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Immune System and Postmenopausal Bone Loss

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Abstract The relation between immune system, estrogen deficiency and postmenopausal bone loss is an intriguing and yet unexplained challenge of the past two decades. Here we summarize the evidences that link estrogen deficiency, T and B cells proliferation and activation, cytokines production, and bone loss with particular regard to humans.

Keywords Estrogen · Menopause · Osteoclast · T cells · B cells · Cytokines · Osteoporosis

Introduction

It is well known that diseases characterized by inflammation and activation of immune system are associated with systemic and local bone loss [1, 2]. Similarly, estrogens are well-known regulators of the immune system and T cells functions [3, 4]. Despite these observations, it is only in the last few years that investigators have paid attention to the interaction between immune system and postmenopausal bone loss and it is only in the recent years that T lymphocytes have been recognized as key regulators of osteoclast (OC) and osteoblast (OB) formation and activity [5, 6].

The role of T cells in bone metabolism is still controversial; the majority of the data have been obtained in mice while little information is available regarding the role of T cells in human bone loss. Human studies show a key role of T cell produced TNF in rheumatoid arthritis [7], multiple myeloma [8, 9], and bone metastasis [10, 11]; our group recently suggested a key role of T cells also in postmenopausal bone loss [12]. Other reports show that estrogen deficiency increases the production of TNF and RANKL by bone marrow cells, including T cells, and that their increase correlates with indices of bone resorption [13–15].

The majority of the data have been obtained in animal models and in vitro cultures, while data on humans are few, this review aims to summarize the evidences that link estrogen deprivation, immune function, and bone loss with particular attention to humans.

Estrogen Loss and OC Formation

Estrogen loss increases bone resorption acting mainly through cytokine-driven increases in OC formation; OC formation occurs when monocytes are co-stimulated by the essential osteoclastogenic factors receptor activator of NFκB ligand (RANKL) and macrophage colony stimulating factor (M-CSF) [16–18]. These cytokines are produced by bone marrow stromal cells [15], OBs [19], and activated T cells [12, 20].

RANKL is a member of the TNF super family that is present as both a transmembrane molecule and a secreted form; it binds to its physiologic receptor RANK, which is 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 OB [16]. The unbalance between RANKL and OPG has been indicated as the pivotal mechanism responsible for estrogen deficiency bone loss [14, 18].

M-CSF induces the proliferation of OC precursors, the differentiation and the fusion of more mature OCs, and increases the survival of mature OCs. RANKL promotes the differentiation of OC precursors into fully mature

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multinucleated OCs and stimulates the capacity of mature OCs to resorb bone.

Additional inflammatory cytokines are responsible for the upregulation of OC formation observed during estrogen deficiency; some of these molecules have a well-established role in osteoclastogenesis and bone loss, while others have not. Among these molecules, the most involved in estrogen deficiency bone loss appears to be TNFα, IL-1, IL-7, and IFNγ.

TNFα

TNFα is a factor which enhances OC formation by up-regulating stromal cells production of RANKL and M-CSF, and by augmenting the responsiveness of OCs precursors to RANKL [10, 21]. Furthermore, TNF directly induces marrow precursor's differentiation into OCs in the absence of RANKL, although according to some studies it is not osteoclastogenetic in cells not previously primed by RANKL [22]. TNF increases after menopause both in mice [23] and in humans [12, 24], and it is produced mainly by activated T cells. A recent paper suggests that in vivo blockade of TNF in postmenopausal osteoporosis reduce bone resorption [25], as in rheumatoid arthritis [26], this suggests that TNFα increase could be one of the mechanism responsible for postmenopausal bone loss.

IL-1

It has been demonstrated that IL-1 plays an important role in bone loss induced by estrogen deficiency, particularly it has been demonstrated that IL-1 levels increase after menopause, that this increase is reversed by estrogen replacement in women [24, 27] and that bone loss does not occur after ovariectomy in mice deficient in receptors for IL-1 [28]. Moreover, treatment with IL-1 receptor antagonist decreases OC formation and bone resorption in ovariectomized mice [29, 30]. A recent study demonstrate that the blockade of both TNF and IL-1 reduce bone resorption in postmenopausal osteoporosis [25].

