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Abstract

Bone remodeling occurs through the interactions of three major cell lineages, osteoblasts, which mediate bone formation, osteocytes, which derive from osteoblasts, sense mechanical force and direct bone turnover, and osteoclasts, which mediate bone resorption. However, multiple additional cell types within the bone marrow, including macrophages, T lymphocytes and B lymphocytes influence the process. The bone marrow microenvironment, which is supported, in part, by bone cells, forms a nurturing network for B lymphopoiesis. In turn, developing B lymphocytes influence bone cells. Bone health during homeostasis depends on the normal interactions of bone cells with other lineages in the bone marrow. In disease state these interactions become pathologic and can cause the abnormal function of bone cells and the inadequate repair of bone after a fracture. This review summarizes what is known about the development of B lymphocytes and the interactions of B lymphocytes with bone cells in both health and disease.

Key words

B-lymphocyte
Bone formation
Bone Marrow
Bone resorption
Cytokines
Hematopoiesis
Osteoblast
Osteoclast
Osteocyte
Plasma Cells

Introduction

A variety of factors influences skeletal health. Genetics and sex steroids have been most studied [1] but, in addition, the immune system can have major effects on the skeleton and may be involved in the development of a variety of skeletal diseases [2]. It is well described that the cells responsible for maintaining the skeleton, and those responsible for the immune and hematopoietic systems in bone marrow interact through multiple mechanisms. These interactions are critical for the health of both organ systems and their study has been termed osteoimmunology [3].

Among the immune cells that have important roles in influencing the structure of the skeleton and the risks for disturbing bone metabolism are B lymphocytes (B cells) [4]. These undergo a significant portion of their development in the bone marrow, where they also interact with bone cells (osteoblasts, osteoclasts, osteocytes, and their precursors) [5-7]. In addition, mature B lymphocytes with memory function recirculate and engraft in bone marrow after their extraskeletal maturation. Numerous subsets of B lymphocytes have been identified. These have a variety of roles in the immune system, including antigen-dependent T lymphocyte priming, the generation of antibodies after their maturation into plasma cells, and the production of cytokines and chemokines, which are immune cell mediators that regulate differentiation, migration, and activation of both immune and bone cells [5-7]. These regulatory factors may act either directly on bone cells or indirectly by modulating the bone marrow microenvironment [8-12]. Conversely, the B lymphocyte lineage, including hematopoietic stem cells (HSCs) and more mature progenitors require support from stromal elements, including osteoblast lineage cells, to function normally [13, 14]. This review is a summary of what is known about the interactions of B lymphocytes with bone cells in both health and disease.

The Interactions of Bone Cells and B Lymphocytes during B Lymphopoiesis

Immune response includes well-orchestrated action of innate and adaptive immune mechanisms executed by different inflammatory and immune cells, including B lymphocytes [15]. There are two main types of B lymphocytes that have different origins and functions in the immune system. The more primitive B lymphocytes, named B-1, are part of the innate immune system. These are generated primarily from the fetal liver and bone marrow progenitors, and sustained through self-renewal in the periphery [16, 17]. Conventional B lymphocytes, named B-2, are antibody-expressing cells that function as a major part of the humoral arm of the adaptive immune system [18]. Postnatal conventional B-2 lymphopoiesis in mammals occurs in the bone marrow, in order to continually generate B lymphocytes for the duration of lifespan [19]. It traverses through distinct stages of differentiation, starting from HSCs and down-stream multipotent progenitors (MPPs), through common lymphoid progenitors (CLPs) and B cell-biased lymphoid progenitors (BLPs) that form early pre-pro-B lymphocytes, followed by pro-B lymphocytes, pre-B lymphocytes, and then immature B lymphocytes that migrate outside the bone marrow [7, 20-22]. Upon activation, the end-stage B lymphocytes known as immunoglobulin (Ig) secreting plasma cells, as well as antigen-specific memory B lymphocytes, may eventually return to the bone marrow [23]

B lymphopoiesis as well as hematopoiesis in general are maintained by the specialized microenvironment, also called bone marrow niches, supported in large part by non-hematopoietic stromal cells that interact intimately with the developing hematopoietic cells [8, 9, 24, 25]. Schofield was the first to propose that accurate spatial organization of the hematopoietic niches is crucial for the reconstituting and differentiating ability of HSCs and their progeny [26]. Within the niche, lineage progression relies on complex interactions that include soluble or membrane-bound factors (including CXCL12, stem cell factor (SCF), thrombopoietin, angiopoietin-1/Tie2, and angiopoietin-like protein 3), cell-cell contacts through adhesion molecules (VCAM-1 (CD106), ICAM-1 (CD54), and Nidogen-1), extracellular matrix (ECM) proteins (osteopontin, decorin, and tenascin C), local conditions (such as the concentration of oxygen and calcium), signaling pathways (Wnt, Notch, TGF-β, Hedgehog, parathyroid hormone (PTH) and retinoic

acid receptors (RARs)) and autonomic nerve stimulation [27-33]. In the bone marrow, HSCs are mostly located within the perivascular niche adjacent to sinusoidal blood vessels. They have the ability to mobilize upon activation by differentiation stimuli toward committed progenitors or to egress into the circulation in response to granulocyte-colony stimulating factor (G-CSF) [34-37]. Many pathological conditions, which affect the bone marrow microenvironment, perturb niche integrity and hematopoiesis, such as blood cell diseases, allogeneic bone marrow transplantation, malignant, autoimmune and inflammatory processes, endocrine, metabolic and genetic disturbances that affect bone tissue, or bone metastases [9, 12, 24, 38-41].

Effects of Osteoblast Lineage Cells on B Lymphopoiesis

Non-hematopoietic stroma is the crucial regulator of the B lymphopoiesis, influencing both commitment to B lymphocyte lineage and progression through the stages of differentiation [9, 24]. Stromal elements supporting B lymphopoiesis are composed of a heterogeneous cell population, mostly of mesenchymal origin, including fibroblasts, osteoblasts, adipocytes, reticular cells, endothelial cells, and perivascular cells [8, 14, 22, 25, 30, 31, 34, 35]. In their supportive role, osteoblast lineage cells comprise a range of differentiation stages, including committed osteoprogenitors, immature pre-osteoblasts, mature osteoblasts, and terminally differentiated osteocytes. The initial concept of the organization of different bone marrow niches suggests that primitive mesenchymal stem cells are more important for HSC maintenance, whereas lineage-restricted mesenchymal cells govern more committed hematopoietic progenitors [35, 40]. Particularly, populations of stromal cells with osteogenic capacity and perivascular location have been implicated in the regulation of the various stages of B lymphocyte differentiation within the bone marrow [8, 25]. Targeted deletion of pivotal B lymphocyte-supporting factors, such as CXCL12 and interleukin 7 (IL-7), at different stages of mesenchymal lineage (driven by paired related homeobox 1 (Prxx1), osterix (Osx), leptin receptor (Lepr) or nestin promotors) revealed their role in B lymphopoiesis [33, 34, 42, 43]. Furthermore, stromal cells supporting HSCs have been separated into peri-sinusoidal and endosteal/peri-arteriolar niches [44]. However, a recent study by Balzano et al, using combined approach of phenotypical characterization and reporter gene expression, proposed the existence of a multispecific

niche with the capacity to support both early progenitors and committed hematopoietic lineages [31]. In the following paragraphs, we will focus on the details of B lymphopoiesis that are influenced by osteoblast lineage cells (Figure 1).

