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Perspective Article

Hylonome

Summary - Bone resorption session

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This session focused on mechanisms of bone resorption in both normal and pathologic states, in particular, in bone metastasis, myeloma, and in chronic inflammatory states such as rheumatoid arthritis. In addition, the results of the Toulouse Bed Rest Study on the effects of immobilization and subsequent ambulation on bone loss and recovery of bone density in young subjects were reported.

Dr. Roodman began the session with a discussion of the mechanisms of bone resorption in myeloma. Bone resorption is a local process in myeloma where osteoclastic bone destruction only occurs at areas adjacent to the tumor and does not normally occur at areas distant from the tumor. These data suggest that there is a local cytokine network induced by myeloma cells that is responsible for the bone destructive process. Several cytokines have been implicated in myeloma bone disease including RANKL, MIP-1α, and IL-6. All these factors can enhance osteoclast (OCL) formation and osteoclastic bone destruction. Osteoprotegerin (OPG) levels are markedly decreased in myeloma patients, further favoring bone resorption. An important feature of myeloma bone disease is that there is no osteoblastic response to the previous bone resorption so that patients develop purely lytic lesions and frequently have negative bone scans in the face of extensive bone disease.

The AML-1 class of transcription factors is abnormally produced by myeloma cells. In myeloma, AML-1A exceeds AML-B, which is the inverse of the normal situation, and this abnormal AML-1A to B ratio also drives expression of other genes potentially involved in myeloma bone disease including IL-3. IL-3 can stimulate OCL formation as well as myeloma cell growth. These data suggest that interacting cytokines (MIP-1α, RANKL, IL-3) are responsible for the increased bone destruction in myeloma. Several important questions were raised in this presentation: 1) Can osteoblasts be stimulated in myeloma to repair the bone lesions following stem cell transplant? Bone lesions in myeloma patients usually do not heal after stem cell transplantation. 2) Can antagonists to the MIP-1α receptors, CCR1 and CCR5, be useful therapeutic agents in myeloma? 3) Can manipulating the levels of the AML-1 class of transcription factors in myeloma have a profound effect on both the tumor burden and bone destruction? Since all of these factors appear to work in combination at low concentrations, blocking any one of them may be sufficient to block myeloma bone disease. Future studies should be directed to understanding the role of the individual OCL activating factors plays in myeloma and overcoming the osteoblast deficit.

Dr. Joseph Lorenzo discussed the characteristics of OCL precursors in mouse bone marrow. Two classes of precursors have been identified that are capable of forming OCL *in vitro*: CFU-GM derived cells and pre-B cells that express the B220+ antigen. The B220+ pre-B cells are capable of differentiating either to mature B cells or toward the myeloid lineage to form OCL. The OCL that formed from B220+ cells express calcitonin receptor and TRAP, and resorb calcified matrices. Following ovariectomy (OVX), the number of B220+ cells increase in the marrow and form increased numbers of OCL. B220- cells are unchanged in the marrow and do not contribute to the increased osteoclastogenesis following OVX.

The IL-1 receptor-1 knockout mouse does not lose bone following OVX. B220+ cells do not increase in IL-1 receptor knockout mice following OVX even though basal OCL numbers are increased. OVX also has little effect on RANK or c-fms expression in wild-type or IL-1 receptor-1 knockout mice. It was suggested that the B220+ OCL precursor is estrogen responsive and that estrogen preferentially suppresses OCL formation by these cells. Future studies will have to confirm this. In addition, whether the B220+ OCL precursor is really a cell in the B cell lineage or simply a multipotent progenitor cell that expresses B220 remains to be resolved.

IL-7 acts early in the OCL differentiation pathway to inhibit osteoclastogenesis. It has no effect on RANKL or OPG mRNA expression, but induces the B220+ OCL pre-

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cursors to differentiate into mature B cells rather than OCL. Thus, IL-7 appears to shunt B220+ OCL precursors away from the OCL lineage and thereby inhibit OCL formation.

Some questions that should be pursued about the OCL differentiation pathway are: how do GM-CSF and IL-7 compete for differentiation of OCL precursors? Why does estrogen deprivation preferentially induce B220+ cells to form OCL rather than CFU-GM derived cells, and are these B220+ OCL precursors truly cells in the B cell lineage?

Dr. John Chirgwin reviewed the "vicious cycle" that occurs in osteolytic bone metastasis, where bone-derived factors released during the bone resorption process, enhance the growth of tumor cells and further increase bone destruction. PTHrP is the primary mediator of the lytic process in breast, prostate and in lung cancer. Dr. Chirgwin also noted the studies of Dr. Zhang and co-workers (J. Clin Invest 2001; 107:1235-1244), showed that prostate cancer cells may enhance osteolysis by secreting soluble RANKL. He reviewed a recent study published in Cancer Cell 2003, by Kang et al., in which transfecting any four of five genes (IL-11, connective tissue growth factor, CXCR4, osteopontin, or MMP-1) into breast cancer cells, increased the capacity of human breast cancer cells that had a low potential to metastasize to bone, to induce osteolytic bone metastasis. These studies raised an interesting question of whether expression of these genes was acquired during the growth of the tumor or if minor clones expressing these factors were already present in breast cancer. The current data suggest that there already are cells within the primary tumor that overexpress these five genes and may eventually be responsible for the bone metastases. Furthermore, the requirement for expression of at least four of these genes to induce bone metastasis raises several interesting possibilities about the mechanism of bone metastasis.

