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Inflammatory osteoclasts, a different breed of bone eaters?

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Like many other populations of tissue-residing myeloid lineage cells, osteoclasts lie at the intersection of health and disease. These bone-degrading cells are critical for both homeostatic bone remodeling and pathological osteolysis (1). Since its discovery, RANKL has been considered the key cytokine for osteoclast differentiation. Lack of RANKL or its downstream signals through RANK causes osteopetrosis, excessive accumulation of brittle bone, in both mouse and man. However, as befits their myeloid lineage, osteoclasts and their precursors express a large number of receptors, allowing them to sense a wide variety of signals from their environment. Inflammation in or near bone induces a robust osteoclastogenic response, and the relative roles of RANKL and inflammatory cytokines such as TNF α , IL-1 β , and IL-6 in this context have been debated.

There are several lines of evidence suggesting that RANKL plays a primary role in inflammatory osteolysis. Treatment of rats with collagen-induced arthritis with OPG, a decoy receptor for RANKL, or TNF-tg mice (which develop spontaneous arthritis due to high levels of TNF α expression) with RANK:Fc prevented bone erosion at inflamed joints (2, 3). Mice globally lacking RANKL with serum transfer arthritis, although showing comparable inflammation and cartilage loss to controls, showed no osteoclast formation in affected joints, and bone erosion was minimal (4). TNF α injection into RANK knockout mice induced only very rare osteoclasts (5). Inflammatory cytokines such as TNF α and IL-1 β enhance RANKL expression by osteoblast lineage cells and synovial fibroblasts, as well as Th17 T cells. Ablation of RANKL expression from synovial fibroblasts alone greatly reduced bone erosion in a model of inflammatory arthritis (6). Perhaps most importantly, denosumab, a humanized anti RANKL-antibody, reduces bone erosion in human rheumatoid arthritis (RA) patients (7).

Despite a clear role for RANKL in inflammatory bone erosion, RANKL-independent, TNF α -mediated osteolysis has been demonstrated in mice, but only in cases of genetic manipulation of factors in addition to RANK or RANKL deficiency. For example, ablation of Nfkb2 allows efficient generation of osteoclasts on the calvarium in response to TNF α in Rank- or Rankl-deficient mice (8). Similarly, deletion of RBP-J allowed bone resorption following TNF injection into Dap12-deficient mice, which show defective RANKL-mediated signaling to NFATc1 (9). In wild type cells, the ability of TNF α to induce

osteoclast differentiation in the presence of RANK/RANKL blockade is enhanced by a variety of cytokines, but in vivo evidence has been lacking.

The work of O'Brien et al (this issue) takes on the question of RANKL-independent bone erosion with some new angles. After confirming that TNF α alone is very weakly osteoclastogenic compared to RANKL in vitro, they turn to the combination of TNF and IL-6, and find that this combination is much more efficient for osteoclast generation (Fig 1). Intriguingly, despite the fact that cultures treated with TNF alone showed no resorptive activity while those in TNF/IL-6 resorbed nearly as well as those generated with RANKL, there was little difference in mRNA expression of many markers of mature osteoclasts (Nfatc1, Itgb3, Dcstamp, Acp5, Atp6v0d2) between TNF and TNF/IL-6 groups. The one marker significantly elevated by IL-6 was cathepsin K, which could potentially be responsible for increased resorption, although a more comprehensive analysis of gene expression might yield additional effectors of resorption dependent on IL-6.

While a role for RANKL itself in the in vitro osteoclastogenesis was stringently excluded using both OPG and Rank-deficient precursors, TNF/IL-6-induced differentiation depended on Nfatc1 and Dap12, both factors also critical for RANKL-mediated differentiation. As the requirement for Dap12 likely reflects its role in calcium signaling upstream of NFATc1, these data indicate that the overall characteristics of the osteoclasts generated by RANKL-dependent or -independent pathways are likely to be similar. Although RANKL and TNF α signal through very similar upstream pathways, IL-6 supplies a unique input that appears to depend on proliferation. Exposure of osteoclast precursors to RANKL leads to cell cycle arrest, and O'Brien et al found that addition of the cell cycle inhibitor hydroxyurea has no effect on their differentiation. In contrast, TNF α /IL-6 mediated osteoclastogenesis was severely impaired when proliferation was blocked by this treatment. Since equal numbers of precursors were used under all conditions and mature osteoclasts are postmitotic, it is not clear why cell proliferation is required downstream of TNF α /IL-6 but not RANKL. Nevertheless, this difference represents the most direct evidence for a novel upstream input into the osteoclastogenic program from IL-6, which deserves more study.

