The Emerging Role of T cells in Post Menopausal Osteoporosis

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Introduction

Estrogen deficiency caused by natural or surgical menopause results in rapid and marked bone loss in humans and rodents through a variety of mechanisms. Although upregulation of osteoclast formation is recognized as the main mechanism by which estrogen deficiency stimulates bone resumption, the responsible cells and cytokines are not completely understood. Recent studies examining the role of immune cells in bone loss have demonstrated a new role for TNF-alpha-producing T cells in increasing osteoclast activity and in augmenting estrogen deficiency-induced bone destruction. This article will discuss how estrogen deficiency can lead to not only an increase in activated, TNF-alpha-producing T cells, but also to an increase in new T-cell development and thymic rebound culminating in increased bone loss.

TNF-alpha, Estrogen Deficiency, and Bone Loss

Postmenopausal osteoporosis is a common disorder stemming from an alteration of bone remodeling induced by estrogen deficiency. Such an alteration consists of an increase in bone resumption over bone formation that results in progressive bone loss. Although multiple genotropic and non-genotropic effect of estrogen on bone and bone marrow cells contribute to the bone-sparing effects of sex steroids [1-3], most of the bone sparing activity exerted by estrogen occurs through modulation of bone cell life span and decreased cytokine-driven osteoclastogenesis [1, 4]. Osteoclast formation occurs when bone marrow monocytes are costimulated by the essential osteoclastogenic factors RANKL and M-CSF [5-7], but additional inflammatory cytokines are responsible for the upregulation of osteoclast formation observed in a variety of conditions such as inflammation, hyperparathyroidism, and estrogen deficiency [8-11].

Among the factors that upregulate osteoclast formation and lead to bone loss in estroprevic humans and rodents is tumor necrosis factor alpha (TNFα) [12]. This estrogen-regulated cytokine promotes osteoclastogenesis by augmenting the production of RANKL [3], the non-redundant cytokine responsible for osteoclast development [7], and by increasing the responsiveness of maturing osteoclasts to this factor [13-15]. Further enhancing the bone-wasting activity of TNFα is the capacity of this cytokine to stimulate the activity of mature osteoclasts [3], inhibit bone formation [12] and augment the production of other cytokines known to be implicated in the pathogenesis of ovariectomy (ovx) induced bone loss, such as IL-1, IL-6, and M-CSF [3].

The essential role of TNFα as an inducer of bone loss in ovx animals has been demonstrated using multiple models. For example, ovx does not induce bone loss in TNF -/- mice [16], transgenic mice insensitive to TNFα due to the overexpression of soluble TNF receptor [17] and in mice treated with the TNF inhibitor, TNF binding protein [18]. Thus, although not essential for baseline osteoclastogenesis, TNF is regarded as a key causal factor in the bone loss observed in estrogen deficiency.

Estrogen Regulation of T-Cell TNFα and Bone Loss

The presence of increased levels of TNFα in the bone marrow of ovx animals and in the conditioned media of peripheral blood cells of postmenopausal women is well documented [19-24]. However, the cells responsible for this phenomenon had not been conclusively identified. Early studies revealed that estrogen-deficiency increases TNFα production by monocyte-enriched peripheral blood mononuclear cells and unfractionated human and murine bone marrow cells [21, 22]. Further studies in humans found that adherent mononuclear blood cells contain CD3+ CD56+ lymphocytes, a TNFα-producing subset of adherent T cells, and that the number of these T cells are decreased by estrogen treatment and inversely correlated to bone density [25].

The initial-human studies are concordant with more recent findings using murine models. In studies using highly purified cells, adherent bone marrow cells were found to contain T cells (~ 10% of total cells) and that ovx increases T-cell content by ~ 2-fold [13]. Importantly, ovx was also found to induce an increase in the production of TNFα by T cells, but not by bone marrow monocytes [13]. The pivotal role of T-cell-produced TNFα was conclusively established by the failure of ovx to induce bone loss in mice with T cells lacking TNF secretion [16]. Ovariectomy was found to enhance T-cell-TNFα production by causing an expansion of the T-cell pool in the bone marrow [16] through a complex mechanism driven by upregulation of antigen-dependent stimulation of T-cell activation [26]. Thus, earlier findings in bone marrow and adherent-cell cultures are consistent with the stimulatory effect of estrogen deficiency on the T-cell production of TNFα observed in more recent studies, leading to the conclusion that the ovx-induced increase in TNFα levels is likely to be due to T-cell-TNFα production.

