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Review

Interaction between bone and immune cells: Implications for postmenopausal osteoporosis

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ABSTRACT

Postmenopausal osteoporosis is a systemic disease characterized by the loss of bone mass and increased bone fracture risk largely resulting from significantly reduced levels of the hormone estrogen after menopause. Besides the direct negative effects of estrogen-deficiency on bone, indirect effects of altered immune status in postmenopausal women might contribute to ongoing bone destruction, as postmenopausal women often display a chronic low-grade inflammatory phenotype with altered cytokine expression and immune cell profile. In this context, it was previously shown that various immune cells interact with osteoblasts and osteoclasts either via direct cell-cell contact, or more likely via paracrine mechanisms. For example, specific subtypes of T lymphocytes express TNFα, which was shown to increase osteoblast apoptosis and to indirectly stimulate osteoclastogenesis via B cell-produced receptor-activator of NF-κB ligand (RANKL), thereby triggering bone loss during postmenopausal osteoporosis. T_h17 cells release interleukin-17 (IL-17), which directs mesenchymal stem cell differentiation towards the osteogenic lineage, but also indirectly increases osteoclast differentiation. B lymphocytes are a major regulator of osteoclast formation via granulocyte colony-stimulating factor secretion and the RANKL/osteoprotegerin system under estrogen-deficient conditions. Macrophages might act differently on bone cells dependent on their polarization profile and their secreted paracrine factors, which might have implications for the development of postmenopausal osteoporosis, because macrophage polarization is altered during disease progression. Likewise, neutrophils play an important role during bone homeostasis, but their overactivation under estrogen-deficient conditions contributes to osteoblast apoptosis via the release of reactive oxygen species and increased osteoclastogenesis via RANKL signaling. Furthermore, mast cells might be involved in the development of postmenopausal osteoporosis, because they store high levels of osteoclastic mediators, including IL-6 and RANKL, in their granules and their numbers are greatly increased in osteoporotic bone. Additionally, bone fracture healing is altered under estrogen-deficient conditions with the increased presence of pro-inflammatory cytokines, including IL-6 and Midkine, which might contribute to healing disturbances. Consequently, in addition to the direct negative influence of estrogen-deficiency on bone, immune cell alterations contribute to the pathogenesis of postmenopausal osteoporosis.

1. Introduction

Postmenopausal osteoporosis is a systemic disease characterized by low bone mass and bone microarchitecture destruction, leading to an increased fracture risk [1]. A decline in estrogen hormone levels after menopause leads to an altered balance between bone formation and bone resorption favoring bone resorption [1]. One cause are the direct effects of estrogen on bone cells. Estrogen increases osteogenic differentiation of mesenchymal stem cells (MSCs) and osteoblast maturation, thereby enhancing bone formation. Furthermore, estrogen inhibits osteoclast formation and induces osteoclast apoptosis, which limits bone resorption. When estrogen is insufficient in the female body, these osteo-anabolic and anti-osteoclastic effects are reduced, leading to ongoing bone destruction [2]. However, estrogen does not only influence bone cells and thus postmenopausal osteoporosis, because this condition is a multifaceted disease affecting the entire body. It is known that estrogen also interacts with various immune cells, leading to a chronic low-grade pro-inflammatory phenotype under estrogen-deficiency [3–5]. Because immune cells can interact with bone cells on various levels, it is reasonable to hypothesize that bone loss after

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menopause is partly due to the interplay between immune cells and bone metabolism. This review will focus on describing the clinical bone and immune phenotype of postmenopausal osteoporotic patients and summarizing preclinical data in various models for postmenopausal osteoporosis. Further chapters will highlight clinical and preclinical data on the interaction between various immune cells and bone metabolism (a research field termed "osteoimmunology") and summarize how inflammation might affect the development of osteoporosis and osteoporotic fracture healing.

