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MEETING REPORTS

Osteoimmunology: Meeting Report from the 32nd 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 (1). This definition can be broad or narrow. While many osteoimmunologists focus on RANKL/RANK signaling pathways in osteoclast precursors of the monocytic lineage, this report will present a sampling of the broader aspect of the role of immune cells and immune mechanisms relevant for bone diseases. A number of interesting new directions and developments in osteoimmunology were presented at the 32nd Annual Meeting of the American Society for Bone and Mineral Research in Toronto.

Hemopoietic Stem Cells

Hemopoietic stem cells (HSCs) are regulated by specialized non-hemopoietic 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 PTH increases the number of HSCs localized in close proximity with endosteal surfaces (2). Attesting to the pivotal role of direct PTH signaling in osteoblasts in the regulation of HSCs, transgenic mice with constitutive PTH receptor signaling in osteoblasts were found to have an expanded HSC niche (2). Moreover, osteoblasts from wild type (WT) mice treated with intermittent PTH produced high levels of the Notch ligand Jagged1, and supported an increase in the number of HSCs, with evidence of Notch1 activation in HSCs *in vivo* (2). These studies indicate that osteoblasts represent a regulatory component of the bone marrow (BM) microenvironment and exert an effect on HSCs through Notch signaling. Recently it has been shown that PTH fails to expand HSCs in *IL-6*(-/-) mice (3). This suggests that IL-6, a cytokine produced by PTH-stimulated osteoblasts, contributes to expanding HSCs, primarily by decreasing HSC apoptosis (3;4). Several studies were presented at this year's meeting that enriched our knowledge about the interaction of bone cells and bone-regulating factors with HSCs.

Gsα is a G protein subunit that mediates cyclic-AMP-dependent signaling downstream of G protein-coupled receptors, including the PTH/PTH-related peptide receptor PPR. Since PPR signaling in osteoblasts expands HSCs, Gsα may have a role in the regulation of the HSC niche. One study showed that Gsα knockout (KO) mice had 37% fewer long term HSCs, corresponding to the most primitive and quiescent hematopoietic population (5). The data showed that this effect was due to impaired Gsα signaling in cells of the osteoblast lineage, which results in impaired IL-7 production.

Sclerostin is a negative regulator of bone growth, secreted by osteocytes, that reduces osteoblast proliferation by inhibiting WNT signaling through binding to LRP receptors (6-8). Sclerostin is encoded by the *SOST* gene. One study showed that *SOST* is also expressed in HSCs, B cells, and granulocytes (9). Accordingly, lymphoid and myeloid differentiation were altered in the absence of *SOST*, though the number of HSCs was not affected.

A dramatic consequence of estrogen deficiency is an increase in the number of BM hemopoietic cells that is secondary to an

expansion of hemopoietic precursors such as multipotent CFUs (CFU-GEMMs), burst-forming units-erythroid (BFU-Es), CFU-GMs, and pro-B lymphocytes (10-12). Conversely, estrogen treatment decreases the pool of early hemopoietic precursors, including HSCs, via estrogen receptor (ER)-α-dependent mechanisms (10;13;14). Research presented at this year's meeting showed that the T cell costimulatory molecule CD40 Ligand (CD40L) plays a pivotal role in this process (15). Ovariectomy (ovx) was shown to induce a 2-3-fold increase in the number of HSCs, B cells, and monocytes in T cell-replete controls but not in T cell-deficient mice. T cells induced the expansion of HSCs by upregulating the expression of Jagged1 on bone marrow stromal cells (BMSCs). The relevance of CD40L was demonstrated by the failure of ovx to increase BMSC expression of Jagged1 and the number of HSCs and mature hemopoietic cells in *CD40L*(-/-) mice.

PTH and T Cells

A role for T cells in effects of PTH in bone was first suggested by Hory *et al*. (16), who reported that transplantation of human parathyroid tissue into nude mice failed to stimulate bone resorption. Subsequent studies by Pettway *et al*. (17) 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. In contrast, T cell-deficient mice and T cell-replete mice have an identical anabolic response to intermittent PTH in the cortical compartment. These observations were confirmed in a study showing that PTH improved the healing of a cortical bone fracture in nude rats, a strain with partial T cell deficiency (18). Some anabolic activity was also observed in trabecular bone; however, the lack of a WT control group prevented the investigators from determining whether PTH has full or blunted activity.

Studies have also shown that T cells are required for continuous PTH treatment to induce cortical and trabecular bone loss. However, it is unknown whether direct PTH stimulation of T cells is required for PTH to induce bone loss. To address this issue, in one study the PPR in T cells was conditionally deleted (19). This research showed that silencing of the T cell PPR blocked the capacity of PTH to induce T cell TNF production. As a result, KO mice were protected against the bone-catabolic effect of *in vivo* PTH treatment. Bone loss was also blocked by specific silencing of T cell TNF production.

