2006 IOF+ANZBMS Combined Meeting

Combined Meeting of the
3rd IOF Asia-Pacific Regional Conference on Osteoporosis *and the*
16th Annual Meeting of the ANZ Bone & Mineral Society
~ 22-26 October 2006, Port Douglas, Australia ~
www.anzbms.org.au/asm/asm2006

SESSION TIME: 0810 – 0950, Monday 23 Oct 2006

Invited Plenary Abstracts

Plenary Lectures 1 - Cellular Symphony of Osteoclastogenesis

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T. Jack Martin (Australia)

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P3 **Bone remodelling and the roles of nuclear receptor**
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Nobuyuki Udagawa (Japan)

P1

Osteoblast and osteoclasts: local communication through several mechanisms

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Cells of the osteoblast lineage regulate osteoclast formation from hemopoietic precursors through contact dependent mechanisms that are controlled by hormones and by the production of locally generated inhibitors. The key effectors are osteoblast-derived RANKL signalling through its receptor, RANK, on haemopoietic cells, and OPG as the decoy receptor that inhibits the process. Local signalling that results in bone formation during remodelling takes place in several ways. Growth factors released from resorbed bone matrix can contribute to preosteoblast differentiation and bone formation. The preosteoblasts themselves, growing in the resorption space, can communicate through cell contact and paracrine signalling mechanisms to differentiate. Osteocytes can sense the need for bone repair by detecting damage and pressure changes, and signalling to surface cells to respond appropriately. Now that it has been shown through mouse genetics that PTHrP generated locally in bone is a crucial physiological regulator of bone formation, and probably also of resorption, we need to understand how local PTHrP release is controlled in bone in the remodelling process. Finally the observation that concurrent treatment with bisphosphonates impairs the anabolic response to PTH, adds to other clues that osteoclast activity is necessary to complement the direct effect that PTH has in promoting differentiation of committed osteoblast precursors. This might involve the generation of a coupling factor from osteoclasts that are transiently activated by RANKL in response to PTH. Both human and mouse genetics provide evidence supporting the view that osteoclasts, despite in some circumstances being unable to resorb bone, e.g. failure of acidification, can nevertheless be associated with normal, or even increased bone formation. An implication is that it may be possible to design resorption inhibitors that do not block PTH anabolic action when given simultaneously.

It is proposed that transiently activated osteoclasts can contribute to the coupling of bone formation to resorption by producing activity that influences preosteoblast participation in bone formation.

Bone formation results from a complex cascade of events that involves proliferation of primitive mesenchymal cells, differentiation into osteoblast precursor cells (osteoprogenitor, preosteoblast), maturation of osteoblasts, formation of matrix, and finally mineralization. It is highly likely that in some forms of osteoporosis, deficient bone formation results from impaired osteoblast replenishment from precursors, and even from a deficiency of progenitors. Recent studies of control of osteoblast differentiation have provided

valuable new insights, including identification of the roles of several key transcription factors in osteoblast differentiation.

The only proven anabolic therapy for bone is PTH. The anabolic effect of PTH is dependent upon intermittent administration, but when an elevated PTH level is maintained even for a few hours it initiates processes leading to new osteoclast formation, and the consequent resorption over-rides the effects of activating genes that direct bone formation.

New approaches to anabolic therapies may come from the discovery that an activating mutation in the LRP5 gene is responsible for an inherited high bone mass syndrome, and the fact that this can be recapitulated in transgenic mice, whereas inactivating mutations result in severe bone loss. This has focused attention on the Wnt/frizzled/βcatenin pathway as an important one in bone formation, and proof of concept has been obtained in experimental models.

P2

Immune mechanisms in osteoclastogenesis

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Signaling through receptor activator of NF-κB ligand (RANKL) induces osteoclast differentiation in the presence of M-CSF. To explore the molecular mechanism of osteoclast differentiation, we performed a genome-wide screening of genes induced by RANKL. We identified that the transcription factor nuclear factor of activated T cells c1 (NFATc1) is specifically induced by RANKL and it plays a central role in RANKL-mediated osteoclast differentiation.