(Insert Figure 1 here)

Niches for B lymphopoiesis

B lymphopoiesis progresses through distinct progenitor niches, following precise positional cues. HSCs typically are located near vascular sinuses or terminally differentiated osteoblasts along the bone surface while downstream progenitors (committed to the B lymphocyte lineage) are found translocating toward the central region of the marrow cavity [21, 22] (Figure 1). Environments that induce B lymphopoiesis from CLP and pre-pro-B lymphocytes are believed to produce the chemokine CXCL12, which attract cells to the sites of IL-7 secretion [45]. CXCL12 (also known as stromal cell-derived factor 1) signals through its receptor CXCR4, which is expressed at various stages throughout B lymphocyte differentiation [43]. Studies using genetically engineered mice lacking CXCL12 or CXCR4 have indicated that the development of B lymphocytes from the earliest progenitors to homing of plasma cells into the bone marrow depends on CXCL12-CXCR4 signaling [22]. Bone marrow provides supportive niches that promote long-term survival of plasma cells, which reside close to CXCL12-producing stromal cells [46]. CXCL12 is expressed in diverse cell types within the bone marrow, including stromal cells, CXCL12-abundant reticular (CAR) cells, osteoblasts, endothelial cells, and nestin-expressing mesenchymal stem cells. Using mice with the green fluorescent protein (GFP) reporter gene knocked into the CXCL12 locus, CAR cells were identified as a population of stromal cells with long processes, which highly expressed CXCL12 [47]. In the context of B lymphopoiesis, it is thought that multipotent progenitors encounter the processes located on CAR cells, positioned close to the bone marrow sinusoids, and develop into pre-pro-B lymphocytes [21, 48]. Further studies demonstrated that these reticular cells resemble the phenotype of mesenchymal progenitors, bearing markers such as VCAM-1 (CD106), CD44, CD51, platelet derived growth factor receptor (PDGFR)α (CD140a), and PDGFRβ (CD140b). They express osteogenic and

adipogenic genes, including peroxisome proliferator-activated receptor γ (PPARγ) and Osx, and possess at least osteogenic and adipogenic differentiation potential [49, 50]. In addition, it was shown that human and rodent $CD146^+$ perivascular reticular cells express alkaline phosphatase and exhibit osteoblastogenic potential [51]. Taken together, these data argue that CAR mesenchymal cells include functional osteoprogenitors. This was further supported by models that deleted CXCL12 at various differentiation stages of mesenchymal lineage cells using *Cre*-recombinase technique. Deletion of CXCL12 in *Prrx1-Cre*-targeted mesenchymal stromal cells led to a substantial reduction in CLPs and pre-pro-B lymphocytes, with significant loss of HSCs, whereas deletion of CXCL12 in *Osx-Cre*-targeted osteoprogenitors did not affect HSC function, but caused constitutive hematopoietic stem and progenitor cell (HSPC) mobilization and reduced number of B lymphoid progenitors [42]. Finally, mice with the *2.3 kb type I collagen (Col2.3)-Cre*-targeted deletion of CXCL12 in osteoblast exhibited decreased CLP number with no effect on B lymphocytes [34].

Pre-pro-B lymphocytes differentiate into early and late pro-B lymphocytes. They reside near IL-7-producing spindle-shaped reticular cells, which are positioned close to vessels and express the mesenchymal markers VCAM-1 (CD106), PDGFRα (CD140a), ICAM-1 (CD54) and BP-1 (CD249) but not the endothelial marker CD31 [8, 22]. In addition, expression of Nidogen-1 was identified as a specific retention signal for pro-B lymphocytes in the peri-sinusoidal niche [31]. A study by Cordeiro Gomes et al showed that CXCR4 is intrinsically required for the differentiation of MPPs to CLPs and BLPs, expressing the lymphocyte antigen complex 6 locus D protein (Ly6D), and for the positioning of $Ly6D^+$ cells near $IL\text{-}7^+$ cells in the bone marrow [43]. The authors further demonstrated that IL-7 is primarily produced by a subset of CAR cells with mesenchymal progenitor properties. In addition, *Osx-Cre*-targeted osteoprogenitors have been shown to express IL-7 [52].

IL-7 signals through the IL-7 receptor (IL-7R), which is expresses by B lymphocyte lineage until the large pre-B stage [53]. Around thirty percent of $IL\text{-}7R^+$ B lymphocyte progenitors co-localize with bone-lining cells and there is a significant reduction in their number upon conditional deletion of osteoblastic

cells [34]. *IL-7*-deficient and *IL-7R*-deficient mice exhibit severely impaired B lymphocyte development, indicating that IL-7 is indispensable for the differentiation of pro-B and pre-B-2, as well as pre-B-1 lymphocytes [21, 53-55]. Female *IL-7*-deficient mice also exhibited lower trabecular bone volume and increased osteoclastogenesis [56, 57], whereas the Col2.3-driven overexpression of human IL-7 in osteoblasts rescued the osteopenia and B lymphopoiesis of *IL-7*-deficient mice [58]. Moreover, in mice, which systemically overexpress IL-7 under the control of the major histocompatibility complex (MHC) class II promoter, pro-B, pre-B, and immature B lymphocytes in bone marrow were greatly increased [59], and there was associated impairment of osteoclast activity and bone metabolism [60]. In contrast, initial findings suggested that thymic stromal lymphopoietin or another, yet unknown, cytokine can substitute for IL-7 in human B lymphopoiesis [61], since B lymphocytes were present in severe combined immunodeficiency (SCID) patients where no IL-7R signaling occurs [62]. However, more recent studies showed that IL-7 induces human pro-B lymphocyte proliferation, suggesting that B lymphocytes in SCID patients correspond to a wave of IL-7-independent fetal B lymphocytes [63]. Balzano et al confirmed that pro-B lymphocytes were associated with IL-7-rich human bone marrow peri-sinusoidal stromal cells, suggesting their dependence on IL-7 [31].

