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Review

The immune system and bone

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ABSTRACT

T cells and B cells produce large amounts of cytokines which regulate bone resorption and bone formation. These factors play a critical role in the regulation of bone turnover in health and disease. In addition, immune cells of the bone marrow regulate bone homeostasis by cross-talking with bone marrow stromal cells and osteoblastic cells via cell surface molecules. These regulatory mechanisms are particularly relevant for postmenopausal osteoporosis and hyperparathyroidism, two common forms of bone loss caused primarily by an expansion of the osteoclastic pool only partially compensated by a stimulation of bone formation. This article describes the cytokines and immune factors that regulate bone cells, the immune cells relevant to bone, examines the connection between T cells and bone in health and disease, and reviews the evidence in favor of a link between T cells and the mechanism of action of estrogen and PTH in bone.

Introduction

The close anatomical relationship between the bone marrow (BM)[1] and bone has been recognized for centuries, but the existence of a functional relationship has emerged only recently. Critical steps include the discovery of the hemopoietic origin of osteoclasts (OCs), the capacity of hemopoietic cells to produce cytokine and growth factors essential for bone cell development. Moreover, BM stromal cells that are critical for the development of hemopoietic lines were found to have the capacity to differentiate into osteoblasts (OBs) and osteocytes. It has later become clear that the relationship is bidirectional as bone cells express surface molecules which are essential for the expansion of hemopoietic stem cells (HSCs) and their progeny. A relationship between the immune system and bone has long been suspected as bone loss is a constant feature of autoimmune and inflammatory conditions. The molecular links between the immune system and bone have emerged clearly only with the discovery of RANKL and its receptor RANK. These molecules were first identified as factors expressed on T cells and dendritic cells (DCs), respectively. RANKL and RANKL were shown to augment the ability of DCs to stimulate naive T cell proliferation and enhance DC survival. They were later identified as the key osteoclastogenic molecules. It is now clear that a host of immune factors including costimulatory receptors, cytokines such as IFNγ and TNF, and immune cells such as T and B lymphocytes play a fundamental role in the regulation of bone cell development and bone turnover, and in the pathogenesis of bone diseases.

This article focuses on cytokines relevant for the immune system and bone, and on the role of the immune system in the mechanism by which two essential calciotrophic hormones, estrogen and PTH, regulate bone homeostasis in health and disease.

Cytokines and immune factors that regulate bone cells

OCs arise by cytokine-driven proliferation and differentiation of monocyte precursors that circulate within the hematopoietic cell pool [1]. This process is facilitated by BM stromal cells (SCs), which provide physical support for nascent OCs and produce soluble and membrane-associated factors essential for the proliferation and differentiation of OC precursors.

The minimal essential cytokines required for OC formation under basal conditions are RANKL and M-CSF. These factors are produced primarily by bone marrow SCs, OBs, and activated T cells

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[1] *Abbreviations used:* BM, bone marrow; OCs, osteoclasts; OBs, osteoblasts; HSCs, hemopoietic stem cells; DCs, dendritic cells; SCs, stromal cells; OPG, osteoprotegerin; SOFAT, secreted osteoclastogenic factor of activated T cells; ovx, ovariectomy; TCR, T cell receptor; NKT, natural killer T; Tregs, regulatory T cells; KO, knockout; APCs, antigen presenting cells; MHC, major histocompatibility complex; cPTH, continuous infusion of PTH; iPTH, intermittent PTH; LRP, lipoprotein receptor-related protein; TCF/LEF, T-cell factor/lymphoid enhancer factor; ICAM-1, intercellular adhesion molecule-1; WT, wild type; *CIITA*, class II transactivator; ROS, reactive oxygen species; BSO, buthionine sulfoximine; NO, nitric oxide; L-NAME, *N*-nitro-L-arginine methyl ester; NAC, *N*-acetyl-cysteine.

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[2]. RANKL is a TNF superfamily member which exists in membrane-bound and soluble forms. RANKL binds to the transmembrane receptor RANK expressed on the surface of OCs and OC precursors. RANKL also binds to osteoprotegerin (OPG), a soluble decoy receptor produced by numerous hematopoietic cells. Thus, OPG, by sequestering RANKL and preventing its binding to RANK, functions as a potent anti-osteoclastogenic cytokine [2]. RANKL promotes the differentiation of OC precursors from an early stage of maturation into fully mature multinucleated OCs. RANKL is also capable of activating mature OCs, thus stimulating the capacity of these cells to resorb bone. M-CSF induces the proliferation of early OC precursors, the differentiation of more mature OCs, the fusion of mononucleated pre-OCs and increases the survival of mature OCs.

Although a RANKL-independent osteoclastogenic activity in T cell conditioned media was reported over a decade ago [3] only recently has the nature of the factor involved been elucidated. Using a biochemical purification strategy Rifas and Weitzmann [4] elucidated a novel cytokine named secreted osteoclastogenic factor of activated T cells (SOFAT), that when expressed recombinantly promotes the differentiation of OC precursors into bone resorbing OCs in a RANKL-independent manner. SOFAT was found to be derived from an unusual mRNA splice variant coded by the threonine synthase-like 2 gene homolog, and has no homology to any other known cytokine of cytokine family. The secretion of SOFAT by activated T cells may play an important role in inflammatory bone loss in conditions such as rheumatoid arthritis.

