Commentary

Impact of cytokines and T lymphocytes upon osteoclast differentiation and function

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Abstract

Historically, the osteoblast has been considered the master cell in the control of osteoclast development and, therefore, bone resorption. Now the interactions between cells of the immune system and bone cells have redefined our thinking on the regulation of bone resorption. Moreover, the crosstalk between these cell types has special significance in inflammatory conditions such as rheumatoid arthritis. This report highlights the contribution that T lymphocytes make in regulating osteoclast formation and bone resorption.

Rheumatoid arthritis, the most common chronic autoimmune disease, ultimately presents with joint destruction as a consequence of an inflammatory process. Whilst the pathogenesis for onset of this disease is not understood, the final steps in the process leading to bone destruction have been recently resolved. It has been a long held view that infiltrating synovial cells are responsible for juxta-articular bone loss, although it is now clear that the osteoclast is the only cell capable of resorbing bone. The recognition of this exclusive role for the osteoclast in all pathologies involving bone loss (osteoporosis, arthritis, periodontal disease) has identified a single cell whose function can be modulated to enhance or reduce bone loss [1].

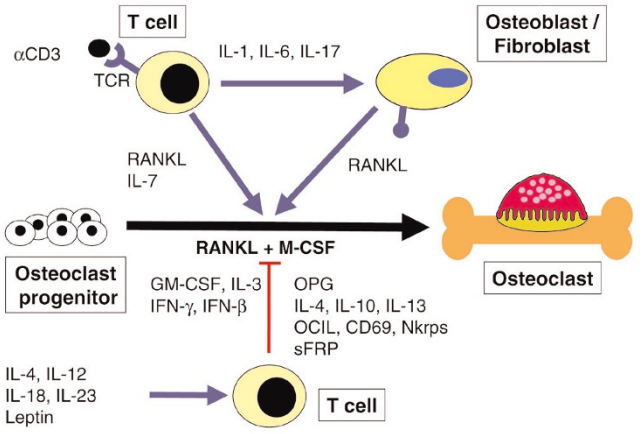
The identification of the osteoclast and its role in bone destruction permits targeted therapy to reduce its resorptive capacity. Such therapies include the use of agents that can interfere with receptor activator of NFκB ligand (RANKL), one of the key cytokines promoting osteoclast differentiation. This may be achieved through the use of recombinant Fc-osteoprotegerin (Fc-OPG) or a humanised anti-RANKL antibody (Denosumab) that is being developed by Amgen. Both have demonstrated efficacy in preclinical models of bone loss, with Denosumab progressing through clinical trials; Fc-OPG was withdrawn from clinical trials due to immune side effects. Other inhibitors of osteoclast activity include the bisposhonates, c-src inhibitors, cathepsin K inhibitors and inhibitors of the chloride channel CLC7 [2]. Notably, bisphosphonates have been successful in limiting bone loss in rodent models of arthritis, although it should be noted that the nitrogen-containing bisphosphonates (which include aldronate, ibandronate, pamidronate and zoledronate) enhance proliferation of γ/δ T lymphocytes, while non-nitrogen-containing bisphosphonates (for example, clondronate) do not [3]. Enhanced inflammation has been noted in rodent models of arthritis when treated with zoledronate, raising a cautionary note to evaluate the bone-protective effects versus the potential of enhanced immune response with nitrogen-containing bisphosphonates in inflammatory conditions.

