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Osteoimmunology: The Nexus between bone and immune system

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1. ABSTRACT

Osteoimmunology is an interdisciplinary research field which combines the existing fields of osteology (bone biology) and immunology under one umbrella. The observation that contributed enormously to the emergence of osteoimmunology as an independent field of investigation was the enhanced bone loss in various inflammatory bone diseases such as rheumatoid arthritis, osteoporosis and periodontitis. T helper cells (Th1, Th2, Treg and Th17) along with various other immune cells (B cells, DC, macrophages etc.) are actively involved in bone homeostasis. The present review thus provides an

overview of the nexus between these two prominent systems (Bone and Immune system) of an organism, which reside in a common niche (bone marrow) and thus cross-communicate to modulate their respective development. Investigations in the field of osteoimmunology thus promise the advent of new era in the field with novel therapeutics for bone loss in various inflammatory conditions. A molecular insight into the field of osteoimmunology can lead to novel approaches for the prevention and treatment of diverse inflammatory conditions such as osteoporosis, rheumatoid arthritis and osteoarthritis.

2. INTRODUCTION

The term 'osteoimmunology' was coined by Aaron and Choi in the year 2000 while highlighting the two-way communication between the skeletal and immune systems, especially observed in various inflammatory bone diseases (1). The interaction between the bone and immune system has long been appreciated, but it's only the latest research related to bone phenotypes found in various immune related knockout mice which highlighted the importance of this interplay, thereby paving path for the emergence of this novel interdisciplinary field called osteoimmunology. Osteoimmunology studies the relationship between the skeleton and immune system (osteo-immune system), with a special emphasis on its various interconnected cells and molecular signaling pathways regulating these processes in a number of inflammatory conditions such as osteoporosis, rheumatoid arthritis (RA), cancer, periodontal disease etc. (2). Bone is an active tissue, being constantly renewed in healthy individuals with participation of the immune system to a large extent. Both the bone and immune cells are derived from common progenitors residing in the common niche called bone marrow. Any imbalance between the processes of bone formation and bone resorption is linked to various inflammatory bone diseases (3). Bone homeostasis is largely regulated by various immune responses under both normal and pathogenic conditions along with aging. The role of immune cells in modulating bone health is now well established; however, the molecular mechanisms responsible for such clinical manifestations have just begun to be unraveled with the advancement of osteoimmunology. Osteoimmunology still is a very naive and emerging field of modern biology, standing at the interface of both immune and bone systems. Thus, advancements in the field will not only improve our understanding about both the systems but will also conceptualize the development of novel treatment options for various bone diseases.

3. BONE CELLS

Bone is a dynamic organ which is continuously been remodeled throughout the life of an organism. This task is achieved by three different types of bone cells viz. osteoblasts (bone forming cells), osteoclasts (bone eating cells) and osteocytes (Figure 1). A detail discussion of the same is dealt under the following heads.

3.1. Osteoblasts

Osteoblasts are bone-forming cells derived from mesenchymal stem cells (MSCs) residing in the bone marrow which form clusters covering the bone surface. They are metabolically highly active, synthesizing the collagenous and non-collagenous bone matrix proteins, which are excreted and then deposited between the osteoblasts and the bone surface. This newly formed matrix, which is not yet calcified, is termed as osteoid. The lag phase between osteoid deposition and its mineralization is approximately 10 days. Differentiation of MSCs into osteoblast depends on the expression of two key transcription factors, Runx2 and its target Osterix, in response to external stimuli. Parathyroid hormone (PTH), Prostaglandin E2 (PGE2), insulin-like growth factor (IGF)-1, bone morphogenic proteins (BMPs), and Wingless and Int-1 (Wnt) proteins are key stimuli for osteoblast differentiation. PGE2 is an important anabolic factor for bone and induces the expression of bone sialoprotein and alkaline phosphatase in mesenchymal cells. BMPs and transforming growth factor (TGF)-β, which shares structural similarities with BMPs, foster osteoblast differentiation by activating intracellular SMADs. Wnt proteins belong to a family of highly conserved signaling molecules which are potent stimulators of osteoblast differentiation. Wnt proteins bind to receptors on mesenchymal cells such as Frizzled and low-density lipoprotein receptor-related protein 5 (LRP5), eliciting activation and nuclear translocation of β-catenin, thereby inducing transcription of genes involved in osteoblast differentiation. Wnt acts not only in close synergy with BMPs but also cross-talks to the receptor activator of nuclear factor κB ligand (RANKL)-osteoprotegerin (OPG) system, which is involved in the differentiation and function of bone-resorbing osteoclasts.

3.2. Osteocytes

Osteocytes are by far the most abundant cell type found within the bone. One cubic millimeter of bone contains up to 25,000 osteocytes, which are considerably connected with each other and the bone surface by small tubes (canaliculi), constituting a large and dense communication network inside the bone. The surface of this network of lacunae containing the osteocytes along with the canaliculi containing the interconnecting filaments of the osteocytes covers an area of 1000 to 4000 square meters. Osteocytes are derived from osteoblasts, which are subsequently entrapped in the bone matrix. Osteocytes however,

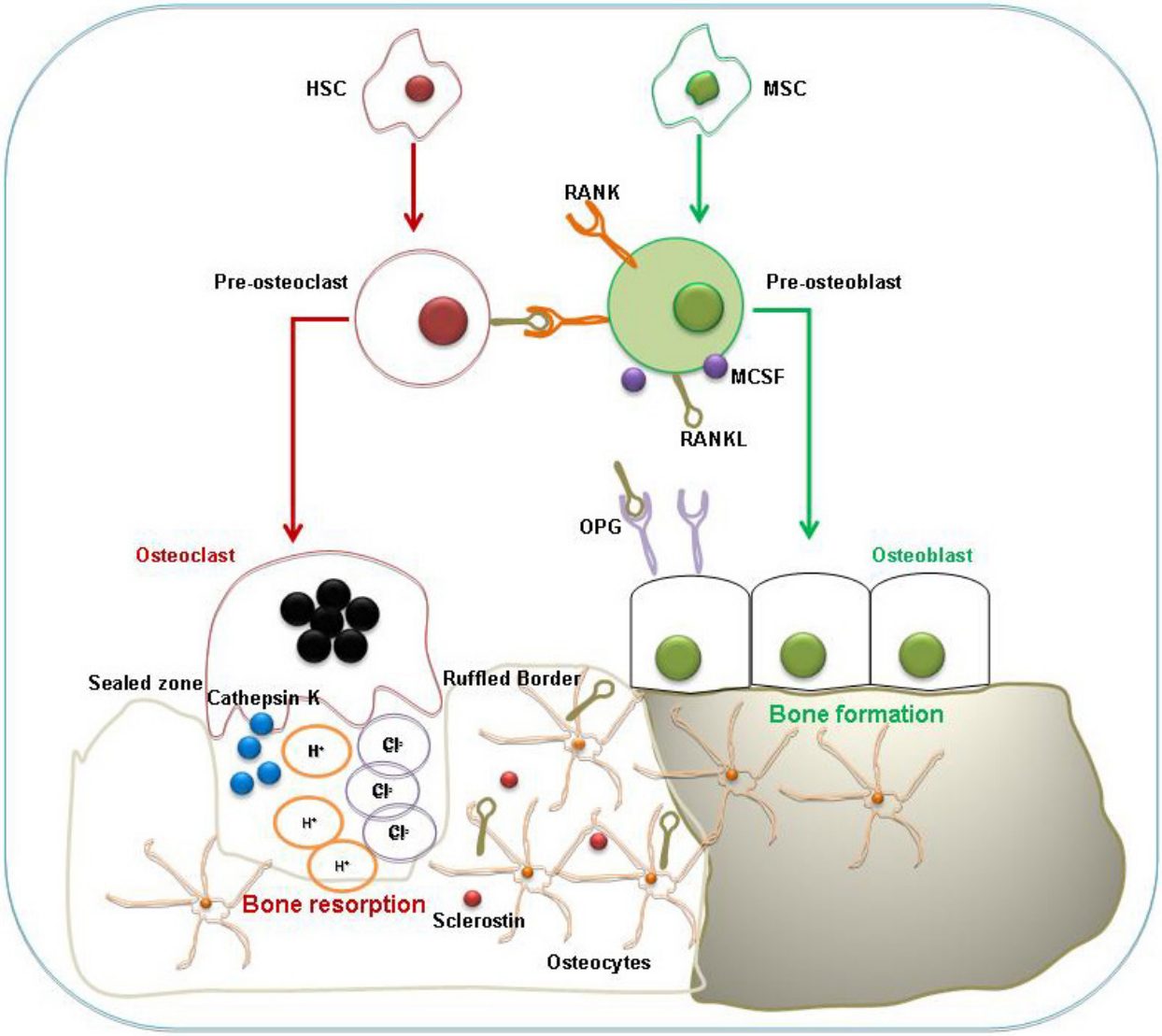

Figure 1. Interaction between Osteoblasts-Osteoclasts-Osteocytes during bone remodelling. Bone is continuously being remodelling by coordinated and sequential actions of bone-resorbing osteoclasts and bone-forming osteoblasts. Osteocytes which represent about 95% of all bone cells, cause bone remodelling which is fine tuned by both osteoclasts and osteoblasts. Osteoblasts are formed from MSCs and produce an extracellular bone matrix of type I collagen and non-collagenous proteins, including osteocalcin, osteonectin and osteopontin. The deposition of calcium hydroxyapatite causes mineralization and stiffness of bone. The HSCs, originated macrophages and monocytes differentiate into osteoclasts, the differentiation and functioning of these functionally activated multinucleated cells-osteoclasts depends on RANKL that acts on osteoclastic RANK in the presence of MCSF. OPG, produced by osteoblastic lineage cells helps in blocking the interaction of RANK with RANKL thereby inhibiting osteoclastogenesis.

also start to express genes that are specific for these cells, not found in other cells such as osteoblasts. One of the most interesting products of the osteocyte is sclerostin, a secreted molecule that binds LRPs and blocks Wnt-stimulated bone formation (4). Consistent with its function as an inhibitor of bone formation, sclerostin overexpression leads to low bone mass, whereas its deletion leads to increased bone density and strength. Loss of function mutations in SOST gene encoding sclerostin entails increased bone mass in humans, a disease termed sclerosteosis. Various local and systemic factors are responsible for regulation of sclerostin expression in osteocytes. For instance, intermittent administrations of PTH, which has strong anabolic effects on the bone, potently inhibits sclerostin expression.

