

Review Article

The Role of T Cells in Osteoporosis, an Update

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Abstract: Emerging evidence highlights the importance of the interplay between the bone and immune systems. That evidence bolsters a longstanding recognition that estrogen deficiency, infection, inflammation, and autoimmune disorders are associated with systemic and local bone loss. Yet, only recently has an understanding emerged that T lymphocytes and their products act as key regulators of osteoclast formation, life span, and activity. This review presents this understanding of the process of T lymphocytes and their products mediating osteoporosis and explores some of the most recent findings and hypotheses to explain their action in bone. A more complete appreciation of the interactions between immune and bone cells should lead to targeted therapeutic strategies for diseases that affect either or both systems.

Key Words: Osteoporosis, T cells, osteoclast, osteoclastogenesis, cytokine, chemokine

Introduction

Osteoporosis, a systemic skeletal disorder, is characterized by reduced bone mineral density (BMD), microarchitectural deterioration of bone tissue, and a consequential increase in bone fragility and susceptibility to fracture [1]. The lifetime risk for a fragility fracture in a 50-year-old white US woman is about 40% and 13% in a white US man [2]. Two types of cells have pivotal roles in bone biology: osteoblasts (OBs), a cell type that builds bone and is derived from a multipotential mesenchymal cell that can alternatively differentiate into marrow stromal cells or adipocytes; and osteoclasts (OCs), a cell type that resorbs bone and is derived from myeloid precursors that give rise to macrophages and dendritic cells [3]. Mature OBs continue matrix deposition and start mineralization, expressing alkaline phosphatase and bone sialoprotein as well as osteocalcin and osteopontin [4]. OCs are large multinucleated cells and the only cells capable of breaking down mineralized bone, dentine, and calcified cartilage [5, 6]. The presence of the receptor activator of nuclear factor- κ B (RANK) and macrophage

colony-stimulating factor (M-CSF) are essential for the formation and fusion of multinucleated OCs [7, 8]. These two factors must be kept in balance to maintain skeletal integrity and calcium metabolism. The most common problem with this balancing act occurs when the rate of resorption exceeds the rate of mineral deposition, resulting in bone loss, such as that seen with osteoporosis. One way to maintain the balance is to keep in check the bone resorption by the OCs.

Though infection, inflammation, and autoimmune disorders are known to be associated with bone loss, only recently has it been recognized that T lymphocytes and their products are the key regulators for bone remodeling [9, 10]. Major advances and discoveries in this field have led to revelations about the molecular mechanisms, various cytokines, and signaling transducers that participate in the regulatory interactions between T lymphocytes and bone cells [11, 12]. Moreover, besides the arsenal of mutual signaling molecules, T lymphocytes and

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bone cells also share a common site of origin, namely bone marrow (BM). They influence each other not only after maturation and activation, but also at the very beginning of their existence. For example, T cells are capable of affecting osteoclastogenesis by secreting various cytokines such as interleukin (IL)-1, IL-6, interferon (IFN)- γ or IL-4 [13, 14]. Herein, we will review the direct and indirect mechanisms involved in osteoporosis and the evidence to support the hypothesis that one of the critical mechanisms involved in osteoporosis is: activated T cells induce the production of osteoclastogenic factors.

Double-edged Sword Effects of T Cells upon Osteoclastogenesis

The BM hosts fully functional and mature T cells that exhibit several distinctive features. In the BM, mature T cells represent about 3%-8% of total nucleated cells [15]. BM not only primes naïve T cells and recruits effector T cells, but also serves as a site for the preferential proliferation of CD4⁺ and CD8⁺ T cells [16-20]. BM T cells contribute to the homeostasis of the immune system and to the bone cells present in BM environment. Depending on whether studies are performed *in vitro* or *in vivo*, T cells exert varying effects on osteoclastogenesis.

Promoting Effects of T Cells on Osteoclastogenesis

Activated T cells may undermine bone homeostasis and stimulate bone destruction under pathological conditions such as estrogen deficiency [21-24]. They have exerted their effect via membrane-bound and secreted RANK ligand or RANKL, an essential stimulating signal for osteoclastogenesis that is involved in activating mature OCs. Transfer of *ctla4*^{-/-} bone marrow cells in *rag1*^{-/-} mice led to a significant decrease in BMD. Consistent results were achieved by direct transfer of purified *ctla4*^{-/-} T cells in *rag1*^{-/-} and *opg1*^{-/-} mice [21]. In another elegant study, Cenci *et al* reported that increased production of TNF- α by T cells in bone marrow mediated the increased bone resorption and bone loss in ovariectomized mice. Ovariectomy-induced bone loss is prevented by administering either estrogen, TNF- α -binding protein, or an anti-TNF- α antibody. TNF- α augments M-CSF- and RANKL-induced OC formation. But in T-cell deficient mice, ovariectomy failed to induce bone loss, stimulate bone resorption, or increase M-CSF- and RANKL-dependent osteoclastogenesis [22]. By utilizing