2. Postmenopausal osteoporosis – bone and immune phenotype

Postmenopausal osteoporosis is characterized by an uncoupling of the basic multicellular unit that is responsible for bone remodeling in the adult organism. Under physiological circumstances, resorption and formation always occur in a coupled manner, leading to bone resorption and formation occurring at the same bone location in the same order. Bone remodeling and, therefore, also osteoblastogenesis and osteoclastogenesis, is controlled by several circulating hormones and local bone-produced factors [6]. Estrogen plays an important role in this process by increasing osteogenic differentiation and decreasing osteoclastogenesis. But of course, many other factors were also shown to regulate bone remodeling. During postmenopausal osteoporosis, bone resorption is increased by up to 70% due to increased osteoclast numbers in the bone, while bone formation might also be increased but to a lesser extent than bone resorption, or might be unchanged or even decreased by up to 14% dependent on the state of the menopause [7]. This leads to the hypothesis that postmenopausal osteoporosis is rather a high-bone turnover disease with a shift in the remodeling balance in favor of resorption [8]. By contrast, senile osteoporosis is often described as a low-bone turnover disease with both decreased resorption and, to a greater extent, decreased bone formation [1].

Postmenopausal osteoporosis can be mimicked in preclinical models by inducing estrogen-deficiency. The mainly used animal model is ovariectomy (OVX) in rodents, however, there are also chemical methods to block the action of estrogen in the model organism. A drawback of the surgical models is that estrogen-deficiency is induced in a very abrupt manner, in contrast to the slow but steady decline during natural menopause. Furthermore, the ovaries produce many more important factors in addition to estrogen, which are reduced together with estrogen after OVX. Even so, the bone phenotype of OVX rodents still resembles the clinical picture in various ways. Following OVX, the animals display a very rapid loss of trabecular bone, resulting in an osteopenic phenotype. The responsible mechanism is a shift in bone remodeling towards bone resorption because of increased osteoclast numbers, mimicking the human situation of high bone turnover in early menopause [9]. Later, trabecular bone loss slows down and cortical bone also becomes affected. After a few weeks, a clear osteoporotic bone phenotype becomes evident. This is due not only to increased resorption, but also impaired osteoblast activity secondary to increased osteoblast apoptosis [10]. A limitation of these animal models is that the total bone loss is less than in human patients [11].

Clinical data regarding the immune phenotype of postmenopausal patients suggest that women after menopause display increased inflammatory cytokines levels, including interleukin 1β (IL-1β), IL-6 and tissue necrosis factors α (TNFα) [12–15]. This was demonstrated both for circulating peripheral blood cells and directly for cells within the bone environment [6,16]. IL-1 levels were shown to correlate with bone resorption, and increased IL-1 levels in postmenopausal patients could be blocked by estrogen treatment [16,17], leading to the conclusion that estrogen-deficiency is indeed the driver behind the increased cytokine expression. In addition to the higher levels of inflammatory mediators, immune cell numbers were shown to be altered in postmenopausal women. The Immunos clinical study demonstrated lower numbers of $CD19^+$ B lymphocytes in a postmenopausal female patient cohort, whereas these cells secreted more granulocyte macrophage colony-stimulating factor (GM-CSF). While T cell numbers were not different in this study, they did display an altered cytokine production profile [18]. Another study found that T cells are more likely to express TNFα in postmenopausal osteoporotic patients who suffer from a fracture [19]. A more recent study showed increased circulating T cells and monocytes in postmenopausal women [14]. Further, patients with manifested postmenopausal osteoporosis display a higher neutrophil-to-lymphocyte ratio in the peripheral blood [20] and mast cells numbers in the bone marrow are increased [21]. In conclusion, postmenopausal osteoporotic women display a chronic low-grade inflammatory phenotype with altered cytokine expression and immune cell profile. However, not only estrogen-deficiency might lead to these alterations, since also aging itself was demonstrated to lead to a chronic inflammatory status called "inflamm-aging" [22]. Influence of aging might even be responsible in great part for postmenopausal osteoporosis and aggravates symptoms of estrogen-deficiency [23].