3.3. Osteoclasts

Osteoclasts are bone eating multinucleated cells containing up to 20 nuclei (5). They are directly attached to the bone surface and build resorption lacunae (Howship's lacunae). Apart from their multiple nuclei, another characteristic of the osteoclast is the ruffled border, a highly-folded plasma membrane facing the bone matrix and designed to secrete and

resorb proteins and ions into the space between the osteoclast and bone surface (Figure 1). The space between this ruffled border and the bone surface is the place where bone resorption occurs. It is sealed by a ring of contractible proteins and tight junctions (sealing zone) because it represents one of the few regions of the human body, where a highly acidic surrounding is found. Bone erosion by osteoclasts comprises two major steps: first, demineralization of inorganic components, and second, removal of organic matrix in the bone. To demineralize the bone, osteoclasts secrete hydrochloric acid through proton pumps into the resorption lacunae; energy for the same is provided by an ATPase allowing the enrichment of protons in the resorption compartment, which, in fact, represents an extracellular lysosome. In addition to protons and chloride, osteoclasts release matrix-degrading enzymes including tartrate resistant acid phosphatase (TRAP), lysosomal cathepsin K, and other cathepsins. Cathepsin K can effectively degrade collagens and other bone matrix proteins. Consequently, inhibitors of cathepsin K block osteoclast function and slow down bone resorption. Osteoclasts are derived from hematopoietic precursor cells called as monocytes which under the influence of specific signals differentiate into mature osteoclasts (osteoclastogenesis). Osteoclastogenesis requires macrophage colony stimulating factor (MCSF) and RANKL as inducing signals. Pre-osteoblasts express both MCSF and RANKL and thus induce osteoclastogenesis, highlighting the close interaction between the processes of bone formation and resorption. During differentiation and maturation processes, osteoclasts acquire specific markers (viz. TRAP) and fuse with each other to form multinucleated giant cells. RANKL (a member of TNF superfamily) is expressed on the surface of diverse cell types including pre-osteoblasts and activated T cells (6). Its expression is induced in osteoblasts in response to various factors such as PTH, vitamin D, and prostaglandins. Moreover, inflammatory cytokines such as TNF, interleukin (IL)-1, and IL-17 induce enhanced expression of RANKL (7, 8). RANKL is indispensable for both the differentiation of osteoclasts and their bone-resorbing capacity by engaging its receptor RANK on monocytic osteoclast-precursor cells. The interaction of RANKL with RANK is regulated by OPG (a secreted glycoprotein), responsible for suppressing osteoclastogenesis both *in vitro* and *in vivo*. Interestingly, OPG expression is accelerated by estrogens, which explains the increase in osteoclast numbers and enhanced bone resorption after menopause. In accordance, RANKL-deficient mice have no osteoclasts and thus have severe osteopetrosis. Thus, exploiting the RANKL-RANK-OPG signaling pathway for various drug candidates had led to recent clinical trials on postmenopausal osteoporosis with the potent anti-resorptive effect of a neutralizing RANKL antibody (Denosumab) (9). Beyond RANKL-RANK interactions, other important pro-osteoclastogenic signaling pathways are based on the triggering receptor expressed on myeloid cells (TREM), which interacts with the tyrosine kinase DAP12 and the osteoclast-associated immunoglobulin-like receptor (OSCAR). Both molecules strongly enhance osteoclastogenesis (10).

4. BONE REMODELING

Bone remodelling requires a continuous change of functions provided by adaptation of the bone to various mechanical/physiological stress, a phenomenon accompanying functional adaptation observed by Wolff 100 years ago (11). This dynamic process of form following function (12) involves two processes viz. bone formation and bone resorption. Remodelling of bone is constituted by spatial and temporal coupling of bone formation by osteoblasts and bone resorption by osteoclasts (13). It has been estimated that 5–25% of bone surface is always on the mission of bone remodelling (14, 12), thereby ensuring mechanical integrity and restoring of micro damages along with creating a balance in the release of calcium and phosphorus in normal host physiology. The entire remodeling process takes about 3 to 6 months. Adults continuously remodel their skeleton, a process even faster in childhood and adolescence. In adults, it takes 7 to 10 years to remodel the entire skeleton, indicating that we fully replace it several times during our lifetime. Most of the bone remodeling happens in the trabecular bone, which promotes the building of an optimal inner microstructure adapted to the individual's mechanical demands. Trabecular bone is the leading structure in the vertebral bodies (up to two thirds of the bone substance) and in long bones such as the femurs (about 50% of the bone substance). The process of bone remodeling requires a tight mutual regulation of osteoblastogenesis and osteoclastogenesis, a phenomenon called coupling, regulated at three different levels: direct interaction between osteoclasts and osteoblasts, by local exchanges between the immune and bone system cells, and also by neuro-endocrine system. The different steps involved in a typical bone remodelling cycle are (Figure 2): activation, resorption, reversal and formation (15) and are discussed in detail as under.

4.1. Activation Phase

This phase is initiated due to the onset of remodelling signals which can be either direct mechanical strain on bones resulting in structural damage or due to action of hormones (Estrogen and PTH). The osteocyte apoptosis and increased osteoclastogenesis occur due to damaged bone matrix (16) such as limb immobilization (17). The osteocytes secrete TGF-β during normal conditions and thereby inhibit osteoclastogenesis. The osteocyte apoptosis lower the TGF-β level which leads to enhanced osteoclastogenesis (18). The activation of protein kinase (C/A) and calcium intracellular signalling

