Osteoimmunology: Interplay Between the Immune System and Bone Metabolism

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T lymphocyte, dendritic cell, osteoblast, osteoclast, TRANCE-RANK, costimulation

Abstract

Studies of bone and the immune system have converged in recent years under the banner of osteoimmunology. The immune system is spawned in the bone marrow reservoir, and investigators now recognize that important niches also exist there for memory lymphocytes. At the same time, various factors produced during immune responses are capable of profoundly affecting regulation of bone. Mechanisms have evolved to prevent excessive interference by the immune system with bone homeostasis, yet pathologic bone loss is a common sequela associated with autoimmunity and cancer. There are also developmental links, or parallels, between bone and the immune system. Cells that regulate bone turnover share a common precursor with inflammatory immune cells and may restrict themselves anatomically, in part by utilizing a signaling network analogous to lymphocyte costimulation. Efforts are currently under way to further characterize how these two organ systems overlap and to develop therapeutic strategies that benefit from this understanding.

INTRODUCTION

The emergence of the niche field of osteoimmunology represents a conceptual rethinking of multiple phenomena, relating biological events in bone and the immune system (1). The root of exploration of this interplay begins with the basic understanding that bone provides a microenvironment that is critical for the development of the hematopoietic stem cells (HSCs), from which all cells of the mammalian immune system derive, and that various immunoregulatory cytokines influence the fate of bone cells.

The reasons bone is an ideal anatomic microenvironment for HSC maintenance and differentiation have become clearer with recent data indicating that bone matrix–generating osteoblasts (OBs) provide key factors to the development of the HSC niche. There is growing evidence that bone continues to play a role in adaptive immunity at steps beyond lymphocyte development. We now know that long-lived memory T and B cells return to specialized niches in the bone marrow. Why these cells are retained in the bone and what molecular and environmental factors they rely on while there represent a series of challenging questions that will clearly benefit from experiments designed in the context of osteoimmunology.

How the immune system exerts its influence on bone is equally interesting. Ontogenically, skeletal development proceeds independently of early development of the immune system. Hence, it is unlikely that there are developmental influences of the immune system on skeletal and marrow cavity formation. However, bone homeostasis and remodeling occur throughout life in all bony animals. Anatomically, bone marrow spaces are loosely compartmentalized, allowing immune cells and bone cells to interact and influence each other (**Figure 1**). Hence, bone homeostasis is often influenced by immune responses, particularly when the immune system has been activated or becomes diseased. During pathological conditions like arthritis, infiltrating lymphocytes and other mononuclear cells provide several key factors that influence bone metabolism by altering the balance between bone-forming OBs and bone-resorbing osteoclasts (OCs). In the past, whether these interactions also influence normal bone homeostasis had been unclear. However, the discovery that activated T cells express the TNF superfamily member TRANCE, coupled with the subsequent finding that TRANCE is a key differentiation factor for OCs, represents critical evidence that a link exists between normal immune cell function and bone metabolism. During their lifetimes, mammals are challenged with various infectious agents, which results in a gradual change in the composition of the T cell compartment toward an accumulation of TRANCE-expressing memory cells that preferentially reside in bone. Hence, with age the immune system might exert a greater influence on bone homeostasis.

Finally, how relevant are osteoimmunologic approaches in biomedical research to the treatment of human disease and the maintenance of good health? Although most research in osteoimmunology currently focuses on inflammatory bone diseases, such as those associated with osteoarthritis or rheumatoid arthritis (RA), it is important to consider the vast public health implications arising from common metabolic bone diseases, such as osteoporosis, that may be caused by or associated with inflammatory molecules. To prevent and treat these conditions, it will be necessary to develop anti-inflammatory agents that have a high degree of specificity so that both the immune and bone systems may function with minimal complications.

With these issues in mind, we first briefly review bone development and remodeling and then focus on several key areas of crosstalk between the bone and immune system that we believe will most benefit from interdisciplinary approaches aimed at elucidating underlying cellular and molecular mechanisms of action. The work thus far of many groups on the physiologic and clinical relevance of

HSC: hematopoietic stem cell

Osteoblast (OB): bone-producing cell that also provides signals for osteoclast formation

Osteoclast (OC): specialized multinucleated giant cell that resorbs bone

TNF: tumor necrosis factor

TNF-related activation-induced cytokine (TRANCE): a TNF family cytokine that is essential for osteoclast development

Osteoporosis: a condition characterized by decreased bone mass and density that causes bones to become fragile

