TNFα and pathologic bone resorption

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Abstract. Chronic inflammatory bone diseases, such as rheumatoid arthritis, periodontal disease and aseptic periprosthetic osteolysis, are characterized by bone loss around affected joints and teeth caused by increased osteoclastic bone resorption. This resorption is mediated largely by the increased local production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNFα). These cytokines may induce resorption indirectly by affecting the production of the essential osteoclast differentiation factor, receptor activator of NF-κB ligand, and/or its soluble decoy receptor, osteoprotegerin, by osteoblast/stromal cells or directly by enhancing proliferation and/or activity of cells in the osteoclast lineage. The importance of TNFα in the pathogenesis of various forms of bone loss is supported by both experimental and clinical evidence. However, TNFα is not absolutely required for osteoclastogenesis, erosive arthritis, or osteolysis, as all these events could occur in the absence of TNFα and whether TNFα promotes osteoclast formation independently of RANK signaling is still a topic of debate. Here we review our current understanding of the mechanisms whereby TNFα increases osteoclastogenesis in vitro and in vivo. (Keio J Med 54 (3): 127–131, September 2005)

Key words: TNF, Cytokines, osteoclasts, NF-κB, inflammation

Regulation of Osteoclast Formation

Osteoclasts are multinucleated cells formed by fusion of mononuclear progenitors in the monocyte/macrophage lineage derived from the colony-forming units granulocyte-macrophage (CFU-GM). Considerable progress has been made in our understanding of osteoclastogenesis through cell culture techniques,1 and the generation of transgenic and knockout mice.2 These studies have identified two distinct signaling pathways whose activation is required for osteoclastogenesis. The first is activated by macrophage-colony stimulating factor (M-CSF), which signals through its receptor c-Fms, and the second by the ligand of receptor activator of NF-κB (RANK) through its receptor, RANK.3,4 Mice genetically deficient in M-CSF5,6 or RANKL7,8 signaling do not form osteoclasts and thus develop osteopetrosis. Osteoclastogenesis is also dependent on intracellular signaling molecules downstream from c-Fms and RANK.9–11 These include the adapter protein, TRAF6,12,13 and the transcription factors, AP-1,14,15 NF-κB16,17 and NFAT.18,19

The progression of osteoclast precursors through the various stages of differentiation from the pluripotent hematopoietic stem cell has been characterized by using a combination of cell surface markers, cell sorting and osteoclastogenesis assays.20 To date, the earliest identified osteoclast progenitor in bone marrow has been phenotyped as a c-Kit+/c-Fms−/CD11b−/RANK− cell, which then differentiates into a c-Kit+/c-Fms+/CD11b+/RANK− early stage progenitor. Stimulation of this cell by M-CSF expressed by osteoblast/stromal cells in the marrow advances differentiation to the c-Kit−/c-Fms+/CD11b+/RANK+ late stage progenitor, which responds to RANKL to complete osteoclast development. RANKL not only delivers a final differentiation signal, but also activates osteoclasts and promotes their survival.20–23
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TNFα, like many other osteoclast-stimulating molecules, promotes RANKL production by stromal cells, and also induces its secretion by T lymphocytes, B lymphocytes, and endothelial cells to induce osteoclast formation indirectly. TNFα also stimulates M-CSF production by murine or human stromal cells. Whether TNFα promotes osteoclast formation independently of RANK signaling has been a topic of considerable debate. Two independent groups demonstrated that TNFα promotes osteoclast formation in vitro despite RANK signaling blockade, while others reported that “permissive” levels of RANKL are required for TNFα-induced osteoclastogenesis. These discrepant findings may reflect different cell populations and culture conditions, but the observation that TNFα can stimulate osteoclast formation from bone marrow cells from RANK and RANKL knockout mice clearly demonstrates RANKL/RANK independent osteoclastogenesis in vitro (and our unpublished observations). Although small numbers of osteoclasts were reported to form in vivo in response to high local concentrations of TNF delivered locally over the calvarial bones of RANK-/ mice, RANK independent osteoclastogenesis under physiological conditions in vivo has not been demonstrated. Thus, the relevance of this action of TNFα in vitro to normal or diseased states remains unclear.

To study the effects of chronic TNFα exposure on osteoclast precursor differentiation and the requirement of RANKL/RANK signaling in this process, we used TNFα transgenic (TNF-Tg) mice, which develop erosive arthritis, and RANK knockout mice. In TNF-Tg mice there is a chronic low-level expression of the human TNFα transgene and as a result the mice develop an erosive arthritis with features similar to those seen in human rheumatoid arthritis (RA). These features include inflammatory bone loss, most notably focal erosions affecting the immediate subchondral bone and bone at the joint margins where osteoclast numbers are increased (Fig. 1). Thus, the mechanisms whereby TNFα induces osteoclast formation can be studied using this model.

Osteoclast and CFU-GM colony formation is increased in cultures of spleen cells from TNF-Tg compared to wild-type mice. However, a more striking finding is that mature osteoclasts are derived from TNF-Tg splenocytes and peripheral blood mononuclear cells (PBMC) one day earlier than from wild-type cells. This accelerated osteoclastogenesis could not be inhibited by TNF blockade in vitro, but was observed in cultures of splenocytes from wild-type mice injected previously with TNFα. From these data, we conclude that TNFα has a priming effect on osteoclast progenitor cells in vivo thereby increasing the number of pre-osteoclasts in peripheral tissues.