DO11.10 mice, a strain in which all T cells recognize a single peptide epitope of chicken albumin, they also investigated the effect of estrogen deficiency upon the birth and death of cytokine-producing T cells. Their studies revealed that estrogen deficiency induced osteoporosis by increasing T cell activation-induced proliferation, and suppressing the apoptosis of active T cells, while the blockade of antigen presenting cell (APC)-induced T cell activation prevented the resulting T cell expansion and bone loss [24]. Consistent with the results of animal studies, a human study of post-menopausal bone loss demonstrated women with post-menopausal osteoporosis have higher T cell activity than healthy post-menopausal subjects [25]. Among T cells, a T helper cell 17 (Th17) cell subset is important in inducing bone loss, which contrasts with the more established T lymphocyte cytokine-expressing subsets- Th1 and Th2. Recent data indicate that the IL-17-producing Th17 cell subset stimulates osteoclastogenesis through osteoclastogenesis-supporting cells, which is the only osteoclastogenic Th cell subset characterized so far [26]. IL-17, a cytokine secreted by the Th17 cell, is well known to induce local inflammation in autoimmune diseases through inflammatory cytokine production. Moreover, IL-17 induces RANKL expression that is crucial for osteoclastogenesis and bone resorption. In addition, it can synergize with these cytokines (IL-1, TNF- α , RANKL), but has direct activity as well. Th17 cells express higher levels of RANKL than Th1 and Th2 cells [26]. Therefore, the infiltration of Th17 cells into the inflammatory lesion links the abnormal T cell response to bone damage. Therefore, given that activated T cells have promoted osteoclastogenesis *in vivo*, this subset is an auspicious therapeutic target for bone loss.

Interdicting Effects of T Cells on Osteoclastogenesis

On the other hand, T cells are capable of mediating anti-osteoclastogenic signals in bone turnover. Hints that this modulation may occur come from *in vitro* studies demonstrating that osteoclastogenesis was inhibited by CD8⁺ T cells [27, 28]. Moreover, after activation, mouse CD8⁺ T cells showed delayed kinetics of RANKL expression, as compared with corresponding CD4⁺ T cell [29]. Depletion of CD4⁺ and CD8⁺ T cells in mice enhanced vitamin D3-stimulated OC formation via a mechanism involving decreased osteoprotegerin (OPG) production [30]. The protective role of T cells on bone

homeostasis was also clearly documented by other *in vivo* studies that showed that both B cell- and T cell-deficient mice had decreased BMD [31]. Furthermore, researchers found OPG production in the BM by B cells stimulated by T cells through CD40L/CD40 interactions prevented osteoporosis. IFN- γ produced by T cells potently suppressed RANKL signaling by accelerating the degradation of TRAF6 by activating the ubiquitin/proteasome system [14]. Additionally, anti-CD3 and anti-CD28-Ab activated T cells and inhibited osteoclastogenesis, while activation of T cells by staphylococcal enterotoxin A, phytohemagglutinin and concanavalin A had inconsistent effects.

T Helper Cells and Osteoclastogenesis

Depending on the manner in which they are activated, T cells can mediate both osteoclastogenesis and anti-osteoclastogenesis [32]. The net effect of T cells on osteoclastogenesis depends on the balance between positive and negative factors expressed by the T cells. While the CD4⁺ Th cell subsets Th1 and Th2 produce IFN- γ and IL-4, respectively, both of which are anti-osteoclastogenic. It is unclear how activated CD4⁺ Th cells in arthritis enhance osteoclastogenesis in the presence of these cytokines. Therefore, a need exists to define the very rare but pathologically important Th cell subset responsible for abnormal bone resorption, such as osteoclastogenic Th cells (Thoc cells). As indicated by Tomoki *et al*, the characteristics of Thoc cells should be the following: First, Thoc cells do not produce a large amount of IFN- γ . Second, they trigger local inflammation and the production of inflammatory cytokines, including TNF- α , to induce RANKL expression on synovial fibroblasts. Third, Thoc cells express RANKL and might directly participate in accelerated osteoclastogenesis [33]. If these Th cells have such characteristics as those above, they may be more disposed to osteoclastogenesis over anti-osteoclastogenesis.