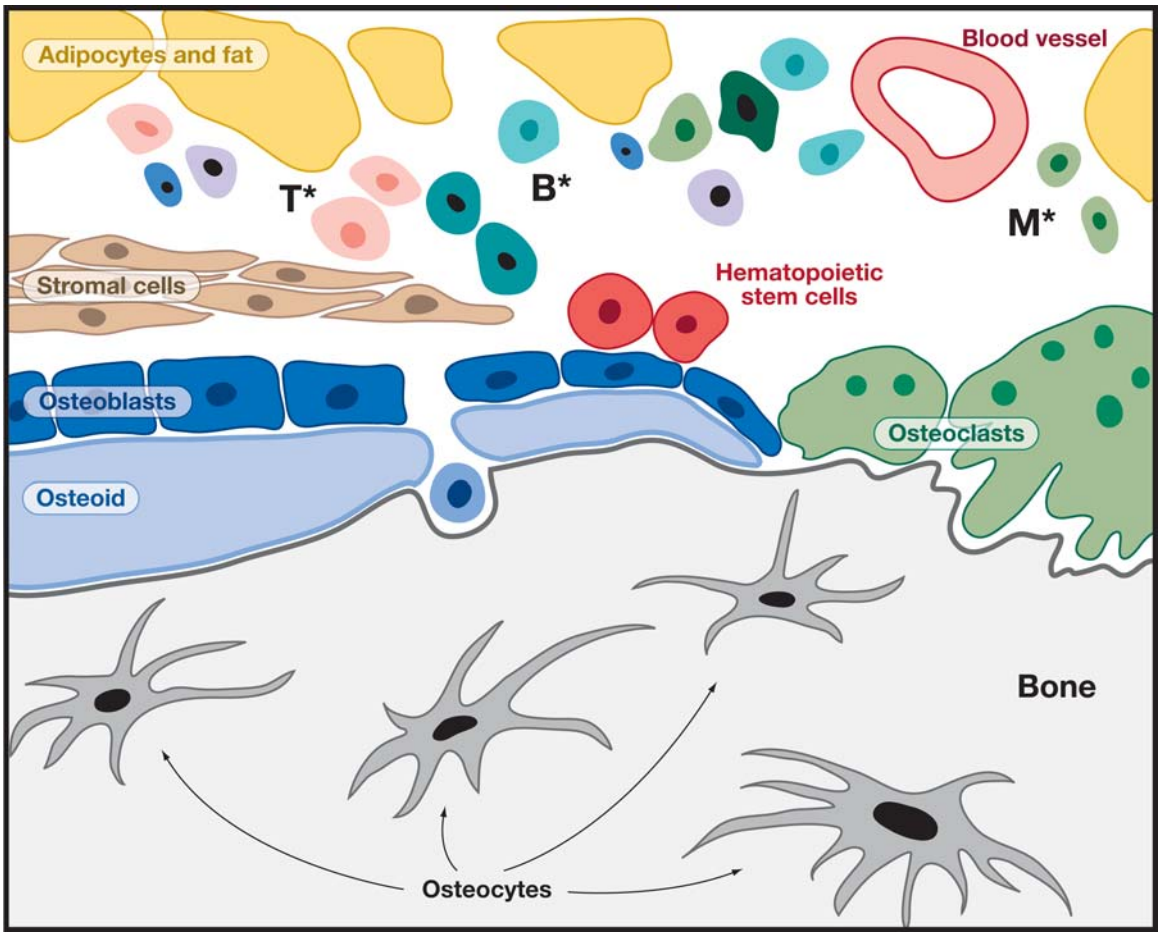

Figure 1

Schematic diagram of the bone microenvironment as a loosely compartmentalized lymphoid organ: T* (memory T cells and circulating T cells), B* (B cells, differentiation of which occurs via interaction with stromal cells; memory B cells also interact with stromal cells; in addition, there are circulating mature B cells), stromal cells (bone marrow stromal cells are of mesenchymal origin, but not fully characterized), M* (monocyte and its derivatives), and osteoid [newly formed, but not yet calcified matrix, composed mostly of type I collagen (~90%) and noncollagenous proteins (~10%)].

TRANCE-RANK signaling has served as a cornerstone of osteoimmunology research. As such, we provide a current review of the role of TRANCE in the osteoimmune system. Additionally, we review the overlapping signaling networks within cells of the immune system and bone and attempt to build a conceptual framework of parallel signaling systems in immune and bone cells.

A BRIEF INTRODUCTION TO BONE

Bone Development

The skeleton is among the largest organs in the body. It is composed of a mineralized framework that is maintained by a complex cellular network (2). In addition to providing structural integrity, the skeleton is a

Receptor activator of NF-κB (RANK): the signaling receptor for TRANCE

Osteoid: uncalcified bone matrix produced by osteoblasts and consisting mainly of collagen, but also of some noncollagenous proteins

BMP: bone morphogenetic protein

storehouse for calcium, a critical ion for a variety of metabolic processes, and it is the site of hematopoiesis. Bone development occurs along two pathways. Endochondral bone, which includes the long bones and vertebrae, begins in the embryo as a cartilaginous template (3) (**Figure 2**). A second type of bone, called intramembranous bone (e.g., the flat bones of the skull, scapula, and ileum), forms directly from the condensation of mesenchymal cells, which directly differentiate into OBs (3).

A second level of organization in endochondral bone is its division into cortical and trabecular or cancellous bone. Cortical bone forms the outer surface of endochondral bones and provides the structural integrity for many of the long bones. It forms up to 80% of the skeleton and is typically dense bone with a well-organized pattern of collagen fibrils that are aligned along stress lines to provide bone with maximum strength (2, 3). Trabecular bone is thinner and less well organized, and it is primarily found traversing the bone marrow space. However, in some bones with a high degree of trabecular bone, like vertebrae, trabecular bone provides much of the structural integrity. A major function of trabecular bone is to provide a large surface area for metabolic processes. Bone turnover, which consists of bone resorption (removal) and its replacement with new bone, occurs much more frequently in trabecular bone than in cortical bone.

OBs initially produce an osteoid matrix that is calcified extracellularly. The major structural protein of bone is type I collagen, which provides bone with a resistance to fracture in a way that is similar to the effect of reinforcing bars in modern reinforced concrete buildings. In addition, osteoid contains a large number of other noncollagen proteins that have a variety of critical functions in bone. The mineral crystal of bone is hydroxyapetite, which is a calcium-phosphate salt, containing hydroxyl ions.

Bone Cells

OBs are derived from a mesenchymal progenitor cell that is multipotential and can also differentiate into marrow stromal cells and adipocytes (4) (**Figure 3**). The signals that regulate the decision of mesenchymal progenitor cells to form OBs are not fully understood. However, a number of critical paracrine signals and transcription factors have been identified. These include the transcription factors Runx2 and osterix, which when absent prevent OB formation, and members of the bone morphogenetic protein (BMP) family (5–7), which initiate the signals for OB differentiation. Most recently, it was demonstrated that Wnt signaling pathways are involved in the decision of the mesenchymal progenitor cell to become either an adipocyte or an OB (8–12).

As matrix calcifies under the influence of the OB-produced enzyme bone-specific alkaline phosphatase, a portion of the OBs are entrapped in the calcified matrix and persist in bone as unique cells called osteocytes. These cells are believed to sense mechanical force on bone and to send signals via cellular processes, termed canaliculi (13), that connect osteocytes to each other and to OBs on the surface of bone.

