

**Peer Review:** This article has been peer-reviewed.

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