

## Interactions between the immune system and bone

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### Abstract

The relationship between the immune system, estrogen deficiency and bone loss is an intriguing and, as yet, unexplained challenge of the past two decades. Here we summarize the evidence that links immune cells, inflammation, cytokine production and osteoclast formation and activity with particular regard to humans.

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### INTRODUCTION

The bone, hematopoietic and immune systems are in deep physical contact and share several common pathways. Inflammatory disease characterized by systemic and local bone loss is an interesting field with which to explore the relationship between activation of the immune system and bone remodelling. In the last few years, investigators have shed light on this topic and T cells have been recognized as key regulators of osteoclast (OC) and osteoblast (OB)<sup>[1]</sup> formation and activity in different pathological conditions, such as osteoporosis<sup>[2]</sup>, rheumatoid arthritis (RA)<sup>[3]</sup>, bone metastasis<sup>[4,5]</sup> and periodontitis<sup>[6,7]</sup>.

Estrogen deprivation induces bone loss. At the same time estrogens are well known regulators of the immune system and T cell functions<sup>[8,9]</sup>. Thus the immune system can be suggested as a key interface between estrogen deprivation and bone metabolism.

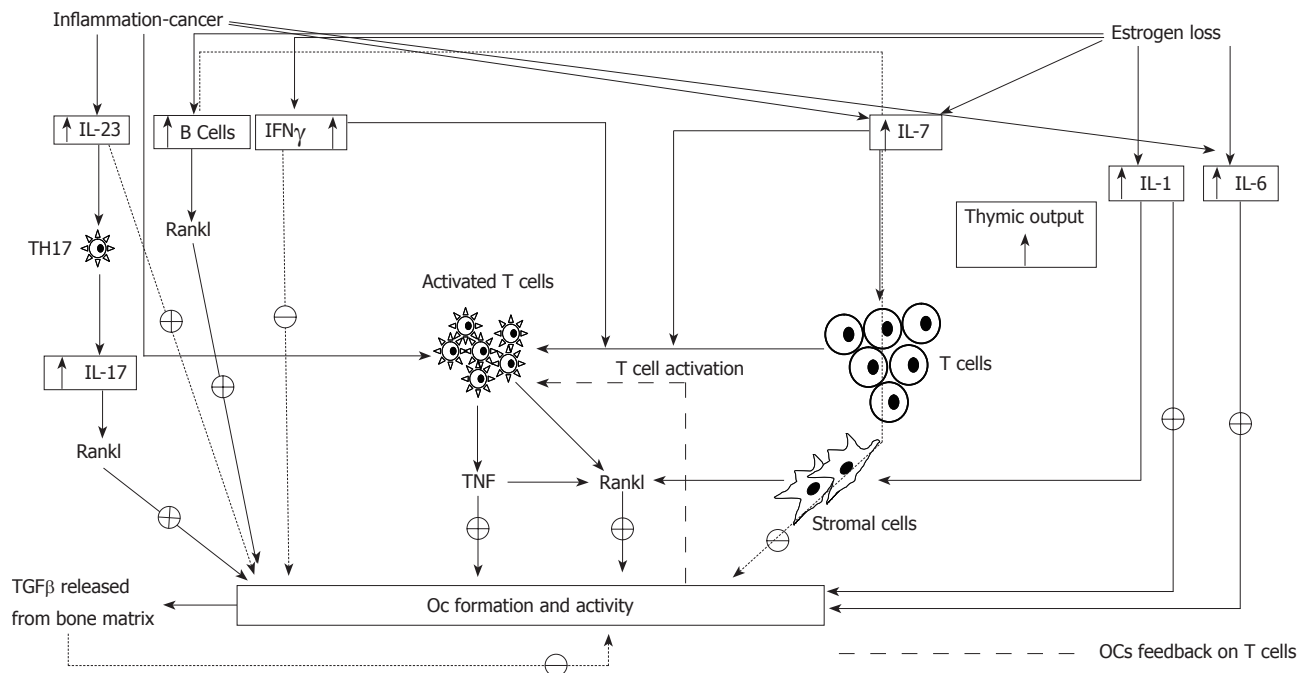
The role of the immune system in bone resorption is still controversial: studies on humans are few and the majority of the data have been derived from animal models and cellular cultures. This review aims to summarize the evidences linking immune activation and bone loss with particular attention to humans.

#### Cytokines and OC formation

The Receptor Activator of NFκB Ligand (RANKL) and Macrophage Colony Stimulating Factor (M-CSF) are produced by bone marrow stromal cells<sup>[10]</sup>, OBs<sup>[11]</sup> and activated T cells<sup>[2,12]</sup>. The co-stimulation by RANKL and M-CSF is essential for the differentiation of monocytes into OCs<sup>[13-15]</sup>.