Preclinical data confirm the increased presence of inflammatory mediators, including IL-1β, IL-6 and TNFα, in the blood and bone marrow of OVX rodents [4,24,25]. Estrogen-deficiency leads to spleen enlargement and to a general increase in circulating lymphocytes [26]. Furthermore, OVX mice displayed increased numbers of TNF-producing T lymphocytes in the bone marrow [27]. This is due to an increased proliferation and life span of T cells in the bone marrow under estrogen-deficient conditions, because bone marrow macrophages and dendritic cells were shown to increase their expression of major histocompatibility complex II and, therefore, antigen presentation to T cells. Moreover, OVX induced increased interferon γ (IFNγ) production in T lymphocytes. In contrast to the clinical data, $B220^+$ B lymphocyte numbers were shown to be increased in the bone marrow of OVX mice, an effect which was abolished by estrogen treatment [28–31]. Regarding cells of the innate immune system, OVX mice displayed a severe impairment in macrophage activation upon bacterial sepsis [32]. However, in an LPS air pouch injection model, OVX mice showed significantly increased macrophage, monocyte and neutrophil infiltration to the site of injury [33]. Furthermore, OVX rats displayed increased numbers of circulating neutrophils, eosinophils and basophils [34]. Confirming clinical data, mast cell numbers in the bone marrow of OVX rodents were shown to be increased [25,35]. In conclusion, rodents subjected to estrogen-deficiency also display a chronic low-grade inflammatory phenotype and these models recapitulate most, but not all, of the clinical features of the immune phenotype during postmenopausal osteoporosis.

3. Interactions between immune and bone cells

3.1. Interaction between T lymphocytes and bone cells

T lymphocytes are hematopoietic cells and mature in the thymus. They have distinct surface receptors and functions dependent on their differentiation state. $CD8^+$ cytotoxic T cells directly lyse infected or mutated cells. $CD4^+$ T-helper (T_h) cells interact with other immune cells like B lymphocytes by surface receptors and secreted cytokines, thereby increasing or decreasing their activation state. T_h cells can be further subdivided by their cytokine expression profile. T_h1 cells are polarized by IL-12 and secrete IFNγ, IL-2 and TNFα, and mainly affect macrophages. For example, TNFα mediates increased RANKL expression by macrophages, thereby stimulating osteoclastogenesis [36]. T_h2 cells are triggered by IL-2 and IL-4 and secrete IL-4, IL-5, IL-9, IL-10 and IL-13, and mainly affect B lymphocytes, mast cells and granulocytes [37]. A further subgroup are T_h17 cells, classified by their high IL-17 secretion [38]. $CD4^+CD25^+$ T_{regs} are regulatory T cells, which modulate the immune system and are responsible for the maintenance of immune tolerance [39]. As mentioned above, several clinical studies demonstrated normal or increased circulating T cell levels in postmenopausal osteoporotic patients with an altered cytokine expression profile. Both human patients and OVX animals displayed increased TNFα expression

in T lymphocytes. TNFα was shown to increase osteoblast apoptosis and to indirectly stimulate osteoclastogenesis via B cell-produced receptor-activator of NF-κB ligand (RANKL), thereby triggering bone loss during postmenopausal osteoporosis [40,41]. Furthermore, T_h17 cells were found to be increasingly present in the bone marrow of OVX mice [42]. This was mediated via the expansion of intestinal T cells under estrogen-deficiency, which then migrate into the bone marrow via C-X-C motif chemokine receptor 3 (CXCR3)- and C-C chemokine ligand 20 (CCL20)-mediated mechanisms. T_h17 cells in the bone marrow were shown to increase recruitment of inflammatory monocytes as osteoclast precursor cells to the bone marrow [43]. IL-17 is known to switch MSC differentiation from adipogenesis to osteogenesis and induce osteogenic differentiation of pre-osteoblastic cells [44,45]. However, IL-17 also induces RANKL secretion by osteoblasts, thereby stimulating bone resorption [46]. It was shown previously that IL-17 upregulates RANK on osteoclast progenitor cells, thereby increasing their sensitivity to RANKL stimulation [47]. Further direct effects of IL-17 on osteoclasts remain unclear, because various experimental studies reported contradictory results [45]. In addition to the indirect osteoclastic effects of IL-17 secreted by T_h17 cells, these cells were also shown themselves to express RANKL [48]. However, RANKL knockout particularly in T cells, did not attenuate OVX-induced bone loss, therefore, direct effects of T cells on osteoclasts remain unclear [49]. Blocking IL-17 signaling protected mice from OVX-induced bone loss [50], indicating the important role of this T cell-derived cytokine during the development of postmenopausal osteoporosis. Interestingly, HIV infection is associated with increased risk for osteoporosis [51], although patients frequently suffer from T-lymphopenia. Studies have shown that HIV-infected T cells produce less OPG, but more RANKL, leading to bone loss in these patients [52]. This further underlines the importance of T cell derived mediators during osteoporosis development.

