At the 31st Annual Meeting of ASBMR, many interesting papers demonstrated various modulators of osteoclast differentiation and function. Topics included negative regulators of osteoclast differentiation, cathepsin K, angiogenesis, IKK2-NF-κB pathways, IL-34, and the TGF-β superfamily. The most interesting osteoclast-related abstracts are summarized below.

**Negative Regulators of Osteoclast Differentiation**

As evidenced by in vitro and in vivo experiments as well as clinical trials using the anti-RANKL antibody denosumab, the RANKL-RANK axis primarily regulates osteoclast development in human as well as murine skeletal tissues (1;2). At the meeting, several important negative regulators of osteoclast differentiation were revealed. Interferon regulatory factor 8 (IRF8), a transcription factor expressed in immune cells, was found to be downregulated in mouse and human osteoclast precursors in response to RANKL stimulation at the early stage of differentiation (3). Gene deletion of IRF8 stimulated, while overexpression of the molecule strongly suppressed RANKL-induced osteoclast differentiation (4). IRF8-deficient mice exhibited severe osteoporosis caused by increased bone resorption, and they showed enhanced bone destruction following lipopolysaccharide administration due to enhanced osteoclast formation. IRF8 suppressed osteoclast differentiation by suppressing the transcriptional activity and expression of NFATc1. These results clearly indicate that IRF8 is a negative regulator of osteoclastogenesis (3).

Interferon-β (IFN-β) is a potent negative regulator of osteoclast differentiation acting downstream of RANKL-RANK signaling, and the negative feedback regulation by IFN-β requires IRF9 expression. IFN-β-induced inhibition of osteoclast differentiation was abrogated by a selective iNOS inhibitor (L-NIL) or in IRF9(-/-) cells, but iNOS induction was rather enhanced in IRF9(-/-) cells. These results suggest that the negative feedback regulation of RANKL-induced osteoclastogenesis through IFN-β is mediated by two different pathways: IRF9 and iNOS/NO (5).

**Cathepsin K Is a Negative Regulator of Bone Formation**

Cathepsin K is highly expressed in osteoclasts and critically regulates type I collagen degradation during bone resorption. Mice globally lacking the cathepsin K gene had mild osteopetrosis and high numbers of poorly functional osteoclasts, and interestingly, these mice also exhibited increased bone formation rates (BFR) (6;7). To investigate which cell types are responsible for the increased BFR in these mice, conditional deletion of the cathepsin K gene was achieved by mating CKLP^fl/fl^ mice, in which exon 5 of the cathepsin K gene was flanked by lox P sites, with Mx1-Cre mice (Mx1-Cre;CKLP^fl/fl^) or osterix-Cre mice (Osx-Cre;CKLP^fl/fl^). When the cathepsin K gene was deleted in osteoclasts by injecting Mx1-Cre;CKLP^fl/fl^ mice with polynosinic-polycytidylic acid, the mice exhibited moderate osteopetrosis with an increase in cancellous bone volume (+163%) and osteoclast number (+400%). Importantly, BFR and osteoblast number in the mice
were also markedly increased (+47% and +214%, respectively) compared to controls, and osteoblasts obtained from the mice showed increased alkaline phosphatase and mineralization activity. In contrast, Osx-Cre:CKLP\textsuperscript{fl/fl} mice failed to show any skeletal phenotype, strongly suggesting that deletion of cathepsin K in the osteoblast lineage has no effect on bone resorption or on bone formation, providing genetic evidence that cathepsin K produced by osteoclasts may impair the coupling message from osteoclasts to osteoblasts (8). These intriguing observations may explain why the mechanisms of action of cathepsin K inhibitors appear distinct from those of bisphosphonates in the skeletal tissues of ovariectomized animals and osteoporosis patients (9-11).

**Angiogenesis is Regulated by Osteoclasts**

A novel role of osteoclasts in skeletal angiogenesis was demonstrated. Angiogenesis is a crucial step for skeletal development, and it was previously reported that matrix metalloproteinase 9 (MMP-9) produced by osteoclasts plays an important role in growth plate angiogenesis (12). Using the fetal mouse metatarsal angiogenesis assay, it was revealed that continuous PTHrP treatment increased both the area covered by endothelial cells and osteoclastic resorption in metatarsal explants, which was abrogated by osteoprotegerin treatment or in osteoclast-deficient op/op mouse bones, showing that the angiogenic effect of PTHrP requires osteoclasts. In addition, the proangiogenic effect of PTHrP is blunted in metatarsal explants from MPP-9(-/-) mice. These results suggest that the presence of osteoclasts and MMP-9 produced by osteoclasts is essential for skeletal angiogenesis (13).