Characterization of Supportive Stromal Cells

Although it was initially postulated that distinct stromal cell subpopulations express CXCL12 and IL-7 [45], it was later shown that $LepR^+$ perivascular stromal cells may co-express IL-7, SCF, and CXCL12, maintaining both HSCs and B lymphoid lineage cells [34, 43, 64]. Further characterization of HSC and differentiating B lymphocyte interactions with various stromal cells subsets was achieved by recent studies at single-cell resolution [31, 65, 66]. By using a series of lineage-specific *Cre*-transgenic mice (*VE-Cad*, *LepR*, *Col2.3*), Tikhonova et al mapped the transcriptional landscape of mouse bone marrow vascular, perivascular, and osteoblast populations, respectively. They found that adipocytic-primed $LepR^+$ cells represent a major reservoir of pro-hematopoietic factors in the bone marrow niche, including CXCL12, SCF, IL-7, IL-15, IL-34, M-CSF, and BMP-4 [65]. Balzano et al indicated a limited diversity of bone

marrow mesenchymal subsets by showing that peri-sinusoidal stroma co-expresses LepR, BP-1, CXCL12, SCF, and IL-7 at the single-cell level [31]. These supporting cells reside in a vicinity of both HSCs and pro-B lymphocytes, and simultaneously express HSC and B lymphoid niche genes. Moreover, they identified murine and human $CD317^+$ bone marrow stromal cell counterparts, expressing CXCL12 and IL-7, co-localized to both HSPCs and pro-B lymphocytes. In addition, Baccin et al, applied combined single-cell and spatial transcriptomics to allocate bone marrow-resident cell types and to characterize the endosteal, subendosteal, arteriolar, sinusoidal, and non-vascular niche composition [66]. Using this approach, they mapped two CAR cell subsets (Adipo-CAR and Osteo-CAR) occupying distinct areas – sinusoidal and arteriolar/non-vascular respectively, and confirmed their ability to establish micro-niches as professional cytokine-producing cells, with high local production of all major HSC-, myeloid-, and lymphoid-support factors. As shown by the recent study of Green et al, there are also differences in niche cellular composition between skeletal sites and this has implications for B lymphopoiesis [67]. The study identified four subsets of osteoprogenitor cells among murine $Lin^-CD31^-Sca\text{-}1^-CD51^+$ population, based on the expression of PDGFRα (CD140a) and PDGFRβ (CD140b). Double-positive cells ($PDGFR\alpha^+PDGFR\beta^+$) were significantly more abandoned in calvaria bone compared with long bones and vertebrae, parallel to the increased proportions of B lymphocyte progenitors. In addition to the expression of osteoblastic genes, this subset expresses significantly higher levels of factors involved in the extrinsic regulation of B lymphopoiesis (*Flt3l, Cxcl12, Il7* and *RANKL/Tnfsf11*) and supports B lymphocyte differentiation *in vitro* [67].

Role of osteoblast lineage cells in B lymphopoiesis

As described, CXCL12 and IL-7 can be produced by cells of the osteoblast lineage, and expression of both factors is positively regulated by signaling through the PTH/PTH-related peptide receptor (PPR) in osteoblasts [32, 43, 52, 68]. When stimulated with PTH *in vitro*, calvarial osteoblasts showed marked increases in CXCL12 and IL-7 levels and supported differentiation from the earliest B lymphocyte progenitors to differentiated IgM^+ B lymphocytes [68]. The ablation of Gsα, component of G protein-

coupled PPR, in Osx^{+} osteoprogenitors (*GsαOsx* KO mice) leads to severe osteoporosis and significantly impaired B lymphopoiesis, with a specific block in the transition from pro-B to pre-B lymphocytes due to reduced expression of IL-7. Furthermore, the defect in B lymphopoiesis can be rescued by exogenous IL-7 or transplantation into a wild-type microenvironment. These results demonstrate *in vivo* that Gsα-dependent signaling pathways in osteoblast lineage cells extrinsically regulate bone marrow B lymphopoiesis, in a manner that is at least partially dependent on IL-7 [52, 69]. In addition, Cheung et al provided evidence that connective tissue growth factor, which is important for skeletogenesis and the regulation of bone marrow stromal cells, potentiates the proliferation of B lymphocyte progenitors and promotes pro-B to pre-B differentiation in the presence of IL-7 [70]. Fujita et al showed that secreted form of osteoblast stimulating factor 5 (OSF-5), produced by mesenchymal and osteoblast cells, inhibits the proliferation of pre-B lymphocytes in mice and humans [71]. Mice with disrupted function of collagen X (ColX), a major hypertrophic cartilage matrix protein associated with endochondral ossification, have aberrant B lymphopoiesis and diminished expression of SCF and CXCL12 [72]. In transgenic mice, which had thymidine kinase targeted to osteoblasts using the Col2.3 kb promoter, osteoblast ablation with ganciclovir resulted in an acute loss of B lymphocytes, specifically pre-pro-B and pro-B lymphocytes [73]. With continued ganciclovir treatment, HSCs were progressively lost and there was dysregulation of bone marrow cellularity. These results indicate that impairment of B lymphopoiesis following osteoblast depletion occurs prior to the loss of HSCs.

Several studies showed that signaling through RARs regulates bone structure and hematopoiesis through intrinsic and extrinsic mechanisms [33, 74-76]. RARγ expression in non-hematopoietic cells is essential for normal hematopoiesis, since *RARγ*-deficient mice developed myeloproliferative-like syndrome, and defects in erythropoiesis and B lymphopoiesis, which could be rescued by transplantation of bone marrow cells into wild-type mice [74]. Deletion of RARγ in *nestin-Cre*-targeted mesenchymal cells impaired bone marrow B lymphopoiesis and thymic T lymphopoiesis, whereas deletion in *Osx-Cre*-targeted mesenchymal cells had no impact on hematopoiesis [75]. Finally, loss of RARγ in *Prrx1-Cre*-

targeted mesenchymal cells of male mice impaired tibial longitudinal growth and caused lower trabecular bone mass, together with elevated pro-B and pre-B lymphocyte numbers, increased CXCL12 expression in bone marrow and enhanced osteoclastogenesis [33, 76].