Cytokines and Growth Factors

PTH acts via osteoblasts to stimulate both osteoclast formation and bone formation (20). The influence of PTH on osteoclast formation is partially dependent on cytokines that act by binding to the gp130 signal transduction unit (*e.g.*, IL-6, IL-11, oncostatin M (OSM)). Since these cytokines stimulate osteoclast and osteoblast differentiation they may also mediate anabolic effects of PTH. A study of the role of the OSM receptor found that, despite the known pro-osteoclastic and pro-RANKL influence of OSM, OSM receptor signaling suppressed the action of PTH that enhances RANKL expression in osteoblasts and stimulates osteoclast formation, indicating significant crosstalk between these pathways (21).

TGF-β1 has been shown to recruit BMSCs to bone-resorptive sites in response to osteoclastic bone resorption for coupled bone formation. However, the factor that is responsible for the differentiation of BMSCs into osteoblasts during their recruitment remains unknown. Using osteoprogenitor-specific IGF-I receptor (IGF-IR)-deficient mice, it was shown that IGF-I released during bone resorption stimulates osteoblast differentiation of BMSCs at bone resorptive sites recruited by TGF-β1 (22).

Clinicians have long sought to use combined treatment with antiresorptive agents and anabolic agents to treat severe osteoporosis. Most attempts have combined treatment with alendronate and teriparatide. Unfortunately, a number of studies have revealed that the anabolic effects of teriparatide or PTH on bone formation are impaired by concurrent use of antiresorptive drugs. The mechanism for this phenomenon

remains unknown. One investigation presented at the meeting showed that osteoblast number was decreased in mice with concurrent treatment with PTH and alendronate (as compared to treatment with a single drug) due to the interruption of BMSC recruitment (23). Further studies revealed that inhibition of active TGF-β1 release by alendronate reduces the recruitment of BMSCs to bone sites and impairs PTH anabolic action in bone.

M-CSF is absolutely required for osteoclastogenesis, and its genetic absence leads to osteopetrosis due to a failure of osteoclast formation. There are two isoforms of M-CSF, soluble and membrane-bound, but their individual biological functions are unclear. Thus it was revealed that membrane-bound M-CSF is essential for normal bone remodeling since, in its absence, bone density is increased (24). Data also showed that the anabolic response to PTH is augmented in mice lacking membrane-bound M-CSF, perhaps because of a reduced resorptive response to this treatment.

Immune Modulators and Inflammation

MHC Class II TransActivator (CIITA) is a master switch for MHC Class II expression and antigen presentation in antigen-presenting cells (APCs) that has recently been found to be expressed in osteoclast precursors. The role of CIITA in regulating osteoclast differentiation and activity was investigated using transgenic mice lines that overexpress CIITA (25). CIITA-transgenic mice displayed a dramatic decrease in trabecular structure, a consequence of significantly elevated osteoclast formation and activity. CIITA-transgenic mice also displayed a global increased activation of the signaling pathways downstream of RANK, indicating the common upstream adapter TRAF6 as a potential target of CIITA. *In vivo* experiments revealed profound suppressive effects of estrogen on chromatin remodeling at the CIITA locus. These data suggest that CIITA regulates osteoclast differentiation and bone homeostasis, and is controlled by estrogen *in vivo*.

The role of Notch signaling in pathologic inflammatory bone resorption is not known. Thus one study examined the role of Notch signaling in osteoclastogenesis and bone resorption under inflammatory conditions (26). The authors found that deletion of RBP-J, the master transcription factor in Notch signaling, resulted in a dramatic increase of TNF-induced osteoclastogenesis. These results show that RBP-J negatively regulates TNF-induced osteoclastogenesis by suppressing induction of NFATc1. These findings identify a key role for the Notch component RBP-J in restraining inflammatory TNF-induced osteoclastogenesis.

Finally, the gut is inhabited by a microbial ecosystem, the gut microbiota, which consists of 10 times as many cells as our own eukaryotic cells. The possible impact of gut microbiota on bone metabolism is unknown. An interesting study evaluated the skeletal phenotype of germ-free mice and conventionally-raised mice (27). Germ-free mice had 49% higher bone volume and higher cortical thickness compared to controls, and also had decreased serum serotonin levels compared to controls. The absence of gut microbiota leads to increased bone mass associated with reduced serum serotonin. Gut microbiota may modulate gut serotonin synthesis and thereby via an endocrine mechanism also bone metabolism.

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