The Effect of Cytokines and Chemokines Secreted from T Cells on Osteoclastogenesis

OCs arise by the cytokine-driven proliferation and differentiation of monocyte precursors that circulate within the hematopoietic cell pool [34]. Lymphocytes are important immune cells that can produce IL-1, IL-6, IL-17, RANKL, and TNF- α . Although osteoclastogenesis can be induced by TNF- α in a RANKL-dependent and -independent manner, IL-1 and IL-6 play roles in bone

resorption via the induction of RANKL. In the inflammatory conditions, lymphocytes also secrete a number of inhibitory molecules that directly inhibit OC formation, including OPG, IL-4, IL-10, IL-13, and IFN- γ . In addition to cytokines, many chemokines are directly or indirectly involved in regulating bone loss under pathological conditions, such as that which occurs during inflammation, infection, and estrogen deficiency.

RANKL and Osteoclastogenesis

RANKL, the ligand binds to the transmembrane receptor RANK, is expressed on the surface of OC, T cells, and other immune cells. RANKL does not affect cell proliferation but does promote the differentiation of OC precursors from an early maturation-stage into fully mature multinucleated OC. Moreover, RANKL can activate mature OCs thus stimulating these cells to resorb bone. A number of recent reviews about the diverse physiologic functions of the RANKL-RANK signaling axis in bone and its central roles in osteoimmunology have been published [33, 35, 36]. Therefore, we will focus on the new insight about this axis. Previous studies have shown RANKL can induce nuclear factor of activated T cells, cytoplasmic 1 (NFATc1), the master regulator of OC differentiation, by activating the TNF-receptor-associated factor (TRAF) 6, NF- κ B and the c-Fos pathways via RANKL-RANK stimulation [37, 38]. Deletion of NFATc1 in young mice resulted in osteopetrosis and inhibition of osteoclastogenesis *in vivo* and *in vitro* [39]. Consequently, NFATc1 regulates a number of OC-specific genes in cooperation with other transcription factors, such as AP-1, PU.1, and microphthalmia-associated transcription factor (MITF). Recently, Kim *et al* had showed that selective inhibition of a motif in RANK using the RANK receptor inhibitor peptide blocks RANKL-induced OC maturation and function both *in vitro* and *in vivo* by regulating cytoskeleton integrity and survival of OCs. The inhibitory action of the RANK receptor inhibitor peptide is TRAF6- and NFATc1-independent, suggesting an alternative pathway may exist for OC maturation and function that is independent of the RANK/TRAF6 axis as well as NFATc1 induction [40]. Also, Irie *et al* found bi-directional ephrinA2-EphA2 signaling regulated bone remodeling at the initiation phase in a way that was dependent on the transcription factor c-Fos but independent of the c-Fos target gene NFATc1 [41]. The ubiquitination of TRAF6 is an important mechanism mediating its signaling functions. But, how the ubiquitination and signaling

function of TRAF6 are regulated under physiological conditions, particularly during osteoclastogenesis, is incompletely understood. Lately, a de-ubiquitinating enzyme- CYLD, was found to negatively regulate RANK signaling by inhibiting TRAF6 ubiquitination and activation of downstream signaling events by interacting physically with the signaling adaptor p62 and thereby being recruited to TRAF6 [42]. c-Src kinase is a rate-limiting activator of OC function, which is central to osteoclast activity but not to differentiation. Lyn was confirmed as the second of the known Src family kinases to impact the OC that blunts osteoclastogenesis by suppressing RANKL-mediated Gab2 phosphorylation via activation of a SHP-1-dependent inhibitory signaling pathway [43]. Previous studies have highlighted the importance of NF- κ B in osteoclastogenesis, which has 5 members: RelA/p65, RelB, c-Rel, NF- κ B1/p50, and NF- κ B2/p52. However, none of the previous studies addressed the role of individual subunits in the OC response to RANKL. By using *Rela*^{-/-}*Tnfr1*^{-/-} mice, Vaira S et al found RelA/p65 promoted osteoclast differentiation by blocking a RANKL-induced apoptotic JNK pathway [44]. RANKL also binds to OPG, a soluble receptor antagonist for RANKL that prevents it from binding to and activating RANK, thus limiting OC formation and bone degradation [33]. OPG expression is regulated by many of the factors that regulate RANKL expression.