3.2. Interaction between B lymphocytes and bone cells

B lymphocytes are cells of the adaptive immune system. They mature in the bone marrow via CXCL12 signaling and their main function is antibody production to fight against pathogens. However, B cells also present antigens for T cell activation and secrete various cytokines like G-CSF [53]. Clinical data suggest that postmenopausal osteoporotic patients display normal or less B lymphocytes in the bone marrow, while their individual G-CSF secretion is enhanced. In OVX mice, B cells appear to proliferate more in the absence of estrogen via increased CXCL12 signaling [54,55] and also start to secrete more G-CSF. G-CSF is known to promote granulocyte differentiation, particularly neutrophils, which might promote the enhanced neutrophil numbers found in estrogen-deficient subjects [56]. Furthermore, G-CSF leads to increased proliferation of osteoclast progenitor cells [56]. In addition to G-CSF, activated B cells were shown to secrete RANKL under inflammatory conditions, thereby activating osteoclast formation [57,58]. By contrast, under physiological conditions, B cells produce approximately 40–60% of the total bone marrow-derived osteoprotegerin (OPG) and thereby inhibit osteoclast differentiation [59]. The influence of B cells on osteoblasts is less well investigated, although there are indications that B cells might inhibit osteoblast differentiation via CCL3 and TNF signaling [60]. In conclusion, B cells appear to play an important role in regulating osteoclastogenesis by the RANKL/OPG signaling system. During postmenopausal osteoporosis, B cells activated by estrogen-deficiency and pro-inflammatory conditions contribute to increased bone resorption by secreting enhanced levels of G-CSF and RANKL. Further clinical data regarding low bone mineral density in Non-Hodgkin's-Lymphoma patients underlined the importance of balanced B cell numbers and activation for bone homeostasis [61].

3.3. Interaction between macrophages and bone cells

Macrophages derive from the monocytic lineage such as osteoclasts and are part of the innate immune system. They are found in almost all body tissues and mainly engulf and digest cellular debris and foreign material by phagocytosis after infection or tissue injury. Another important function of macrophages is the recruitment and activation of other immune cells by the secretion of inflammatory mediators and the presentation of antigens to T cells. However, besides their pro-inflammatory reactions to acute infection and tissue injury, macrophages are also important for the resolution of inflammation and orchestrate tissue regeneration. For this, their phenotype may switch from the more pro-inflammatory state (termed M1) to an anti-inflammatory and pro-regenerative state (termed M2) [62]. However, it is believed that there are many more sub-classes of macrophage activation than just M1/M2. As mentioned previously, monocytes are increasingly present in the bone marrow of postmenopausal patients [14] and OVX mice displayed altered bone marrow macrophage activation and M1/M2 switch upon inflammatory stimulus [33]. Preclinical studies have shown that macrophages can influence bone cells either via paracrine signaling or via direct cell-cell contact, making an involvement of macrophages in the bone phenotype of postmenopausal osteoporosis very likely. Together with the paracrine factors, M1 macrophages secrete high levels of reactive oxygen species (ROS), nitric oxide (NO) and several pro-inflammatory cytokines, including IL-1, IL-2, IL-6, TNFα and IFNγ [63]. M2 macrophages, which are activated via IL-4, IL-10 and IL-13, secrete anti-inflammatory molecules such as CCL18, CCL22, IL-10 and pro-osteogenic molecules, including bone morphogenic protein 2 (BMP-2), transforming growth factor β (TGFβ) and osteopontin [64]. All of these factors can directly influence bone cells, some increasing osteogenic differentiation and some decreasing it (e.g. IL-1, IL-6). Conditioned medium experiments indicated that non-activated monocytes promote MSC recruitment and increase their osteogenic potential, however, the involved paracrine factors remain unclear [65]. Co-culture experiments have shown that direct cell-cell contact of non-polarized macrophages and bone marrow MSCs induced oncostatin M expression, which then increased osteoblast differentiation [66]. Further studies revealed that macrophages induced osteogenic differentiation of bone marrow MSCs via prostaglandin E2 synthesis independent of the macrophage polarization status, but with the strongest effects by M1 macrophages [67,68]. However, other studies suggested a greater osteogenic effect of M2 macrophages and rather an inhibition of osteogenic differentiation by M1 macrophages [69,70]. Co-culture of M1 macrophages and MSCs also reduced OPG expression, indicating an indirect influence on osteoclast formation. Direct effects on osteoclast formation might be mediated by pro-inflammatory cytokines, including IL-6 and TNFα, which were shown to increase osteoclastogenesis. Regarding postmenopausal osteoporosis, it was found that M1 polarization is increased whereas M2 polarization is disturbed in bone marrow macrophages from OVX mice. This could be reversed by estrogen treatment, indicating that estrogen is indeed the main driver [71]. Furthermore, it was shown that M2 macrophages differentiate into mature osteoclasts in the presence of RANKL only in the absence of estrogen. This was mediated via estrogen receptor alpha signaling on macrophages. In conclusion, macrophages might act differently on bone cells dependent on their polarization profile and, therefore, their secreted paracrine factors. This might have implications for the development of postmenopausal osteoporosis, because macrophage polarization is altered during disease progression. Additionally, macrophages might play an important direct role in the pathogenesis of postmenopausal osteoporosis by driving towards osteoclastic differentiation under estrogen-deficiency.