Of course, in vitro observations are more meaningful when supported by in vivo models of disease. In this case, O'Brien et al turned to the serum transfer arthritis model in inducibly RANK-deficient mice, using Mx-1 cre (Rank^{-/-}). After demonstrating that IL-6, as well as the related cytokines Osm and Lif which signal through the same GP130-dependent pathway, are highly induced in this model, they analyzed osteoclast formation with TRAP staining of histologic sections and bone erosion by microCT. Inflammation and cartilage erosion were unaffected by the loss of RANK, while osteoclasts and bone erosion were decreased. However, unlike in global RANK knockout mice, neither formation of TRAP⁺ cells in inflamed joints nor bone erosion was eliminated in this experiment. A strength of this study was the rigorous way in which the authors sought to demonstrate that this residual osteoclast activity was not likely to have been caused by incomplete ablation of RANK in their model. First, they demonstrated that expression of Rank mRNA in bone marrow was reduced by > 99%, and RANKL was unable to induce any osteoclast formation from Rank^{-/-} marrow in vitro. Then, they generated Rank^{-/-} reporter mice by crossing these with the mTmG line, in which Cre-mediated recombination converts cells from red to green

fluorescence. TRAP positive osteoclasts within bone erosions in arthritic Rank^{-/-} reporter mice were all green, indicating that Cre had acted to recombine the reporter locus. Although it is possible that the mTmG locus has more efficient recombination than the Rank locus in vivo, the extremely low expression of Rank mRNA in bone marrow makes this highly unlikely.

An explanation for the appearance of osteoclasts in mice with inducible (this study) rather than congenital deletion of Rank (4) has not arisen from these experiments. One possibility is a difference in arthritis severity between the two studies, which could be caused by variability in strain backgrounds or in batches of serum utilized. Despite the differences in degree of residual resorption between the studies, the totality of the evidence indicates that RANKL indeed plays a strong role in inflammation-induced bone erosion. However, variability in human patient populations is certainly larger than in our mouse models, and differences in outcomes in mouse studies should perhaps be celebrated rather than criticized. Careful comparison of methodologies and consideration of additional variables in housing, diet, microbiome, and other factors in animal studies with divergent outcomes may point to reasons for suboptimal therapeutic outcomes in individual patients.

Since pharmacologic inhibition is never complete in real life clinical situations, it is not possible to determine whether refractory bone erosions in RA patients treated with RANKL-blocking antibody denosumab result from RANKL-independent mechanisms. However, studies such as that of O'Brien et al demonstrating the possibility of inflammatory cytokine driven osteolysis suggest that it should be considered, thereby highlighting the importance of anti-inflammatory treatments, whether chemotherapeutic or biologic. Since all RA patients receive some form of anti-inflammatory treatment for primary symptom relief, it is unlikely that the inflammation-directed therapies will be affected by the findings of O'Brien et al. However, recognition of RANKL-independent osteoclast generation may affect the approach to drugs targeting bone erosion directly. A small molecule inhibitor of cathepsin K, odanocatib, is in final stages of FDA approval for the treatment of osteoporosis (10). Since the osteoclasts generated by TNF α /IL-6 had strong induction of cathepsin K, it is very likely that cathepsin K inhibition will be more effective in blocking inflammatory osteolysis than RANKL inhibition, as it will target both populations of osteoclasts.

The question of whether inflammatory osteoclasts, generated in RANKL-dependent or -independent inflammatory conditions, are significantly different from homeostatic osteoclasts or those generated in other disease states such as bone metastasis remains open. Now that differences such as a requirement for cell proliferation have been demonstrated, a rationale for pursuing this idea is present. The field of macrophage/myeloid lineage biology has been flooded in recent years by definitions of many functional subsets, often determined by microenvironmental context, and significant plasticity to move from one subset to another. Given the commonality in surface receptors and important signaling pathways between osteoclasts and other myeloid cells, it seems likely that subtypes of osteoclasts exist, analogous to functional macrophage subsets. Since osteoclasts are tightly bone apposed, and present in relatively small numbers compared to the total number of cells in the bone and bone marrow, it will be challenging to demonstrate subtypes experimentally.

Nevertheless, advances in lineage tracing and in vivo imaging may yet provide new opportunities for understanding these fascinating bone eating cells.

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Figure 1.

Cells in the inflammatory pannus found around RA joints, including synovial fibroblasts (SF), Th17 T cells, macrophages (Mac), and osteoblasts (Ob) contribute to an environment high in cytokines such as RANKL, TNF α , and IL-6. TNF α and IL-6 can work together, completely independent of RANKL, to induce differentiation of osteoclasts that secrete cathepsin K (catK) and resorb bone near the joint.