IL-1 acts by increasing RANKL expression by bone marrow stromal cells and directly targets OC precursors and promotes OC differentiation in the presence of permissive levels of RANKL. The effect of TNF on osteoclastogenesis is upregulated by IL-1 [31].

IL-7

Recently a central role in bone remodeling has also been postulated for IL-7 [10, 32–34], which is a cytokine known for its ability to stimulate T and B cell number and reaction to antigenic stimuli in humans [35]. We and others demonstrated that IL-7 is also produced by T cells in postmenopausal women [36] and in cancer patients [10].

Some studies have demonstrated that IL-7 promotes osteoclastogenesis by upregulating T cell-derived osteoclastogenic cytokines; including RANKL [32, 36, 37] and that the production of IL-7 is upregulated by estrogen deficiency [32, 33, 38]. A recent study in mice suggest that IL-7 increases OC formation by increasing OC precursor generation, presumably through an action on the cells attached to bone rather than on cells contained in the bone marrow [38]. On the contrary, recent studies on IL-7 receptor-deficient mice suggest that IL-7 has an anti-osteoclastogenic effect in vivo, in particular the authors suggest that IL-7 deficiency in mice caused increased OC number in bone and decreased bone mass and that OVX-induced bone loss in these mice occurred in trabecular, but not cortical bone [39, 40].

As regards humans, the results are less controversial, in particular it has been suggested that IL-7 is osteoclastogenic in psoriatic arthritis [41] and in patients affected by solid tumors [10, 42, 43], also in healthy volunteer the expression of IL-7 receptor on T lymphocytes correlates with their ability to induce osteoclastogenesis from human monocytes [44].

IFNγ

The effect of IFNγ on OC formation and activity is controversial. IFNγ behave like an anti-osteoclastegenic cytokine in vitro [45], in vivo in nude mice [46], and in a knockout model in which the onset of collagen-induced arthritis is more rapid as compared with wild-type controls [47].

These data are not confirmed by studies in humans and in experimental models of diseases that indicate an increased level of IFNγ during estrogen deficiency and endotoxin-induced bone disease [48, 49].

In humans, IFNγ is positively correlated with bone erosions in leprosy and rheumatoid arthritis [50, 51]. Moreover data from randomized controlled trials have shown that IFNγ does not prevent bone loss in patients with rheumatoid arthritis [52, 53], nor the bone wasting effect of cyclosporin A [54].

In humans, it has been suggested to employ IFNγ in the treatment of osteopetrosis. In this condition, IFNγ is able to restore bone resorption [55].

Taken together, the data in humans suggest that, in some conditions, including estrogen deficiency, IFNγ stimulates bone resorption. These discrepancies could be explained by the fact that IFNγ influences OC formation both via direct and indirect effects [48]: it directly blocks OC formation

targeting maturing OC [56] and induce antigen presentation and thus of T-cell activation in vivo. 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.

Estrogen Loss and Immune System

Plenty of data suggest that sex hormones have an important role in the regulation of immune function. Estrogen receptors have been demonstrated on human blood mononuclear cells, splenocytes, thymocytes, and peripheral T cells more than 20 years ago [57, 58]. The addition of estradiol to in vitro cultures of human lymphocytes can enhance immunoglobulin secretion [59], and in vivo 17β-estradiol treatment causes an augmentation of antibody production against double-stranded DNA in mice [60].

Estrogen loss is effective in expanding the pool of B lineage cells and particularly of B220 + IgM- cells in mice [34, 61], these cells has been regarded as OC precursors at least in animal models. Estrogen deficiency is supposed to increase the number of B cells trough the increased levels of IL-7.