3.4. Interaction between neutrophils and bone cells

Neutrophils are generated from myeloid precursors in the bone marrow controlled by several cytokines, mainly G-CSF. Mature neutrophils are terminally differentiated, short-lived cells containing numerous granules and circulate in the blood, from where they are

rapidly recruited to infection and inflammation sites to eliminate pathogens via phagocytosis, degranulation and the formation of neutrophil extracellular traps (NETs). Neutrophils can also synthesize inflammatory mediators such as C-X-C and C-C chemokines, thereby enabling their interchange with other cells, including bone cells [72]. As indicated above, postmenopausal females display an increased neutrophil-to-lymphocyte ratio associated with low bone mineral density [73,74]. Direct effects of estrogen on neutrophils are supported by the fact that neutrophil numbers fluctuate during the menstrual cycle [75] and that OVX mice displayed increased neutrophil infiltrations during inflammatory processes [33,34,76,77]. *In vitro* studies showed that estrogen can influence neutrophil activity, chemotaxis, apoptosis, and the production of ROS and NO [78–80]. Overall, neutrophils could be involved in postmenopausal osteoporosis development, because their number, activity and functions are affected by estrogen and they express mediators that can favor osteoclastic bone resorption, including IFNγ, IL-6 and RANKL. Confirming the effects of neutrophils on osteoclasts, insufficient neutrophil recruitment to the inflamed gingiva in a periodontitis model induced T_h17 cells to produce more IL-17, known to induce osteoclastic bone resorption [81]. In rheumatoid arthritis (RA), activated neutrophils were shown to express RANKL that stimulates osteoclastic bone resorption in the inflamed joint [82,83]. Interestingly, also neutrophils of COPD patients, who frequently suffer from osteoporosis, highly express RANKL, which was correlated with a decrease in bone mineral density [84]. Activated neutrophils were also found in bone biopsies of osteomyelitis patients with bone erosions. Here, infiltrated neutrophils correlated with increased osteoclast numbers and highly expressed IL-8, which was shown to induce osteoclast formation [85]. Interestingly, in an in vitro model of chronic gouty arthritis, neutrophils were shown to directly adhere to osteoblasts, thereby inducing osteoblast retraction without affecting osteoblastic matrix mineralization, but stimulating osteoclastic matrix resorption [86]. In summary, activated neutrophils appear to induce osteoclast formation under inflammatory conditions, both directly and indirectly. However, also a lack of neutrophils influences bone, because patients with severe chronic neutropenia suffer from low bone mineral density probably resulting from a high bone turnover and an increased expression of the pro-inflammatory cytokines IL-1β and TNFα [87]. Therefore, while neutrophil presence might be important for bone homeostasis, however, highly activated neutrophils could be involved in the development of bone loss. Neutrophils also affect osteoblasts, because an in vitro co-culture model of neutrophils, endothelial cells and osteoblasts revealed that neutrophils induce the expression of osteogenic markers, including alkaline phosphatase, osteocalcin, collagen type 1, BMPs and TGFβ in osteoblasts. Moreover, osteoblastic mineral deposition was increased, thus indicating a possible osteogenic effect of neutrophils in bone [88]. Neutrophils also influence MSCs, because MSCs co-cultured with activated neutrophils differentiated into osteoblasts, which was influenced by altered cytokine levels of IL-1α and TGFβ [89]. By contrast, further in vitro experiments revealed that neutrophils inhibit the production of extracellular matrix by MSCs [90]. Moreover, neutrophil expansion by G-CSF induced the apoptosis of MSCs and osteoblasts in vitro through neutrophil-produced ROS [91]. Certainly, osteogenic effects might strongly depend on the activation status of neutrophils. In conclusion, neutrophils express and secrete inflammatory mediators, which can directly or indirectly affect MSCs, osteoblasts and osteoclasts. However, further investigations are needed to elucidate the molecular mechanisms of the cellular crosstalk in bone, particularly under estrogen-deficient conditions.