In line with the concept that B lymphopoiesis within the niche greatly rely on direct interactions with osteoblast lineage cells, mice lacking transcriptional factor Runx2 or Osx, which are required for osteoprogenitor differentiation, failed to develop a mineralized skeleton and die soon after birth with no hematopoiesis in long bones [77-80]. Osx^+ osteoprogenitors promote transition of pro-B to pre-B lymphocytes through insulin-like growth factor 1 (IGF-1) production. Loss of Osx^+ cells or Osx-targeted deletion of IGF-1 led to a failure of B lymphocyte maturation [40]. These mice exhibited a reduced number of pre-B, immature B, and mature B lymphocytes with accumulation of up-stream progenitors, suggesting that osteoprogenitor derived IGF-1 is important in the regulation of the later stages of B lymphopoiesis. In contrast, mice deficient in osteocalcin, a marker of mature osteoblasts, had a phenotype of high bone mineral density and unaltered hematopoiesis [81]. Finally, osteocytes, which are terminally differentiated bone cells, embedded within the mineralized matrix, may also be involved in the regulation of early B lymphocyte development [24]. Mice deficient in sclerostin, encoded by the *Sost* gene, which is predominately expressed by osteocytes and is a Wingless (Wnt) pathway-antagonist (inhibiting osteoblast function), displayed osteopetrotic bones with reduced bone marrow cavities [82, 83]. Mice with Wntless (Wls) deficiency, which blocks Wnt protein secretion, in *Col1-cre*- or *nestin-cre*-targeted transgenic mice exhibit defective B lymphopoiesis and abnormal T lymphocyte infiltration in the bone marrow [84]. In addition, *Sost* KO mice have a block in early (pro-B and pre-B) lymphocyte development, probably due to impaired function of osteoblasts and decreased expression of SCF and CXCL12 [82, 83, 85].

Maturation of B lymphocytes and egress to the periphery

With the progression of differentiation, pre-B-2 lymphocytes downregulate IL-7R expression and migrate close to stromal cells that are scattered throughout the bone marrow [8, 35]. Studies in which pre-B lymphocytes were injected into hydroxyurea-treated mice suggest that pre-B lymphocytes home to cells

that express galectin-1 (Gal-1) [64]. Osteoblast lineage and reticular cells can produce Gal-1, which serves as a ligand for pre-B cell receptor (pre-BCR), involved in the proliferation and differentiation of mouse pre-B-2 lymphocytes. Immature B lymphocytes localize near endothelial cells, lining blood vessels, prior to exiting into the circulation [86]. It is of interest to note that more than 80% of developing B lymphocytes are extinguished in the process of negative selection in order to establish central B lymphocyte tolerance [21]. Egress of the remaining immature B lymphocytes from bone marrow through blood and into the spleen is directed by sphingosine 1 phosphate (S1P)-mediated chemoattraction and can be impaired with a small molecule inhibitor of the S1P receptor 1 (S1PR1) or deletion of S1PR1 from B lymphocytes [87, 88].

Osteoblast-derived factors as well as some other extrinsic stimuli, predominantly provided by non-hematopoietic cells (e.g. cytokines, chemokines, growth factors, cell-cell interactions, and extracellular matrix components), must be delivered to B lymphocyte progenitors at the appropriate stage of development to activate intrinsic mechanisms (e.g. transcription factors and cell cycle regulators). These control their survival, expansion, and expression of lineage-associated genes [7, 8, 19, 21, 49]. DNA-binding proteins, such as Ikaros and PU.1, are required primarily for the formation of more primitive lymphoid progenitors, whereas other factors, such as E2A and EBF1 directly promote B lymphocyte-specific gene-expression program and indirectly control commitment through induction of the paired box 5 (Pax5) transcription factor. This transcriptional regulator completes the B lymphocyte commitment process by repressing lineage-inappropriate gene expression and reinforcing B lymphocyte specific gene expression [18].

The functional interplay between B lymphocyte and osteoclast lineage cells was demonstrated by investigation of *Pax5*-deficient mice [9, 89, 90]. Pax5 is a member of a multigene family that regulates pro-B to pre-B transition. In the absence of Pax5, B lymphocyte development in the bone marrow is blocked at the early stage of pro-B lymphocytes, which are multipotential and able to differentiate down several hematopoietic maturation pathways including osteoclasts [89]. The development of multilineage differentiation potential by Pax5 inactivation confirms that B lymphocyte lineage commitment needs to be continually maintained by Pax5 [18].

Contribution of Other Cell Lineages to the Regulation of B Lymphopoiesis

Besides osteoblast lineage cells, myeloid lineage cells, including osteoclasts and macrophages. within the bone marrow environment have been reported to regulate B lymphopoiesis. Administration of zoledronic acid, an anti-resorptive agent that suppresses osteoclast activity, led to decreased production of CXCL12 and IL-7 from stromal cells and a reduction in early B lymphocyte progenitors in mice [91]. Originally, it was believed that zoledronic acid did not have a direct effect on B lymphocyte progenitors or osteoprogenitors *in vitro*. Hence, the reduction in early B lymphocyte progenitors was supposed to be achieved through modulation of osteoclast activity. However, more recent work has demonstrated direct effects of bisphosphonates on B lymphocyte function [92]. Investigation by Singbrandt et al revealed an interesting *in vivo* regulatory network coordinating erythropoiesis, B lymphopoiesis, and osteoclast function [93]. The study showed that adult mice, which were treated with a clinically relevant dose of erythropoietin (Epo), had expanded erythropoiesis due to stimulation of committed erythroid progenitors as well as loss of the trabecular bone volume and impaired B lymphopoiesis within the bone marrow microenvironment. Inhibition of the osteoclast activity with bisphosphonate therapy blocked the Epo-induced bone loss and reduced the magnitude of the erythroid response to Epo.

Several recent studies have highlighted a role of macrophages in the regulation of bone tissue homeostasis and hematopoietic niche integrity [25, 94]. Three subset of bone marrow resident macrophages have been described, including erythroblastic island macrophages, HSC niche macrophages, and osteal macrophages (named osteomacs) [94]. It was demonstrated *in vivo* that loss of macrophages negatively affected the growth and survival of osteoblasts, and stimulate egress of HSCs into the blood stream [95-97]. Mobilization of HSCs by G-CSF is mediated by macrophages (since osteoblast and B lymphocytes do not express G-CSF receptor) and is associated with suppressed endosteal bone formation, decreased expression of CXCL12, impaired B lymphopoiesis, and increased level of apoptotic B lymphocytes in bone marrow [95-98]. On the other hand, myeloid-derived suppressor cells (MDSC) may negatively regulate B lymphopoiesis [99]. Expansion of bone marrow MDSCs in aging and chronic inflammation can lead to

failure of B lymphocyte generation through an increase in TGF-β production and enhanced apoptosis of immature B lymphocytes [100].

(Insert Figure 2 here)

Effects of B Lymphocytes on Bone Cells

In addition to the regulation of B lymphopoiesis by osteoblast lineage cells within bone marrow niches, the regulatory loop of B lymphocytes influencing bone turnover is equally important. This is achieved by shared cytokines, transcription factors, and molecular pathways that have significant impact on the function of both immune and bone cells, such as the receptor activator of nuclear factor κ-B ligand (RANKL)/RANK/osteoprotegerin (OPG) system [24, 38, 101] (Figure 2).