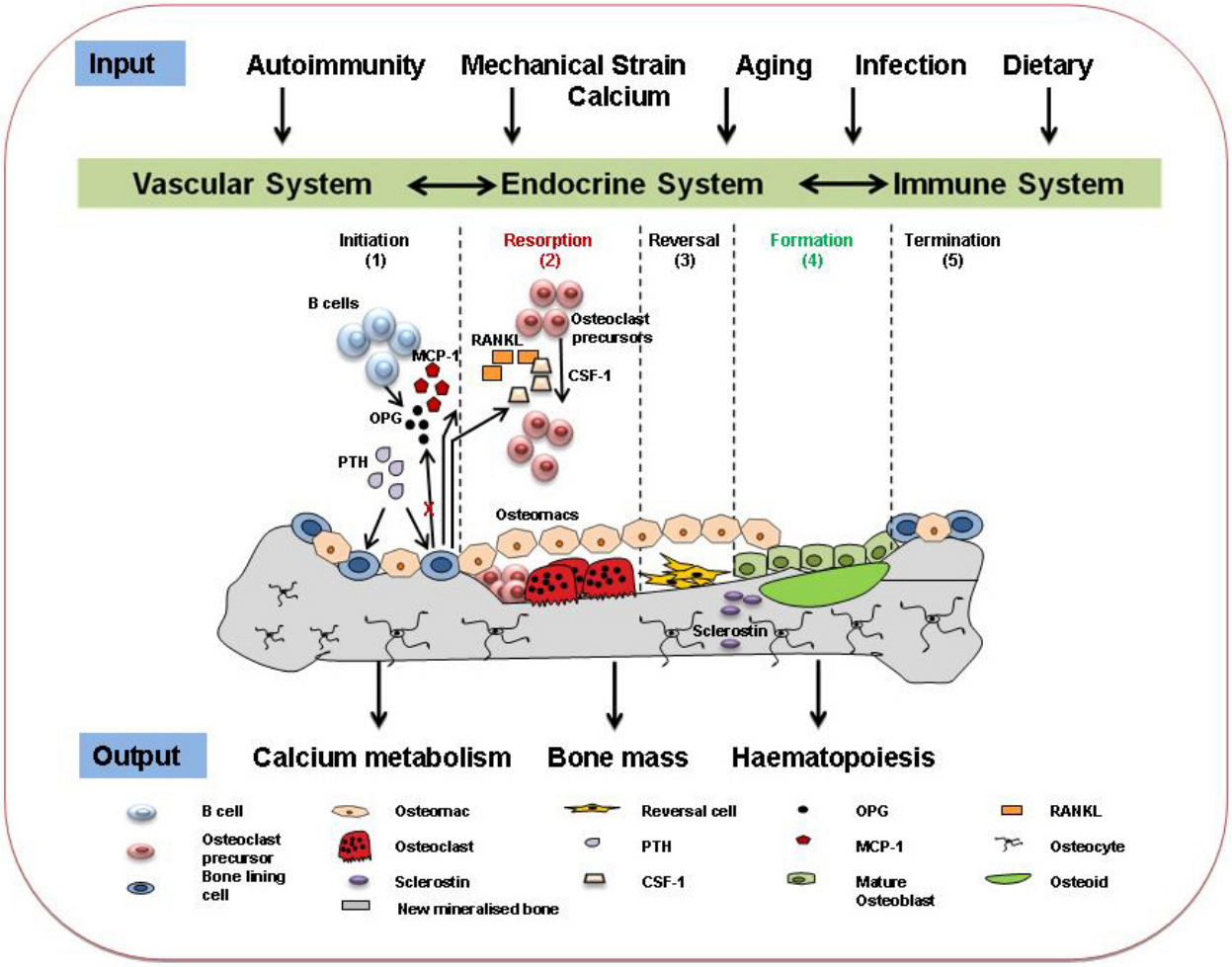

Figure 2. Schematic representation of Bone Remodelling cycle. The bone remodelling can be kicked off by a number of factors such as autoimmunity, mechanical strain, infection, dietary calcium etc. affecting vascular, endocrine as well immune system of the individual. The resting bone prior to activation is covered with bone lining cells (viz. pre-osteoblasts), which are in close association with osteomacs before the onset of activation. B cells present in bone marrow secrete osteoprotegerin which suppress osteoclastogenesis. The activation phase of bone remodelling defines the endocrine processes, where PTH binds to the PTH receptor on pre-osteoblasts. The osteocyte apoptosis occurring due to damaged mineralized bone matrix results in decreased concentration of TGF-β and its resultant effect on osteoclastogenesis. Resorption is initiated as MCP-1 is released from osteoblasts in response to PTH signaling, recruiting pre-osteoclasts which lead to a decreased concentration of OPG and at the same time there is increased expression of CSF-1 and RANKL promoting proliferation and differentiation of mature osteoclasts. These osteoclasts facilitate degradation of bone matrix due to creation of localized microenvironment. Reversal phase is demarcated due to the removal of undigested collagen from bone surface. During Formation process degraded bone matrix and mature osteoclasts initiate formation signals. The sclerostin expression is reduced due to osteocyte activation by PTH. In Termination phase sclerostin over expression returns to normal levels thereby inhibiting bone formation. The mineralization of newly formed osteoid occur and bone surface returns to resting phase with bone lining cells in close collaboration with osteomacs and remodelling of bone tissue gets completed.

pathways are initiated due to binding of PTH to its receptors (19), creating an environment responsible for secretion of molecules recruiting osteoclast precursors for the induction of osteoclastogenesis and in long run bone resorption.

4.2. Resorption Phase

The osteoblasts recruit precursors of osteoclasts to the bone remodelling site in response to signals initiated by osteocytes or direct endocrine activation signalling. The osteoblasts cause expression of monocyte chemoattractant protein (MCP)-1, a potent chemoattractant for osteoclast precursors thereby enhancing RANKL-induced osteoclastogenesis (20). The expression of different osteoclastogenic cytokines viz. RANKL, OPG and CSF-1 by osteoblasts is modulated in response to PTH. This modulation leads to over production of CSF-1, RANKL and reduced expression of OPG leading to enhanced bone resorption rate (21). RANKL induces differentiation and proliferation of osteoclast precursors to multinucleated osteoclasts leading to prolonged life of mature osteoclasts (22). Matrix metalloproteinases (MMP)-13 has also been found to be secreted due to mechanical (23) and endocrine

(24) remodelling signals. Osteoclasts adhere to these integrin-binding sites via $\alpha_v\beta_3$ integrin molecules (25) forming an isolated microenvironment beneath the cell called as “sealed zone” (Figure 1). The elevated hydrogen ions concentration (acidic environment) in the sealed zone results in dissolution of mineralized matrix resulting in formation of Howship’s resorption lacunae (26). The residual organic bone matrix gets degraded at a faster rate due to accumulation of collagenolytic enzymes (viz. cathepsin K) (27).

4.3. Reversal Phase

Bone resorption is followed by reversal phage in which mononuclear cells start removing remnants of collagen to prepare the bone surface for subsequent bone formation by the osteoblasts. Osteomacs (Osteal macrophages), a special subtype of macrophage residing in bony tissues, have been associated to be primarily responsible for removal of matrix debris during reversal phase. MMPs, the enzymes necessary for the matrix degradation are mainly produced by macrophages (28). Macrophages also initiate the expression of osteopontin (29), which is incorporated into mineralized tissue. But lately it has now been observed that mesenchymal bone lining cells cause deposition of collagenous matrix along with osteopontin rich cement lines within the Howship lacunae (30).

4.4. Formation phase

The different mechanisms giving rise to the coupling phenomenon for propagating the transition of bone formation to bone resorption has always remained controversial, as earlier studies postulated that bone matrix store these coupling molecules and liberate during bone resorption. The different factors like IGF (I and II) and the modulation of other factors like TGF-β primarily recruit different MSCs to bone resorption sites (31). While as in case of mice and humans having functionally defective osteoclasts, which are unable to resorb bone and bone formation by osteoblasts tend to prevail only in the absence of matrix bound growth factors, leading to the postulation that osteoclasts themselves produce the coupling factors (32). The differentiation of osteoblast progenitors at the resorption lacunae cause secretion of molecules that ultimately lead to formation of new bone surface. The main component of bone consists of collagen type I and the remaining organic part is composed of proteins, including proteoglycans, glycosylated proteins viz. tissue non-specific alkaline phosphates, Gla-containing proteins, small integrin-binding ligand and lipids (33). For bone to attain its final form, hydroxyapatite is integrated to form the newly deposited osteoid. The precise molecular mechanism underlying the different processes of bone mineralization still remains to be fully elucidated.

4.5. Termination Phase

The bone remodelling cycle concludes when there has been an attainment of equilibrium between bone resorption and bone formation and thereby triggering signals through osteocytes which tend to cease this ongoing dynamic equilibrium. Towards the end of the remodelling cycle, loss of sclerostin expression causes initiation of osteoclastogenesis. Following bone mineralization, mature osteoblasts either undergo apoptosis or revert to a bone lining phenotype or become embedded in the mineralized matrix and differentiate into osteocytes. Developmental bone growth, post developmental maintenance, repair of bone, and provision of calcium from the bone depend on a dynamic process called bone remodeling. To maintain structural integrity of the skeleton, bone needs to be constantly remodeled along with repairing the micro-cracks that develop in both cortical and trabecular bones due to normal wear and tear.

5. IMMUNE CELLS

The immune system is a remarkably versatile defense system which has evolved for protection of animals from invading pathogens. Both the adaptive and innate immunity mediate this task efficiently. Both the immune and bone cells reside within the same physiological niche of bone marrow, thereby creating diverse crosstalk options between both the systems. Thus, the interactions between immune-bone cells continue to be of great scientific interest to both researchers and clinicians. We will now discuss the individual components of the immune system and their effects on the bone health (Figure 3) in the following heads.

5.1. T Cells

T cells are the key players during various adaptive immune responses. Naïve Th cells can differentiate into various subsets depending upon the environmental stimuli (antigens, cytokine etc.) they receive during their activation. Based on these, Th cells are now well categorized into four established subsets viz. Th1, Th2, Treg and Th17 cells. The bone protective role of resting T cells was clearly demonstrated by the finding that T cell-deficient mice have enhanced osteoclastogenesis and thus reduced bone density with respect to controls animals (34). Unactivated Th cells have been reported to suppress osteoclast formation both *in vitro* (35) and *in vivo* (34). Under basal homeostatic conditions Th cells do not secrete RANKL (36). In contrast, activation of T cells during inflammatory conditions leads to enhanced production of RANKL and TNF-α, thereby promoting osteoclastogenesis and subsequent bone loss in various inflammatory and autoimmune conditions (37) such as periodontitis (38, 39), cancer (37) and

