The role of the immune system in the physiopathology of osteoporosis

Valentina Cagnetta
Vittorio Patella

Department of Clinical Methodology
and Surgical Techniques, Orthopedics Section II,
University of Bari "Aldo Moro", Bari, Italy

Address for correspondence:
Valentina Cagnetta, MD
Department of Clinical Methodology and
Surgical Techniques, Orthopedics Section II
University of Bari "Aldo Moro"
Piazza Giulio Cesare, 11
70100 Bari, Italy
Phone/Fax: +39 080 5592719
E-mail: valentinaca@hotmail.it

Summary

The close anatomical relationship between the immune system, estrogen deficiency and bone loss has been recognized for centuries but the existence of a functional relationship has emerged only recently. The role of the immune system in the development of senile osteoporosis, which arises primarily through the effects of estrogen deficiency and secondary hyperparathyroidism, is slowly being unraveled. This review focuses the evidence that links immune cells, inflammation, cytokine production and osteoclast formation and their activity. The under standing of the interplay of inflammation and osteoclast can lead to the development of new drugs for prevention and treatment of bone loss.

KEY WORDS: osteoporosis; immune system; osteoclast; cytokines; T cells; B cells.

Introduction

Bone provides structural integrity for the body, it is the site of hematopoiesis and it is a storehouse for calcium and phosporus (1). Likewise, the immune system provides organism with protection from invading pathogens (2). Multiple overlapping and interacting mechanisms have envolved to regulate both systems. The osteoblasts are critical regulator of the hematopoietic stem cell niche from which all blood and immune cell derive, the osteoclast appear to share a common origin with the myeloid precursor cells that also gives rise to macrophages and myeloid dendritic cells. Multiple soluble mediators of immune cell function including cytokine, chemochines and growth factor also regulate osteoblast and osteoclast activity (3). 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. Estrogen deprivation induces bone loss. At the same time estrogens are well known regulators of the immune system and T cell functions (4, 5). Thus the immune system can be suggested as a key interface between estrogen deprivation and bone metabolism. This review aims to summarize the evidences linking immune activation and bone loss with particular attention to humans.

Estrogen loss and immune system

The immune system plays a critical role in the pathophysiology of postmenopausal osteoporosis. Estrogen deficiency induces bone loss and at the same time estrogen mild increase in production of proinflammatory cytokines, such as IL-1, IL-6, and TNF-alpha, systemically and locally, which promotes osteoclastogenesis in the bone marrow because they are well known regulators of the immune system and T cell function (6). The most important cytokine in the context of estrogen deficiency-induced bone loss has been shown to be TNFα produced by bone marrow T-lymphocytes that are stimulated through a complex mechanism involving antigen-presenting cells and various cytokines including IL-7, IFN-gamma, and TGF-beta. Proinflammatory cytokines also suppress osteoblastogenesis, while the crosstalk between T-lymphocytes and marrow stromal cells regulates the osteoclastogenic activity of the latter. Estrogen plays a fundamental role in skeleton growth and bone omeostasis and 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 (7).
D'Amelio et al. demonstrated that T cells from post- menopausal 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 (8). They 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.
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 (9, 10). The relevance of TNF in the mechanism by which estrogen causes bone loss has been demonstrated using multiple animal model. For example in the murine model ovariectomy increases the number of bone marrow T cell-producing TNF (11-13). The effects of ovx on antigen presentation and the resulting changes in T-cell activation, proliferation, and lifespan are explained by a stimulatory effect of ovx on the expression of the gene-encoding class II transactivator (CIITA). The product of the CIITA gene is a non-DNA binding factor that functions as a transcriptional co-activator, when recruited to the MHCII promoter by interaction with promoter-bound factors (14). CIITA expression is required and sufficient for the stimulation of antigen presentation in bone marrow monocytes with the final effect of up-regulation of expression of MHCII on macrophages (9, 15, 16). This process proved essential since data show that ovariectomy induces rapid bone loss in wild type mice but failed to do so in TNFα-deficient [TNFalpha (-/-)] mice and in T cell-deficient nude mice.

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 (17).
Estrogen withdrawal has effects on the B cell compartment. B cells have recently been directly implicated in the regulation of bone resorption as they represent a major source of OPG. Historically, an extensive *in vitro* data set has led to the widely accepted precept the mayor source of BM OPG is the OB and/or its immediate precursor, the BM SC. More recent data have show that B cell are more likely the dominant producers of OPG in the bone microenvironment *in vivo* (18). This conclusion was arrived at following an extensive series of investigations into the bone phenotype of B cell Knockout (KO) mice (18). B cell KO mice present at baseline with an osteoporotic phenotype (19), a consequence of enhanced osteoclastic bone resorption. Examination of the RANKL/OPG ratio B cell KO bone marrow identified a specific deficiency in OPG mRNA and in the protein expression (18). Reconstitution of young B cell KO mice with B cell by means of adoptive transfer, completely rescued mice from development of osteoporosis, by normalizing OPG production (18). B cells play an important role in regulating basal OC formation and in regulating bone omeostasis.