T-Cell Activation During Estrogen Deficiency

Until recently it was unclear how ovx leads to increases in the numbers of activated T cells producing TNFα in the BM [16]. Emerging data suggests that the expansion of the CD4 T-cell pool is primarily the result of increased T cell activation induced by a complex pathway [26, 27]. Specifically, it appears that ovx increases T-cell activation by enhancing antigen presentation by bone marrow monocytes and dendritic cells through upregulated expression of major histocompatibility complex class II (MHCII). Estrogen repression of antigen presentation has also been described in the vagina [28, 29], uterine stromal cells [30] and in models of delayed-type hypersensitivity [31, 32]. Ovx increases antigen presentation by enhancing the expression of MHCII through upregulation of class II transactivator (CIITA) [26] . The product of the this gene is a non-DNA binding factor that functions as a transcriptional coactivator when recruited to the MHC II promoter by interaction with promoter-bound factors [33 , 34]. Ovx upregulates antigen presentation indirectly, through modulation of TGFβ [27], an estrogen induced anti-inflammatory cytokine that has been previously shown to have a key role in ovx-induced bone loss [35-37]. The blunted bone marrow levels of TGFβ directly augments CD4 T-cell activation, proliferation and TNFα production [27].

Data suggests that ovx increases the T-cell reactivity to a pool of self and foreign antigens physiologically present in healthy animals, rather than a T-cell response directed against a specific antigen. This is consistent with the fact that clones of T cells expressing T-cell receptor (TCR) directed against self antigens not expressed in the thymus survive negative selection during T-cell maturation [38-41]. Such clones are known as autoreactive or self-reactive T cells and reside in peripheral lymphatic organs of adult individuals [42]. In addition, foreign antigens of bacterial origin are

physiologically absorbed in the gut and the vagina. As these peptides come in contact with immune cells locally and systemically, they induce low-grade T-cell activation [42]. Thus, a moderate immune response is constantly in place in healthy humans and rodents due to presentation by MHCII and MHCI molecules of both self and foreign peptides to CD4 and CD8 T cells [43]. Interaction with self-peptide-MHC complexes enhances the sensitivity of mature T cells to foreign antigen; this contact with self maintains T-cell sensitivity [44, 45]. The purpose of this autoreactive response is to facilitate immune cell survival and renewal. This has led one group of researchers to hypothesize that ovx may lead to higher expression of MHC class II presenting self-peptides, resulting in T cells that are normally tolerant to physiologic levels of self-antigens to become activated in the bone marrow [26, 46, 47].

Despite the wealth of information that has now been generated regarding the contribution of T cells to ovx-induced bone loss, it remains unclear where T cells are activated and exerting their effects. There are three potential mechanisms regarding the location of T-cell activation and effector function during ovx-induced bone loss. In the first model, T cells get activated in the secondary lymphoid organs and then travel through the blood to the bone marrow to exert their effector functions. Once in the bone marrow, activated T cells upregulate osteoclast formation and induce bone loss through upregulated production of RANKL and TNF. In the second mechanism, T cells are only activated and exert their effects in the bone marrow. In support of this model, the percentage of activated T cells is much higher in the bone marrow than in other secondary lymphoid organs and this feature is both cytokine and antigen driven [48, 49]. As a result, the bone marrow is the lymphoid organ with the highest percent and number of proliferating T cells, apart from the thymus [50]. In the third model, T cells are activated in both the bone marrow and secondary lymphoid organs, but only induce osteoclastogenesis in the bone marrow. Although osteoclast precursors and osteoclastogenic cytokines are present in peripheral lymphoid organs, osteoclasts are not typically found in these organs. Thus, it is unlikely that T cells contribute to ovx-induced bone loss by facilitating osteoclast formation and/or activation in peripheral lymphoid organs. It remains to be determined which model of T-cell activation and effector function is in operation during ovx-induced bone loss. A greater understanding of T-cell localization may lead to the development of therapeutics able to block the homing and or localization of T cells to the surface of bone.

T-Cell Ontogeny and Estrogen-Deficiency-Induced Thymic Rebound

The thymus is essential for establishing the diversity of the peripheral T-cell pool. T cells originate in the thymus from bone marrow precursors [51] which derive from the Hematopoietic Stem Cell pool (HSC) ($Lin^{-}Sca^{-}1^{high}c\text{-}Kit^{high}IL\text{-}7R\alpha^{-}$) [52]. Poorly understood thymic derived signals direct the migration and the seeding of these bone marrow precursors into the thymus at distinct temporal windows [53]. One of the earliest identifiable intrathymic, bone marrow-derived T-cell precursors is the early thymic progenitor cell (ETP: $Lin\text{-}cKit^{high}IL\text{-}7R^{high}CD44^{+}CD25^{-}$) [54, 55]. This cell is part of the double negative 1 fraction (DN1) of T-cell precursors ($CD4^{-}CD8^{-}\ CD44^{+}CD25^{-}$). DN1 cells proceed through three additional distinct stages of differentiation: $CD44^{+}CD25^{+}$ (DN2), $CD44^{-}CD25^{+}$ (DN3) and CD44-CD25- (DN4) [56] before acquiring the pre-T-cell receptor (pre-TCR). Once the immature T cells express the pre-TCR, they become CD4 and CD8 double positive (DP), rearrange the TCR-alpha chain, and progress through positive and negative selection, after which surviving naïve CD4 or CD8 single-positive T cells are exported to the periphery.

