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## *COMMENTARIES*

# Do Osteoclasts Have Dual Roles: Bone Resorption and Antigen Presentation?

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**Commentary on**: Li H, Hong S, Qian J, Zheng Y, Yang J, Yi Q. Cross talk between the bone and immune systems: osteoclasts function as antigen-presenting cells and activate CD4+ and CD8+ T cells. *Blood*. 2010 Jul 15;116(2):210-7.

**Osteoclasts are the only cells that can efficiently resorb bone. They are multinucleated and form by the terminal differentiation and fusion of a mononuclear hematopoietic precursor cell under the influence of the cytokines receptor activator of NF-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF) (1). Osteoclast precursor cells in the bone marrow are multipotential and also give rise to macrophages and dendritic cells (2;3). Macrophages are phagocytic cells that are mediators of the innate immune response, which is the first level of defense against pathogens (4). In addition, they are involved in tissue remodeling and the adaptive immune system. The latter is a more targeted immune response, which is mediated by T- and B-lymphocytes. Dendritic cells are specialized "professional" antigen-presenting cells, with unique characteristics that separate them from macrophages (5). However, like macrophages, they process foreign antigens and "present" peptides derived from these antigens along with additional key molecules on their cell surface. This permits them to interact with specific classes of T-lymphocytes in specialized areas of lymph nodes and initiate a cascade of events that facilitates the organism's ability to contain and destroy invading pathogens. A recent paper by Li *et al*. (6) argued that, in addition to their ability to resorb bone, differentiated osteoclasts are also capable of presenting antigen to T-lymphocytes and initiating adaptive immune responses. These authors believe that understanding the varied roles of osteoclasts in the bone marrow may provide insights into the way bone and immune cells interact and potentially provide new therapeutic targets to combat human diseases that are caused by abnormal immune system function.**

Normally, bone is continually reforming itself (7). Bone resorption is accomplished by osteoclasts. These are multinuclear giant cells that efficiently resorb bone. Osteoclasts often form in bone near sites of inflammation, which is a potent stimulator of their activity (8). One of the unanswered questions in bone biology research is why, generally, osteoclasts only form in bone. It is now well-described that the osteoclast precursor cell is abundant and circulates widely in blood. This cell, which is included in the monocyte fraction of the leukocyte pool, also appears capable of differentiating into the macrophage and the dendritic cell (2;3). The cytokines that are critical for stimulating osteoclast differentiation from the osteoclast precursor are RANKL and M-CSF (9;10). These cytokines are also abundant at sites of inflammation (8). However, osteoclasts are not seen in inflammatory tissues except on the surface of bone (11). Hence, additional signals are required for their formation. One source of these "co-stimulatory" signals is the interaction of ITAM receptors on osteoclast precursor cells with specific ligands that are abundant only in the bone microenvironment (4;12-14). Immune cells also produce multiple

cytokines that affect osteoclast formation and function (1). However, the ability of osteoclasts to influence immune cells has only recently been recognized (15).

In the paper by Li *et al*. (6), the authors ask the question of whether osteoclasts can function as antigen-presenting cells. Antigen presentation is a critical step of the adaptive immune response. This process primes and activates T-lymphocytes and permits the specific destruction of invading pathogens through the development of cell and humoral immunity (16). "Professional" antigen-presenting cells, like macrophages and dendritic cells, phagocytize foreign antigens and digest and process these intracellularly. They then express the processed antigen peptides on their cell surface along with major histocompatibility complex (MHC) proteins. T-lymphocytes with specific T-cell receptors (TCR) interact with the presented antigen peptides and the MHC. This, in turn, activates the T-lymphocytes and, together with additional co-stimulatory factors and cytokines, directs the development of an adaptive immune response. Critical to this process is the expansion of the reactive T-lymphocytes by cell replication.

Li *et al*. examined the ability of mature osteoclasts to process antigen, express MHC proteins and cytokines, and activate T-lymphocyte replication. Osteoclasts were generated from human peripheral blood mononuclear cells that were selected by density gradient centrifugation (Ficoll-Paque, GE Healthcare) and a short (two-hour) incubation on plastic. The adherent cells were then stimulated to form osteoclasts by incubation with RANKL and M-CSF for 14 days. The weakness of this isolation method is that it is really not specific for isolating various subgroups of circulating monocytes and there was no attempt by the authors to characterize the heterogeneity of the population that they stimulated to form osteoclasts. Neither was there any quantization reported that documented the percentage of cells in the cultures that expressed osteoclast-specific markers. Hence, it is difficult to know the uniformity of the population of cells that the authors subsequently studied. The authors also performed a number of experiments in which they isolated cells using non-enzymatic dissociation conditions and analyzed the released populations for expression of antigens by flow cytometry. The problem with this approach is that it is the experience of this author that mature multinucleated osteoclasts are very resistant to release by these conditions. Unfortunately, Li *et al*. do not report the percentage of cells that remained on the culture plates after non-enzymatic dissociation. It is possible that the cells analyzed by flow cytometry were a sub-population of the cells in the cultures after 14 days. Hence, assumptions made about the characteristics of the released cells may not be generalizable to the entire population of cultured cells.

In any event, Li *et al*. show that the cells generated in the cultures formed multinucleated osteoclasts, which resorbed bone. The released cells also expressed both MHC I and MHC II proteins as well as the cofactors CD80, CD86 and CD40, which are necessary for antigen presentation, after stimulation with cytokines. However, except for CD11c, levels of these proteins on the surface of the osteoclast cultures were less than on standard preparations of dendritic cells that were similarly treated. The osteoclast cultures also produced characteristic cytokines in response to lipopolysaccharide (LPS) stimulation, which mimicked those produced by dendritic cells. Most importantly, the osteoclast cultures activated cell replication in T-lymphocytes in an antigen-specific and MHC protein-specific manner, which is the *sine qua non* of antigen-presenting cell function. However, the capacity of osteoclasts to induce this response was reduced compared to that of true dendritic cells.

The results of these experiments are tempered by my concerns about the uniformity of the osteoclast cultures, as outlined above. However, they are provocative and suggest that, like macrophages and dendritic cells, to which they are related in their origins, osteoclasts can present antigens to T-lymphocytes and are involved in T-lymphocyte homeostasis. The results of the Li *et al*. study are

supported by Kiesel *et al*., who found that murine osteoclast cultures could present antigens to CD8 T-lymphocytes and stimulate T-lymphocyte replication (17). However, as with the Li *et al*. paper, the heterogeneity of the cells studied by Kiesel *et al*. was never fully assessed. It is curious that memory T-lymphocytes preferentially home to bone marrow and proliferate there (18;19). Perhaps the antigen-presenting ability of osteoclasts is involved in this phenomenon. Clearly, additional experiments are needed. These would include better techniques to purify the osteoclast precursor cells and additional *in vivo* studies to document a role for osteoclast antigen presentation in either normal or abnormal T-lymphocyte or bone cell functions.

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