M-CSF induces the proliferation of OC precursors, differentiation and fusion of more mature OCs and increases the survival of mature OCs. RANKL promotes the differentiation of OC precursors into fully mature multinucleated OCs and stimulates the capacity of mature OCs to resorb bone.

RANKL is a member of the TNF superfamily, present both as a transmembrane and in secreted form. It binds to its physiological receptor RANK expressed



**Figure 1** Multiple cytokines have a role in the regulation of OC formation and activity. Their complex interactions are important in explaining bone loss after menopause or in inflammatory diseases.

on the surface of OC lineage cells. Its action is opposed by osteoprotegerin (OPG), a neutralizing soluble decoy receptor, produced by marrow stromal cells and OBs<sup>[15]</sup>. Estrogen deficiency induces the imbalance between RANKL and OPG; this phenomenon is important in the genesis of post-menopausal bone loss<sup>[15,16]</sup>.

Some studies questioned the central role of RANKL and suggested the hypothesis that activated T cells could induce osteoclastogenesis by an independent mechanism since saturating concentrations of OPG failed to neutralize more than 30% of OC formation induced by activated T cells<sup>[17]</sup>. Rifas and Weitzmann discovered a novel cytokine called Secreted Osteoclastogenic Factor of Activated T cells (SOFAT) in activated T cell medium. This cytokine induces both osteoblastic IL-6 production and functional OC formation in the absence of OBs or RANKL, insensitive to the effects of OPG.

The demonstration that SOFAT is a potent inducer of IL-6 production by OBs suggests that it could play a significant role in the local inflammatory response and also could exacerbate bone destruction in rheumatoid arthritis indirectly through multiple IL-6-mediated events<sup>[18]</sup>.

Estrogen deficiency induces bone loss through a complex modification of cytokine production balance. Primarily, researchers observed increased TNF $\alpha$  production by T cells both in ovariectomized mice<sup>[19]</sup> and post-menopausal women<sup>[2,20]</sup>. TNF $\alpha$  enhances OC formation by up-regulating stromal cell production of RANKL and M-CSF and by increasing the responsiveness of OC precursors to RANKL<sup>[5,21]</sup>. Besides, other studies showed a key role of T cell-produced TNF $\alpha$  in rheumatoid arthritis<sup>[3,22]</sup>, multiple myeloma<sup>[23,6]</sup> and bone metastasis<sup>[4,5]</sup>. The effect of TNF $\alpha$  on osteoclastogenesis is up-regulated by IL-1<sup>[24]</sup>; this cytokine production increases after meno-

pause<sup>[20,25]</sup>, enhancing RANKL expression by bone marrow stromal cells and directly promoting OC differentiation. In fact, treatment with IL-1 receptor antagonist decreases OC formation and bone resorption in ovariectomized mice<sup>[26,27]</sup>, whereas the blockade of both TNF $\alpha$  and IL-1 reduce bone resorption in post-menopausal osteoporosis<sup>[28]</sup>.

IFN $\gamma$  role in osteoclastogenesis has been hard to define: this cytokine has an anti osteoclastogenic effect *in vitro*<sup>[29]</sup> and *in vivo* in nude mice models<sup>[30,31]</sup>. Studies in humans indicate an increased level of IFN $\gamma$  during estrogen deficiency<sup>[32,33]</sup>, in leprosy and rheumatoid arthritis with bone erosions<sup>[34,35]</sup>. Moreover, data from randomized controlled trials have shown that IFN $\gamma$  does not prevent bone loss in patients with RA<sup>[36,37]</sup> nor the bone wasting effect of cyclosporin A<sup>[1]</sup>. These data are explained by the finding that IFN $\gamma$  influences OC formation both via direct and indirect effects<sup>[32]</sup>. It directly blocks OC formation targeting maturing OC<sup>[38]</sup> and also induces antigen presentation and thus T cell activation. When IFN $\gamma$  levels are increased *in vivo*, activated T cells secrete pro-osteoclastogenic factors and this activity offsets the anti-osteoclastogenic effect of IFN $\gamma$ <sup>[39]</sup>.

T cells also produce interleukin-7 (IL-7), a cytokine able to enhance B and T cell number and reactivity to antigenic stimulus<sup>[40,41]</sup>. Some studies have demonstrated that IL-7 promotes osteoclastogenesis by up-regulating T cell-derived osteoclastogenic cytokines including RANKL<sup>[42,44]</sup> and that the production is up-regulated by estrogen deficiency. *In vivo* IL-7 blockade is proven to suppress T cell expansion and TNF $\alpha$  and IFN $\gamma$  production, preventing bone loss due to estrogen deprivation<sup>[45,46]</sup>. In healthy humans, the expression of IL-7 receptors on T lymphocytes is strictly related to their ability to induce OC formation from peripheral blood

mononuclear cells (PBMC)<sup>[47]</sup>. A comprehensive summary of these pathways can be found in Figure 1.