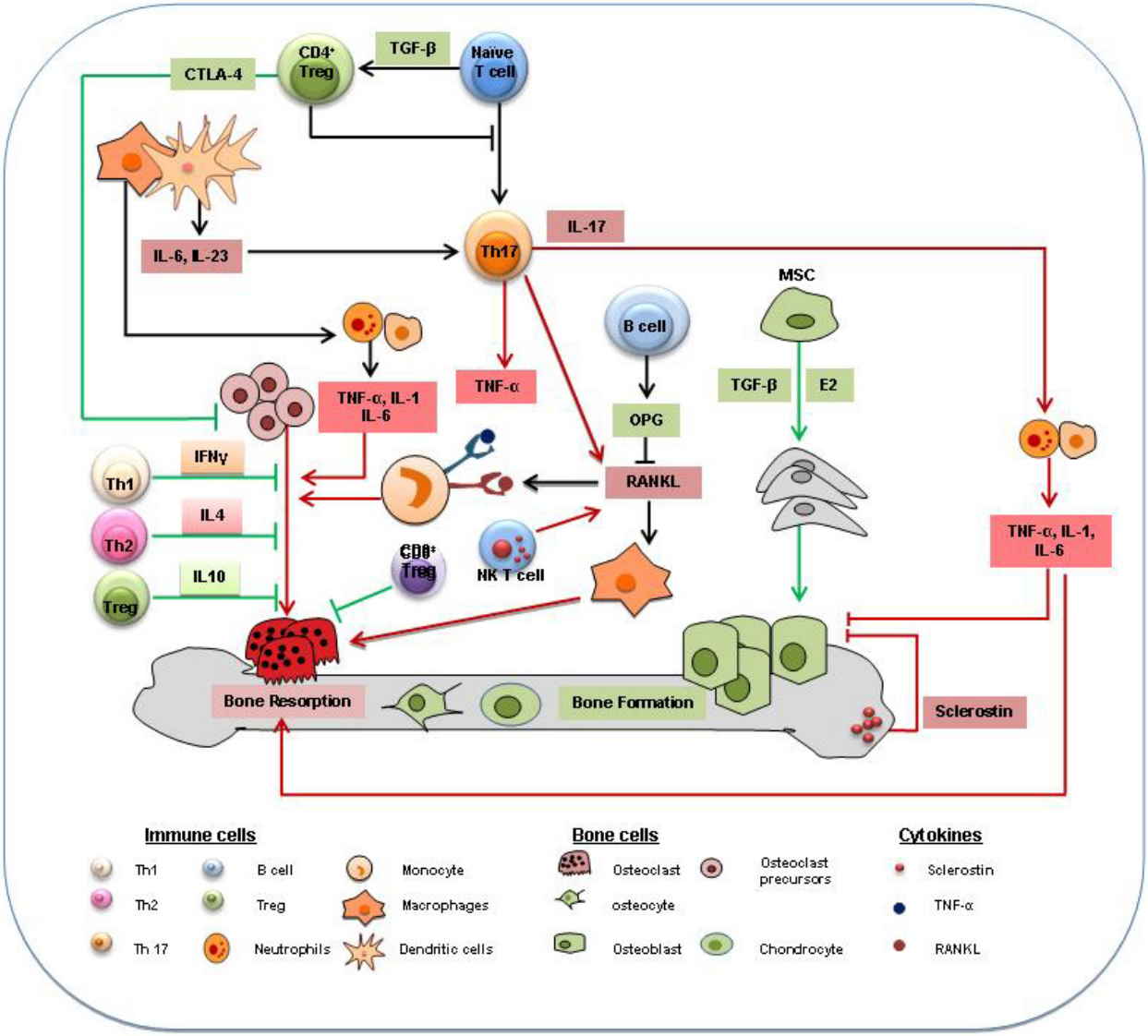

Figure 3. The mechanism of bone loss through Osteo-immune network. The immune skeletal interface works through deep interaction between different immune cells and bone cells giving rise to a typical immune skeletal interface. The Th cell subsets (Th1, Th2, Th17 and Tregs) play an important role in modulating bone health. Th1 and Th2 secrete IFN-γ and IL-4 respectively leading to the inhibition of osteoclastogenesis. Treg cells further secrete IL-10, CTLA4 (which can bind to CD80/CD86 on osteoclast precursors) which promote apoptosis and inhibition of bone resorption. On the other hand, Th17 cause expression of different osteoclastogenic cytokines (IL-1, IL-6, IL-17, TNF-α), thereby leading to enhanced osteoclastogenesis and bone loss. B cells produce OPG which block the expression of RANKL, the main key factor responsible for osteoclastogenesis. The sclerostin produced by osteocytes inhibit the osteoblast bone formation process. Other immune cells viz. macrophages, DCs and monocytes secrete different proinflammatory cytokines such as TNF-α, IL-1 and IL-6 leading to increased expression and differentiation of osteoclastogenesis leading to enhanced bone loss.

osteoporosis (40, 41). However, not all types of T cells are osteoclastogenic, as $CD8^+$ T cells have been reported with a bone protective role. $CD8^+$ T cells have been shown to suppress osteoclastogenesis via secretion of various soluble proteins such as OPG (42). However, use of blocking antibodies against OPG had no effect in reversing the suppression mediated by $CD8^+$ T cells, signifying the role of another factor(s) (39). Recently, a central role for $CD8^+$ T cells in bone tumor burden have also been demonstrated, thereby protecting the bone from metastasis (43).

5.2. Th1 and Th2 Cells

Naive $CD4^+$T cells proliferate and differentiate on antigenic stimulation into distinct effector T cell subsets. Based on their respective cytokine production profiles, differentiated $CD4^+$T cells were earlier classified into either Th1 or Th2 subsets (44). Th1 cells are mainly involved in the eradication of intracellular pathogens with the help of IL-2, IL-12, IFN-γ, TNF-α and TNF-α secreted by them. On the other hand, Th2 cells are involved in the elimination of parasitic

infections, extracellular microorganisms and allergic conditions by the secretion of IL-4, IL-5, IL-6, IL-9, and IL-13 cytokines (45). Both Th1 and Th2 cells are now know to inhibit osteoclast formation by various groups through secretion of their signature cytokines IFN-γ and IL-4 respectively (Figure 3) (46). In the last decade, the discovery of two new subsets of $CD4^+$Th cells have been reported; Th17 cell subset (46) and regulatory T (Treg) cell subset, defined on the basis of expression of master transcription factors Roryt and FoxP3 respectively (47, 48). Of these two Th17 cells are now confirmed to be the main osteoclastogenic T cells.

5.3. Th17 cells

Naive T cells when activated in the presence of TGF-β and inflammatory stimuli in humans or TGF-β and IL-6 in mouse differentiate into Th17 cells. Th17 cells secrete IL-17A and IL-17F the signature cytokines of Th17 cells along with IL-22, and IL-26 (49). Th17 cells also have been reported to produce certain amounts of IFN-γ (50). Th17 cells induce osteoclastogenesis through secretion of IL-17, a known osteoclastogenesis promoting cytokine via induction of RANK (51), in both stromal cells and osteoblasts. However, the effect of IL-17 is not only limited to its direct effect on osteoclastogenesis supporting cells, IL-17 also supports and escalates local inflammation by inducing the secretion of various inflammatory cytokine (TNFα, IL-1 etc.) from supporting immune cells (Figure 3) (52). These cytokines in lieu enhance the expression of RANKL on osteoclastogenesis supporting cells and thus activate osteoclast precursor cells through RANKL signaling. Th17 have now been proven to have a dominant role in various bone diseases, such as arthritis and osteoporosis (53, 54).

5.4. $CD4^+$ Treg cells

$CD4^+$ regulatory T cells are now known to inhibit osteoclast differentiation and function under both *in vitro* and *in vivo* conditions and thereby suppress inflammatory bone loss in mice (55, 56). Tregs suppress osteoclastogenesis mainly via two mechanisms: firstly, via direct cell-cell contact, secondly through contact independent mechanisms involving production of various signature cytokines (57, 58). Kim *et al.* reported that Tregs inhibit osteoclast differentiation from peripheral blood mononuclear cells in a cytokine-dependent manner via secretion of TGF-β and IL-4 cytokines (59). Tregs have also been recently implicated in suppressing osteoclast differentiation and bone resorption in estrogen deficient postmenopausal osteoporosis model through production of IL-10 and TGF-β1 (57). Another reported mechanism by which Treg cells control immune function is by the secretion of cytotoxic T-lymphocyte antigen 4 (CTLA-4), which binds to CD80/CD86 on mononuclear osteoclast precursor cells and inhibits inflammatory responses (Figure 3) (58, 59). Further Treg cells have been shown to inhibit collagen induced arthritis in mice (60). Treg cells have also been reported to directly inhibit osteoclastogenesis and suppress formation of resorption pits *in vitro* by $CD11b^+$ monocytes which have been treated with M-CSF and RANKL.

5.5. $CD8^+$ Treg cells

Although $CD8^+$Treg cells have been documented in both humans and mice (61) they have not been extensively studied till date mainly due to their low abundance (0.2. to 2% of $CD8^+$ T cells) in both the circulation and periphery. In comparison, the well-studied $CD4^+$ Treg cells comprise approximately 5–12% of $CD4^+$ T cell population in various lymphoid organs. The $FoxP3^+CD8^+$Treg cells and the $FoxP3^+CD4^+$Treg cells both have several distinct and overlapping functions. Both $CD8^+$Treg cells and $CD4^+$Treg express CD25 cell marker along with the marker of regulatory T cells i.e. $FoxP3^+$ transcription factor. Recently it has been shown that the osteoclast-induced $FoxP3^+CD8^+$Treg cells secrete cytokines that suppress both the formation and activity of osteoclasts (62). The $FoxP3^+CD8^+$Treg cells do not affect the survival of osteoclasts, but affects the activity of mature osteoclasts by suppressing their actin ring formation. The ability of osteoclasts to induce $FoxP3^+CD8^+$Treg cells and the ability of $FoxP3^+CD8^+$Treg cells to subsequently regulate osteoclast function establishes a bidirectional regulatory loop between these two cells types in the bone marrow. Interestingly, this regulatory loop does not require the presence of various proinflammatory cytokines. Indeed, the ability to isolate functional $FoxP3^+CD8^+$Treg cells from mice, in the absence of any inflammatory disease, indicates that these cells have an important role in maintaining skeletal homeostasis (62).