While RANKL and M-CSF are essential for physiologic OC renewal, additional cytokines, are responsible for the upregulation of OC formation observed in a variety of conditions such as inflammation and estrogen deficiency [5,6]. One such factor is TNFα (TNF), a cytokine that enhances OC formation by upregulating the SC production of RANKL and M-CSF [7,8], and by augmenting the responsiveness of OC precursors to RANKL [9,10]. The ability of TNF to increase the osteoclastogenic activity of RANKL is due to synergistic interactions at the level of NFκB and AP-1 signaling [10]. Another target of TNF is the RANKL receptor RANK, whose expression in OC precursors is synergistically upregulated by TNF and RANKL [11]. TNF not only augments OC formation, but also stimulates OC activity [12] thus further driving an imbalance between bone formation and bone resorption. It should also be mentioned that TNF has been found to directly induces BMM differentiation into OCs in the absence of RANKL [13,14], Another relevant effect of TNF is that of inhibiting osteoblastogenesis [15], thus blocking the expected homeostatic response of new bone formation. TNF impairs the function of bone-forming osteoblasts by suppressing mature osteoblast function such as the production of a matrix that is competent for mineralization and by blocking the differentiation of new osteoblasts from their progenitors [15].

The relevance of TNF in ovariectomy (ovx)-induced bone loss has been demonstrated using multiple models. For example, ovx does not induce bone loss in TNF−/− mice and mice lacking the TNF receptor p55 [16], transgenic mice insensitive to TNF due to the overexpression of soluble TNF receptor [17] and mice treated with the TNF inhibitor TNF binding protein [18].

Like TNF, IL-1 promotes RANKL expression by BM SCs and OBs and stimulates OC lifespan and activity. IL-1 directly targets OC precursors and promotes OC differentiation in the presence of permissive levels of RANKL. Furthermore, IL-1 mediates, in part, the osteoclastogenic effect of TNF by enhancing SC expression of RANKL and by directly stimulating differentiation of OC precursors [19]. TNF and IL-1 have potent anti-apoptotic effects in OCs prolonging OC lifespan and contributing toward accelerated bone resorption [20].

Another cytokine relevant for OC formation is IL-7 [21]. IL-7 is a powerful lymphopoietic cytokine that has previously been recognized as a potent inducer of bone destruction in vivo [22]. How IL-7 leads to bone loss is controversial, and it's mechanisms of action are only now beginning to be elucidated. IL-7 is a stimulator of both B and T cell lineages, and it has been suggested that IL-7 induces bone loss by a mechanism involving the expansion of cells of the B lineage, in particular $B220^{+}IgM^{-}$ B cell precursors [22–25], as estrogen deficiency has been reported to potently induce the expansion of these cells [22,25]. How B lineage cells may lead to bone destruction is not presently understood but may involve overexpression of RANKL, a property of activated B cells [26]. IL-7 is also established to regulate multiple stages of T cell metabolism [27]. IL-7−/− mice are severely lymphopenic [28] and IL-7 receptor −/− mice have been reported to display increased bone volume and bone mineral density [22]. In contrast, IL-7 transgenic mice have expanded BM cavities with focal osteolysis of cortical bone and eroded bone surfaces [29]. This data suggests that IL-7 may induce bone loss by T cell and B cell mediated mechanisms. Indeed, IL-7 has been reported to induce production of RANKL by human T cells [30], and injection of IL-7 into mice in vivo induces bone destruction [22,31] by eliciting the secretion by T cells of the key osteoclastogenic cytokines RANKL and TNF [31]. In addition, levels of IL-7 are significantly elevated following ovx [32]. Attesting to the key role of IL-7 in the bone destruction associated with estrogen deficiency, in vivo IL-7 blockade, using neutralizing antibodies, is effective in preventing ovx-induced bone destruction [32]. Furthermore, IL-7 induced osteoclastogenesis and bone loss is compounded by suppression of bone formation leading to uncoupling of bone formation from resorption.

An important, yet controversial, OC regulating factor is IFNγ. This factor was initially described as an anti-osteoclastogenic cytokine because is a potent inhibitor of osteoclastogenesis in vitro [33]. The notion that IFNγ is an inhibitor of bone resorption was reinforced by the finding that silencing of IFNγR−/− signaling leads to a more rapid onset of collagen induced arthritis and bone resorption [34] as compared to WT controls, and by the report that IFNγ decreases serum calcium and osteoclastic bone resorption in nude mice [35,36].

However, observations in humans and in experimental models of disease indicate that IFNγ promotes bone resorption and causes bone loss in a variety of conditions. Studies with IFN−/− and IFNR−/− mice have revealed that among these conditions are estrogen deficiency and endotoxin-induced bone disease [37,38]. Mice lacking either IFNγ production and/or IFNγR expression are protected against ovx-induced bone loss [37,38], endotoxin-induced bone loss [37], and alveolar bone loss [39]. Moreover, in erosive tuberculoid leprosy and psoriatic arthritis IFNγ production correlates positively with tissue destruction [40,41]. In addition, randomized controlled trials have shown that IFNγ does not prevent bone loss in patients with RA [42,43], nor the bone wasting effect of cyclosporin A [44]. Furthermore, IFNγ has been reported to be efficacious in the treatment of osteopetrosis through restoration of bone resorption, both in humans [45] and rodents [46]. These latter findings conclusively demonstrate that in some conditions, including estrogen deficiency, the net effect of IFNγ in vivo is that of stimulating osteoclastic bone resorption.

The complex effects of IFNγ can be explained by the fact that IFNγ influences OC formation both via direct and indirect effects [37]. IFNγ directly blocks OC formation through targeting of maturing OC. This effect is best observed in vitro [33,47]. However, IFNγ is also a potent inducer of antigen presentation and thus of T cell activation. Therefore, when IFNγ levels are increased in vivo, activated T cells secrete pro-osteoclastogenic factors and this activity offsets the anti-osteoclastogenic effect of IFNγ.

Another cytokine that has recently been linked to inhibition of OC formation is IL-23. Specifically this factor inhibits OC formation in the presence of $CD4^{+}$ T cells [48], presumably inhibiting the