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MEETING REPORTS

Osteoimmunology: Meeting Report from the 33rd Annual Meeting of the American Society for Bone and Mineral Research

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Introduction

Osteoimmunology is a new discipline that studies the crosstalk between the immune system and bone. This definition can be broad or narrow. While many osteoimmunologists focus on RANKL/RANK signaling pathways in monocytes, cells integral to immunity, this report will present a sampling of the broader aspect of the role of immune cells and immune mechanisms in the regulation of bone turnover in health and disease. A number of these interesting new directions and developments in osteoimmunology were unveiled at the 33rd Annual Meeting of the American Society for Bone and Mineral Research in San Diego and are briefly summarized below.

Adipogenesis and the Immune System

It is now known that immune system dysfunction resulting from obesity, as reflected by a marked decrease in immune cell populations, precipitates a susceptibility to life-threatening diseases. One investigation by Chan *et al*. examined whether obesity damages the bone marrow niche where progenitors critical to bone remodeling and immunity originate (1). The study showed that obesity alters all the major bone marrow lineages including T cells and B cells. Moreover, exercise, as modeled by low intensity vibration, improved the size of the B cell population potentially through modulating hematopoietic progenitors originating from the bone marrow.

Recently interferon gamma (IFN-γ) was found to induce osteoblastogenesis *in vitro* and bone formation *in vivo*. Since this anabolic effect could be exerted by inducing osteoblast differentiation of mesenchymal stem cells (MSCs) at the expense of adipogenesis, Bermeo-Serrato *et al*. assessed whether IFN-γ had an anti-adipogenic effect both *in vitro* and *in vivo* (2). The data show that IFN-γ acts as an adipogenic antagonist, supporting the hypothesis that the anabolic effect of IFN-γ on bone involves the reciprocal regulation of adipogenesis and osteogenesis in the bone marrow microenvironment.

The nuclear receptor PPARγ inhibits osteogenesis by favoring adipogenesis from common mesenchymal progenitors. PPARγ agonists may cause bone loss and accumulation of marrow adiposity in mice and in postmenopausal women, suggesting a link between PPARγ and bone turnover. To investigate this issue, Fu *et al*. made use of *PPARγ*(-/-) mice (3). They found that these mice exhibit severe lipodystrophy, a decrease in hematopoietic stem cells (HSCs), an increased proportion of osteoclast progenitors and extramedullary hematopoiesis. These data suggest that PPARγ regulates bone turnover both by a direct effect on cell differentiation and an indirect effect on cell fate determination through adipocyte and adipokine secretion. These specific characteristics provide support for a key role of PPARγ at the crossroads of osteogenesis, adipogenesis and hematopoiesis/osteoclastogenesis.

Hematopoietic Stem Cells and the Immune System

HSCs are regulated by specialized non-hematopoietic cells spatially organized in a

niche that is essential for their self-renewal and differentiation. The niche comprises a variety of cells, including osteoblasts. In 2003, Calvi *et al*. revealed that parathyroid hormone (PTH) increases the number of HSCs localized in close proximity to endosteal surfaces (4). More recently it has been shown that PTH fails to expand HSCs in interleukin 6 (*IL-6*(-/-)) mice (5). This suggests that IL-6, a cytokine produced by PTH-stimulated osteoblasts, contributes to expanding HSCs, primarily by decreasing HSC apoptosis. This year the same group reported that soluble IL-6 receptor (sIL-6R) can replace IL-6 (6). Their findings demonstrate that sIL-6R is sufficient to support the hematopoietic cell expansion and the anabolic actions of PTH in bone, and link together IL-6-targeted signaling in the bone and marrow microenvironments toward a better mechanistic understanding of PTH anabolic actions.

A significant advance in HSC biology is the recognition that activation of Wnt signaling in both stromal cells and HSCs is required for the expansion of the hematopoietic niche. However, the source and the nature of the Wnt ligands required to activate Wnt signaling in these cells remains unknown. Since PTH induces T cell production of Wnt10b, a Wnt ligand critical for PTH action in bone, Li *et al*. investigated the role of T cell-produced Wnt10b in PTH-induced HSC expansion (7). They found that activation of the PTH receptor (PTHR1) in T cells and the resulting production of Wnt10b and stimulation of Notch signaling are required for PTH to stimulate HSC expansion and engraftment and improve survival after bone marrow transplantation.