Induction and activation of NFATc1 is regulated by calcium-dependent phosphatase calcineurin. Immunoreceptor tyrosine-based activation motif (ITAM) signaling mediated by dual membrane adaptors, Fc receptor (FcR) common γ subunit (FcRγ) and DNAX activating protein (DAP12) is essential for RANKL induction of osteoclast differentiation. FcRγ and DAP12 associate with multiple immunoreceptors such as OSCAR and TREM-2 and activate calcium signals leading to the induction of NFATc1. The importance of ITAM-mediated signaling in the skeletal system is underscored by the observation that the combined deficiency of FcRγ and DAP12 results in severe osteopetrosis due to impaired osteoclast differentiation. RANKL-induced osteoclast differentiation is finely regulated through costimulatory signals provided by multiple immunoreceptors. Thus, RANKL and M-CSF are not sufficient to activate the signals required for osteoclast differentiation. Recent advances in the understanding of osteoclastogenic signal transduction will also be discussed in the context of osteoimmunology.

References:
Dev Cell 3, 889; 2002, Nature 428, 758, 2004; J Exp Med 202, 1261, 2005

P3

Bone remodelling and the roles of nuclear receptor

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Nuclear receptors (NRs) are ligand-dependent transcription factors, and form a gene superfamily. Their fat soluble ligands like steroid hormones and vitamin D are well known to support bone formation and bone remodeling through gene regulations mediated through NRs. However, the physiological roles of NRs in bone tissues still remain unclear.

To investigate the physiological roles of VDR, we had generated VDR deficient mice by a conventional gene targeting, and found that $VDR^{-/-}$ knockout (VDRKO) mice showed features typical of vitamin D-dependent type II rickets like bone loss by impaired mineralization (Yoshizawa T. et al., *Nat. Genet.*, 16, 391, 1997).

Likewise, the male-specific significance of AR in bone remodeling has been demonstrated by ARKO mice (Kawano H. *et al.*, *PNAS*, 100, 9416, 2003).

However, primary target cells of such NR functions in bones have not yet been defined. To directly examine physiological impact of AR in osteoclasts, we used a Cre/*lox*P system to disrupt AR gene in osteoclasts. The osteoclast-specific ARKO (Oc-ARKO) mice had normal appearance, but exhibited clear loss of bone mass with enhanced bone remodeling. Osteoblast-specific VDRKO (Ob-VDRKO) mice unexpectedly exhibited clear increases in bone mass as well as bone mineral density with less bone remodeling. Thus, the present study reveals the physiological impact of AR and VDR function in bone.

P4

Mesenchymal/haemopoietic interactions in osteoclastogenesis

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Osteoclasts, the multinucleated cells that resorb bone, originate from monocyte/macrophage lineage haemopoietic cells. Mesenchymal osteoblastic cells or bone marrow stromal cells are involved in osteoclast differentiation. Osteoclast precursors express RANK (a receptor of RANKL), recognize RANKL expressed by osteoblasts through cell-cell interaction and differentiate into osteoclasts in the presence of M-CSF. OPG, produced mainly by osteoblasts, is a soluble decoy receptor for RANKL. Deficiency of OPG in mice induces osteoporosis caused enhanced bone resorption. Elevated osteoblastic activity was suppressed by bisphosphonate administration in OPG-deficient mice. These results suggest that bone formation is accurately coupled with bone resorption. Collagen sponge disks containing BMP-2 were implanted into the dorsal muscle pouches in OPG-deficient mice. TRAP-positive osteoclasts and ALP-positive osteoblasts were observed in BMP-2-disks preceding the onset of calcification for one week. F4/80-positive osteoclast precursors were similarly distributed in both BMP-2- and control disks. OPG and soluble RANK inhibited BMP-2-induced osteoclast formation but not the appearance of ALP-positive cells in OPG-deficient mice. A small number of osteoclasts were observed in RANKL-containing disks in the absence of BMP-2 in the OPG-deficient mice. We then examined how osteoblasts are involved in osteoclastogenesis other than RANKL expression, using RANKL-deficient mice. RANKL-deficient mice showed severe osteopetrosis due to loss of osteoclasts. Injection of RANKL into RANKL-deficient mice induced many osteoclasts in bone but not soft tissues. Most of TRAP-positive osteoclasts localized in contact with ALP-positive cells in BMP-2-disks in RANKL-deficient mice injected with RANKL. These results suggest that mesenchymal osteoblasts determine the place of osteoclastogenesis from haemopoietic stem cells in bone.