3.5. Interaction between mast cells and bone cells

Mast cells are part of the innate immune system and derive from the myeloid stem cell lineage traceable in connective tissues throughout the entire body. Mast cell progenitors are released from the bone marrow and express cluster of differentiation 34 and the surface marker c-Kit (ligand stem cell factor), whereby the latter strongly influences mast cell maturation in the target tissue by the interplay with the local growth factor environment [92,93]. Mature mast cells are characterized by their large number of secretory granules full of pre-formed mediators (e.g., histamine, IL-6, IL-8, TNFα, platelet-derived growth factor (PDGF), tryptases, chymases), which can be rapidly released upon stimulation, as during inflammation or allergic reactions [94]. Moreover, mast cells are also capable of the *de novo* synthesis and release of mediators, including IL-6, TNFα and vascular endothelial growth factor. For several mast cell mediators, osteo-catabolic or osteo-anabolic effects are described, suggesting a possible role of mast cells in the regulation of bone turnover [95]. Interestingly, patients suffering from systemic mastocytosis, a disease characterized by abnormally high numbers of mast cells, frequently display an osteoporotic bone phenotype [96,97]. It is thought that a high bone turnover status drives the decline in bone mass, because patients with indolent systemic mastocytosis display increased osteoclast and osteoblast parameters and serum markers [98,99], as well as higher IL-6 serum levels [100]. Clinical data suggest that mast cell numbers are also increased in the bone marrow of postmenopausal patients [21,101]. Confirming clinical examinations, OVX rodents exhibit more mast cells in the bone marrow compared to sham mice [102,103]. Mast cell accumulation in OVX mice was directly linked to an increase in osteoclast numbers [104] and mast cells were often located in close proximity to osteoclasts [105], suggesting interactions of mast cells and osteoclasts. Supporting this, mast cell-deficient Mcpt5-Cre R-DTA flox mice were protected from OVX-induced bone loss by the prevention of increased osteoclast numbers and activity, thus indicating that mast cells stimulate osteoclastogenesis in estrogen-deficiency [105]. Confirming these results, supernatants of stimulated mast cells induced osteoclastogenesis in vitro when estrogen was absent, which was not the case in the presence of estrogen, suggesting an inhibitory effect of estrogen on the osteoclastogenic-potential of mast cells [105]. Certainly, it remains unclear which mast cell mediators are responsible for the osteoclast-stimulating effects, particularly under estrogen-deficient conditions. Histamine is a major candidate, because both histamine-deficient mice and blocking the histamine receptor H1 in rats prevented the OVX-induced bone loss by reducing osteoclast activities [106]. Additionally, blocking the histamine H1 receptor in postmenopausal females improved bone content compared to placebo treatment [107]. *In vitro* experiments corroborate these findings, because blockade of the histamine H1 receptor reduced the osteoclastogenic potential of mast cell supernatants [35]. Nevertheless, there are several other mast cell mediators, including TNFα, IL-6 and RANKL, which could be involved in the stimulating effects on osteoclasts. However, their specific contribution to the development of postmenopausal osteoporosis needs to be elucidated further in detail, for example, by the use of mast cell mediator-specific knockout models. In contrast to osteoclasts, less is known about the interactions of mast cells and osteoblasts, although several mast cell mediators are known for their osteo-anabolic or osteo-protective effects, including prostaglandin D2, substance P, IFNγ and IL-10 [108,109]. Furthermore, mast cell released PDGF was shown to mediate the differentiation of bone marrow stroma cells into pre-osteoblastic fibroblasts in an experimental rat model [110]. *In vitro* experiments revealed that mast cell chymase alters osteoblast properties including adhesion, morphology, and function [111]. Mast cell-deficient $Kit^{W/W\text{-}v}$ mice displayed reduced osteoblast activity [112]. Moreover, female mice lacking the mast cell chymase Mcpt4 displayed an increased bone mass, levels of bone formation markers and bone formation rate [113]. These findings suggest that mast cell mediators can influence osteoblasts and bone formation, which might also have implications for postmenopausal osteoporosis, however, specific knowledge is currently rather limited.