RANKL/RANK/OPG system in B lymphopoiesis

RANKL (encoded by the *Tnfsf11* gene) is a cytokine with important functions in osteoclastogenesis and B lymphopoiesis (Figure 2). RANKL signals through RANK (encoded by the *Tnfrsf11a* gene) expressed on osteoclast progenitors as well as on B lymphocytes [102]. Mice lacking RANK or RANKL show severe osteopetrosis due to impaired osteoclastogenesis and are characterized by a reduction of mature $B220^{+}IgM^{+}$ and $B220^{+}IgD^{+}$ B lymphocytes in the spleen and lymph nodes [102, 103]. Low number of B lymphocytes in these mice maybe due to the osteopetrotic bone microenvironment and inadequate bone marrow cavities as well as a cell autonomous requirement for RANKL/RANK signaling during B lymphocyte development [24, 90]. Transplantation of $RANKL^{-/-}$ fetal liver hematopoietic cells into recombination-activating gene 1 (*RAG1*)-deficient host (which lack mature B and T lymphocytes) resulted in a pro-B to pre-B lymphocyte developmental block, whereas transplantation of wild-type hematopoietic cells into $RANKL^{-/-}$ recipients resulted in normal B lymphocyte development [103]. *RANKL*-deficient mice show ectopic hematopoietic islands containing proliferating progenitors at the outer surfaces of vertebral bodies. The spleens of *RANK*-deficient mice are enlarged, with a reduction in B lymphocytes, whereas all other hematopoietic cell lineages are normal (T lymphocytes, macrophages) or elevated (granulocytes,

erythrocytes) [102]. However, *Mb1-Cre*-targeted deletion of RANK in B lymphocytes from the pro-B cell stage onwards did not result in defective B lymphopoiesis [104]. Collectively, findings in genetically modified mice with impaired RANKL/RANK signaling point to a prominent block in the early (pro-B/pre-B stage) B lymphocyte differentiation. Moreover, *RANK*-deficient patients present with hypogammaglobulinemia associated with an impairment in immunoglobulin secreting plasma cells [105]. Conversely, mice deficient in OPG (encoded by *Tnfrsf11b* gene), a decoy receptor for RANKL, have enhanced osteoclastogenesis, leading to severe osteoporosis, and an enlarged population of pro-B lymphocytes in the bone marrow, and mature B lymphocytes in the spleen and lymph nodes [106, 107]. Pro-B lymphocytes lacking OPG also exhibit enhanced proliferation when stimulated with IL-7 [107]. However, despite a greater peripheral number, these lymphocytes show functional defects *in vivo*, including impaired co-operation with T lymphocyte and inappropriate immunoglobulin isotype switching.

B lymphocytes as a source of RANKL and OPG

Within the bone marrow environment, RANKL is produced by a range of osteoblast lineage cells, as well as B lymphocytes [9, 12, 38, 108] (Figure 2). Under normal conditions, B lymphocyte-derived RANKL is required for B lymphocyte development but is not integral for maintaining bone tissue homeostasis, likely due to the contributions of other RANKL-producing cells [24], particularly osteocytes [109, 110]. Nevertheless, activated B lymphocytes overexpress RANKL [111], which may have serious effects on bone metabolism [9, 24]. In human and animal B lymphocytes, OPG production can be significantly upregulated *in vitro* by costimulatory signaling through CD40 and, both, CD40 and CD40L KO mice displayed an osteoporotic phenotype and a significant deficiency in bone marrow OPG [112-114]. Li et al quantified OPG production in the bone marrow B lymphoid compartment, and found that B lineage cells are responsible for 64% of total bone marrow OPG production [112]. In particular, mature B lymphocytes and plasma cells have been shown to produce the highest amount of OPG per cell [24, 90, 112]. Intracellularly, the RANKL/OPG axis is regulated by mechanistic target of rapamycin complex 1 (mTORC1) [115]. Deletion of a negative regulator of mTORC1, tuberous sclerosis complex 1 (TSC1), in

CD19-Cre-targeted B lymphocytes increased RANKL and decreased OPG expression *via* negative regulation of β-catenin, causing reduced trabecular bone mass and enhanced osteoclastogenesis.

Additional clarification of the relevance of B lymphocyte-derived OPG on bone cells was made by studies in genetically modified mice. Mice with global deletion of OPG *(Tnfrsf11b*$^{-/-}$ mice*)* have enhanced osteoclast formation and bone resorption rates [116]. In contrast, mice with selective deletion of OPG in B lymphocytes (generated by crossing *CD19-Cre* mice with *Tnfrsf11b*$^{fl/fl}$ mice) had normal rates of osteoclast formation and bone resorption [117]. This result argues that B lymphocytes may not be a significant source of the OPG that influences bone cells. In contrast, mice with deletion of OPG in osteocytes, late osteoblasts and possibly earlier stage osteoblasts (produced by crossing *Dmp1-Cre* and *Tnfrsf11b*$^{fl/fl}$ mice) phenocopied, almost entirely, the enhanced osteoclast formation and bone resorption of global *Tnfrsf11b*$^{-/-}$ mice [117, 118]. However, deletion of OPG more exclusively in osteocytes (produced by crossing *Sost-Cre* and *Tnfrsf11b*$^{fl/fl}$ mice) failed to demonstrate a bone phenotype that mimicked global *Tnfrsf11b*$^{-/-}$ mice [117]. In summary, it appears that in homeostasis B lymphocytes produce the most OPG per cell but osteoblasts are the major source of OPG that affects bone mass *in vivo*.

B lymphocyte role beyond the RANKL/RANK/OPG system

B lymphocytes may regulate bone homeostasis by secreting factors other than RANKL and OPG, as a part of the adaptive immune response (Figure 2). In particular, B lymphocytes and plasma cells produce different cytokines and chemokines (including TNF-α, IL-6, IL-10, TGF-β, CCL3, CCL7), which are involved in the control of bone remodeling and regeneration either by acting on bone cells directly or by modulating the bone microenvironment [119-123]. In addition, B220^{+} B lymphocytes express Wnt1, one of the Wnt-family glycoproteins essential for normal skeletal development and homeostasis [124]. Inactivating mutations in the *Wnt1* gene are associated with osteoporosis and osteogenesis imperfecta in affected families. On the other hand, data suggests that lipopolysaccharide-pretreated B lymphocytes suppressed osteoblastogenesis from rat bone marrow stromal cells through the activation of Notch signaling [125]. In addition, B lymphocytes play a pathogenic role in skeletal diseases by secreting inflammatory

cytokines, matrix metalloproteinases, and RANKL as well as by producing pathogenic autoantibodies [122, 126]. Taken together, it is clear that B lymphocytes have multiple, often contradictory, effects on bone metabolism which depend on particular physiological and pathological circumstances (Figure 2).