TNF- α and Osteoclastogenesis

TNF- α is one of the critical cytokines in the pathogenesis of bone loss, as shown by many gain- and loss-of function genetic models, as well as by the clinical efficacy of anti-TNF- α therapy [45]. Notably, transgenic mice that express human TNF- α spontaneously develop destructive arthritis. In patients, the anti-TNF- α antibody has been shown to suppress bone damage, suggesting TNF- α directly acts on bone independent of its action on the immune system [46]. Indeed, TNF- α stimulates OC formation and bone resorption *in vivo* and also enhances the formation of OC-like cells in BM culture. The ability of TNF- α to stimulate osteoclast formation in mixed stromal cell/osteoclast precursor cell cultures was dependent on IL-1 [47], whereas TNF- α -induced osteolysis was found to be M-CSF-dependent [48]. Recently, studies showed TNF- α contributed to inflammatory bone loss by enhancing the osteoclastogenic potential of osteoclast precursor cells through inducing paired Ig-like receptor-A (PIR-A), a co-stimulatory

RANK. Also, as the expression of PIR-As and PIR-A ligands was impaired, bone erosion and osteoporosis induced by aberrant TNF- α expression were ameliorated in mice [49]. Lee et al reported that macrophage-elicited osteoclastogenesis in response to bacterial stimulation requires Toll-like receptor 2-dependent TNF- α production [50]. Interestingly, WP9QY, a peptide that mimics this TNF receptor (TNFR) contact site and inhibits TNF- α -induced activity when bound to RANKL, prevented the increased osteoclastogenesis and bone loss induced in mice by ovariectomy or low dietary calcium. That latter case involved both wild-type and TNFR double-knockout mice, suggesting a peptide that mimics a TNFR ligand contact site blocks bone resorption by interfering with the recruitment and activation of OCs by both RANKL and TNF [51]. Clearly, this receptor could be a promising target for future therapeutic development.

IFN- γ and Osteoclastogenesis

IFN- γ , primarily produced by Th1 cells and NK cells, inhibits bone resorption *in vitro* by regulating the OC progenitors and also inhibits the ability of 1,25-dihydroxyvitamin D3, PTH, and IL-1 to stimulate the formation of OCL in cultures of human BM [52]. Recombinant IFN- γ rescues the defect in osteoclastogenesis in peripheral white blood cells from malignant osteopetrosis patients *in vitro* [53]. However, the effects of IFN- γ are controversial. Preexposure of OC precursors to RANKL renders them resistant to the inhibitory effects of IFN- γ by inducing terminal differentiation [54]. IFN- γ -producing human Th1 cells, but not IFN- γ -negative T cells, directly induce the differentiation of human macrophages into OC via expression of RANKL [55]. IFN- γ receptor^{-/-} mice fail to undergo ovariectomy-induced (ovx-induced) bone loss *in vivo*. Furthermore, IFN- γ production increases bone resorption induced by LPS in mice [56] and positively modulates actinobacillus actinomyces-temcomitans-specific RANKL⁺ CD4⁺ Th cell-mediated alveolar bone destruction [57]. Together, these data suggest that IFN- γ has both anti-osteoclastogenic and pro-osteoclastogenic properties *in vitro* and *in vivo*. Recently, Gao et al reported that IFN- γ promotes osteoclast formation through stimulation of antigen-dependent T cell activation and, that under conditions of estrogen deficiency, infection, and direct T cell activation by suppression of TGF- β signaling, the net effect of IFN- γ is the inducing of bone resorption and bone loss [58].

IL-17 and Osteoclastogenesis

IL-17, produced by the novel T helper subset named Th17, belongs to a family of related but unique cytokines with at least six members. The identification of six IL-17 family members (IL-17A-F) may extend the role of this novel cytokine family in the pathogenesis of chronic bone injury. IL-17A induces the production of pro-inflammatory mediators, such as IL-1, TNF- α , and the expression of RANKL to promote osteoclastogenesis and bone resorption. The effects of IL-17 on osteoclastogenesis and bone resorption are enhanced by TNF- α , which is also produced in the inflamed joints in patients with rheumatoid arthritis [59]. Inhibition of IL-17A in an antigen-induced arthritis model prevented the joint and bone destruction and decreased the production of RANKL, IL-1 α , and TNF- α in the involved lesions [60]. In fibroblast-like synoviocytes, IL-17 and TNF- α synergize to induce IL-23 p19 expression [61]. Not only can IL-17A synergize with these cytokines, it has direct activity as well. IL-17 production from activated T cells was required for the spontaneous development of destructive arthritis in mice deficient in the IL-1 receptor antagonist [62]. Collagen-induced arthritis was markedly suppressed in IL-17^{-/-} mice, suggesting IL-17 was responsible for the priming of collagen-specific T cells and collagen-specific IgG2a production [63]. Moreover, IL-17 receptor signaling has been identified as a critical pathway in turning an acute synovitis into a chronic destructive arthritis [64]. These observations strongly implicate IL-17A as an important mediator of arthritis. The results in human studies are consistent with the animal studies. IL-17 is spontaneously produced by RA synovial membrane cultures, and IL-17-producing cells were found in the T cell-rich area. High levels have been detected in the synovial fluid of patients with RA [65]. In addition, IL-17 protein has been detected in synovial tissue from children with inflammatory arthritis [66].