Dr. Chirgwin discussed osteoblastic (OBL) bone metastasis and showed the utility of the neonatal calvarial assay to screen OBL stimulating factors. Studies show that endothelin-1 is a potent inducer of bone formation in these assays as well as *in vivo*, and that endothelin-1 is responsible for the osteoblastic metastases seen when the ZR75 breast cancer cell line is transplanted into nude mice. An important observation is that tumors that produce osteoblastic factors also enhanced bone resorption. The model Dr. Chirgwin proposed is that osteoblastic factors increased the number of immature osteoblasts present in the tumor microenvironment. These immature osteoblasts express high levels of RANKL, which stimulates bone resorption. Bone resorption then releases and activates growth factors from the bone matrix that further stimulate OCL formation and tumor cell growth. The implication is that osteoblastic factors induce osteoclastic bone resorption as part of their effects on the osteoblast. In support of this concept are the studies in patients with prostate cancer that have been treated with an endothelin receptor antagonist. These patients had decreases in markers of bone resorption even though endothelin-1 is an osteoblast stimulatory factor.

Dr. Paddy Ross examined the role of guanine-nucleotide exchange factors (GEFs) in OCL biology. He discussed the role of the actin cytoskeleton in osteoclastic bone resorption and noted that in contrast to other cells, OCL lack focal adhesions but have podosomes, which are unique to OCL. Podosomes contain Pyk2 rather than focal adhesion kinase. An interesting feature of podosomes was that av-b3 integrin does not co-localize with actin in the podosomes in contrast to focal adhesions, in which proteins co-localize with actin. Members of the GEF family, which catalyze GDP to GTP exchange, are present in OCL and include VAV-1, 2, and 3. These GEFs bind Rac and Rho. The VAVs are activated by Src or SYK kinase. The VAV-3 knockout mouse and the VAV-1 and 3 double knockout mouse have a bone phenotype which is similar to the Src knockout mice. OCL from these mice have decreased OCL spreading, a poorly formed actin ring and do not resorb bone. Integrin signaling is suppressed in OCL precursors from the VAV knockout mice. These data suggest that in OCL, cytokines can simulate formation of actin and the cytoskeleton, and that integrin signaling is important in VAV signaling.

Dr. Steve Goldring discussed inflammatory conditions associated with bone loss and noted that the pathophysiology underlying bone loss in rheumatoid arthritis (RA) is very similar to that seen in cancer (myeloma), with uncoupling of the remodeling sequence. The pannus that forms in the rheumatoid joint is key to the process. The OCL that form on the bone-pannus interface are TRAP positive, express calcitonin receptors, express cathepsin-K and have all the features of OCL. Cytokines and chemokines are produced by the pannus, which recruit OCL precursors to the site of inflammation. The pannus then produces factors such as RANKL, PTHrP and IL-17, which induce OCL formation. The important role of the OCL in the bone destructive process associated with RA was demonstrated by studies that showed that OPG inhibited bone erosion in models of rheumatoid arthritis. In support of this concept, RANKL knockout mice do not develop focal erosions in serum transfer models of arthritis. IL-1 and TNFα are important inducers of osteoblast apoptosis and may explain the decreased osteoblast response in rheumatoid arthritis. These studies suggest that therapeutic agents that are active in rheumatoid arthritis may also be active in treating bone metastases and myeloma. Agents that enhance bone formation in RA may also be active in patients with bone destruction from cancer.

Dr. Jörn Rittweger discussed the Toulouse immobilization study. In this study, three groups of subjects were studied. The control group, which was maintained at bed rest at -6^{o} head down tilt, the second group had the head down tilt but underwent exercise, and the third group had the head down tilt but received pamidronate. He reported that muscle calf cross-sectional area was decreased in all three groups, and that bone loss occurred in all three groups, but was decreased by 50% in groups 2 and 3 from 4% versus 2% over the 30-day study. There were large individual variations in bone density in the subjects. Interestingly, bone loss in dif-

ferent sites within the same tibia differed markedly and did not correlate. In contrast, bone loss between the left and right tibia were highly correlated. Bone loss continued even when the patients were allowed to ambulate. The rapid increase in bone mineral density that occurred after mobilization at the diaphysis most probably reflected mineralization rather than bone formation. These studies provide models for developing strategies for combatting bone loss during space travel and prolonged immobilization and bed rest.