Concomitant with the onset of puberty, the thymus involutes significantly and is characterized by reduced numbers of intrathymic T cells, decreased production of growth factors such as IL-2 and IL-7, and a dramatic reduction in cortical and medullary tissue, although the T-cell-developmental program remains intact. [57]. By middle age most parenchymal tissue is replaced by fat, and in both mice and humans fewer T cells are produced and exported to secondary-lymphoid organs as aging progresses [58], thus decreasing the frequency of naïve T cells. Peripheral-T-cell numbers are maintained by low-grade proliferation of naïve T cells in response to low-affinity antigens, increasing numbers of memory T cells that are maintained and expanded via cytokine-driven mechanisms (IL-7, IL-15), and activation and expansion of naïve and memory T cells that occurs in response to cognate-antigen stimulation [57].

While the thymus involutes significantly at the onset of puberty, recent studies now suggest that significant thymic output remains throughout adult life and the age-associated decline in thymic activity appears to be only quantitative and not qualitative [59]. Lymphocytic-thymic tissue has been documented in adults up to 107 years of age [60] and recent evidence has shown that functional thymic tissue remains in humans until at least the sixth decade of life [61-63]. Studies have also shown that in adults and rodents, thymocytes exist in all stages of maturation and that the thymus continues to contribute naïve T cells capable of responding to a wide variety of antigens to the peripheral lymphocyte pool until late in life [63-65]. This continuous low-level export of T cells into the periphery may provide a constant source of T-cell diversity, as even though there is an age associated decrease in recent-thymic-emigrant T cells, a reduction in TCR diversity is not observed until 70 years of age [66].

Further evidence for the functional capacity of the thymus in adults has been observed in the setting of severe T-cell depletion, such as with HIV infection and bone marrow transplant, in which thymic rebound is essential for restoration of T-cell homeostasis. For example, HIV has been shown to suppress thymic function, while treatment of these patients with HAART has increased levels of circulating CD4s due increased thymic abundance and a resurgence in thymic activity, even in older patients [64]. In addition, after bone marrow transplant there is a resurgence in thymic activity that is necessary for the restoration of peripheral naïve-T-cell populations, providing further evidence that given the proper signal thymic rebound can occur [67]. The mechanism driving this resurgence of thymic activity is not completely understood, but one factor believed to be involved is IL-7. The levels of this cytokine increase during cytoreductive therapies, when consumption by lymphocytes decreases [68], or after ovx [69]. Importantly, IL-7 is sufficient to enhance thymopoiesis in young mice [70], but this cytokine plays a more relevant role in aged mice [71, 72].

While puberty marks the onset of thymus involution, increased generation and maturation of thymocytes and enhanced thymic output follow ovariectomy [47]. Attesting to the potency of the regulatory effects of sex steroids, sex-steroid deprivation has been shown to restore thymic function in aged mice and rats [65, 73] while estrogen treatment induces thymic atrophy by promoting thymocyte apoptosis and an arrest of differentiation at the DN1/DN2 transition, [74-76]. In accordance with the notion that estrogen deficiency induces a rebound in thymic function, it has been found that ovx increases the expansion of thymic T cells and the export of naïve T cells from the thymus via an IL-7-dependent mechanism [47]. Indeed, increased thymic T-cell output accounts for ~ 50 % of the increase in the number of T cells in the periphery, while the remaining 50 % is due to increased peripheral expansion. This study also has shown that thymectomy decreases by ~ 50 % the bone loss induced by ovx, thus demonstrating that the thymus plays a previously unrecognized causal effect in ovx-induced bone loss[47].

Although a role for increased thymic output in postmenopausal bone loss in humans awaits demonstration, increased thymic output could be particularly relevant for the bone loss of young women undergoing surgical menopause [77] or for the rapid bone loss characteristic of women in their first 5-7 years after natural menopause [4]. Indeed, an age-related decrease in thymic T-cell output could mitigate the stimulatory effects of sex-steroid deprivation and explain why the rate of bone loss in postmenopausal women diminishes as aging progresses [78].

Conclusions

The difficulty in accessing intact human-bone marrow makes studies of immune cell: bone cell interaction difficult to observe, however through the use of murine models a greater understanding of the mechanisms involved is currently underway. These positive findings in the mouse will likely lay the foundation for future studies and therapies in humans.

Great advances have been made in understanding the mechanisms involved in the bone destruction that occurs during estrogen deficiency. It is now understood that T cells play not only a pivotal role in the functioning of immune system, but they also play a causal role in estrogen-deficiency-induced bone destruction through increased de novo T-cell production, T-cell activation, expansion, and cytokine production. Despite this wealth of knowledge, the exact nature of the specific antigens involved, the relevant reactive T-cell subsets, and their anatomical distributions, have yet to be elucidated. By achieving a greater understanding of the nature and distribution of the activated T cells involved in ovx-induced bone loss, it may ultimately lead to novel therapeutic approaches to preventing or ameliorating postmenopausal osteoporosis.

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