**Constitutive Activation of IKK Is Sufficient for Osteoclast Differentiation**

NF-κB is a collective term referring to dimeric transcription factors that belong to the Rel family, and NF-κB is involved in various aspects of physiologic and pathologic events. The essential role of NF-κB in osteoclast development was first observed in knockout mice. Although targeted disruption of either \textit{Nfkbia} or \textit{Nfkbi} alone did not affect skeletal development, double knockout of these genes induced osteopetrosis in mice due to a defect in osteoclast differentiation (14;15). These results suggest that there are redundant roles of the canonical and noncanonical NF-κB pathways in osteoclast differentiation. Interesting results were presented on the role of the NF-κB pathways in osteoclastogenesis. It was shown that constitutively active IKK2 (IKK2\textsuperscript{SEE}) was sufficient for osteoclast differentiation independent of RANKL (16). Osteoclasts were induced by IKK2\textsuperscript{SEE} introduction even in the presence of osteoprotegerin or in RANK knockout cells, indicating that the effect is independent of RANKL-RANK pathways. Double knockout cells missing \textit{Nfkbia} or \textit{Nfkbi} were resistant to IKK2\textsuperscript{SEE}-induced osteoclastogenesis, while NEMO, IKK\alpha, or RelB deficiency did not affect IKK2\textsuperscript{SEE}-induced osteoclast differentiation. In addition, adenoviral transfer of the IKK2\textsuperscript{SEE} gene induced massive osteolysis in mice (16). It was also reported that tyrosine-mutated or ubiquitin-like domain-deleted IKK2 failed to support RANKL-induced osteoclast differentiation (17). These results indicate an essential role of IKK2-NF-κB pathways in osteoclast differentiation.

**The Role of Splenic IL-34 in CSF-1-Independent Osteoclastogenesis**

Osteoclast differentiation is critically regulated by two cytokines produced by osteoblasts: RANKL and macrophage colony-stimulating factor (M-CSF). However, the defective osteoclast differentiation in M-CSF-deficient op/op mice is recovered in an age-dependent manner, although the mechanisms underlying this effect remain unknown (18). Recently, interleukin-34 (IL-34) was discovered as a novel ligand for the M-CSF receptor, and highly expressed in the spleen (19). It was shown that IL-34 substituted for M-CSF in a murine osteoclastogenesis assay (20). When a highly potent 1,25-dihydroxyvitamin D\textsubscript{3} analog, 2MD, was administered to
3-week-old op/op mice, many tartrate-resistant acid phosphatase (TRAP)-positive osteoclasts were observed in tibiae. Prior to the appearance of osteoclasts in bone, the expression of IL-34 in the spleen was increased in response to 2MD, which was followed by the appearance of TRAP-positive mononuclear cells. In addition, 2MD failed to induce TRAP-positive osteoclasts in bone in splenectomized op/op mice. These results suggest that IL-34 is a novel regulator of osteoclast differentiation and that the spleen is a source of osteoclast progenitors, which are differentiated in response to IL-34 induced by 1,25-dihydroxyvitamin D₃ (20).

Role of the TGF-β Superfamily in Osteoclastogenesis

The direct effect of TGF-β and bone morphogenetic proteins (BMPs) on osteoclast differentiation was reported. It was previously found that TGF-β promoted RANKL-induced osteoclastogenesis (21,22). At this year's meeting, it was shown that exogenous BMP2 synergizes with sub-optimal levels of RANKL to enhance in vitro differentiation of osteoclast precursors (23). In addition, when TGF-β signals were blocked either by administration of a specific inhibitor or by introducing a dominant negative mutant of the TGF-β type II receptor, RANKL-induced osteoclastogenesis was almost completely suppressed both in vitro and in vivo, and the effect of TGF-β on RANKL-induced osteoclast differentiation was partially explained by the direct interaction between Smad2/3 and TRAF6 (24).

Conclusion

More than 10 years have passed since the discovery of the RANKL-RANK system as a master regulator of osteoclast differentiation. However, many questions remain answered. In particular, the cellular and molecular interaction between bone formation and bone resorption has attracted a great deal of attention. For example, it was reported that osteocytes produce osteoprotegerin in a Wnt-β-catenin signal-dependent manner, indicating a tight connection between osteocyte activity and bone resorption (M. Kneissel, plenary symposium II). It will become increasingly important to understand osteoclastic bone resorption in relation to entire skeletal homeostasis, which will open a new field of research into the osteoclast.

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splenic IL-34 in CSF-1-independent osteoclastogenesis induced by active vitamin D_{3}. J Bone Miner Res. 2009;24(Suppl 1). [Abstract]