How B-lineage cells may lead to bone destruction is not presently understood, but may involve over expression of RANKL in activated B cells [62]. Furthermore, early B220 + IgM- precursor cells have been found to be capable of differentiating into OCs in response to M-CSF and/or RANKL in vitro [63, 64] and may thus contribute to increase the pool of OC precursors.

Clinically, both cellular and humoral immune responses have been found to be higher in hormone replacement therapy users than non-users [65], we recently demonstrated that T cells from postmenopausal women are less prone to immune stimulation as respect to pre-menopausal healthy women [12]. Similarly, there are reports suggesting that hormone replacement therapy might influence the development and course of autoimmune disorders and neoplastic diseases [66].

Estrogen withdrawal upregulates T-cell TNF production by a complex pathway which involves the thymus and the bone marrow: in the bone marrow, ovx promotes T-cell activation by increasing antigen presentation by macrophages and dendritic cells [49, 52].

It is well known that the thymus undergoes age-related atrophy coincident with enhanced circulation of sex steroids from puberty onwards. The impact of this atrophy is most profound in clinical conditions that cause a severe loss in peripheral T cells capable of regenerating sufficient numbers of naïve CD4+ T cells that is indirectly correlated with age. Recent animal [67] and human studies [68] demonstrated that castration results in complete and enhanced regeneration of the aged mouse and human thymus and restoration of peripheral T-cell phenotype and function. These findings have underscored the role of sexual hormones in the regulation of T cells activation and reinforce the role of T cells in postmenopausal bone loss.

It has recently been reported that 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 [14] and that in postmenopausal women, production of cytokines representative of T helper 1 lymphocytes are increased, and this effect is reversed by supplemental estrogen.

Taken together these data demonstrate that estrogen lose causes an increase in T-cell activation and in production of pro-osteoclastogenic cytokines; moreover, experimental model demonstrated that this effect is the driver of increased bone resorption after menopause as nude mice appears not to lose bone after ovariectomy [23].

The complex pathway trough which estrogen acts on immune cells and bone is summarized in Fig. 1.

T Cells and Osteoclasts: Reciprocal Interactions

Most osteoclastogenic cytokines also regulate macrophages or dendritic cells which share with OC their bone marrow precursors during development, in particular it has been demonstrated that circulating OC precursors exist primarily within the monocytic fraction of peripheral blood [12, 69–71] and their presence in the circulation serves both as a reservoir for replenishing pre-osteoclast populations in the bone marrow as needed and as a potentially abundant source of pre-osteoclasts that can be recruited into bone or joint tissue in response to reparative or pathological signals, for this reason OCs have been regarded as immune cells that are recruited in bone in response to RANKL and costimulatory molecules expressed on accessory cells.

In the recent years, several investigators have paid attention to the role of T cells in regulating osteoclastogenesis; the majority of the studies both in animal and in human models suggested that T cells induced OC formation, increased their lifespan and activity, while other workers suggested that T cells could inhibit OC formation in vitro. Some studies support the hypothesis that T cells activation after estrogen withdrawal induce OC formation and bone loss: in particular data from mice show that adoptive transfer of wild-type T cells restores the capacity of ovariectomy to induce bone loss, while transfer of T cells from TNF null mice does not [23, 49, 72, 73]. Other studies argued against a pivotal role of T cells in bone loss induced by ovariectomy in mice models [74–76]. In