Can B lymphocytes differentiate into osteoclasts?

The question of whether cells of the B lymphocyte lineage can become osteoclasts has been controversial. Some studies reported that $B220^{+}IgM^{-}$ B lymphocytes could differentiate into osteoclasts *in vitro* in the presence of 1,25(OH)2 vitamin D3 and ST2 stromal cells, or macrophage colony-stimulating factor (M-CSF) and RANKL [127-129]. These authors proposed that the $B220^{+}$ B lymphocyte population contains an estrogen-regulated subset of osteoclast progenitors, playing a role in the accelerated osteoclastogenesis of estrogen deficiency [127, 129]. However, it was later demonstrated that osteoclast formation by $B220^{+}$ bone marrow cells was most probably the result of contamination by myeloid cells [130]. It also was suggested that myeloma cells, which are malignant plasma cells, typically residing in the bone marrow, could differentiate into bone resorbing osteoclasts [90]. *In vitro* experiments showed that myeloma cells fuse to form polykaryons with osteoclast like properties, including expression of the osteoclast-specific marker tartrate-resistant acid phosphatase and the ability to resorb bone-like substrates [131, 132]. It is now established that osteoclast resorption releases myeloma cells from dormancy, while bone lining cells promote dormancy [133].

Lineage tracing experiments examining the connections between the origins of osteoclasts and B lymphopoiesis have been performed. Studies labeling B lymphocytes with a fluorescent marker under the influence of the CD19 promoter, which identifies cells at or after the pro-B stage, failed to label osteoclasts *in vivo* [134]. However, more recently it was demonstrated that $CD115^{+}$ pro-B lymphocyte could be an osteoclast progenitor [135]. CD115 is the receptor for M-CSF (CSF1R/cFms) and is critical for osteoclast formation [135, 136]. These studies used the MP1 promoter to fluorescently label cells rather than the CD19 promoter because it has higher expression in early B lineage progenitors [137]. Using this promoter, it was demonstrated that osteoclasts were labeled *in vivo* [135]. Significantly, the osteoclastogenic potential of

this early progenitor was enhanced by Epo, which also increased RANKL production [135]. These data argue that an early progenitor exists, which has both B lymphocyte and osteoclast lineage potential. Enhanced production of a resorptive environment by elevated levels of Epo could be a mechanism by which the marrow space in bone is enlarged to accommodate enhance erythropoiesis during period of hypoxia [138]. Conversely, loss of Epo production in chronic kidney failure may be involved in the bone disease associated with this condition [135].

B Lymphocytes in Bone Pathology

Considering their close anatomical and functional interactions, it is clear that B lymphocytes have important roles in various states of disturbed bone homeostasis. Their effect may be predominantly pathologic or protective, depending on the context. In the following paragraph, several well-studied bone disorders are described, with the focus on B lymphocyte function (Table 1).

Bone fractures

Fracture healing is a regenerative process in which bone is restored without scar tissue formation. The healing cascade initiates with a cycle of inflammation, cell migration, proliferation, and differentiation of progenitors into mature osteoblasts and osteoclasts. During the bone healing process, immune cells infiltrate into the hematoma and release cytokines, eliciting inflammation [139]. Because inflammation precedes bone regeneration, it has long been considered that the immune system is crucial in bone fracture healing [140]. Clinicians have reported that bone healing is delayed in patients treated with immunosuppressants and the incidence of nonunion is more frequent in HIV patients [141-143].

Studies of mice with a repairing fracture showed that during soft callus formation, immune cells are found only in the endosteal region, close to the fractured bone ends. The avascular cartilage filling the fracture callus at this time was devoid of T and B lymphocytes [120]. Upon subsequent hypertrophy of the cartilage the region was revascularized and T and B lymphocytes reappeared in the callus in areas of newly formed woven bone. The observed large number of T and B lymphocytes present during the remodeling process correlated with the rising numbers of osteoblasts and osteoclasts in regions of newly formed bone.

Recent clinical studies showed that strong activation takes place in draining lymph nodes of fractured bones. Prolonged lymphoid activation correlates with healing and bone union, whereas early termination of nodal reactivity characterizes failure in reparation [144, 145].

In the context of bone fracture healing, B lymphocytes were previously thought to not have a significant role since there was no impairment in bone healing in μMT/μMT mice, that are deficient in mature B lymphocytes because they lack BCR μ chains [146]. On the other hand, T and B lymphocyte deficient *RAG1*$^{-/-}$ mice showed improved callus mineralization [147]. Although this study implies a negative effect of the adaptive immune system on fracture healing, B lymphocytes are known to increase at the injury site and in the peripheral blood [120, 148]. This suggests that mid-to-late bone regeneration depends on incoming lymphocytes capable of shifting the cytokine milieu towards OPG and against RANKL [149]. Overall, increasing the local concentration of OPG by incoming B lymphocytes would strongly down regulate osteoclast activity and favor bone formation, reinforcing the idea that lymphocytes actively participate in bone regeneration. In agreement, plasma cells, which are the subset of B lymphocytes with a high capacity to secrete OPG [150], were also found in the late stages of fracture healing.

It has recently been shown that low production of IL-10 by B lymphocytes is associated with delayed fracture healing [151]. The major source of IL-10 in bone healing is the $IgM^{+}CD27^{+}$ memory B lymphocyte subset, and co-culture of these cells with T lymphocytes suppressed the production of IFN-γ and TNF-α [151]. These findings suggest that a portion of B lymphocytes exhibit immunosuppressive function, which, considering the importance of inflammatory phase during fracture healing, could be harmful for bone regeneration. The functions of other B lymphocyte subsets in bone regeneration remain largely unknown and further studies are required.

Metabolic bone disease

The calcified skeleton has multiple critical functions. It is a storehouse for calcium, facilitates bone marrow development, and provides structural integrity [152]. Failure of the structural role of the skeleton results in bone fractures and associated morbidities. When fractures occur with minimal impact or if, for a

variety of reasons, an individual is at increased risk of developing fractures from minimal impact, that individual is defined as having osteoporosis. Clinically it is often defined as a bone mineral density T score of -2.5 or less [152]. This disease is a serious health problem that affects more than 20 million Americans, accounts for approximately 1.5 million fragility fractures yearly and has an annual cost of over 22 billion dollars [153].