The pro-resorptive roles of T lymphocytes

In the pathogenesis of rheumatoid arthritis, lymphocyte and synovial cell expansion is observed. These occur prior to bone destruction, suggesting that these cells may be responsible for osteoclast formation and activation. RANKL and macrophage-colony stimulating factor (M-CSF) are the principal factors involved in the differentiation of the osteoclast (Figure 1) and RANKL is expressed by activated T lymphocytes [1]; lymphocytes express soluble RANKL, which may result from shedding of its membrane-bound form or the secretion of an isoform of RANKL that may be produced from an alternative mRNA transcript. *In vitro*, the process of T cell activation can be recapitulated following stimulation of cells with phenyl methyl acetate/concanavalin A and engagement of the T cell receptor (Figure 1). Such activated cells express RANKL and, importantly, support osteoclast formation and activation. A second mechanism by which T lymphocytes may support osteoclast formation directly is as a consequence of IL-7 production, and this appears to be mediated by a RANKL-independent process

GM-CSF = granulocyte macrophage-colony stimulating factor; IFN = interferon; IL = interleukin; M-CSF = macrophage-colony stimulating factor; OPG = osteoprotegerin; RANKL = receptor activator of NFκB ligand; sFRP = secreted Frizzled-related protein; TGF = transforming growth factor; TNF = tumour necrosis factor.

Figure 1



Osteoclast differentiation. Cells of the mylomonocytic lineage (appropriate sources for *in vitro* differentiation are cells from bone marrow, monocytes, spleen or RAW 264.7 cells) under the influence of macrophage-colony stimulating factor (M-CSF) and receptor activator of NFκB ligand (RANKL) differentiate into osteoclasts. Depicted above this differentiation pathway is the potential role for T lymphocytes in enhancing or fulfilling this process. Upon activation of osteoclasts following engagement of the T cell receptor (TCR), T lymphocytes may produce several factors that promote osteoclast formation (RANKL and IL-7) or the production of RANKL by fibroblast and stromal cells (for example, IL-1, IL-6, IL-17). Below the differentiation pathway, the inhibitory actions of T lymphocytes are presented. T lymphocytes produce a vast array of inhibitory molecules, and several of these are elevated in response to IL-4, IL-12, IL-15, IL-18, IL-23 and osteoprotegerin (OPG). GM-CSF, granulocyte macrophage-colony stimulating factor; sFRP, secreted Frizzled-related protein; OCIL, osteoclast inhibitory lectin.

[4]. Finally, T lymphocytes express tumour necrosis factor (TNF)-α and this acts in concert with RANKL to promote osteoclast formation. The essential role of T lymphocyte-derived RANKL in rodent models of arthritis has been identified through adoptive transfer experiments that highlight the essential contribution of T cells to bone loss.

In addition to the ability of T lymphocytes to directly support osteoclastogenesis, T lymphocytes also secrete pro-resorptive cytokines, including IL-1, IL-6 and IL-17, each of which can stimulate RANKL expression by osteoblasts and fibroblasts, permitting osteoclast formation by a contact-dependent process (Figure 1) [1,2,4]; unlike T cells, there is no convincing evidence that osteoblasts or fibroblasts produce soluble RANKL. Notably, IL-17 production further discriminates $CD4^+$ T cell populations, with the cells producing it being designated as Th_{17} cells. Th_{17} cells also express TNF, IL-6 and granulocyte macrophage-colony stimulating factor (GM-CSF) and are responsive to IL-23, which regulates their expansion and survival. The importance of IL-17 and IL-23 in rheumatoid arthritis and other inflammatory diseases has been highlighted in studies using knockout mice. IL-17 or IL-23 deficient mice were resistant to experimental autoimmune encephalomyelitis and to collagen-induced arthritis [5,6]. These roles suggest that Th_{17} cells function as important immunomodulators of osteoclastic bone resorption [7].

T lymphocytes protecting against bone loss

In contrast to the pro-resorptive roles of T lymphocytes, several other T cell-derived interleukins and cytokines have the capacity to inhibit osteoclast formation and activity, and these may be expressed by naïve or activated T cells (Figure 1). GM-CSF, IL-3, IFN-γ, IFN-β, OPG, IL-4, IL-10, IL-13, osteoclast inhibitory lectin and secreted Frizzled-related proteins (sFRPs) potently inhibit osteoclast formation (Figure 1) [2]. The molecules highlighted in Figure 1 are discussed below.