5.6. Natural killer (NK) T cells

NK T cells are involved in the clearance of virus-infected, transformed or aberrant cells (63). NK cells release a rapid wave of cytokines and growth factors that influence the initiation and development of immune responses mediated by both T and B cells (64). The activation of the invariant NKT (iNKT), a subset of NK T cells increases the development, maturation and activity of osteoclasts (65). NK T cells have also been detected in the inflamed synovial tissue, and constitute up to 20% of all lymphocytes in the synovial fluid of patients with established RA (66). Recent reports clearly indicate that $CD56^{bright}$ NK T cell subsets have upregulated expression of various adhesion molecules and chemokine receptors that contribute to the preferential recruitment of NK T cells into inflamed synovium of RA patients (67), thereby enabling cells to engage and subsequently activate

monocytes through various receptor-ligand interaction pathways (66, 68). NK T cells present in the synovial fluid of RA patients efficiently trigger formation of osteoclasts from monocytes precursors. NK T cells also express both MCSF and RANKL (Figure 3), responsible for the induction of osteoclastogenesis which is further upregulated by IL-15 (69, 70).

5.7. *γδ T Cells*

The majority of T cells in the circulation express αβ-T cell receptor (TCR) chains, but a small subset of T cells expresses a unique TCR, containing a gamma (γ) chain paired with a delta (δ) chain, thereby, forming a γδ TCR heterodimer giving rise to a population of T cells called as γδ T cells. Γδ T cells comprise only about 1–10% of cells in the human peripheral circulation, however their numbers are more abundant in tissues such as the skin (70). γδ T cells are different from αβ T cells in that their function is largely innate-like rather than adaptive and their TCR specificity is directed almost exclusively towards nonpeptide antigens. They have now been linked with various inflammatory conditions such as autoimmunity, allergy, certain hematological tumors (70, 71) and infectious disease (70). They express various growth factors such as connective tissue growth factor (72) and fibroblast growth factor (83) that are critical in healing of skeletal fractures. The population of γδ T cells is quite heterogeneous and unique as these cell populations are lost in patients on treatment with amino-bisphosphonates (73, 74).

5.8. B Cells

B cells have a very intricate and versatile relationship with various immune cells along with the bone cells (75). B cells differentiate from hematopoietic stem cells (HSCs) in the bone marrow. Osteoblastic lineage cells present in the bone marrow niches support the differentiation of both HSCs and B cell differentiation. The differentiation of B cells is regulated by various transcription factors, as several knock outs of these transcription factors have resulted into attenuated bone phenotypes. These studies thus highlight the close nexus between osteoclast and B cell differentiation. Activated B cells have an important role in various inflammatory diseases resulting in altered bone physiology. Both B cells (76, 37) and B-cell derived plasma cells have been reported to regulate osteoclastogenesis (77), via expression of RANKL (78), decoy receptor 3 (DcR3) (79), or as an indirect consequence of IL-7 secretion (80) which is a potent stimulator of bone resorption (81). Malignant B-cell-derived plasma cells in multiple myelomas inhibit osteoblast differentiation by production of cytokines such as dickkopf-related protein 1 (DKK1) and sclerostin (77). B cell lymphopoiesis is enhanced during estrogen deficiency (82), whereas estrogen treatment inhibits B cell lymphopoiesis (83). Thus, B cells have an important role in ovariectomy induced bone loss (81). Interestingly, immature B220 expressing B cell populations can even trans-differentiate into osteoclast differentiation pathways under in vitro conditions (84), thereby providing source of osteoclast precursors in ovariectomy induced bone loss. Expression of RANKL by $B220^{+}$ cells has been reported to be enhanced in ovariectomized mice (85). Also, B cells isolated from the bone marrow of estrogen deficient postmenopausal women has been reported to secrete RANKL (86), further strengthening the notion that B cells have an active role in osteoporosis. Peripheral blood B cells have been reported to inhibit osteoclast formation by secretion of TGFbetain a human in vitro model of osteoclastogenesis (87) by stimulating OPG production (88). Depletion of B cells in an animal model of periodontitis aggravates bone loss, suggesting the role of B cells in limiting bone resorption under certain pathological conditions (89). Mice deficient in B cells have increased bone resorption and are thus osteopenic. B cells are the major source of OPG in the bone microenvironment and thus its deficiency leads to enhanced osteoclastogenesis (34). Conditional B cell RANKL knock out studies in mice reveal the contribution of mature B cells in ovariectomy induced bone loss, as mice lacking RANKL in B lymphocytes were partially protected from enhanced osteoclastogenesis (90) with a simultaneous decline in OPG (Figure 3) production. Thus, the resulting imbalance in the ratio of RANKL/OPG favors bone resorption by enhancing osteoclastogenesis (91). Further the crosstalk between B cells and T cells further regulates production of various bone-active cytokines, since B cells suppress osteoclastogenesis when activated by Th1 cytokines, but promotes osteoclastogenesis when stimulated by Th2 cytokines (92). Both T and B cells also cooperate in limiting basal bone resorption in vivo by regulating the secretion of OPG by B-lineage cells via CD40/CD40L costimulatory pathways (93).

5.9. Dendritic Cells

Dendritic cells (DCs) are highly potent antigen presenting cells (APCs) which play an important role in both the initiation and regulation of T cell mediated immunity to pathogens and tumors along with preventing immune responses against self-tissues (94). Normally DCs are not found in the bone and they do not contribute to bone remodeling, since DC deficient animals have not been reported with any skeletal defects (95). In contrast, both mature and immature DCs have been reported to be present in various compartments of synovial and periodontal tissues in inflammatory conditions (96, 97). Interestingly, active disease sites in RA and periodontitis are hotspots for DC and T cells interaction thereby regulating inflammation induced bone loss through modulation of T cell activity (96, 97). Recent

report by Rivollier's group (98) had shown that human peripheral blood monocytes-derived DCs can trans-differentiate into osteoclasts in the presence of MCSF and RANKL under *in vitro* conditions, suggesting the role of DCs in osteoclastogenesis. The role of DCs in osteoclast development was clarified by *in vitro* studies using pure $CD11c^{+}CD11b^{-}$DC subset in DC-osteoclast development (5), derived from total BM cultures in the presence of granulocyte-macrophage colony stimulating factor (GMCSF) and IL-4 (99). The results suggested that murine $CD11c^{+}$DC can develop into functional osteoclasts during immune interactions with $CD4^{+}$ T cells and are potent in inducing bone resorption (99). These findings clearly indicate the key role of $CD11c^{+}$DC subset(s) in osteoclastogenesis. DCs may also undergo osteoclast like trans-differentiation following stimulation via the RANK-RANKL pathway (100). Additionally, mature DCs can also drive the activation and expansion of IL-17 secreting Th17 cells, thereby enhancing osteoclastogenesis (101).

5.10. Neutrophils

Neutrophils are one of the most abundant forms of white blood cells (WBCs) found in the blood stream of mammals. Neutrophils are a crucial arm of the innate immunity during the early phases of inflammation resulting from bacterial infections, environmental exposure and certain cancers. Neutrophils show massive infiltration at sites of bony lesions in both human and animal models of bone diseases such as human periodontitis (102) and arthritis (103, 104). Activated neutrophils participate in the inflammatory process by synthesizing significant amounts of proteins and lipids (105). Neutrophils have also been documented to express high levels of RANKL (106) in both human and murine systems and thus activate osteoclastic bone resorption (107). Neutrophils have further been reported to regulate osteoblast functions thereby leading to increased bone resorption (108, 109).

5.11. Macrophages and osteomacs

Macrophages are mononuclear cells of the myeloid lineage, and are responsible for maintaining homeostasis and tissue repair. Macrophages fuse with each other to form multinucleated cells called as polykaryons which thereby easily resorb larger materials than the mononuclear pre-fusion cells. This unique ability of macrophages has put them at a central point in the evolution and function of the skeletal and immune systems as multinucleated macrophages can either form giant cells like granulomas at chronic inflammatory sites or giant multinucleated osteoclasts in the bone. The decision to undergo osteoclastogenesis is governed solely by the local cytokine milieu with RANKL (110). Macrophages, derived from blood (monocytes), are actively involved in tissue inflammation site against noxious insult; they are capable to perform a wide array of functions. Inflammatory macrophages have the ability to kill through apoptosis, clearance of proliferating resident stromal, infiltrating leukocytes, and parenchymal cells (111).

Osteomacs are tissue macrophages present on or within the endosteal and periosteal surfaces of the bone. They compose approximately 10–15% of the bone tissue and play an important role in development, homeostasis, and repair of bone tissue (112). Osteomacs can be identified with the help of the pan macrophage protein F4/80 in mice, which are absent on osteoclasts. The myeloid marker CD68 defines the expression of osteomacs in humans (113). Osteomacs have been associated with full functional differentiation, including mineralization of osteoblasts during *in vitro* cultures. Osteomacs under *in vivo* conditions form a canopy at bone modelling sites over matrix-producing osteoblasts. The reduction/loss of macrophages *in vivo* leads to a complete loss of endosteal osteomacs along with their associated osteoblasts, defining that osteomacs play an essential role in maintenance of mature osteoblasts (114).