If our interpretation is correct, and systemic TNFα increases the number of pre-osteoclasts in peripheral tissues like the spleen and blood, then these cells should be identifiable by phenotypic surface markers. FACS analysis showed that spleens from TNF-Tg mice had 4 to 7-fold more CD11b+ cells than wild-type mice, but TNFα had no effect on CD11b expression in pre-osteoclasts. Spleen cells isolated from TNFα-treated mice proliferated faster in response to M-CSF compared to PBS-treated mice in vitro, but direct administration of TNFα to spleen cells did not affect their proliferation. Since osteoclast precursors differentiate from myeloid progenitors in the bone marrow, we examined their frequency in the bone marrow of TNF-Tg mice to determine if chronic exposure to TNF alters the generation of pre-osteoclasts. Compared to their wild-type littermate controls, TNF-Tg mice have significantly increased CD11b+ pre-osteoclasts. Furthermore, TNF treatment of bone marrow cells from wild-type mice leads to an increase in the frequency of c-Fms+/CD11b+ cells and increases c-Fms mRNA expression, suggesting that TNF promotes the differentiation of marrow osteoclast precursors thereby increasing the pool size of these cells (Fig. 2). TNF treatment of CD11b+c/Gr-1−/low osteoclast precursors purified from bone marrow by FACS analysis increased their c-fms mRNA expression 4-fold, suggesting that these cells...
express TNF receptors at this stage in their development (our unpublished observations). Whether these pre-osteoclasts actively leave the bone marrow to go to peripheral tissues and whether TNF affects this process have yet to be determined.

Having demonstrated that transgenically-expressed and exogenous TNFα increase peripheral pre-osteoclast frequency, we examined if this increase was reversible via in vivo TNFα blockade with the TNFα inhibitor, etanercept. This treatment reduced the number of CD11b+ splenocytes, and their osteoclastogenic and CFU-GM colony formation potential to wild-type levels. These findings are consistent with our observation that patients with RA and psoriatic arthritis have a marked increase in the number of pre-osteoclasts in their PBMC population compared to normal and osteoarthritis controls. Importantly, this increase also appears to be reversible with anti-TNFα therapy, and may be a dominant mechanism by which this treatment inhibits erosions in these patients.

We previously investigated RANK-independent osteoclastogenesis in animal models of wear debris-induced osteolysis and fracture healing, but found no osteoclast formation in the absence of RANK signaling. To investigate if the effects of chronic exposure to TNFα are dependent on RANK signaling, we treated TNF-Tg mice with a RANK antagonist, RANK:Fc (a soluble fusion protein consisting of the extracellular domain of RANK fused to the Fc domain of IgG1), and crossed TNF-Tg mice with RANK−/− mice. We found that CD11b+ cells increased in both models. However, the elevated levels of TNFα did not compensate for the absence of RANK signaling because the precursors were unable to differentiate into mature osteoclasts. Furthermore, we found no erosions in mice with established arthritis that had been treated with RANK:Fc, which suggests that RANK blockade can arrest erosive disease (our unpublished observations).

Thus, chronic exposure to TNFα in vivo increases osteoclastogenesis through two distinct mechanisms (Fig. 2) in which TNFα first affects osteoclastogenesis at the osteoclast precursor stage in the bone marrow by priming these cells to differentiate into c-Fms+/CD11b+/RANK+ precursors through a RANKL/RANK independent mechanism. These osteoclast precursors then enter the blood and peripheral tissues where they can respond to TNFα by both direct and indirect mechanisms to become mature osteoclasts at sites of bone resorption. For example, TNFα can induce a variety of cells, including synovial cells, T cells and osteoblast/stromal cells, to increase their expression of RANKL, which binds to RANK on the surface of these precursors and induces their differentiation. TNFα can also bind to its receptor on the surface of these precursors and directly induce their differentiation to mature osteoclasts, thus enhancing the RANKL-induced indirect action. The dominance of RANK blockade in the model in Figure 2 is consistent with results from other animal models of arthritis, osteoporosis, hypercalcemia of malignancy and tumor metastasis to bone. These findings suggest a clinical correlate whereby patients with active erosive arthritis might have increased numbers of CD11b+ cells with enhanced osteoclastogenic potential in their blood. It is possible that patients with active disease or “flares,” could be identified by an increase in circulating pre-osteoclasts. Furthermore, it is possible that patients that are non-responsive or refractory to anti-TNF therapy could be identified by changes in this population following therapy.

Fig. 2 RANK dependent and independent mechanisms of TNF-mediated osteoclastogenesis. During the early stages of osteoclastogenesis, TNF increases the pool size of marrow osteoclast precursors by promoting their proliferation and differentiation in response to M-CSF and by stimulating c-Fms expression, which is independent of the RANK pathway. These osteoclast precursors then differentiate into mature osteoclasts in the presence of RANKL, and this process is accelerated by TNF. The role of TNF at this later stage of osteoclast differentiation is RANKL/RANK dependent.

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