OPG acts as a decoy receptor to RANKL to limit its biological action, while several of the other cytokines perturb JAK/STAT signalling. It should be noted that GM-CSF has dual functions depending upon the presence of RANKL. When RANKL is absent, GM-CSF enhances progenitor proliferation, thus promoting the total numbers of osteoclasts when such progenitors are exposed to RANKL. However, when RANKL and GM-CSF are co-administered to osteoclast progenitors, differentiation is favoured towards dendritic cells and not osteoclasts, and this process is further enhanced when IL-4 is additionally supplied. This lineage switch between osteoclasts and dendritic cells has been described *in vitro*, where cytokine delivery can be precisely defined. The situation *in vivo* is less clear, and the local concentrations of many cytokines and growth factors undoubtedly influence differentiation pathways.

The Wnt pathway is essential for osteoblast differentiation, and its central role in regulating bone mass is exemplified by individuals with high bone mass phenotypes having activating mutations in the Wnt pathway. Wnt signalling is also involved in T cell development and osteoclastogenesis, and the secreted Wnt decoy receptors sFRP-1 and sFRP-3 are able to inhibit osteoclast formation; both of these Wnt antagonists are expressed by T cells.

Finally, the C-type lectins CD69, osteoclast inhibitory lectin and Nkrps, which are markers of activated T cells and are expressed as membrane-bound molecules, also inhibit osteoclastogenesis. Their mechanism of action is not known but they may act through other C-type lectin receptors [2].

In addition to cytokines that are expressed by T lymphocytes to inhibit osteoclast formation, several cytokines have been reported to act upon T lymphocytes, affecting their action upon bone. These include IL-12, IL-18 and IL-23, each of which has been demonstrated to inhibit osteoclast formation in a T lymphocyte-dependent process. Each of these interleukins promotes T cell production of GM-CSF and IFN-γ; however, the relative contribution of both interleukins differs. IL-18 potently enhances GM-CSF production and

blockade of GM-CSF production is sufficient to override the inhibitory action of IL-18. In contrast, IL-12 predominantly increases the production of IFN-γ, which is the principal osteoclast inhibitor in response to IL-12 treatment. IL-12 and IL-18 act synergistically upon T lymphocytes to inhibit osteoclast formation and when administered together at synergistically low doses both GM-CSF and IFN-γ are produced [8].

Leptin has both central and peripheral roles in influencing bone mass, with leptin in the bone microenvironment stimulating T cell production of OPG, the secreted decoy receptor for RANKL. IL-4 acts both as a direct inhibitor of osteoclast formation as well as through T cells to promote a hitherto unrecognised osteoclast inhibitor that is expressed on the T cell surface.

In addition to interleukins that effect osteoclast formation and activity, estrogen and transforming growth factor (TGF)-β also act through T lymphocytes to affect bone density. Mice whose CD4 cells express a dominant-negative TGFβII receptor (such that these cells do not respond to TGF-β) have a lower bone mineral density than wild-type animals [2].

Conclusion

The identification of the osteoclast and its role in joint destruction has enabled the development of therapies aimed at reducing its resorptive capacity, some of which have been demonstrated to be effective in animal models of arthritis. However, the recently discovered links between bone and the immune system have highlighted the interdependence between T cells, osteoblasts and osteoclasts. This is particularly crucial in the pathogenesis of rheumatoid arthritis, with several cytokines and growth factors that are secreted by, or modulate the activities of, T lymphocytes also affecting osteoclast formation and activity.

Of interest now and for future studies is the effect of T cells upon osteoblasts and fibroblasts and how, as a result of altered bone resorption and formation, newly acquired or resorbed bone and differentiated osteoblasts and osteoclasts may, in turn, affect the ontogeny of T cells or modulate their immune response or cytokine repertoire.

Competing interests

The author declares that he has no competing interests.

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