OCs are specialized multinucleated giant cells that resorb bone (14). They are hematopoietic in origin and derive from a myeloid precursor that also gives rise to macrophages and dendritic cells (DCs) (**Figure 3**). The signals that stimulate OCs to form and resorb bone involve a series of transcription factors and paracrine cytokines, which are discussed in detail later in this review. OCs attach themselves to bone through a specialized structure called the sealing zone. This structure allows them to create a resorption space that is isolated from the extracellular space. OCs can acidify the resorption space to solubilize the mineral component of bone (14). To remove the organic components of bone, OCs produce lysosomal enzymes, including cathepsin K, that are released into

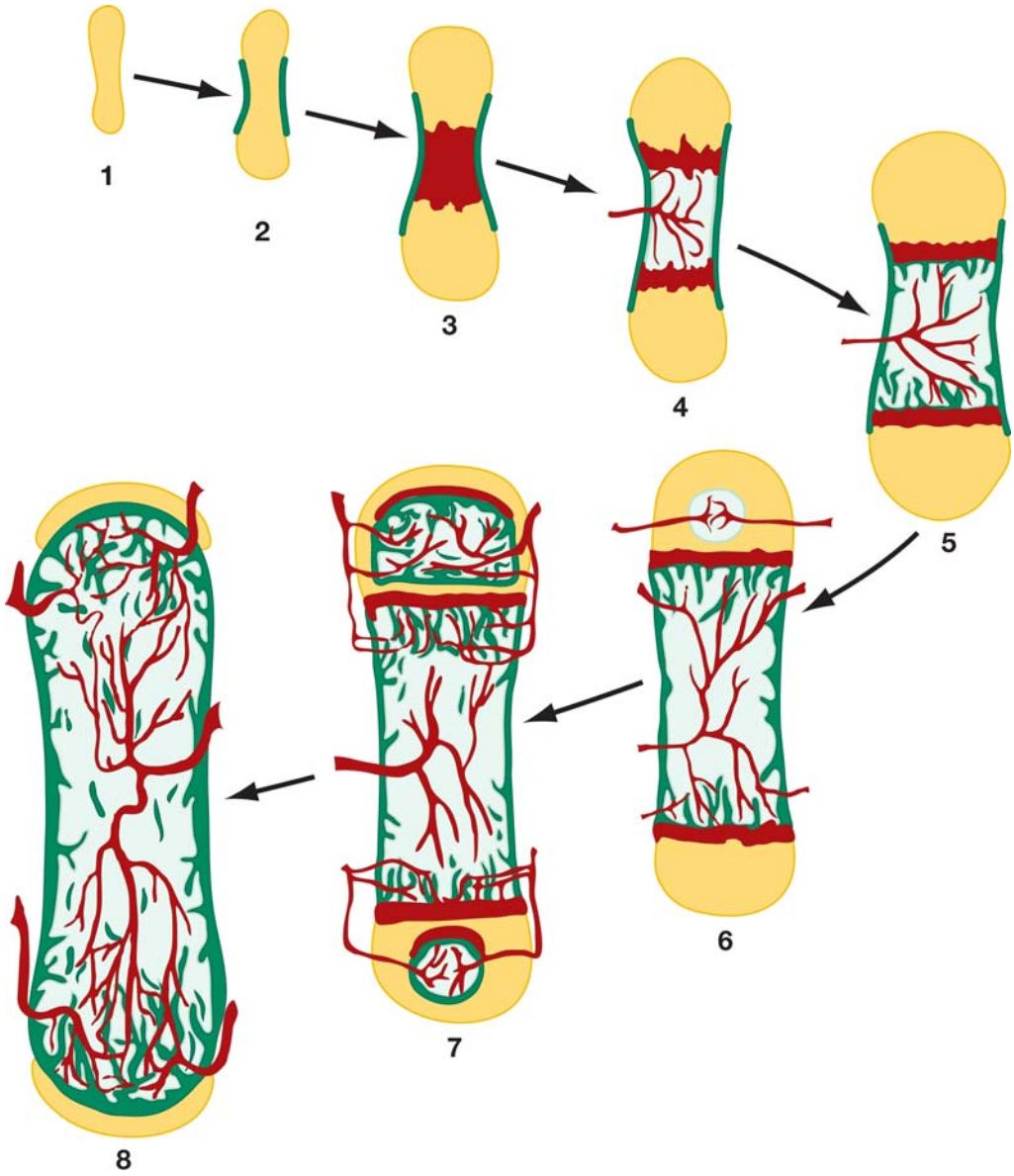

Figure 2

Schematic diagram of endochondral bone development. During embryogenesis, the initial step in long bone development is the patterning of the future bone by cartilage (1). Shortly thereafter, a collar of intramembranous bone forms around the center of the cartilaginous shaft (2), and simultaneously the remaining cartilage near the center of the developing bone calcifies (3). The calcified cartilage is then removed (4), and vascular cells invade the developing marrow space to establish a blood supply. The next step in this process is the production of mineralized bone by OBs (5). As the development of the embryo progresses, mineralized tissue, produced by OBs, replaces cartilage in the majority of the embryonic bone (6–7). However, an area of cartilage is maintained at either end of growing bones after birth. This forms a structure called the epiphyseal plate or growth plate, which in humans facilitates bone growth through childhood and into adolescence. Linear growth in humans ends after puberty when the growth plate is lost as a result of the actions of sex steroid hormones (8).