Cytokines and OC formation

OCs arise by citokine-driven proliferation and differentation of monocyte precursors that circulate within the hematopoietic cell pool. The minimal essential cytokines required for OC formation under basal conditions are RANKL and M-CSF. The Receptor Activator of NFkB Ligand (RANKL) and Macrophage Colony Stimulating Factor (M-CSF) are produced by bone marrow stromal cells (20), OBs (21) and activated T cells (2, 22). The co-stimulation by RANKL and M-CSF is essential for the differentiation of monocytes into OCs (23, 24).
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 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 (23).
Estrogen deficiency induces the imbalance between RANKL and OPG; this phenomenon is important in the genesis of post-menopausal bone loss (24, 25).
Some studies questioned the central role of RANKL. *In vivo* RANKL-deficient mice have significant osteopetrosis and no osteoclasts, but a normal number of monocyte/macrophages (26). These mice also exhibit failed tooth eruption, which is a common defect associated with developmental osteopetrosis, and diversion of hematopoiesis to the spleen and liver because a functional bone marrow cavity fails to form in the absence of osteoclasts (26, 27). Marrow stromal and osteoblastic cells produce RANKL, and regulation of its mRNA expression in murine marrow cell cultures correlates with activation of osteoclastogenesis (28).
RANK-deficient mice were demonstrated to phenocopy the defect in osteoclast development that was observed in the RANKL-knockout mouse, confirming the exclusive specificity of RANKL for osteoclast-expressed RANK (29).
Estrogen deficiency induces bone loss through a complex modification of cytokine production balance. Primarily, researchers observed increased TNFalpha production by T cells both in ovariectomized mice (11) and post- menopausal women (2, 30). TNFalpha enhances OC formation by up-regulating stromal cell production of RANKL and M-CSF and by increasing the responsiveness of OC precursors to RANKL (6, 31).
Like TNF, interleukin (IL)-1 promotes RANKL expression by bone marrow stromal cells and OBs and stimulates OC lifespan and activity. IL-1 directly targets OC precursors and promotes OC differentation in the presence of permissive levels of RANKL. Recent evidence has also shown that IL-1 mediates, in part, the osteoclastogenic effect of TNF and does so by enhancing stromal cell expression of RANKL and by directly stimulating differentiation of OC precursors (32). TNF and IL-1 engage initially distinct signaling pathway that converge with the activation of the transcription factor NF-*k*B and the stimulation of the miogeno-activated protein kinase (MAPK) system. Thus, the combined effect of these two cytokines provides a potent signal to osteoclastogenesis, inhibition of OB function, and regulation of the lifespan of skeletal cells.
An important yet controversial OC regulating factor is interferon (IFN)-gamma. This cytokine has an anti osteoclastogenic effect *in vitro* (33) and *in vivo* in nude mice models (34, 35). Studies in humans indicate an increased level of IFNgamma during estrogen deficiency (36, 39), in leprosy and rheumatoid arthritis with bone erosions (37, 38). Moreover, data from randomized controlled trials have shown that IFNgamma does not prevent bone loss in patients with RA (39, 40) nor the bone wasting effect of cyclosporin A (41). These data are explained by the finding that IFNgamma influences OC formation both via direct and indirect effects (36). It directly blocks OC formation targeting maturing OC (42) and also induces antigen presentation and thus T cell activation. When IFNgamma levels are increased in vivo, activated T cells secrete pro-osteoclastogenic factors and this activity off- sets the anti-osteoclastogenic effect of IFNgamma (43).
Another cytokine relevant for OC formation is interleukin-7 (IL-7). Some studies have demonstrated that IL-7 promotes osteoclastogenesis by up-regulating T cell-derived osteoclastogenic cytokines including RANKL (44, 45) and that the production is upregulated by estrogen deficiency. *In vivo* IL-7 blockade is proven to suppress T cell expansion and TNFalpha and IFNgamma production, preventing bone loss due to estrogen deprivation (46, 47). 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 (48).

Osteoporosis and inflammation

Several inflammatory diseases such as rheumatoid arthritis, sistemic lupus erythematosus, inflammatory bowel disease, celiac disease, cystic fibrosis and chronic obstructive pulmonary disease have been associated to bone resorption. The link between osteoclast, macrophage colony stimulating factor and pro-inflammatory cytokines, especially TNFalpha and IL-1 explain the association between inflammation and osteoporosis. 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 (49). 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 naive T cells to differentiate into Th17, capable to produce RANKL, TNFalpha and IL-17, a cytokine that increases RANKL expression by OBs (50).
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 (51-53). 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 re- sponse contributes to bone resorption by up-regulating of RANKL expression through Toll-like receptor path- ways. These data align with the known ability of T cells to produce RANKL in the presence of immune stimulus and to increase osteoclastogenesis (54).

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 (55).

Conclusion

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 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 postmenopausal osteoporosis should be regarded as an inflammatory disorder sustained by a chronic mild decrease in T cell tolerance. 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. The fresh insights gained from osteoimmunology will eventually lead to targeted therapies for diseases that affect either or both systems.

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