4. Bone healing in postmenopausal osteoporosis

The compromised bone quality in osteoporosis predisposes patients

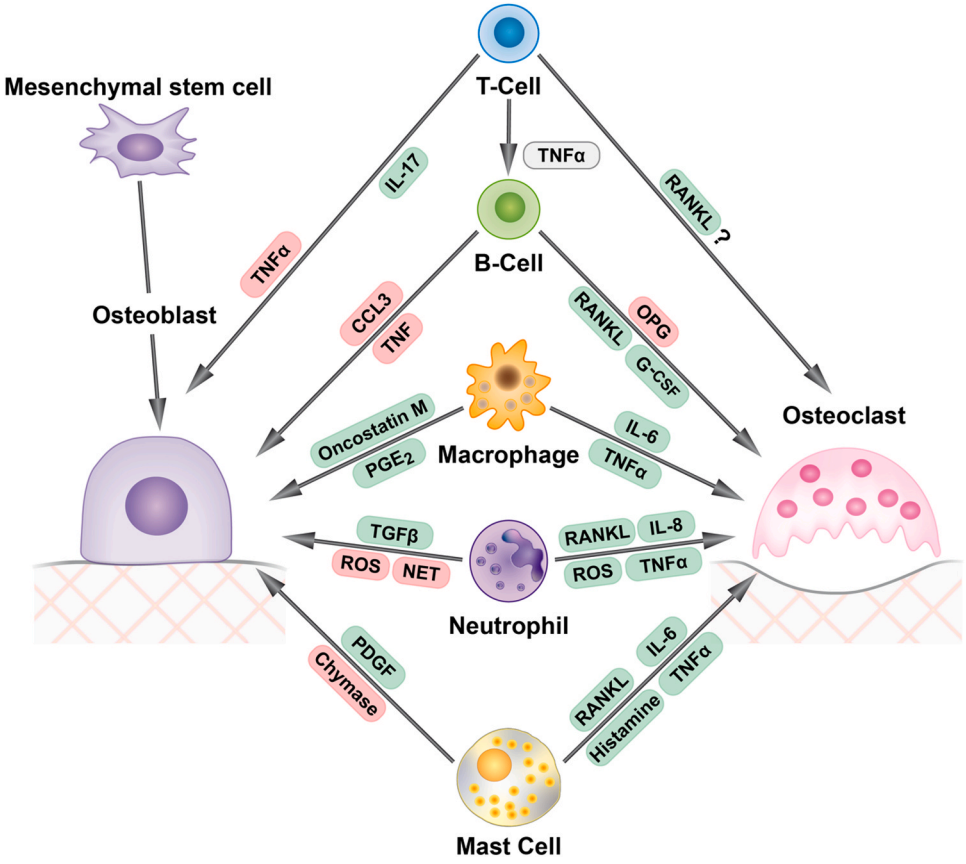

Fig. 1. Schematic illustration of immune cell interactions with bone cells. Selected mediators are displayed, which were shown to play an important role during the interaction of immune cells with mesenchymal stem cells/osteoblasts and osteoclasts. Abbreviations: Interleukin (IL), osteoprotegerin (OPG), tumor necrosis factor α (TNFα), receptor activator of nuclear NFκB ligand (RANKL), reactive oxygen species (ROS), prostaglandin E2 (PGE2), transforming growth factor β (TGFβ), platelet-derived growth factor (PDGF), neutrophil extracellular traps (NETs), C-C chemokine ligand (CCL). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