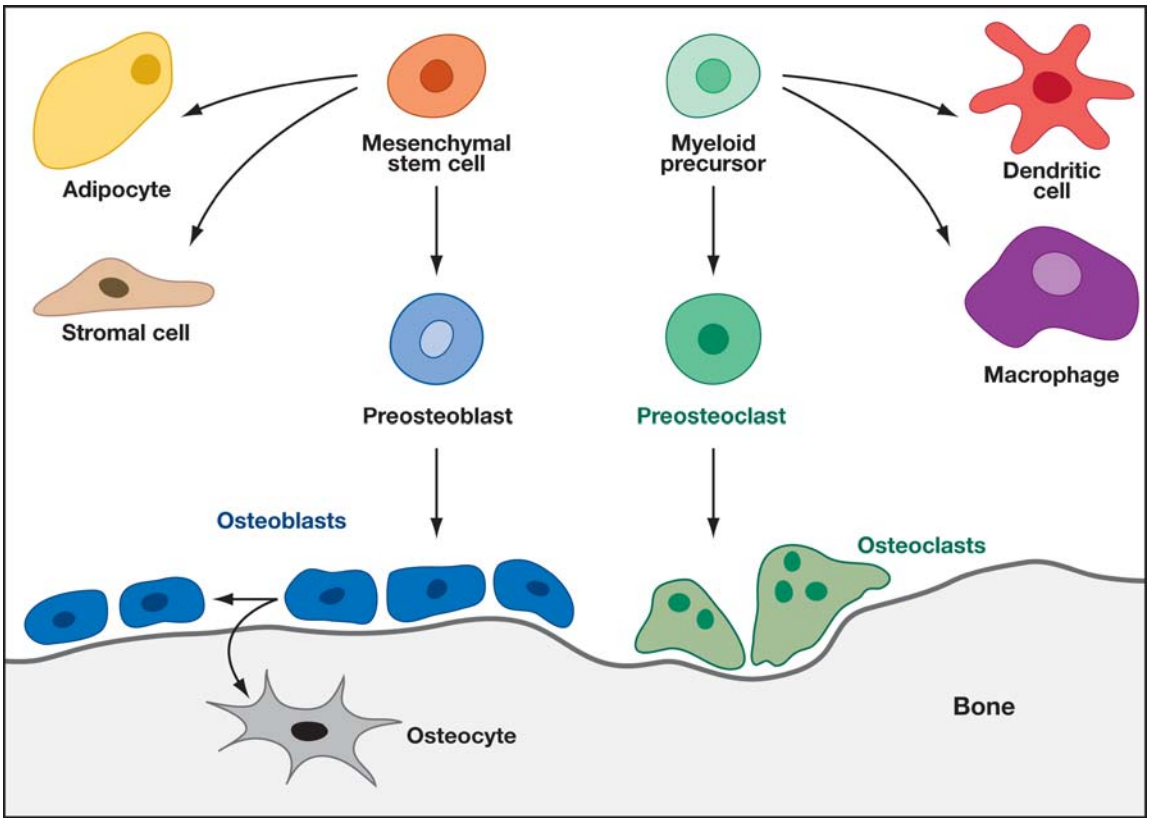

Figure 3

Schematic summary of bone cell differentiation. Mesenchymal stem cells, which also give rise to myoblasts, adipocytes, chondrocytes, and some as yet uncharacterized stromal cells (also called marrow stromal cells, marrow fibroblasts, or the reticular network), differentiate into preosteoblasts and then become OBs on the bone surface. OBs either incorporate into bone as osteocytes or remain on the surface as lining cells. A common myeloid lineage precursor, which can also give rise to macrophages and dendritic cells, commits to becoming a preosteoclast and then fuses to become a mature, multinucleated osteoclast.

the resorption space (14). To facilitate the resorption process, OCs polarize their structure and form a unique element called the ruffled border, which allows the surface area available for active transport of H^+ ions through a unique vacuolar proton pump. The products of resorption traverse the OC by a process that is termed transcytosis and leave the OC through the basolateral membrane opposite the resorption space. OCs are highly motile, move across the bone surface, and resorb relatively large areas of bone. OCs die by apoptotic processes that appear to be regulated by paracrine-acting cytokines and possibly factors in bone matrix (14).

The Coupling of Bone Resorption and Formation

The skeleton is a dynamic organ that is constantly remodeling. To accomplish this function there must be a tight coupling between bone resorption and formation. Although investigators have proposed a local coupling factor linking bone resorption to subsequent formation, its nature remains elusive

(15). During growth and continuing to mid-adulthood in humans, bone mass increases (16). Genetics plays a major role in the peak bone mass that is achieved. Sex steroid hormones are also critical regulators of the skeleton (2). Loss of either estrogens in women or androgens in men is associated with an enhancement of resorption rates in bone without an equivalent increase in bone formation. In women, loss of estrogens with menopause causes roughly a doubling of the rate of bone loss and increases the risk of developing osteoporosis. Because women generally live longer and have a lower peak bone mass than men, they are more prone to develop osteoporosis (2).

INTERPLAY BETWEEN OBs AND THE IMMUNE SYSTEM

The Role of OBs in the HSC Niche

OBs on the endosteal surface of bone, which is adjacent to the marrow cavity, function as critical support cells for HSCs in bone marrow (17–19). Investigators have shown that HSCs are adjacent to OBs and that their number is ~2.3-fold higher in mice upon deletion of the BMP receptor 1A (BMPR1A). Significantly, BMPR1A-deficient mice also have a similar increase in OB number (17). The OBs involved are on the marrow surface of the endosteum and are likely early in their commitment to the OB lineage. Adhesion of HSC and OBs appears to be mediated by interaction of N-cadherin and β-catenin (17).

Similarly, it was demonstrated that expansion of the OB population in bone by stimulation of the PTH/PTHrP (parathyroid hormone/parathyroid hormone–related protein) receptor on osteoblastic cells increased the number of HSCs in bone marrow (18). This effect appeared to be mediated by Jagged-1-Notch-1 signaling because Jagged-1 levels were increased in mice with OB-targeted activation of the PTH/PTHrP receptor. In addition, the increase in the number of HSCs in cultures of cells from transgenic mice with OB-targeted activation of the PTH/PTHrP receptor was abrogated by inhibitors of Notch signaling. In a converse experiment, researchers found that targeted destruction of osteoblastic cells in mice led to a decrease in HSCs in bone marrow (19). Interactions of HSCs and OBs are also mediated by interactions of Tie2 on HSCs and Angiopoietin-1 on OBs. This signaling system inhibits cell division in HSCs while maintaining their capacity for self-renewal (20).