Another group focused on the role of osteocytes in the regulation of HSC expansion. Osteocytes regulate osteoblast and osteoclast activity, in part via G-protein-coupled receptor signaling. Thus, osteocytes may directly or indirectly regulate hematopoiesis through the stimulatory subunit of G-protein, Gsα. Fulzele *et al*. showed that mice lacking Gsα in osteocytes had severe osteopenia and displayed hematopoietic abnormalities that resembled myeloproliferative disease characterized by a dramatic and significant increase in leukocytes, neutrophils, and platelets in the peripheral blood and increased myeloid cells in the bone marrow and spleen (8). These results are the first evidence for osteocyte-mediated regulation of hematopoiesis via Gsα signaling-induced alteration of the bone marrow microenvironment.

Another novel regulator of HSCs discussed at the meeting was nuclear factor of activated T cells c2 (NFATc2). NFATs comprise a family of transcription factors that are critically involved in T cell activation and modulation of bone remodeling. NFATc1 has been identified as the master transcription factor for osteoclasts and also suppresses osteoblast function. By contrast, the role of other NFAT members such as NFATc2 is poorly understood. Based on the observation that NFATc2 is highly expressed in HSCs and regulated in a lineage-specific manner, Rauner *et al*. (9) reasoned that NFATc2 is an essential regulator of both hematopoiesis and bone mass. Confirming this hypothesis, they showed that mice lacking NFATc2 displayed enhanced bone mass due to stimulated osteoblastic function and suppressed osteoclast formation. The data also showed that NFATc2 is critical in the maintenance of hematopoiesis in adult organisms. Thus, NFATc2 is a central player in the intimate relationship of hematopoiesis and bone homeostasis within the bone/bone marrow microenvironment.

Bone marrow stromal cells are known to support hematopoiesis. One interesting study addressed the question of whether circulating osteoblast precursors are equally capable of supporting HSCs (10). That investigation found that human peripheral blood pre-osteoblastic cells have the capacity to support and maintain hematopoiesis in mice and are as potent as bone marrow stromal cells.

Another fascinating study by Mehrotra *et al*. examined the capacity of HSCs to trans-differentiate into osteoblastic cells and contribute to fracture repair (11). Remodeling of skeletal bone requires the recruitment and proliferation of stem cells

with the capacity to differentiate into functional osteoblasts that deposit and mineralize extracellular bone matrix. Given the close association of bone and bone marrow, it has been suggested that bone marrow may serve as a source of these progenitors. To test the ability of HSCs to give rise to osteo-chondrogenic cells, these investigators used a single HSC transplantation model in conjunction with a tibial fracture model. The data demonstrate that HSCs can differentiate into hypertrophic chondrocytes, osteoblasts and osteocytes and contribute to fracture healing. Together, these findings strongly support the concept that HSCs can generate bone cells and suggest the therapeutic potential of HSCs in fracture repair.

Similar conclusions were reached by a study by Meng *et al*. (12). In this work the investigators transduced CD34+ cells with a virus encoding various reprogramming factors. The transduced cell expanded for more than 1 month. *In vitro* studies showed that the transduced cells can be induced to differentiate into osteoblasts, adipocytes and chondrocytes when cultured in corresponding differentiation media for 3 weeks. In short, the study described an effective approach to transdifferentiate HSCs into mesenchymal cells.

Finally, a critical inducer of hematopoietic tissue development during embryogenesis is bone morphogenetic protein 4 (BMP4). However, evaluating the importance of BMP4 in hematopoiesis is complicated by early embryonic lethality in mice lacking BMP4. To define the role of BMP4 in bone marrow hematopoiesis, Tsuji *et al*. established BMP4 conditional knockout mice with inactivated BMP4 in adult bone marrow (13). They found that the loss of BMP4 in bone marrow cells significantly reduced the HSC population.