### **Inflammation and bone loss**

Both acute and chronic inflammatory diseases in the periosteal tissues are well known to cause bone damage as inflammation increases the OC number and activity. The activation of T cells in autoimmunity or during infection increases RANKL production and promote osteoclastogenesis. The process can be reverted by the administration of OPG in several pathological conditions such as RA and periodontitis<sup>[48]</sup>. Particularly in autoimmune arthritis, T cell subsets have been examined and Th17 cells, a specialized inflammatory subset, have been identified as responsible for osteoclastogenesis regulation. An inflammatory milieu induces naïve T cells to differentiate into Th17, capable to produce RANKL, TNF $\alpha$  and IL-17, a cytokine that increases RANKL expression by OBs<sup>[49]</sup>.

Researchers recently began to investigate a possible direct role of dendritic cells (DC) in inflammation-related bone damage. DCs are known for their role of antigen presenting cells (APCs) and do not appear to play a role in bone homeostasis in non-pathological conditions, but some data suggest that DC could act as OC precursors in an inflammatory milieu, transforming into DC-derived-OC according to phenotypic and functional characterization studies. Moreover, DCs modulate T cell activity through RANK/RANKL pathway and other cytokines associated with osteoclastogenesis<sup>[50-52]</sup>. There is a lack of definitive evidence about the physiological relevance of this phenomenon *in vivo* but DCs could act as an osteo-immune interface, contributing to bone loss in inflammatory diseases.

On the other hand investigators have focused on the role of B-lymphocytes in periodontal inflammation. The host immune response is partly responsible for the bone destruction in cases of periodontitis and the RANK/RANK/OPG signalling axis is important both in bone and immune system communication. Data suggest that B-lymphocyte involvement in the adaptive immune response contributes to bone resorption by up-regulating of RANKL expression through Toll-like receptor pathways. These data align with the known ability of T cells to produce RANKL in the presence of immune stimulus and to increase osteoclastogenesis<sup>[53]</sup>.

Other studies focused on psoriatic arthritis, a chronic inflammatory disease characterized by joint erosions mediated by OCs. These OCs seem to derive from CD14+CD16+ circulating monocytes, present at higher level in patients than in healthy controls when exposed to OC-promoting microenvironment (M-CSF and RANKL). OCs do not derive from this population in healthy controls; thus CD16 can be considered a marker of OC precursors in arthritis<sup>[54]</sup>.

### **OC modulates T cell activity**

OC precursors circulate within the mononuclear fraction of peripheral blood<sup>[2,4,55-57]</sup>. This population acts not only as a reservoir for replenishing the pre-OC pool in the

bone marrow but also as a potentially abundant source of pre-OCs that can be recruited into bone or joint tissue in response to reparative or pathological signals. On this basis OCs can be considered as immune cells attracted in bone by stimulatory cytokines, expressed on accessory cells and undergoing specific differentiation.

For a long time, osteoimmunology focused on OC regulation by T cells. Recently investigators have paid attention to the feedback action of OCs on T cells. Kiesel *et al.* observed that OCs are able to present antigenic peptides to T cells and induce FoxP3 expression in CD8+ T cells, thus originating Treg CD8+ cells able to regulate inappropriate activation of the immune response<sup>[58]</sup>.

Senthilkumar *et al.*<sup>[59]</sup> suggest that the cellular responses in cell-to-cell interactions between T cells and OCs are regulated through reciprocal CD137/CD137L and RANK/RANKL interactions. CD137 is a co-stimulatory member of the TNF receptor induced by T cell receptor activation, characterized by the ability to transduce signals in both directions, through the receptor and into the cell that expresses the ligand. Its ligand CD137L is expressed on APCs and OC precursors; *in vitro* CD137L ligation suppresses osteoclastogenesis through the inhibition of multi-nucleation. On the other hand, RANKL expressed on T cells bind to RANK on OCs, producing a reverse signal in T cells able to enhance apoptosis.

Periprosthetic osteolysis is another important research field to understand the reciprocal interactions of OC and T cells. Periprosthetic osteolysis patients show T cell-dependent osteoclastogenesis in PBMC cultures; in fact the process was inhibited by RANK-Fc and T cell depletion<sup>[60]</sup>. In periprosthetic tissues local CD8+ T cells showed a regulatory phenotype, expressing CD25 and FoxP3, while CD4+ T cells did not express activation markers. These data suggest that in an early stage T cells promote osteoclastogenesis, while subsequently OCs activate FoxP3/CD8+ T cells which inhibit CD4+ effector T cells.

