

## Session Summary: IBMS Sun Valley Workshop: Musculoskeletal Biology

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### Osteoimmunology and Bone Marrow

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Osteoclasts are derived from hematopoietic stem cells in the bone marrow, where they give rise to lineage-negative multi-potent progenitor cells. Under the control of specific transcription factors, multi-potent progenitor cells differentiate to committed lineage-positive myeloid and lymphoid precursor cells. Transcription factors essential for osteoclast differentiation from myeloid precursors have been well-established, but the role of B cell transcription factors in this process has not been well-studied. Pax5, a B cell transcription factor, is expressed in the B lymphocyte lineage except in terminally differentiated plasma cells. *Pax5(-/-)* mice have significantly decreased trabecular bone volume with markedly increased osteoclast numbers on the bone surface. *Pax5(-/-)* spleen cells, in the absence of any cytokines, spontaneously develop into cell lines that have the capacity to differentiate into osteoclasts, dendritic cells and macrophages under appropriate culture conditions. FACS analysis, using a panel of cell surface markers, indicates that these *Pax5(-/-)* cells have characteristics of myeloid cells. Conditioned medium from *Pax5(-/-)* cells supports the proliferation of wild type bone marrow cells. In contrast to remarkably increased osteoclast formation, the number of osteoblasts in *Pax5(-/-)* mice is only slightly reduced. Thus the severe bone loss in *Pax5(-/-)* mice is likely due to the increase in osteoclastogenesis. These findings suggest that depletion of Pax5 switches lymphoid progenitors into myeloid progenitors, perhaps due to production of soluble inhibitory cytokines. Thus Pax5 determines the fate of lymphoid progenitors and regulates B cell lineage development.

Osteoimmunology is based primarily on two lines of experimental evidence. One is that activated T cells and other immune cells produce RANKL to stimulate osteoclast formation through the canonical RANKL/RANK/NF- $\kappa$ B/NFAT signaling pathway. Another is involvement of the immunoreceptor tyrosine-based activation motif (ITAM) signaling pathway mediated by the adaptor protein DAP12 or FcR $\gamma$  in osteoclasts and precursors. Upon activation, the tyrosines in ITAM are phosphorylated by members of the Src kinase family. The tyrosine kinase Syk is then recruited to initiate a signal transduction cascade, which includes phospholipase C $\gamma$ , calcium mobilization, and NFAT. Under physiologic conditions, mice that are deficient in a single adapter protein, either DAP12 or FcR $\gamma$ , have moderate or mild osteopetrosis, while DAP12/FcR $\gamma$  double knockout mice develop severe osteopetrosis. However, all ITAM adapter-deficient mice including single or double knockout mice show resistance to ovariectomy-induced bone loss. These data indicate that ITAM signaling mediated by these adapter protein complexes can compensate partially for each other under physiologic conditions but not under pathologic conditions. Specific ligands that activate these immunoreceptor signals have not been identified. It is also unknown whether ligands in the bone microenvironment are subject to change in different physiological conditions, which will be a major focus of future study.

Blood vascular endothelium cells affect osteoclast formation by producing RANKL, M-CSF, and VEGF. Osteoclasts influence tumor angiogenesis by producing angiogenic growth factors such as osteopontin. Whether or not osteoclasts affect vascular formation in non-neoplastic diseases has not been studied. Micro-array analysis of purified osteoclast precursors from peripheral blood of TNF transgenic (TNF-Tg) mice, a mouse model of chronic inflammatory arthritis, demonstrates that osteoclast precursors from TNF-Tg mice express a significantly increased level of VEGF-C, a potent growth factor of lymphatic endothelium. Osteoclastogenic cytokines RANKL, TNF and IL-1 all stimulate VEGF-C production through the classic NF- $\kappa$ B pathway. The major function of the lymphatic system is to drain lymph from interstitial space but lymphatic drainage and its role in

bone disorders has not been studied before. A near-infrared lymphography is developed to assess the lymphatic flow from the footpad to draining popliteal lymph nodes (PLNs) in a mouse leg. The blockade of lymphangiogenesis in TNF-Tg arthritic mice with VEGFR3 receptor neutralizing antibody reduces the volume and lymphatic vessel area of draining PLNs, but it significantly increases the severity of synovitis and joint tissue damage. This is associated with reduced lymphatic drainage. Thus, the lymphatic system plays a beneficial role in the development of arthritis, perhaps by facilitating drainage of interstitial fluid and immune cells to local draining lymph nodes, thereby promoting the clearance of catabolic cytokines and inflammatory cells.