The Role of Immune Cells in the Bone Effects of PTH, Estrogen Deficiency, and HIV

A role for T cells in the effects of PTH on bone was first suggested by Hory *et al*. (14), who reported that transplantation of human parathyroid tissue into nude mice failed to stimulate bone resorption. Subsequent studies by Pettway *et al*. (15) suggested that T cells play a role in the bone-anabolic response to PTH. More recent studies have shown that intermittent PTH treatment induces a blunted anabolic response in the trabecular bone of T cell-deficient mice. However it is unknown whether direct activation of PTHR1 in T cells is required for intermittent PTH (iPTH) to induce Wnt10b production and bone growth. To investigate this matter, Bedi *et al*. generated a strain of mice with a silent PTHR1 in T cells and treated these animals with iPTH for 4 weeks (16). The anabolic activity of PTH was severely blunted in mice lacking PTHR1 signaling in T cells due to the failure of PTH to stimulate Wnt10b production by T cells. The data thus demonstrate that PTHR1 signaling in T cells plays an essential role in the anabolic activity of iPTH by promoting T cell production of Wnt10b, a factor required for iPTH to stimulate osteoblastogenesis, bone formation and bone accretion.

Postmenopausal osteoporosis may be an inflammatory disorder mediated by T cells. Since IL-17 is produced by Th17 and NK cells, DeSelm *et al*. asked if IL-17 participates in its pathogenesis (17). IL-17 promotes osteoclastogenesis by stimulating RANKL expression by osteoblastic cells. The authors found that deletion of the principal IL-17 receptor (IL-17RA) protects mice from ovariectomy (ovx)-induced bone loss. Further supporting a central role of IL-17 in its pathogenesis, ovx-induced osteoporosis is prevented by a blocking antibody targeting the cytokine. The source of IL-17 is likely IL-17-expressing NK1.1 cells as they increase 10-fold within the marrow following ovx. NK cell-produced IL-17 and its effector molecules are candidate therapeutic targets in postmenopausal osteoporosis.

Bone loss is common in conditions associated with immune activation but also in immunodeficiency, including HIV/AIDS. However, contrary to expectation, anti-retroviral therapy (ART) exacerbates rather than ameliorates bone loss. Ofotokum *et al*. examined bone resorption at early time

points following ART initiation (18). They found a spike in bone resorption peaking at 12 weeks. Interestingly, T cell recovery with ART is established to reach a significant magnitude at 12 weeks, the same time point at which the surge in resorption peaked. As lymphocytes are known to mediate bone loss in inflammatory contexts by secreting RANKL and/or TNF, this suggested a possible link between immune reconstitution and bone resorption. To investigate the role of immune recovery the investigators mimicked ART-induced immune reconstitution *in vivo* by means of T cell adoptive transfer into T cell null *TCRβ*(-/-) mice. T cell reconstitution initiated a surge in bone resorption concurrent with pronounced bone loss within the same 12-week window. As in humans on ART, RANKL and TNF were significantly elevated in T cell-reconstituted mice. Taken together, these data suggest that the majority of bone loss associated with ART may occur in the early period of therapy as a consequence of immune reconstitution.

Osteocytes and the Immune System

Some skeletal actions of PTH might be mediated by direct effects of the hormone on osteocytes. One study was conducted using mice that lack PTHR1 in osteocytes (19). The removal of PTHR1 only from osteocytes led to a site-specific reduction in the RANKL/OPG ratio, resulting in increased cancellous but not cortical bone mass. These and other data presented in the study indicate that the actions of PTHR1 in osteocytes are required to maintain basal levels of Wnt signaling and RANKL, and that the primary effect of the absence of the receptor in osteocytes is a reduction in osteoclast activity in cancellous bone.

Osteocytes are thought to control the response of bone to changes in mechanical forces. Indeed, the bone loss due to mechanical unloading has been attributed to both increased bone resorption and decreased bone formation. However, the mechanism by which osteocytes may mediate these responses remains unclear. In one study this issue was investigated using conditional knockout mice lacking osteocytic production of RANKL (20). The data showed that osteocytes produce RANKL and that this production increases in response to mechanical unloading. Moreover, the increased RANKL production by osteocytes is a major contributor to the bone resorption and bone loss associated with mechanical unloading.

Immunomodulators and Growth Factors

BMP2 action in osteoblasts plays fundamental roles in both periosteal and trabecular function. Studies by Yang *et al*. conducted in mice lacking BMP2 production by early osteoblasts revealed that these mice have progressive osteopenia that mimics bone aging and a 50% to 70% reduction in blood vessels associated with the periosteum and the trabeculae due to impaired vascular endothelial growth factor A (VEGFA) production (21).