Bone as a Reservoir for Memory B and T Cells

The bone marrow has long been recognized as the site of early lymphocyte development, but more recent findings indicate that end-stage and memory lymphocytes preferentially inhabit the bone marrow. Upon completing differentiation in germinal centers, antibody-producing B cells, or plasma cells, down-regulate CXCR5 and upregulate CXCR4,

Hormone and Bone Health

Estrogen therapy for the treatment of osteoporosis in women had been the standard of care until the release in 2002 of the findings of the Women's Health Initiative. This study, which was among the largest ever funded by the United States National Institutes of Health, demonstrated for the first time that oral estrogen replacement therapy prevented the development of osteoporotic fractures in postmenopausal women. However, the study also showed that this therapy, both with and without progesterone, was associated with an increased risk of thromboembolic disease and strokes. In addition, it was reported that women who took the combination of oral estrogen plus progesterone had an increased risk of breast cancer and myocardial infarctions. Although estrogen therapy is effective for the prevention of postmenopausal osteoporosis, the United States Food and Drug Administration has recommended that it should only be considered for the treatment of women at significant risk of osteoporosis who cannot take nonestrogen medications, and it should be used at the lowest doses and for the shortest duration possible to reach treatment goals.

facilitating migration from the spleen to the bone marrow, where stromal cells highly express the CXCR4 ligand, CXCL12 (21–23). Bone marrow stromal cells, which appear to be different from mature OBs, retain plasma cells via expression of adhesion molecules VCAM-1 (vascular cell adhesion molecule 1) and E-selectin (22, 23). BrdU incorporation studies have demonstrated that plasma cells found in the bone marrow have life spans typically in excess of 90 days (21, 24), suggesting the existence in bone marrow of specialized survival niches. Although it has been reported that individual bone marrow plasma cells exhibit no intrinsic survival advantage over splenic plasma cells (24), bone marrow may be capable, unlike the red pulp of the spleen, of providing the necessary space for plasma cells to thrive as a population (25). Given the short half-life of bone marrow plasma cells when cultured in vitro, the bone marrow microenvironment may contain a combination of factors, including IL-5, IL-6, SDF-1α, TNF-α, and CD44 ligands, that are required to sustain resident bone marrow plasma cells (26). Of considerable significance is the role of bone as a source of the B cell survival factor BAFF/BLYS (B cell–activating factor/B lymphocyte stimulator). A BAFF/BLYS receptor, BCMA (B cell maturation factor), has recently been shown to be critical for long-term plasma cell survival (27, 28). Recent findings in multiple myeloma patients show that in addition to neutrophils and monocytes, OCs serve as a greater source of BAFF/BLYS than do bone marrow stromal cells (29). Although there is little evidence that the bone marrow microenvironment is required for optimal function of plasma cells, additional research is required to clearly determine the anatomical advantage for plasma cells of relocating from secondary lymphoid organs to this site.

T lymphocyte precursor cells leave the bone marrow and relocate to the thymus for further differentiation. Mature T cells function in the secondary lymphoid organs and at sites of infection. Investigators have recently shown, however, that in the absence of secondary lymphoid tissues the bone marrow can support productive cytotoxic T cell and memory cell generation (30). Although the importance of bone marrow as a secondary lymphoid organ during normal immune responses requires further study, it has potential relevance to host defense against bacterial infections in bone (e.g., osteomyelitis), where OBs may facilitate immune responses by producing a variety of immunomodulatory molecules (31).

Memory T cells form after the expansion and contraction of both $CD4^+$ and $CD8^+$ primary response T cells. In normal mice, memory $CD8^+$ T cells preferentially reside and homeostatically proliferate in the bone marrow (32). One homing study indicates that long-lived central memory $CD8^+$ T cells have a special affinity for the bone marrow, employing VCAM-1 for sticking and L-selectin for rolling within the bone marrow microvessels (33). The advantage of central memory $CD8^+$ T cells relocating to the bone marrow may involve the presence of the crucial survival factor IL-15 (30, 33).

Regulation of OBs by Immune Cells and Cytokines

A variety of cytokines are known to regulate osteoblastic cells. TNF-α inhibits the differentiation of OBs (34). IL-1, TNF-α, and IFN-γ inhibit collagen synthesis in OBs (35–38). IL-4 and IL-13 suppress prostaglandin synthesis in bone and are reported to be chemoattractants for OBs (39, 40). IL-4 acts as a direct stimulator of proliferation and inhibitor of differentiation in an osteoblastic cell line (41). Similarly, IL-4-overexpressing mice exhibit a decrease in bone formation and differentiated OBs on the bone surface (42). The role of cytokines in OB apoptosis has also been studied. TNF-α is potently proapoptotic for OBs (43). Activated T lymphocytes also produce products that drive differentiation of human bone marrow stromal cells toward an osteoblastic phenotype (44). B7-H3 is an Ig

superfamily member that is expressed on the surface of antigen-presenting cells (APCs). Recently, B7-H3 was found to be expressed on developing OBs, with its expression increasing during cell maturation (45). Furthermore, B7-H3-knockout mice have decreased cortical bone mineral density compared with littermate controls (45).

INTERPLAY BETWEEN OCs AND THE IMMUNE SYSTEM

The first observation that immune cells could influence the activity of OCs came from the finding that supernatant from normal human phytohemagglutinin-stimulated peripheral blood monocytes contained factors that stimulated bone resorption (46). This activity was named OC-activating factor (OAF). When it was eventually purified and sequenced, the principal stimulator of bone resorption in these crude OAF preparations was found to be the cytokine IL-1 (47). Subsequently, a long list of cytokines have been identified to have effects on bone. Stimulators of bone resorption include IL-1, TNF-α, IL-6, IL-11, IL-15, and IL-17. Inhibitors of resorption include IL-4, IL-10, IL-13, IL-18, GM-CSF, and IFN-γ. TGF (transforming growth factor)-β and prostaglandins can have either stimulatory or inhibitory effects on resorption, depending on the conditions under which these factors are examined (48).

Production of cytokines by immune cells has been linked to human diseases that involve bone. Perhaps the most extensive studies have been on the role of cytokines in the development of osteolytic lesions observed in RA and other inflammatory bone diseases, including periodontal disease. Although the immunologic perspective toward RA is typically limited to how RA is initiated (i.e., by failure of immunological tolerance) and its resultant synovitis (the conventional definition of disease culmination), a hallmark of RA is the rapid erosion of periarticular bone, which is often followed by general secondary osteoporosis (or osteopenia). OCs are found to be prevalent at the site of focal erosion and are critical for bone erosion (49, 50).