to suffer an osteoporotic fragility fracture with a worldwide incidence of around 9 million fractures annually [114]. In the clinics, osteoporotic fractures are frequently associated with implant failure, healing complications, prolonged hospitalization, and increased mortality [115,116]. The reasons for the impaired healing in osteoporotic bones are not entirely known, but biological factors such as vascularity, hormones, growth factor and cellular availabilities affected by osteoporosis might critically impact the healing process besides surgical complications due to the weak bone quality [117]. Estrogen deficiency directly influences the fracture healing process. OVX mice display a decreased cartilage formation and angiogenesis [118,119], as well as disturbances in pathways of endochondral and intramembraneous bone formation resulting in a decreased mechanical competence of the fracture callus [120]. Further, osteoclast numbers are increased in the callus of OVX mice [121]. Disruptions in the inflammatory response are also known to impair the healing process. Both the depletion of certain immune cell populations, as well as an overwhelming immune response, as in acute or chronic inflammatory conditions, negatively affects bone healing [122]. Notably, experimental data showed that estrogen-deficiency also alters the immune response towards fracture. OVX rodents displayed increased systemic levels of pro-inflammatory cytokines, including TNFα, IL-6 and Midkine (Mdk), early after fracture [123–125]. Additionally, locally at the fracture site, IL-6 and Mdk expression was significantly increased in OVX mice [126], whereas in OVX rats, TNFα and IL-6 expression were significantly reduced and IL-10 increased [124]. These results clearly indicate an imbalanced immune response in OVX rodents. Mdk serum levels were also increased in postmenopausal patients after long-bone fracture compared to age-matched male fracture patients [123]. Other clinical examinations measured inflammatory mediators during the time course of fracture healing or in comparison to healthy controls and identified changes in systemic IL-6, IL-31 and C-reactive protein levels [127–129]. Besides altered pro-inflammatory mediators, an increased neutrophil infiltration was observed in OVX mice [126]. Neutrophils are the first cells that invade the fracture hematoma that are responsible for cell and debris phagocytosis and the secretion of cytokines that attract other immune cells. Locally increased neutrophil numbers have been shown to impair bone repair after severe trauma, probably controlled via IL-6 [130]. However, neutrophil depletion also impaired the fracture healing outcome [131], which is why balanced neutrophil functions appear to be a prerequisite for successful bone repair. Interestingly, Mdk-antibody treatment reduced neutrophil numbers and the IL-6 concentration in OVX mice [126]. Mdk is known as a pro-inflammatory cytokine regulated by estrogen that attracts neutrophils in other inflammatory settings [132]. Regarding changes in immune cells, Inoue et al. described that $Gr1^+$ immune cells were longer present in the callus of OVX mice compared to sham mice [133]. Moreover, increased numbers of mast cells have been found in the fracture callus of OVX mice compared to sham mice [134]. The changes in inflammatory mediators and cells observed after OVX might contribute to the impaired healing outcome in osteoporotic bones, because the initial immune response is considered to be essential for the following repair phase.

5. Reciprocal effects of bone cells on immune cells

Besides the effects of immune cells on bone cells, there are also reciprocal effects from osteoclasts and osteoblasts on cells of the immune system, although this has not been extensively studied yet in the context of postmenopausal osteoporosis. During inflammatory conditions, osteoclasts were shown to influence $CD4^+$ T lymphocytes. Especially the so-called “inflammatory osteoclasts” originating from dendritic cells instead of monocytic cells, influence $CD4^+$ T lymphocytes in an antigen-dependent manner and alter their TNFα production [135,136]. Further, osteoclasts can activate proliferation and cytokine

secretion of $CD8^+$ T cells [137,138]. Less is known about the influence of osteoblasts on immune cells. However, there is convincing evidence that osteoblasts are an important source of activated complement proteins under inflammatory conditions [139,140], thereby also activating cells of the immune system, especially neutrophils [141]. This might be the case also for other immune mediators and immune cells.

6. Conclusion

Estrogen influences the proliferation, differentiation, activation and homing of many immune cells. Under estrogen-deficient conditions, both in rodents and humans, chronic activation of the immune systems leads to a low-grade inflammatory phenotype with altered cytokine and immune cell profiles. Many immune cells were shown to directly or indirectly influence bone cells via factors including OPG/RANKL, inflammatory cytokines such as IL-6 and TNFα and other mediators secreted by immune cells (Fig. 1). Several experimental studies have demonstrated the tremendous influence of immune cell-mediated factors on bone cells and that blocking of the immune cell-bone cell interaction was able to abolish or attenuate OVX-induced bone loss and OVX-induced delayed bone repair. Therefore, in addition to the direct negative influence of estrogen-deficiency on bone, immune cell alterations contribute to the pathogenesis of postmenopausal osteoporosis.

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Declarations of interest

None.

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