IL-10 may down-regulate osteoclastogenesis through inhibition of the expression of NFATc1, c-Fos and c-Jun and inhibition of calcium signaling downstream of RANK by inhibiting transcription of triggering receptor expressed on myeloid cells 2 (TREM-2). However, there has been no study of the role of IL-10 in osteoclastogenesis *in vivo* and under inflammatory conditions. At this year's meeting, studies in IL-10 knockout mice showed that IL-10 inhibits osteoclast formation under physiological and pathological conditions and suggest a model where downregulation of IL-10 contributes to RANKL-mediated osteoclastogenesis (22).

MSCs are known to possess anti-inflammatory and immuno-modulatory functions. Takano *et al*. examined the regulatory function of MSCs for the development of inflammation and bone destruction in rats with adjuvant-induced arthritis (23). Their data show that MSCs significantly suppressed inflammation and bone destruction in this model.

Endodontic infections occur as a consequence of polymicrobial infection of the dental pulp and root canal system, ultimately resulting in destruction of

periapical tissues including the periodontal ligament, cementum and bone surrounding the tooth apex. However, the interplay between cells and cytokines involved in the destructive processes associated with inflammatory periapical bone loss has not been fully elucidated. A study by da Silva *et al*. showed that inflammatory periapical bone resorption is correlated with osteoclast and inflammatory cell numbers, and the severity of the lesions was directly correlated with levels of IL-1α, IFN-γ, IL-10, IL-17, MIP-1α, and cathepsin K, with the RANK/RANKL/OPG pathway, but not with TNF-α (24).

Dual specificity phosphatase 1 (DUSP1) dephosphorylates and inactivates p38 mitogen-activated protein kinase (MAPK). DUSP1 is upregulated by various proinflammatory stimuli and is essential for constraining p38 MAPK signaling and expression of p38-dependent genes such as TNF-α. p38 MAPK plays a central role in both differentiation and activation of osteoclasts. The aim of a study by Vattakuzhi *et al*. was to investigate whether DUSP1 may function as a negative regulator of inflammatory osteolysis (25). Data obtained by using DUSP knockout mice showed that DUSP1 negatively regulates osteoclast activation, and that variations in its expression or function may play a role in inflammatory osteolysis.

The Nod Leucine-rich Repeat with a Pyrin domain 3 (NLRP3) inflammasome is an intracellular protein complex responsible for the maturation of IL-1 into its active form, and for proteolytic inactivation of poly(ADP-ribose) polymerase 1 (PARP1), a negative regulator of osteoclast development. NLRP3 is involved in low-grade aseptic inflammation in metabolic diseases. NLRP3 senses cues triggered by crystalline particulates or degradation products of endogenous extracellular matrix. Since mineral and organic bone degradation products can traffic through bone-resorbing osteoclasts, Bonar *et al*. investigated the role of NLRP3 *in vitro* and *in vivo* (26). They found that NLRP3 activation by bone-relevant danger associated molecular patterns (DAMPs) stimulates cytokine maturation, while constitutive NLRP3 activation increases bone resorption, resulting in very low bone mass. Thus, NLRP3 is a major regulator of bone resorption, perhaps mediating pro-inflammatory signals originating from the bone matrix during normal bone remodeling.

Finally, TNF-α plays a key role in the pathogenesis of inflammatory osteoclastogenesis and bone resorption. Mechanisms that regulate the direct osteoclastogenic properties of TNFα to limit pathological inflammatory bone resorption are mostly unknown. Zhao *et al*. found that deletion of RBP-J, the master transcription factor in Notch signaling, in bone marrow macrophages resulted in dramatic induction of osteoclastogenesis by TNF-α in the absence of exogenous RANKL (27). RBP-J suppressed NFATc1 induction by attenuating c-Fos activation and suppressing induction of Blimp1, thereby preventing downregulation of the transcriptional repressor IRF-8 that blocks osteoclast differentiation. These findings identify a key role for Notch-RBP-J signaling in restraining inflammatory TNF-α-induced osteoclastogenesis and bone resorption and provide mechanisms by which RBP-J suppresses NFATc1 induction. Notch-RBP-J signaling plays a more prominent role in inhibiting osteoclastogenesis in inflammatory settings than under physiological conditions.

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