### **Estrogen loss and immune system**

The role of estrogen in the regulation of immune function has been demonstrated in animals and humans: immune cells are more responsive to antigenic stimulus in hormone replacement therapy users than non-users<sup>[61]</sup>.

Our group demonstrated that T cells from postmenopausal women show blunt reaction to immune stimulation in respect to pre-menopausal healthy women. At baseline, T cells are more active than in healthy post- and pre-menopausal controls: this implies their greater ability to produce RANKL and TNF $\alpha$ , thus inducing OC formation and activity<sup>[2]</sup>. We have also demonstrated that OC formation is abolished in T cell-depleted PBMC cultures and this phenomenon is reversed only by the addition of M-CSF and RANKL in cultures.

Grcevic *et al.*<sup>[62]</sup> suggested a probable role for resting T cells in blunting OC formation; CD4+ and CD8+ T cell-depleted mice have an increased OC formation rate since OPG production is suppressed. T cell-deficient

mice show an increased number of OC in basal conditions and a reduced bone density when compared to controls<sup>[42]</sup>.

Estrogen withdrawal up-regulates TNF $\alpha$  production by T cells through a complex pathway involving the thymus and bone marrow. In the bone marrow, ovariectomy promotes T cell activation by increasing antigen presentation by macrophages and DCs<sup>[33,63]</sup>. Several studies showed the central role of T cells-produced TNF in bone loss induced by estrogen deficiency; in the murine model ovariectomy increases the number of bone marrow T cell-producing TNF<sup>[19,64,65]</sup>. In fact, ovariectomy stimulates the expression of the gene encoding Class II TransActivator (CIITA), a transcriptional coactivator acting on the MHCII promoter, with the final effect of up-regulation of expression of MHCII on macrophages<sup>[33,66,67]</sup>. This process proved essential since data show that ovariectomy induces rapid bone loss in wild type (wt) mice but failed to do so in TNF $\alpha$ -deficient [TNF $\alpha$  (-/-)] mice and in T cell-deficient nude mice. Bone loss was restored by adoptive transfer of wt T cells but not by reconstitution with T cells from TNF $\alpha$  (-/-) mice<sup>[19]</sup>, the bone-wasting effect of TNF $\alpha$  is mediated by the p55 TNF $\alpha$  receptor. In fact, ovariectomy caused bone loss in wt mice and in mice lacking p75 TNF $\alpha$  receptor but failed to do so in mice lacking the p55 TNF $\alpha$  receptor<sup>[19]</sup>.

Moreover, RANKL-expression on lymphocytes and marrow stromal cells is significantly elevated during estrogen deficiency in humans and correlates directly with increases in bone resorption markers and inversely with serum estrogen levels<sup>[16]</sup>.

These data demonstrate the causal relationship between estrogen deprivation, T cell activation with increased production of cytokines, and bone resorption.

Estrogen withdrawal has effects on the B cell compartment as well. In ovariectomized mice B220+ IgM-population expands and can differentiate into OC<sup>[68-70]</sup>. Activated B cells over-express RANKL, contributing to bone resorption<sup>[71]</sup>.

## CONCLUSION

Bone is a dynamic tissue that is constantly formed and resorbed; bone turnover is due to continuous and cyclic bone resorption followed by apposition. These processes are due to the coordinated actions of OC and OB. The action of these two cell types is regulated by paracrine and endocrine factors. In physiological conditions, OB and OC activity is coupled so that the amount of resorption is equal to the amount of formation. However, in pathological conditions or during senescence, resorption is higher than formation, leading to bone loss. OC and OB formation, as well as the coupling between these two cell types, are mediated mainly through cytokines.

Cytokines have pleiotropic functions and regulate several organs and systems. In the last decade several investigators have paid attention to the relationship between estrogen withdrawal, cytokines production, the

immune system and skeleton. Today the majority of the data have been obtained in animal models but in recent years, new evidence has been accumulated in humans towards a profound link between estrogen deprivation, immune system deregulation and bone loss. If this relationship is confirmed by future work, postmenopausal osteoporosis should be regarded as an inflammatory disorder sustained by a chronic mild decrease in T cell tolerance.

The relationship between the immune system and bone is complex and depends upon cytokine production and cell to cell contacts. T cells and OCs appear to be the main players in the mechanisms of bone loss. Future studies are needed to fully understand these relationships in humans.

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