In addition to inflammatory bone diseases, altered immune responses or cytokine production may cause other osteolytic diseases. Estrogen withdrawal after menopause is associated with a rapid and sustained increase in the rate at which bone is lost. This phenomenon seems to result from an increase in bone resorption that is not met by an equivalent increase in bone formation. Interestingly, activated T cells may cause rapid bone loss under conditions of estrogen deficiency by enhancing TNF-α production (51). In a series of experiments involving ovariectomy (OVX)-induced bone loss in mice, an animal model for postmenopausal bone disease, it was reported that nude mice did not lose bone mass after OVX, suggesting that T cells are critical for this response (51). However, similar experiments using nude rats (52), RAG2- or TCR-α-deficient mice, and SCID rats demonstrated OVX-induced trabecular bone loss that was equivalent with that seen in wild-type mice (Y. Choi, Y. Kadono, and J. Lorenzo, unpublished data). Curiously, loss of cortical bone upon OVX was different between T cell–deficient and wild-type models and depended on the bone that was examined. These results suggest there may be compartmental and bone-specific effects of T cell depletion on OVX-induced bone loss. Additional experiments are required to determine how T cells are involved in this response of bone. These studies will likely require the use of mutant mouse models deficient in specific immunoregulatory molecules to mechanistically examine the causes of OVX-induced bone loss.

Special consideration must be given to the genetic background of any mutant mice examined as part of future studies. The reduction in bone density observed as a result of OVX is roughly only 30%–50%, whereas the difference in average bone density observed between 129/J and C57BL/6 mice is significant, with 129/J exhibiting ~40% higher bone density than C57BL/6. Together, 129/J and

C57BL/6 mice constitute most of the knockout mice analyzed.

The role of cytokines in malignancy-related bone disease has also been studied extensively (53). Hematological malignancies such as lymphoma, multiple myeloma, and adult T cell leukemia are associated with increased OC formation and activity, possibly through dysregulation of various cytokines, including IL-1, TNF-α, and IL-6. Unlike bone disease associated with solid tumors, which is typically mediated by PTHrP, hematological malignancies are often characterized by an uncoupling of resorption from formation and the frequent development of purely lytic bone lesions.

It should be noted, however, that most cytokines believed to play a role in regulating bone cells are produced by nonimmune cells, like fibroblasts, as well as by immune cells, and exert pleiotropic effects on various cell types. Despite extensive research over the past two decades on this topic, the molecular and cellular significance of these cytokines in vivo, specifically in the context of an immune response, has only recently begun to be elucidated with the identification of the TRANCE-RANK-OPG axis (54–60).

Osteoprotegerin (OPG): the decoy receptor for TRANCE found in soluble form

Osteopetrosis: increased bone density resulting from an imbalance between the formation and breakdown of bone

THE TRANCE-RANK-OPG AXIS AND OSTEOIMMUNOLOGY

Characterization of the functions of TRANCE and its receptors [RANK and osteoprotegerin (OPG)] have contributed significantly to the emergence of osteoimmunology, specifically with respect to examination of the interplay between active immunity and maintenance of bone homeostasis (58–60). Because there are a number of recent reviews on the diverse physiologic function of the TRANCE-RANK-OPG axis (58, 59), we focus here on its role in the context of osteoimmunology (**Figure 4**).

Regulation of Bone Homeostasis by the TRANCE-RANK-OPG Axis

Bone homeostasis is maintained via functional balance of two cell types: OBs, which build bone, and OCs, which resorb bone (14, 60). In an ongoing cycle, OCs remove bone, and subsequently OBs fill the cavity with new bone. This balance enables the continuous remodeling of the bone matrix necessary to maintain skeletal strength and a reservoir for hematopoiesis. Much recent effort has gone into determining the developmental processes underlying OC differentiation and activation.

OPG was initially identified as a soluble decoy-like factor, capable of inhibiting osteoclastogenesis in vitro (61, 62) and inducing osteopetrosis when transgenically overexpressed in mice (62). Furthermore, OPG-deficient mice were described as osteoporotic and found to have an excess of OCs (63). Interestingly, these mice are also susceptible to arterial calcification, highlighting a potential genetic link between osteoporosis and vascular calcification (63).

The gene identified as encoding the ligand for OPG was determined to be identical to the gene originally characterized as encoding the activated T cell factor TRANCE (54–56). TRANCE is capable of inducing OC differentiation, maturation, and activation in vitro and, importantly, can do so in the absence of bone marrow stromal cells (55–57). Many well-known osteotropic factors, including IL-1, IL-6, and IL-11, are now believed to exert most of their osteoclastogenic activity by inducing TRANCE expression on OBs (60, 64). Not surprisingly, TRANCE-deficient mice are severely osteopetrotic owing to a cell nonautonomous defect in OC development (65, 66). These mice also exhibit failed tooth eruption, a common defect associated with developmental osteopetrosis, and diversion of hematopoiesis to the spleen and liver because a functional bone marrow cavity fails to form in the absence of OCs (65, 66).