6. OSTEOIMMUNOLOGY: INTERACTION OF BONE AND IMMUNE SYSTEMS

Bone and immune system is integrated functional unit (osteoimmune system) by virtue of their common niche, leading to permanent interaction between them at various anatomical and vascular contacts (115). This novel field of osteoimmunology had now convinced the scientific community that there exists a two-way communication between the bone and immune systems. This intricate relationship between the dual systems has been intriguing scientists since the early 1970s, giving birth to the novel field of "osteoimmunology" (116). However, still the role of immune cells in normal bone physiology is not completely understood and is a matter of intense research. Lymphocytes through the secretion of various factors regulate bone remodelling and play an important role in bone homeostasis (Table 1). Thus, researchers currently are more interested in the various types of immune-bone cell interactions responsible for enhanced osteoclastogenesis observed in bone pathologies. The nexus between both the immune and bone systems ("Osteoimmunology") can only be appreciated by understanding the very basics of bone pathologies in various bone pathologies such as osteoporosis, RA and osteoarthritis (Figure 4). We have dealt these under the following separate heads.

6.1. Osteoimmunology of osteoporosis (post-menopausal)

Reduction in ovarian production of estrogens (or oestrogens) in postmenopausal phase is the main cause for initial phase of rapid bone loss

Table 1. Role of cytokines/factors in Osteoimmune system

Cytokine	Source	Effect on OCs	Effect on immunity	Function in bone Homeostasis	Ref.
IL-1	Macrophage and DCs	OC↑	Pro-inflammation	Directly activates RANK signaling to promote osteoclastogenesis	(51)
IL-3	Activated T cells	OB↑	Blocks RANKL induced osteoclast	Inhibits osteoclastogenesis	(60, 199–202)
IL-4	Th2	OC↓	Humoral Immunity	Inhibits osteoclastogenesis	(201)
IL-6	Macrophage, DCs	OC↑	Pro-inflammation, Th17 induction	Activation of osteoclastogenesis	(203)
IL-7	BMSC	OC↑	Promotion of T/B cell development	B cell development	(80)
IL-10	Treg	OC↓	Anti-inflammation	Suppress bone resorption	(58)
IL-17	T cells	OC↑	Pro-inflammatory cytokine	RANKL expression and vigorous pro-inflammatory potency	(2)
IL-18	Macrophage	OC↓	Th1 differentiation, IFN-γ Induction	Inhibits TNF-α mediated osteoclast formation in a T cell independent manner	(204)
IL-23	Macrophage and DCs	OC↑	Th17 induction	Indirect osteoclast activation	(135)
IL-27	Macrophage and DCs	OC↓	Th1and Treg↑; Th17 induction	Inhibits osteoclast formation, blocking RANK dependent osteoclastogenesis	(205)
GM-CSF	Th1	OC↓	Pro-inflammation	Inhibits osteoclastogenesis	(52)
IFN- γ	Th1, NK cells	OC↓	Cellular immunity	Inhibits osteoclastogenesis	(203)
OPG	Osteoclasts	OC↓	Decoy receptor for RANKL	Inhibits osteoclastogenesis	(46)
RANK	Osteoclasts, DCs	OC↑	DCs activation	Osteoclast differentiation and activation	(52)
RANKL	Osteoblast, Th cells	OC↑	DCs maturation and osteoclast differentiation	Direct osteoclast activation through RANK	(206)
TNF-α	Th17, Macrophage DCs	OC↑	Pro-inflammatory cytokine	Indirect osteoclastic activation through RANKL	(206)
TGF-β	Multiple cell lines	OC↑	Blocks activation of lymphocytes and monocytes derived phagocytosis	Indirect osteoclast activation, Inhibits osteoblast differentiation	(52, 207)

Abbreviations: IL: Interleukin; OC: osteoclast; OB: Osteoblast; DCs: Dendritic Cell (s); RANK: Receptor activator for nuclear factor κB; RANKL: Receptor activator for nuclear factor κB ligand; IFN-γ: Interferon-γ: BMSC: Bone marrow stromal cells; TNF-α: Tumor necrosis factor; TGF-β: Transforming growth factor; GMCSF: Granulocyte macrophage colony stimulating factor; OPG: Osteoprotegerin; NK cells: Natural killer cells.

in women, with an annual bone loss rate of 3–5%, responsible for initial trabecular bone loss. These differences in physiology and in bone geometry along with absence of menopause in male's leads to an increased and enhanced frequency of osteoporotic fractures in postmenopausal women than in males of same age (117). The postmenopausal osteoporosis prevalence and severity are modulated by nutrition, smoking, body mass index (BMI), genetic factors and ageing etc. The early occurrence of bone loss in postmenopausal scenario leads to increased levels of PTH and follicle stimulating hormone (FSH) (118). Estrogen deficiency has been associated with increased bone loss by decreasing apoptosis of osteoclast thereby leading to enhanced osteoclastogenesis (119). However, the presence of estrogen has anti-inflammatory and bone protective role (120) by inhibiting self-renewal of osteoblast progenitors (121), reducing production of OPG from stromal cell (122), enhancing production of M-CSF (123) and RANKL (124), and decreasing the anti-anabolic factor sclerostin (125).

T cells and other immune cells play a critical role in postmenopausal osteoporosis. Various studies have been done in ovariectomized (ovx) mice which indicate that in T cell deficient nude mice, ovx could not induce cortical and trabecular bone loss (126–130). These observations were further confirmed, when administration of Abatacept (which causes blockage of T cell simulation and T cell anergy and apoptosis) in ovx mice resulted in depletion of T cells, thereby preventing against ovx induced bone loss (131–133). It has been observed that both $CD4^+$ and $CD8^+$ cells pay a pivotal role in ovx induced bone loss. $CD4^+$ cells consist of Th1, Th2, Th17 and Treg subsets. Th17 cells are the main cells causing osteoclastogenesis by the production of higher levels of IL-17, RANKL, TNF-α and lower levels of IFN-γ (134, 135). Tregs through their production of IL-4, IL-10 and TGF-β1 can suppress the effector functioning of Th17 cells. Tregs can also lead to suppression of bone loss by inhibiting differentiation of monocytes into osteoclasts under both *in vitro* and *in vivo* conditions (136, 137). Estrogen have also been reported to enhance the functioning of Tregs, as