The pleiotropic effects of IL-17 on effector cells from the innate immune system contribute to the cartilage and bone damage seen in autoimmune arthritis. IL-17 synergizes with IL-1 and TNF- α to induce IL-8 and G-CSF, which stimulates neutrophil recruitment to the joint and granulopoiesis. Importantly, once the arthritis is initiated, IL-17 maintains disease independent of TNF- α [67]. Intriguingly, in contrast to monocytes activated *in vitro* with LPS, activated monocytes *in vivo* induced IL-17 expression in an IL-1 α /TNF- α -independent

manner, suggesting different pathways for Th17 responses *in situ* depending on the site or route of accessory cell activation [68]. Surprisingly, in collagen-induced arthritis, Notley *et al* reported that TNF- α was an important negative regulator not only of IL-17 but also of IFN- γ production by T cells, suggesting that this forms a part of a negative feedback loop that limits the intensity and/or duration of Th17 and Th1 responses [69]. However, by using intra-articular injection of lentivirus expressing shRNA for BAFF (TNF superfamily member B cell-activating factor) gene silencing, Lai *et al* showed that BAFF gene silencing inhibited proinflammatory cytokine expression, suppressed generation of plasma cells and Th17 cells, and markedly ameliorated joint pathology [70]. These effects indicate IL-17 is a potential new target for treating bone injury.

M-CSF, Some Other Cytokines and Osteoclastogenesis

M-CSF was first identified as an essential factor for osteoclastogenesis. It transmits its signal to the cell through the specific receptor cFMS, which is a member of the receptor tyrosine kinase superfamily. M-CSF, as a hematopoietic growth factor, is crucial for the proliferation, differentiation, and survival of OC progenitors and increases the survival of mature OC, mainly by activating extracellular-signal-regulated kinase through growth-factor-receptor-bound protein 2 and AKT through PI3K [33, 71]. Any dysregulation of RANKL and M-CSF expression leads to pathological conditions as both RANKL and M-CSF are essential for physiologic OC renewal. Some other cytokines, which are either produced or regulated by T cells, are responsible for the upregulation of OC formation. It is known that IL-1, IL-6 and other members of the gp130 cytokine family, IL-7, IL-15, IL-17 and IL-23 directly or indirectly promote osteoclastogenesis, whereas IFN- γ , IL-3, IL-4, IL-10, IL-13 and IL-12 alone and in synergy with IL-18, inhibit OC formation [72]. However, some cytokines function as both osteoclastogenesis and anti-osteoclastogenesis, such as IL-7 and IFN- γ . For a detailed list, see the review by Lee *et al* [72].

Chemokines and Osteoclastogenesis

Recruitment and homing of myeloid cells often occur under the direction of chemokines and their receptors. Chemokines, which can be divided into the CXC, CC, C, and CX3C subtypes based on

their sequence motif containing the first cysteine residue, act through G-protein-coupled receptors to initiate cytoskeletal rearrangement, adhesion, and directional migration. IL-8, a CXC chemokine produced by OC, stimulates osteoclastogenesis and bone resorption independent of the RANKL pathway, so as to be, in part, dependent on the up-regulation of nitric oxide synthase expression in the OC [52]. There could be some additional chemokines involved in regulating OC formation.

Concluding Remarks

Remarkable progress has been made using animal models to elucidate the cellular and molecular mechanisms governing homeostasis, the cross-talk between the immune system and bone, and the effects of sex steroids, infection, and inflammation leading to bone loss due to dysregulation of T lymphocyte function. If these relationships are equally relevant in humans as they are in rodents, osteoporosis may soon become classified as an inflammatory or autoimmune condition. And if these discoveries can be translated into humans, a door will be opened to exciting new potential therapeutic agents and strategies that target the bone-immune interface to ameliorate bone disease in numerous osteoporotic conditions. However, despite extensive cross-regulation between bone metabolism and the immune system, a number of questions remain, such as the mechanisms by which these systems cross-regulate. As the bone and the immune system are so closely intermingled, all factors that regulate immune cells should be investigated for their effect on bone and vice versa. The fresh insights gained from osteoimmunology will eventually lead to targeted therapies for diseases that affect either or both systems.

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