RANK, the signaling receptor for TRANCE, was initially identified through

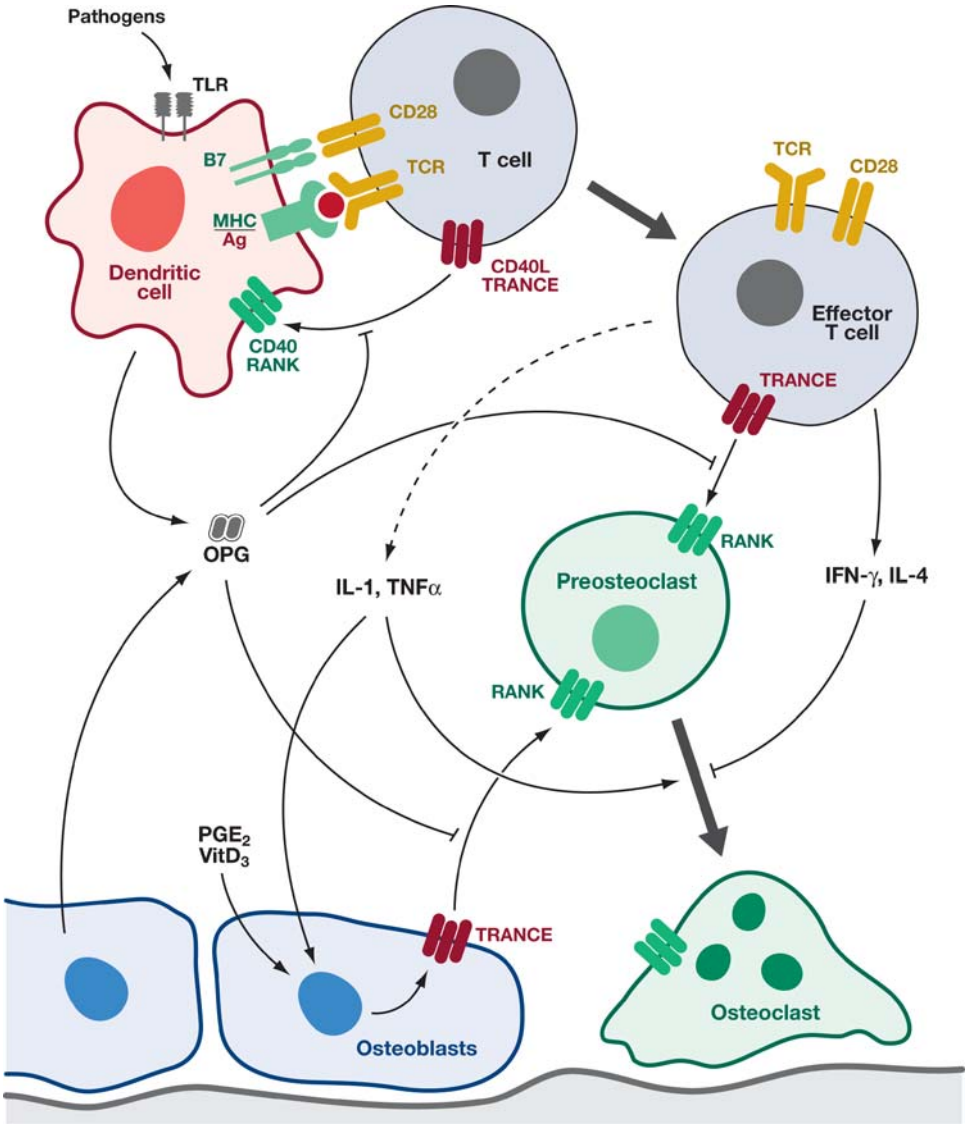

Figure 4

A model for the cellular interactions linking T cell immunity and bone homeostasis. Pathogenic stimuli or self antigens are phagocytosed and presented to naive T cells by DCs. T cells provide activating signals to DCs through CD40L and in return receive optimal activating and costimulatory signals through MHC:TCR and B7:CD28 interactions, respectively. The activated T cells are induced to express TRANCE, which provides further activating and survival signals to the DCs. The DCs may negatively regulate TRANCE:RANK signaling through upregulation of the TRANCE decoy receptor, OPG. Inflammatory cytokines (IL-1, TNF-α) produced during successful T cell immune responses, as well as calciotropic factors (PGE2 or VitD3), induce TRANCE expression by OBs, which cooperate with effector T cells to induce OC differentiation via provision of TRANCE to OC precursors. TRANCE signaling in mature OCs induces bone-resorbing function. OBs block TRANCE binding through secretion of OPG, whereas IFN-γ and IL-4 produced by effector T cells inhibit RANK signaling. Without proper regulation, excessive bone resorption leads to osteoporosis, arthritic joint erosion, and periodontal tooth loss.

TRAF: TNF receptor–associated factor

TRAF6: a TRAF family adapter essential for RANK signaling as well as IL-1R/TLR family signaling

a large-scale analysis of genes expressed in DCs (67). RANK expression at the RNA level is detected in most cell types or tissues examined (67). RANK-deficient mice were demonstrated to phenocopy the defect in OC development observed in the TRANCE-knockout mouse, confirming the exclusive specificity of TRANCE for OC-expressed RANK (68). In humans, gain-of-function mutations in RANK are associated with familial expansile osteolysis and with expansible skeletal hyperphosphatasia, which is characterized by increased OC and OB activity, resulting in fragile bones, pain, and deformity (69–73).

Efforts aimed at elucidating the signaling mechanisms involved in TRANCE-mediated osteoclastogenesis have been informative (58, 60, 64). RANK signal transduction is mediated by adapter proteins called TNF receptor–associated factors (TRAFs) (74–76). Of the six known TRAFs, RANK interacts with TRAFs 1, 2, 3, and 5 in a membrane-distal region of the cytoplasmic tail and with TRAF6 at a distinct membrane-proximal Pro-X-Glu-X-X-(aromatic/acid residue) binding motif (74–77). Genetic experiments show that TRAF6-deficient mice have severe osteopetrosis, implying that the key signals sent through RANK in OC precursors are mediated by the adapter molecule TRAF6 (78, 79; Y. Choi, N. Kim, and Y. Kadono, unpublished data).

Downstream of TRAF6, TRANCE signaling in OCs activates PI3K, TAK1, c-Src, JNK1, p44/42 ERK, p38 MAPK, Akt/PKB, and mTOR, and subsequently a series of transcription factors including NF-κB, c-Fos, Fra-1, and NFATc1. This aspect of TRANCE signaling has been recently reviewed elsewhere (58–60, 64, 80). In addition to the signaling pathways mentioned above, TRANCE stimulation also triggers reactive oxygen species (ROS) production (81). ROS, such as superoxide anions, hydroxyl radicals, and H_2O_2, have been associated with many cellular responses, including metabolic bone diseases found in aged osteoporotic women (82). Recent reports suggest that ROS act as a key second messenger during osteoclastogenesis (81), such that TRANCE stimulation induces the production of ROS in OC precursors via the small GTPase Rac1 and the ROS-inducing factor NADPH oxidase (Nox) 1. How ROS cross-regulates the signaling pathways necessary for OC differentiation is unclear, although one interesting hypothesis is that ROS may potentiate mitogen-activated protein kinase (MAPK) activation by inactivating protein tyrosine phosphatase activity in a manner similar to mechanisms recently described in B cells (83).

Modulation of Immunity by the TRANCE-RANK-OPG Axis

The significance of TRANCE-RANK-OPG signaling in regulating the immune system continues to emerge. Initial studies of TRANCE- and RANK-deficient mice demonstrated the importance of these signals for secondary lymphoid organ development, as these animals display a lack of peripheral lymph nodes and abnormalities in B cell follicle formation and marginal zone integrity in the spleen (58, 59). In this section, however, we focus on the role TRANCE-RANK plays in shaping the immune response in the adult immune system.