The mechanisms by which B lymphocytes regulate bone metabolism in health and disease involves the production of factors including RANKL, OPG, and TGF-β [154, 155]. Human B lymphocytes were shown to inhibit osteoclast formation in a human *in vitro* model of osteoclastogenesis, in part through secretion of TGF-β, a cytokine that is reported to stimulate production of OPG [154, 156]. Notch signaling has also been implicated as a mediator of the effects of activated B lymphocytes on osteoblastogenesis [125]. Conversely, mTORC1 and sclerostin expression are involved in the ability of osteoblasts to regulate B lymphopoiesis [117, 157, 158]. In mouse studies, μMT/μMT and $\lambda 5^{-/-}$ mice, which lack mature B lymphocytes, were found to have a low bone mass relative to wild-type mice and in the case of μMT/μMT mice this phenotype was reversed by B lymphocyte reconstitution [112, 159]. The osteoporotic phenotype of μMT/μMT mice was associated with an increase in levels of serum c-terminal telopeptide (CTx), a measure of total bone resorption rates. B lymphocytes seems to be involved in osteoporosis associated with aging, inducing the development of an inflammatory environment that leads to increased bone resorption and bone fracture risk. In an animal model, bone mineral density (BMD) in aged mice decreases as well as the total number of B lymphocytes in the bone marrow and spleens [160]. In addition, bone marrow and spleen isolated B lymphocytes produce significantly higher amount of both RANKL and OPG in aged compared to young mice [160].

Osteoporosis

Postmenopausal osteoporosis develops when estrogen production by the ovary ceases. To mimic this effect in mice, ovariectomy is performed. Loss of estrogens in this model is accompanied by an increase in B lymphopoiesis [161]. Experiments that examine the role of B lymphocytes in the bone loss that follow

ovariectomy-induced estrogen withdrawal have produced conflicting results. One group found that ovariectomy in μMT/μMT mice produced identical bone loss to that of wild type mice [162]. In contrast, another group examined mice that lacked B lymphocyte-derived RANKL [163]. They found that these animals were partially protected from ovariectomy-induced bone loss, which occurred in cortical but not cancellous bone. Additionally, unlike μMT/μMT mice, animals lacking RANKL expression on B lymphocytes did not have a bone phenotype in the absence of ovariectomy. Since μMT/μMT mice only lack mature B lymphocytes, the results of these two studies [162, 163] argue that RANKL expression on immature B lymphocytes is critical for the cancellous bone loss that occurs with estrogen withdrawal. Expression of RANKL on B lymphocytes seems to be involved in the increase in B lymphocyte number in bone marrow that occurs with estrogen loss in mice [161] since this response was reduced in mice lacking B lymphocyte-derived RANKL [163]. The increase in B lymphocytes in bone marrow and the bone loss that occur after estrogen withdrawal in mice also appear dependent on the expression of RANKL by mature osteoblasts and osteocytes since these responses were absent in mice that lacked RANKL in these cells [164].

As already mentioned, IL-7 is required for normal murine B lymphopoiesis [55]. In addition, IL-7-deficient mice have markedly increased osteoclast number and decreased trabecular bone mass compared to wild type controls [57]. Paradoxically, administration of IL-7 to wild-type mice caused an increase in B lymphocyte number and an increase in osteoclast mediated bone loss, possibly through production of additional cytokines [165]. As with studies in μMT/μMT mice [162], trabecular bone loss after ovariectomy was similar in wild type and *IL-7*-deficient mice [57]. IL-7 mRNA levels in bone increase with ovariectomy, and this effect may be linked to alterations in osteoblast function with estrogen withdrawal [166, 167]. IL-7 was also found to be a direct inhibitor of osteoclastogenesis in cultured murine bone marrow osteoclast progenitors [168]. In addition, targeted overexpression of IL-7 in osteoblasts rescued the osteoporotic bone phenotype of *IL-7*-deficient mice [169].

In human studies, it was found that RANKL expression on both total bone marrow cells and B lymphocytes, was increased in women with estrogen withdrawal [170, 171]. One study of differentially expressed genes in the B lymphocytes in the blood of postmenopausal women identified 169 upregulated and 69 downregulated genes in women with low bone mass compared to women with high bone mass [172]. A similar study looked at circulating B lymphocytes in the blood of 10 high and 10 low bone mass postmenopausal women by gene chip array expression profiling [173]. Twenty-nine genes were identified as differentially expressed and involvement of these genes in a network containing estrogen receptor 1 and mitogen activated protein kinase 3 was identified. A study of B lymphocyte subsets in 26 postmenopausal women with osteoporotic (OP) fractures and 24 postmenopausal healthy controls [174] found differences in several B lymphocyte populations and a correlation between differences in B lymphocyte subsets and bone mineral density.

An examination of differentially expressed genes in bone marrow cells from ovariectomized and sham-operated mice identified 23 significant signaling networks that were differentially affected, including B lymphocyte development, B lymphocyte phosphoinositol 3 kinase (PI3K), and B lymphocyte Fc receptor (FcγRIIB) [175]. These authors then analyzed the associations in 706 postmenopausal women between bone mass and single nucleotide polymorphisms (SNPs) of genes whose expression was increased (IL7R and CD79A) or decreased (GPX3 and IRAK3) by ovariectomy in mice. They found an association of a SNP in glutathione peroxidase 3 (GPX3), a gene involved in the detoxification of free radicals, with femoral neck BMD and associations of two SNPs in the Ig-α protein of the B cell antigen component gene (CD79A) with lumbar spine BMD [175].

Inflammatory arthritis

B lymphocytes as integral elements of the immune response are important contributors to the development of inflammatory joint diseases like rheumatoid arthritis [126]. During inflammatory response, they can affect bone through multiple mechanisms. Subsets of inflammatory B lymphocytes produce significant quantities of RANKL and TNF [176-178], which can stimulate osteoclast-mediated bone

resorption [179] through mechanism dependent on decreased Fra1 [180] and ATM [181]. In addition, enhanced expression of RANKL by inflammatory B lymphocytes can be stimulated by direct activation of the BCR and not necessarily as a consequence of the inflammatory cytokine milieu at sites of inflammation. [179, 182]. B lymphocytes in rheumatoid arthritis also inhibit osteoblast differentiation through production of paracrine factors like CCL3 and TNF [183].

B lymphocytes in their role as progenitors of plasma cells are involved in the production of antibodies, which have direct effects in stimulating osteoclast-mediated resorption. In particular, anti-citrullinated protein antibodies (ACPAs), especially those against citrullinated vimentin on the surface of osteoclasts, can directly stimulate osteoclast to resorb bone [184]. In human studies markers of bone resorption were increased in patients with early rheumatoid arthritis who had ACPAs [185]. In mice, autocrine production of IL-8 by osteoclasts was found to be a critical intermediate in the bone loss that results from the actions of ACPAs [186]. In addition, ACPAs can activate monocytes to produce inflammatory cytokines [187], which also stimulate osteoclast mediated bone resorption [2]. ACPAs and other autoantibodies, notably rheumatoid factor, form immune complexes that potently activate neutrophils. They, in turn, produce cytokines and release cytotoxic compounds generating citrullinated epitopes in the rheumatic joint, contributing to renewed ACPA generation [188].