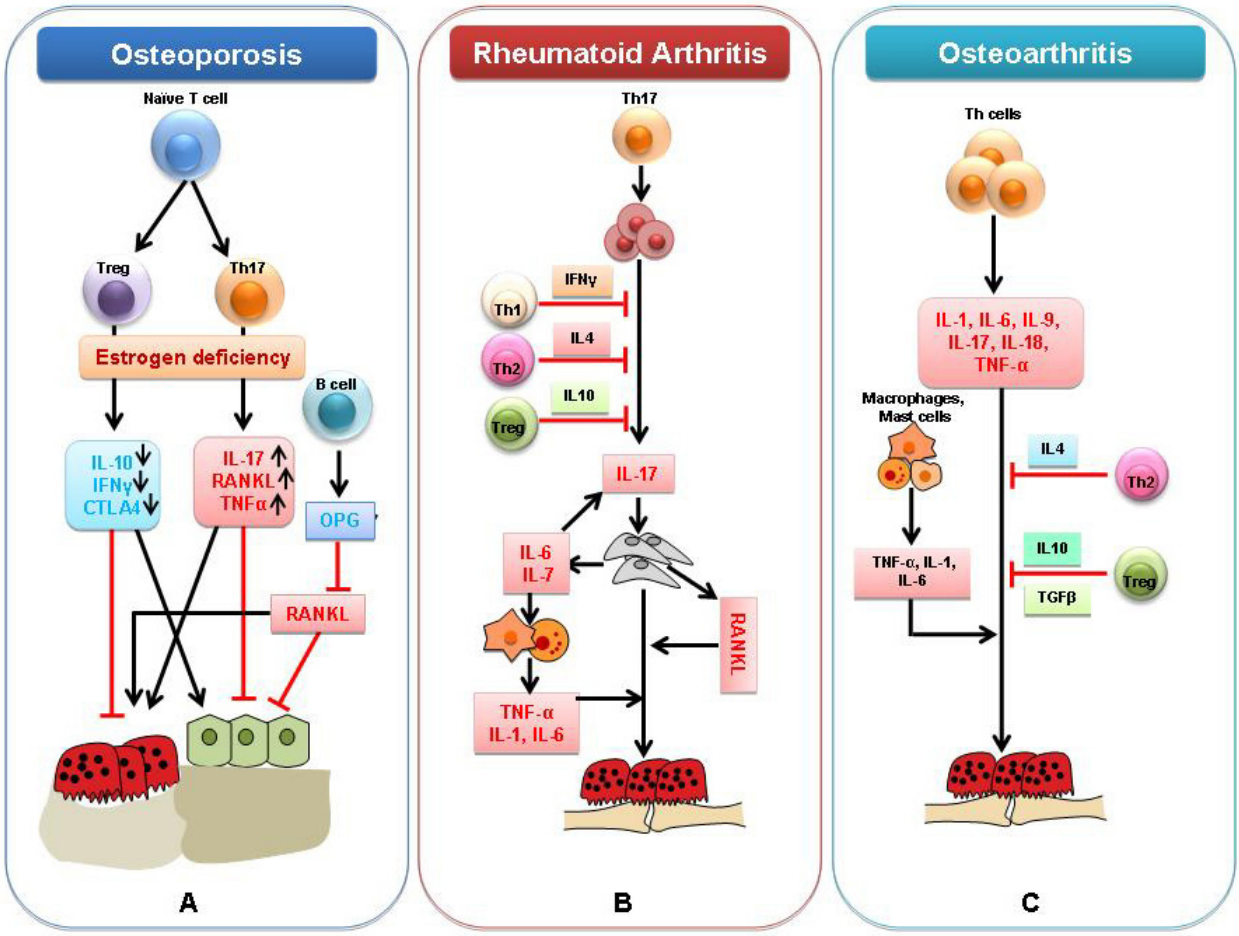

Figure 4. Osteoimmunology of Inflammatory bone diseases. A) The estrogen deficient osteoporosis is caused due to the over expression of Th17 cells leading to the enhanced concentration of different pro-inflammatory cytokines (IL-1, IL-6, IL-17 and TNF-α) and RANKL. The differentiation and expression of Th1, Th2 and Tregs get suppressed during estrogen deficiency (leading to decreased production of anti-inflammatory cytokines like IFN-γ, IL-4, IL-10 and CTLA-4), resulting in enhanced osteoclastogenesis and bone loss. The production of B cells producing OPG is also decreased which could have been an ideal factor for inhibiting RANKL associated bone loss. B) In RA, Th17 act on osteoclast precursors secreting greater amounts of IL-17, which along with other pro-inflammatory cytokines produced by inflamed synovium, macrophages and neutrophils (IL-6, IL-7, TNF-α, and RANKL) enhance the degradation of bone at synovial joints. Simultaneously the levels of different immune cells (Th1, Th2 and Tregs which hamper advancement of arthritis by secretion of IFN-γ, IL-4, IL-10 respectively) is greatly reduced. C) OA is represented by elevated levels of different helper T cells Th1, Th9 and Th17 secreting different pro-inflammatory cytokines viz., IL-1, IL-9 and IL-17 respectively. These cytokines in association with other inflammatory cytokines secreted by mast cells, neutrophils and macrophages at the site of synovial membrane or fluid enhance pathogenesis of bone leading to osteoarthritis condition.

transgenic mice having over expression of Tregs were found with high bone mass due to inhibition of bone resorption by Tregs, thereby preventing ovx induced bone loss (138).

Estrogen is a crucial regulator of osteoblast differentiation and activity. Estrogen enhances osteogenic differentiation of cultured MSCs, promote differentiation of osteoblasts and prolong lifespan of both osteoblast and osteocytes by suppressing apoptosis (139). Estrogen upregulates the expression of RANKL in osteoblastic cell lineage and induces the expression of OPG, a naturally occurring antagonist of RANKL (140). Estrogens have also been reported to interfere with osteoclast signalling downstream of RANK, thereby preventing osteoclast differentiation and promoting osteoclast apoptosis via increased production of TGF-β by osteoblasts. Under conditions of acute estrogen deficiency (post-menopausal condition in woman's), both osteoclast differentiation and activity is increased due to higher expression of RANKL (141) and other pro-osteoclastogenic cytokines (TNF-α, IL-1 and IL-6) by MSCs and lymphocytes. Postmenopausal osteoporosis demonstrates many phenomenon relating it to inflammatory disease (142), leading to designing of future therapies to ameliorate bone loss. Estrogen deficiency have been related for setting up of a chronic inflammatory state which in turn leads to bone loss, and this breaking up of the inflammatory cascade at any point can prove to be effective in alleviating bone loss in different animal models (143, 144). Likewise, different pharmacological and genetic ablation of IL-1 and TNF (143, 145), IL-7 (146), IFN-γ (144) and IGF-1 (147), or over expression

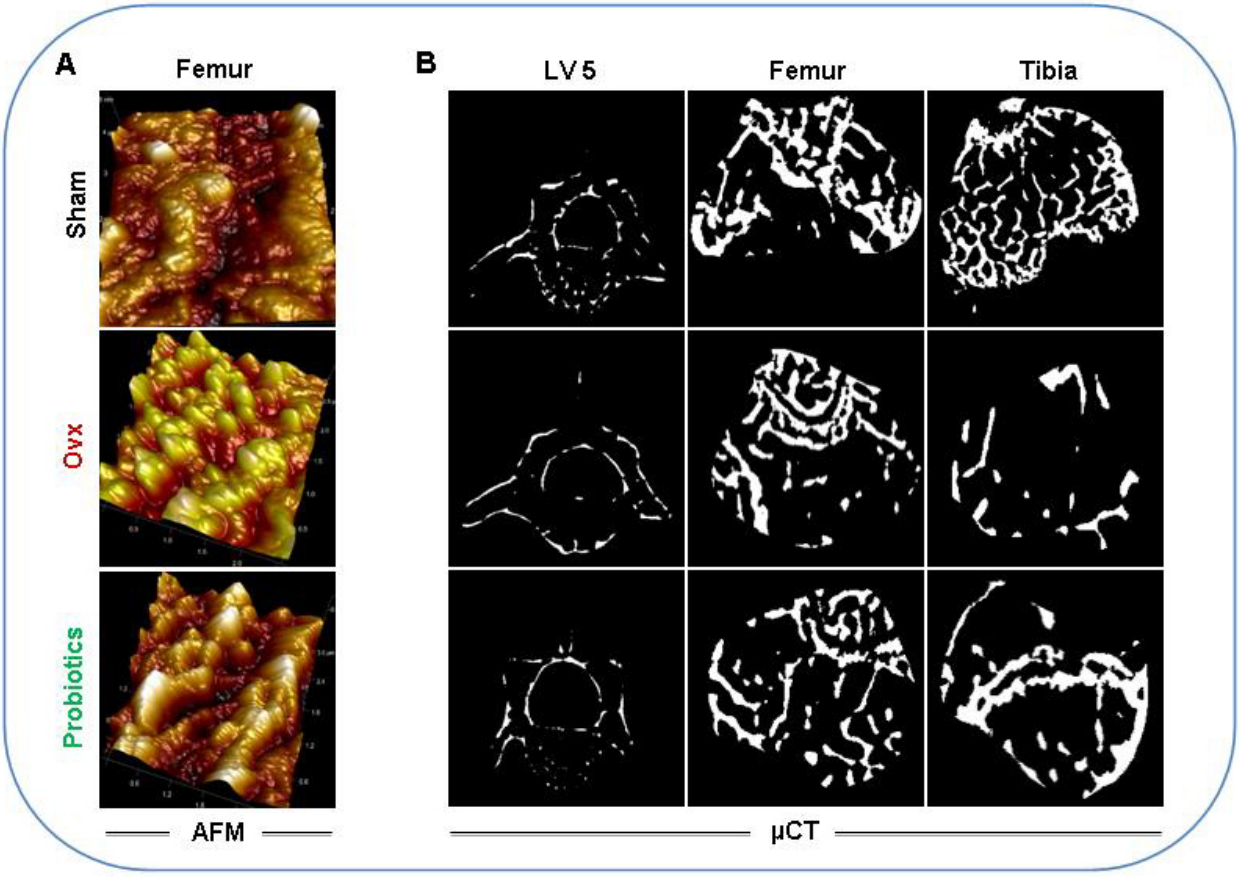

Figure 5. Representative images of normal (Sham, without Ovx) and osteoporotic (Ovx) mice from our lab (unpublished data). A). Atomic Force microscopy (AFM) of femur and B). μCT images of the 3D reconstruction of different trabecular bones in normal/Sham and Ovx mice. (LV5-Lumber Vertebrae 5, Femur and Tibia bones).

of TGF-β by various somatic gene therapy (148), can prove to be very effective against estrogen deficiency induced bone loss in mice.

With the advent of Probiotics boom in the research fraternity in the last few years, probiotics are now been employed as novel therapeutics for treatment of post-menopausal osteoporosis. This approach can be highly invaluable for prevention of increased bone joint destruction in patients where inflammation and bone loss cannot be prevented by established older methods. Recent studies have defined a novel treatment where gut microbiota can play an important role in reducing the bone loss in case of sex steroid deficient mice. The direct clinic approach of administration of pre-and probiotics can be a novel approach for treating bones loss by modulating the host immunity and thus inhibiting osteoclastogenesis (149, 150). Furthermore, the administration of a single or mixture of *Lactobacillus* species have been found to be effective in protecting cortical bone loss in estrogen deficient mice, by reducing the levels of different bone resorption and inflammatory markers (TNF and IL-1β, respectively) thereby increasing the number of Treg cells (151). Results (unpublished data, Figure 5) from our lab too indicate that various novel strains of probiotics, when administered in ovx-mice inhibit bone loss by modulating the Treg-Th17 cell axis. This mechanism of probiotics or gut microbiota induced blunting of inflammation can prove to be an effective and cheap alternative for future therapeutics in the field (149, 150).

