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SESSION TIME: 1500 - 1630, Tuesday 24 Oct 2006

Workshop Abstracts
Workshop B - Inter and intracellular signalling

W4 **Prostaglandins in cross talk in bone**
Lawrence Raisz (USA)

W5 **Signals in life and death of the osteoclast**
Sakae Tanaka (Japan)

W6 **What makes the osteoclast tick?**
Geoff Nicholson (Australia)

W4

Prostaglandins in cross talk in bone

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Prostaglandins (PGs), particularly prostaglandin E_2 (PGE_2), are potent multifunctional regulators of many cellular functions in bone. PGE_2 has been shown to stimulate bone resorption and formation, but can also have inhibitory effects. Most of the hormones, cytokines and growth factors that influence bone cell function also affect PG production in the osteoblasts, and some affect production in cells of the osteoclast lineage. A number of potential amplification loops have been identified. Bone morphogenetic protein-2 (BMP-2), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF) and Interleukins-1 and 6 have been shown to activate the inducible cyclooxygenase (COX-2) and increase PG production and may also be stimulated by PGs themselves. PGs are local regulators, but it is not clear whether they act largely by paracrine or intracrine pathways. The intracellular location of the synthetic enzymes and some receptors support the latter possibility. PGE_2 can affect osteoclast production and function directly, independent of any effects on osteoblasts. An inhibitory effect on osteoclast function could be important in mediating the effect of high calcium concentration, which also induces COX-2, on osteoclast activity. Studies using transgenic mice with deletions of COX-2 or specific PGE_2 receptors as well as studies using selective PG receptor agonists have helped to analyze the multiple and complex effects of prostaglandins on bone cells, but much remains to be learned concerning their specific roles in human physiology and pathology.

W5

Signals in life and death of the osteoclast

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The homeostasis of the skeletal tissues is maintained by a well organized regulation of bone formation and bone resorption. Osteoclasts are terminally differentiated cells primarily involved in bone resorption. The life span of osteoclasts is relatively short both in vitro and in vivo, and once differentiated, they rapidly die in the absence of supporting cells such as osteoblasts or bone marrow stromal cells, or growth factors such as

interleukin (IL)-1, receptor activator of NF-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF). Anti-resorptive drugs such as estrogen, raloxifene and bisphosphonates are known to reduce the life span of osteoclasts. Recent studies have revealed that the rapid cell death of osteoclasts is caused by apoptosis. Apoptosis is a form of programmed cell death that is characterized by specific morphological and biochemical properties. Depolarization of mitochondrial transmembrane potential, chromatin condensation and cytochrome *c* release from mitochondria into cytoplasm were observed in the apoptotic osteoclasts, implying that cytokine deprivation triggers the osteoclast apoptosis through the mitochondrial pathway. We previously reported that a proapoptotic Bcl-2 family member Bim plays an essential role in the osteoclast apoptosis. In this workshop, I will show the mechanism of posttranslational regulation of Bim in more detail, and also focus on the role of other Bcl-2 family members on the apoptosis of osteoclasts and their bone-resorbing activity.

W6

What makes the osteoclast tick?

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The current explosion in knowledge of osteoclast biology mostly relates to their differentiation and we continue to have a relatively poor understanding of how mature osteoclasts are regulated. Twenty years ago the factors known or suspected to act directly on mature osteoclasts were limited to calcitonin (and other stimulators of cAMP), M-CSF, $\alpha v\beta 3$ ligands and an unknown factor expressed by osteoblasts/stromal cell (now identified as RANKL). This list now includes Ca^{++}, H^{+}, ROS, IL-1, TNFα, glucocorticoids, cannabinoids, TSH, and others. Although no convincing evidence exists that gonadal steroids act directly on osteoclasts, FSH does do so to activate resorption.

Traditional osteoclast isolation methods invariably resulted in contamination with osteoblasts/ stromal cells, lymphocytes etc, so direct effects on osteoclast function could not be determined in the absence of indirect effects. Although we are now able to generate relatively pure osteoclast cultures by treating haemopoietic precursors with RANKL and M-CSF, considerable overlap exists between the various processes so that it is often not possible to separate effects on differentiation from those on mature osteoclast resorption and survival. However, by generating osteoclasts en mass in plastic flasks then dissociating and transferring the cells to dentine/bone substrate, it is now possible to investigate direct effects of various factors on osteoclast resorption and survival.

In this model, we have used CFU-GM-derived human osteoclasts and find that they survive for many days in media containing FBS in the absence of RANKL, M-CSF or IL-1. However, the presence of RANKL is absolutely required for resorption. Lower concentrations of M-CSF (25 ng/mL or lower) act synergistically with RANKL to enhance osteoclast activation and resorption, associated with increased activation of AP-1 and NFκB. This suggests the existence of cross-talk between RANK and fms signaling pathways, possibly mediated by Map kinases or src-TRAF6 interaction. The pathways mediating this interaction and other recent findings will be discussed in the workshop.