B cells participate in autoimmune pathogenesis through a variety of mechanisms, including the generation of auto-antibodies, cytokine secretion, antigen presentation, and regulation of other cells. B cell depletion has been used to treat rheumatoid arthritis (RA) patients, but the underlying mechanism is not clear. Contrast enhanced MRI of TNF-Tg arthritic mice reveals that concomitantly with the progression of synovitis from the ankle to the knee, draining PLNs become dramatically enlarged by an expansion of sinusoidal spaces. PLN enlargement is accompanied by an increase in total B cell numbers. Immuno-staining demonstrates that a large amount of B cells are localized within the lymphatic sinuses of PLNs of TNF-Tg mice with severe joint lesions. To examine if B cell depletion could reduce joint tissue damage, TNF-Tg mice are treated with anti-CD20 antibody for 6 weeks. Anti-CD20 treatment depletes more than 80% of B cells in PLNs and significantly reduces the progression of knee synovitis compared to placebo-treated animals. These findings lead to a model explaining the association of B cells, RA development and lymphatic drainage: during the progression phase of RA development, joint inflammation initiates enlargement of draining PLNs, with progressive recruitment and/or local accumulation of B cells. A specific subset of B cells is relocated from B cell follicles to lymphatic spaces, leading to the collapse of the node and obstruction of drainage. Depletion of B cells within lymphatic sinuses of PLNs could treat joint tissue damage by improving lymphatic drainage. To characterize this subset of B cells, multiple color flow cytometry analysis of PLNs from TNF-Tg and wild type mice indicates that TNF-Tg PLNs have an increased specific population of B cells, which are CD21<sup>hi</sup>, CD23<sup>+</sup>, IgM<sup>hi</sup>, IgD<sup>+</sup>, CD24<sup>hi</sup>, CD1d<sup>hi</sup>. This B cell population is restricted to PLNs and iliac nodes early in the disease, and expands dramatically as knee arthritis progresses, but does not involve other peripheral lymph nodes or the spleen. The role of this specific B cell population in RA pathogenesis and the lymphatic draining function is currently under investigation.

Bisphosphonates have been used to treat various disorders with bone implications. They are also used to prevent osteolysis around septic implants in osteomyelitis patients. The mechanisms responsible for the interactions between bisphosphonates and bone infection in osteonecrosis of the jaw are controversial. A murine model of implant-associated osteomyelitis is established. Animals with osteomyelitis are treated with alendronate and osteoprotegerin, gentamycin, etanercept (TNFR:Fc) and PBS. Infection, immunity, osteolysis, vascularity, and popliteal lymph node size are assessed using a combination of *in vivo* imaging, biochemistry and histology approaches. All treatments have no effect on humoral immunity, angiogenesis, or chronic infection. Alendronate and osteoprotegerin decrease osteoclast numbers, cortical bone osteolysis and the size of local draining lymph nodes, but they significantly increase the incidence of high-grade infections in early time points of osteomyelitis establishment. Micro-CT analysis of alendronate- and osteoprotegerin-treated mice shows that the bone void around infected implants is due to both osteoclastic resorption of cortical bone and inhibition of periosteal reactive bone formation. Thus while anti-resorptive agents do not exacerbate chronic osteomyelitis, they can increase bacterial growth during early infection. The mechanisms by which anti-resorptive agents increase bacterial load at the early stage of osteomyelitis is not clear. It may be related to decreased local lymphatic drainage, which prevents the removal of necrotic bone that harbors bacteria.

Overall, these new findings highlight the important impact of interaction among bone cells, immune cells and vascular endothelial cells within the bone marrow or other lymphoid organs

such as draining lymph nodes on physiologic and pathologic bone resorption. Identification of cellular and molecular pathways mediating these interactions will lead to the discovery of novel therapeutics against pathologic bone destruction.