6.2. Osteoimmunology of rheumatoid arthritis

Rheumatoid arthritis is a type of chronic inflammation related disease of synovial membrane, cartilage and bone affecting more than 1% of world population and has been associated with significant morbidity and increased mortality (152). RA onset and prevalence occurs by the infiltration of activated T and B cells into synovial membrane leading to sustained inflammatory state. These cells have been linked with driving bone loss in RA by increasing secretion of RANKL and TNF (153). T cells are in close knit relationship with RA pathogenesis due to the genetic association of T cells with MHC class II (154), as increased numbers of T cells have been found in

inflamed synovium in various arthritis models (154). The increase in the production of IL-7, an essential component for the production and differentiation of T cells (155) have been for long associated with adult and juvenile rheumatoid arthritis (730). The blockade of IL-7 leads to inhibition of collagen induced arthritis (CIA) by abrogating both the activation and production of inflammatory cytokines (156). Th17 cells upon induction by IL-6, TGF-β and IL-23 have been associated with inflammatory arthritis in rodents (157–159). An over expression of cytokine IL-17 produced by perivascular Th17 cells have been detected in rheumatoid synovial membranes (Figure 4) (160). $CD4^+$ and $CD8^+$ Treg cells characterised by expression CD25 and Foxp3 are potent immunosuppressing or anti-inflammatory T cells. Tregs now have established role in suppressing inflammatory bone diseases (161, 162). It was found that that in case of RA patient's inhibition of TNF-α was shown to be associated with over expression of $FOXP3^+CD4^+CD25^+$ regulatory T-cell population through TGF-β and IL-10 dependent pathways (163).

The role of immune system in the aetiology of RA is well established and as such the field of rheumatology has pioneered the use of novel immunomodulatory drugs for suppression of inflammatory processes along with protection from both systemic and local bone erosion. Different biological therapies have revolutionised the treatment scenario of RA by being more effective than conventional synthetic disease modifying anti-rheumatic drugs (DMARDs). One of new therapy is by inhibiting GMCSF, which is primarily responsible for polarizing macrophages, production of inflammatory cytokines and activation of phagocytosis involved in synovial inflammation (164, 165). GMCSF also contributes to the differentiation and expression of Th17 cells and dendritic cells producing different inflammatory cytokines like IL-1, Il-6, IL-17 and TNF-α (166, 167), contributing to pathogenesis of RA (168). Another target for novel therapy can be the modulation of phosphate-3-kinase pathway (PI3K), responsible for growth, survival and apoptosis as well leukocyte adhesion and migration. PI3K is also involved in the differentiation of T cells in mice and it has been found that PI3Kδ nude mice have significantly reduced number of Th2 and Treg cells (169, 170) generating different survival signals in absence of B cell receptors (171–173).

The blocking of TNF-α expression has been found effectively alleviating inflammation and bone loss in RA mice models (174) and as such different therapeutics of TNF antagonists have proved to be suitable for the treatment of patients with RA (175). Since immune activation has been associated with the aetiology of RA, a new era of utilization of powerful immunomodulatory drugs targeting T cell co-stimulation have been proposed and used in different cases of RA. Abatacept, a newly designed drug based on natural CTLA-4 works by dampening inflammatory responses. Also, CTLA-4 being associated with Tregs can not only be helpful in inhibition of osteoclastogenesis and suppressing bone loss by dampening various upstream inflammatory cascades, but can also account for direct production of anti-osteoclastogenic factors.

6.3. Osteoimmunology of osteoarthritis

Osteoarthritis (OA) is an age related inflammatory bone disease associated with synovial joints (176, 177). It is one of the most costly and prevalent form of arthritis effecting wide range of population (178). OA represents the alteration of structure of synovial joint (179), periarticular ligament, subchondral bones (180) and progressive deterioration of articular cartilage (179). Various studies have revealed roles of different subsets of T cells in pathogenesis of OA. The percentage of different $CD4^+$ and $CD8^+$ T cells found in OA have been even found to be in close similarity to that of RA patients (181, 182). There has been elevated percentage of Th1, Th9 and Th17 cells in OA patients as compared to normal patients (181, 183, 184). These cells secrete pro-inflammatory cytokines (TNF-α, IL-9 and IL-17) in synovial membrane or fluid leading to pathogenesis of bone (Figure 4). There is significant reduction in the number of Treg cells in OA, secreting different anti-inflammatory cytokines (IL-10 and TGF-β) at the sites of synovial membrane (185). Activated T cells by direct cell-cell contact or by expression of soluble mediator's viz. IL-17 and IL-18, stimulate overproduction of monocytes and produce inflammatory cytokines (IL-1β and TNF-α) which inhibit formation of type II thereby leading to cartilage damage (186). IL-17 induces osteoclastogenesis by the production of inducible nitric oxide synthase, IL-1 and cyclooxygenase-2 in articular chondrocytes (187. 188). In case of OA, T cells have also been reported to cause synovial damage by the production of collagenase and excessive apoptosis of chondrocytes (189, 190).

It is the complexity of OA progression and initiation that is creating hurdles for the development of effective therapeutics for it. Calcitonin therapy has been found to be very effective for instigating beneficial effects on periarticular bone remodelling and chondroprotection (191). Other bone active reagents such as cathepsin-K or RANKL inhibitors and strontium ranelate have been found to be very effective in OA due its effect on bone resorption and formation (191). The different intracellular, like mechanistic target of rapamycin (mTOR)-complex, extracellular (antigens) stimulations and cell metabolism (e.g., amino acid metabolism) define fate and activity of T cells (192, 193). Manipulation of these factors like mTORC1/mTORC2 which effects development and differentiation of Th17 cells can lead to possibility of

regulating OA (193). Since various metabolites are associated with T cell activation and differentiation especially in case of Th1 and Th17 cells, thus the regulation and alteration of different metabolism and profile of amino acids such as glutamate and arginine family and their metabolites can help in regulation of OA (4). Furthermore, intestinal microbiota also plays a vital role in T cell responses of Th17 cells (194, 195), thereby indicating a critical role of gut microbiota in pathogenesis and opening pandora's box for future therapeutics for OA (196, 197)/

7. CONCLUSION

The evolution of osteoimmunology as an independent field of modern biology has provided new paradigms with new expectations for development of novel strategies for curbing various inflammatory bone diseases. There is a rise in the existence of different skeletal diseases occurring due to defective bone remodeling because of skewed immune system. Inflammatory bone diseases like RA, OA and post-menopausal osteoporosis arising as a result of disruption of the homeostatic nexus between bone and immune system contribute to enhanced bone loss. The process of bone remodelling is a tightly coordinated cellular activity with intricate communication between both the components of bone and immune cells. The intimate cross-talk between osteoclasts and osteoblasts is an integral element in bone remodelling, along with other cell types including osteocytes, bone lining cells, osteomacs and endothelial cells which play important role at various stages of the process. Any imbalance or dysregulation between these cellular activities paves the path for initiation of various bone diseases such as osteoporosis, OA and RA.

Different strategies have been devised for the inhibition of inflammation induced bone loss. A direct approach of inhibiting osteoclastogenesis via modulating or interfering with different inflammatory pathways could provide an alternate and beneficial method for treating inflammatory bone loss. One of the effective treatments as such has been interfering with the inflammatory processes itself to combat bone loss/damage. It is now well established that current therapies provide remission of inflammation with diminutive relief to the patients suffering from such diseases. Thus, new and effective future therapeutics are much awaited for long term relief from various bone loss mediated pathologies. Thus, a better understanding of the nexus between both the systems should be at heart of future research in the area. Research in the field of osteoimmunology would thus pave path for novel therapeutics with different opportunities of looking beyond bisphosphonates etc. for treating different inflammation related bone losses. One such promising candidate in the last few years has been from the emerging field of "Probiotics". Harnessing the potential of novel probiotics (198) for modulating bone health via the host immune system (often referred by us as "Osteo-microbiology") could be the most promising approach for combating the menace of inflammatory bone diseases. Thus, a better understanding of the process of bone remodeling and the molecular controls involved are vital for improving the current treatment options for various inflammatory bone diseases. Thus, elucidating the molecular basis of bone remodeling in osteoporosis, OA, RA etc. would be of great clinical interest and significance in bone biology.

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Abbreviations: BMP, bone morphogenic protein; BMSC, bone marrow stromal cells; CD40L, CD40 ligand; GCSF, granulocyte colony-stimulating factor; GMCSF, granulocyte MCSF; HSC, hematopoietic stem cell(s); IFN, interferon; MCSF, macrophage colony-stimulating factor; MMP, matrix metalloproteinase; NF-κB, nuclear factor κB; OPG, osteoprotegerin; OSCAR, osteoclast-associated receptor; Ovx, ovariectomy; PI3K, phosphatidylinositide-3-kinase; RANK, receptor activator of NF-κB; RANKL, RANK ligand; TCR, T-cell receptor; Th, T-helper; TRAF, TNF receptor-associated factor;

DC ,dendritic cell; TRAP, tartrate-resistant acid phosphatase; RA , rheumatoid arthritis; OA, Osteoarthritis

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