In addition, a proinflammatory microenvironment leads to changes in the stromal cells, which acquire a pathogenic behavior, and induce a plasma cell-like phenotype in B lymphocytes and cytokine secretion in T lymphocytes. In turn, B and T lymphocytes influence the stromal cells, inducing cell proliferation, molecular changes and cytokine production, creating a positive feedback loop and leading to disease chronicity [189]. Furthermore, the inflamed synovial tissue contains enlarged B lymphocyte clusters and ectopic germinal centers [190]. Although B lymphocyte-depleting therapy would appear to represent a good therapeutic option, several clinical trials recommended caution due to serious side effects, which were mostly due to associated immunosuppression [191, 192].

Periodontal diseases

Periodontal disease is a common problem affecting a large proportion of the population [193]. If allowed to progress it produces alveolar bone loss and associated tooth loosening [194]. The original observation that B lymphocytes were involved in this condition comes from histologic examination of lesions [195]. Subsequently, it was found that as lesions progressed, B lymphocyte infiltration increased concomitantly with plasma cells producing a variety of antibody classes against oral pathogens [196]. It is now accepted that periodontal disease is largely driven by B lymphocytes and plasma cells [122]. Data supporting this conclusion comes from experiments showing that transfer of B lymphocytes, which were primed against an oral pathogen, into naive rats enhanced bone loss after exposure of the recipients to the pathogen [197]. Similarly, in mouse models of partial B lymphocyte deficiency (*IgD*-deficient mice) or mature B lymphocyte deficiency (μMT/μMT mice), alveolar bone loss did not occur after the mice were inoculated with an oral pathogen (porphyromonas gingivalis) [198, 199]. In humans it was shown that anti-B lymphocyte therapy of patients with rheumatoid arthritis significantly decreased the incidence of periodontal disease [200]. There are also reports that certain subsets of B lymphocytes, producing IL-10 inhibit inflammation in periodontitis [201, 202].

Production of RANKL by B lymphocytes seems critical to the development of bone loss in periodontal disease as injection of OPG to mice prevented alveolar bone loss in a mouse model [203, 204]. Similarly, in humans, histologic examination of periodontal lesions demonstrates increased RANKL expression [203, 204]. Production of the cytokines: B cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) also seem involved in the development of periodontal disease since their levels are increased in human lesions and their inhibition reduced bone loss in mouse models of the disease [205] Another novel cytokine that is reported to augment periodontal bone loss is secreted osteoclastogenic factor of activated T cells (SOFAT), which is a RANKL-independent osteoclastogenic cytokine, produced by T and B lymphocytes [206].

Human immunodeficiency virus (HIV) bone disease

Bone loss and an increased risk of fragility fractures are characteristic of individuals with HIV infection [207, 208]. Importantly, this condition is also associated with alterations in subsets of B lymphocytes [209]. For example, immature/transitional B lymphocyte expansion was associated with bone loss in HIV-infected individuals with severe $CD4^+$ T lymphopenia [210]. Studies of an HIV rat model [211] demonstrated that these animals had osteopenia, increased osteoclast number and size, and an associated increase in serum levels of the bone resorption marker CTx. Bone marrow cells from these mice had increased levels of transcripts for RANKL and decreased levels of transcripts for OPG. In human studies, HIV infected patients were found to have a 20% lower number of OPG expressing B lymphocytes and a 60% higher level of B lymphocytes expressing RANKL [212]. This study also found an inverse correlation between femoral neck and total hip BMD and the RANKL/OPG ratio in B lymphocytes [212]. Hence, these data argue that alterations in RANKL/OPG expression in B lymphocytes are involved in the development of bone disease in HIV-infected patients

Multiple myeloma

Multiple myeloma is a hematologic malignancy of clonal plasma cells, typically presented with bone marrow infiltration and osteolytic lesions, as well as monoclonal immunoglobulins in the serum and/or urine [213, 214]. Bisphosphates or similar anti-resorptive therapies in combination with proteasome inhibitors and immunomodulatory drugs, as well as HSC transplantation have joined the traditional arsenal (corticosteroids, alkylating agents, and anthracyclines) to target malignant plasma cells, however no successful treatment efficiently eradicate these cells from the bone marrow and patients eventually relapse, leading to further bone destruction. Recently, great advances were made in understanding the mechanisms of myeloma cell homing and activation, stressing two crucial events in the disease pathogenesis – creation of bone marrow niches for malignant plasma cells, and myeloma cells reactivation and expansion [215]. Both of this processes involves complex interactions with multiple cell lineages in the bone marrow microenvironment, such as osteoblasts, osteoclasts, fibroblasts, adipocytes, myocytes, endothelial cells, lymphocytes, dendritic cells, and macrophages, together with the extracellular matrix and soluble factors [215]. In brief, myeloma cells are thought to home preferably to endosteal niches through the interaction of

CXCL12 chemokine with its CXCR4 receptor, similar as normal plasma cells. The endosteal niche adopts myeloma cells arriving in the bone, which then cycle between a mitotically active and dormant state. The nutrient-rich milieu (IL-6, IGF, CXCL12, hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), APRIL) provides crucial signals for myeloma cell survival and activation. In parallel, myeloma cells can inhibit osteoblast function by interfering with several osteogenic signaling pathways, including Wnt, BMP, and Notch [216]. Finally, secretion of chemokines (CCL3, CCL4), RANKL, and IL-6 by myeloma cells induce osteoclasts, which in turn reverse myeloma cell dormancy by remodeling the endosteal niche [133]. Thus, myeloma cells are able to disrupt the process of homeostatic bone remodeling and shape the microenvironment to favor tumor growth resulting in niche remodeling, osteoblast suppression, and osteolytic bone disease.

Summary and future directions:

Our understanding of the interactions between. bone cells and B lymphocytes has greatly progressed over the last 20 years. We now understand that osteolineage cells are involved in maintaining the bone marrow niche for B lymphopoiesis [67, 217] and that there is a reciprocal regulatory relationship between osteolineage cells and many hematopoietic elements of bone marrow, which regulate the normal development of both lineages and can become dysfunction in disease states. Most recently, advances in the ability to analyze the expression profile of single cells and to map their location in the bone marrow in relationship to other cells has greatly advanced our understanding of the relationships and the complexities this microenvironment [67, 217]. It is likely that future technologic advancements will provide additional insights into the functional consequences of the bone marrow's organization and the role that the individual cells have in influencing other lineages. Hopefully, this knowledge will provide us with a better understanding of both the normal and abnormal functioning of B lymphocytes and bone cells so that we can design better therapeutic approaches to treat diseases that affect either system.

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