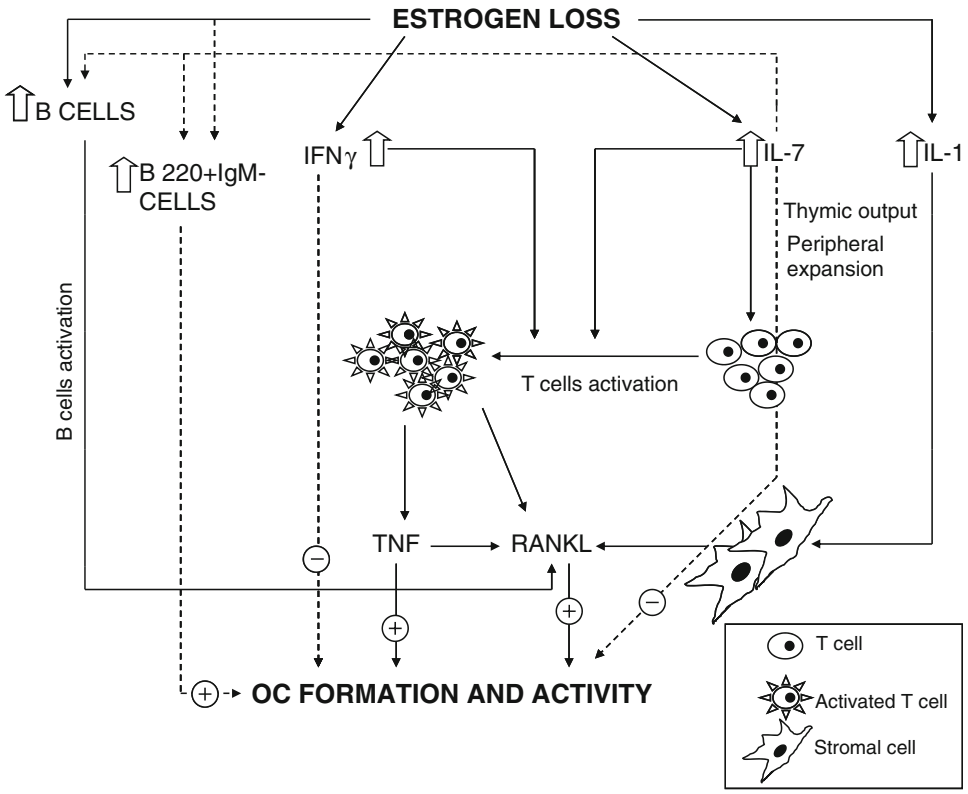

Fig. 1 Schematic representation of the main mechanisms by which estrogen deficiency influences immune system and bone loss. The *dotted lines* represent the pathways most controversial, while the *continuous lines* represent the pathways with more concordant data and also data obtained in humans

particular, Lee et al. [76] suggested that nude mice lose trabecular bone as well as wild-type after ovariectomy and that T cells may have important effects on the cortical rather than on trabecular compartment.

In postmenopausal osteoporosis, we demonstrated that T cells are activated to a greater degree at baseline as respect to healthy post- and premenopausal controls and that this implies their greater ability to produce RANKL and TNFα thus inducing OC formation and activity, we have also demonstrated that in the absence of T cells from peripheral blood mononuclear cells cultures OC formation is abolished, this phenomenon is reversed only by the addition of M-CSF and RANKL in cultures.

A recent paper by Senthilkumar et al. suggested an interesting reciprocal interaction between OC and T cells, mediated trough a direct interaction involving CD137/Cd137L and RANK/RANKL; in particular the authors suggested that the binding between RANK expressed on OC and RANKL expressed on activated T cells activate a signal-mediated mechanism that inhibit T cells proliferation (CD137 L), suggesting that OC can interact with T cell exactly as an immune cell [77].

These summarized evidences suggest that T cells and OC are strictly related, and that their interaction could partially explain the relation between estrogen deficiency, activation of immune system and bone loss, anyway the interaction between T cells and OC are very complex and only at the beginning of their knowledge.

Conclusions

In the last decade, several investigators have paid attention to the relationship between estrogen, the immune system, and the skeleton. Today the majority of the data have been obtained in animal models, but in the recent years new evidences have been accumulated in humans toward a profound link between estrogen deprivation, immune system deregulation, and bone loss. If this relationship will be confirmed by future works, postmenopausal osteoporosis should be regarded as an inflammatory disorder sustained by a chronic mild decrease in T-cell tolerance.

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