To date, most reported data indicate that TRANCE modulates immunity through DCs (**Figure 4**). DCs are the most potent professional APCs and are required to initiate T cell–mediated immunity in vivo (84). DCs differentiate from the hematopoietic monocyte/macrophage progenitor cell lineage and, as close relatives of OCs, can be generated in vitro by treating a common precursor cell with GM-CSF. GM-CSF suppresses c-Fos and Fra-1 (85, 86), which are key transcription factors for OC differentiation. These results highlight a mechanism of developmental divergence between these two cell types. Upon receipt of inflammatory or activating stimuli, DCs home to the T cell areas of the lymph nodes to activate antigen-specific

T cells. Productive activation relies on numerous DC-specific factors, including alteration of the chemokine receptor repertoire, upregulation of costimulatory molecules, and cytokine production. These modifications are induced by exogenous inflammatory stimuli as well as by signals transmitted by TNF family members, TNF-α and CD40L.

TRANCE signaling has also been implicated in DC function, particularly with regard to regulation of DC survival. Activated DCs are relatively short-lived cells, with a half-life as low as 1–2 days upon arrival in the lymph node (87), yet in situ imaging studies suggest that individual T-DC couplings may last 37 h or longer (88–90). TRANCE-prolonged DC survival is attributed to upregulation of the antiapoptotic protein Bcl-xL (91) through a pathway requiring the NF-κB components p50 and c-Rel (92). Treatment of DCs with TRANCE also activates the antiapoptotic serine/threonine kinase, Akt/PKB, through recruitment of PI3-K by TRAF6 and Cbl-b to RANK, in a mechanism dependent on the kinase activity of c-Src (75, 93). TRANCE-prolonged DC survival also has in vivo relevance, as pretreatment of peptide-pulsed DCs with TRANCE prior to subcutaneous injection into recipient mice results in significantly elevated DC persistence in draining lymph nodes and in enhanced Th1 cytokine production and T cell memory formation (94). DC vectors intended for use in immunotherapy persist longer when pretreated with TRANCE (95), and enforced autocrine TRANCE-RANK signaling but not CD40L-CD40 signaling on DCs enhances antitumor immunity (96). *Opg*$^{-/-}$ DCs potentiate in vitro mixed lymphocyte reaction (MLR), despite CD86, MHC class II, and antigen-presentation levels identical to syngeneic *opg*$^{+/-}$ DCs (97).

Blockade of TRANCE signaling in vivo results in a slightly reduced CD4$^+$ T cell response to LCMV infection, although the response is severely inhibited in the absence of CD40 signaling (98). These experiments highlight the requirement for TNF family member signaling in the generation of antiviral immunity, as well as the degree to which TRANCE-RANK and CD40L-CD40 function overlap. However, physiologic signaling through RANK is more limited in scope than that through CD40 in that treatment of immature DCs with TRANCE cannot initiate activation, and TRANCE signaling does not complement the *cd40*$^{-/-}$ defect in germinal center formation and B cell affinity maturation (91, 98). This disconnect is likely not explained by intrinsic signaling differences, as RANK and CD40 activate the same set of signaling cascades, but instead is explained by differential expression patterns and kinetics. For example, on T cells CD40L is rapidly and transiently expressed and is limited only to the CD4$^+$ subset (99). In contrast, TRANCE is expressed on both CD4$^+$ and CD8$^+$ T cells (100) and is capable of binding both its functional (RANK) and decoy (OPG) receptors. These interactions are also likely to succeed CD40L-CD40 signaling, as CD40L is a key inducer of RANK and OPG expression by DCs (101). The physiologic role of CD40L-CD40 versus TRANCE-RANK signaling in DC function may, therefore, depend on the phase of the immune response. CD40L-CD40 signaling may be more prominent during the initiation and effector phases, when many cellular components of the immune system are strongly activated. By contrast, TRANCE-RANK signaling may be more important during the waning phases to ensure that T memory formation is established and then to wind down remaining T-DC interactions, possibly through OPG interference with TRANCE signaling. The severe phenotype of TRANCE- and RANK-knockout mice has thus far not allowed a thorough examination of the role of TRANCE in memory cell formation.

Evidence also suggests that TRANCE may be important for survival of interstitial DCs engaged in antigen surveillance during the interim period separating immune responses. Human CD34$^+$ immature DCs express both TRANCE and RANK and can therefore

provide an autocrine survival signal. Peripheral maturation of these DCs leads to a downregulation of TRANCE, suggesting a requirement for an independent source of TRANCE to validate DC activation (102).

TRANCE may also be involved in actively inducing tolerance. TRANCE signaling has been directly implicated in the induction of oral tolerance in mice. Feeding low-dose ovalbumin to mice concomitant with intravenous TRANCE treatment results in T cells refractory to rechallenge and correlates with in vitro production of the suppressive cytokine IL-10 by mucosal DCs (103). Another study has demonstrated that TRANCE-mediated signaling is required to prevent the onset of autoimmune disease in a TNF-α-inducible mouse model of diabetes and that blockade of TRANCE-RANK interactions parallel a diminution of $CD4^+CD25^+$ regulatory lymphocytes, which are necessary to prevent CTL-mediated islet cell destruction (104). In a recent study of murine cardiac allograft tolerance, TRANCE-RANK signaling was shown to be important for the generation of regulatory T cells via intratracheal delivery of alloantigen (105). It remains unclear, though, whether TRANCE directly triggers T lymphocyte suppression or, alternatively, acts through DC intermediaries. TRANCE is also induced preferentially, among key costimulatory molecules, on T cells activated by tolerogenic DCs (106). Further study of this issue should yield insights into the generation and maintenance of T lymphocyte tolerance.

In addition to regulation of DCs, TRANCE might influence B cell development. In OPG-deficient mice there is an expansion of pro-B cells in the bone marrow, whereas the opposite has been observed in TRANCE- or RANK-deficient mice (65, 68, 97, 107), suggesting that TRANCE-RANK interaction might be involved in the proliferation of pro-B cells. Of interest, pro-B cells also expand in the bone marrow of ovariectomized mice (108), in which TRANCE expression on OBs is thought to be increased. Future studies are required to elucidate the molecular mechanisms of how TRANCE might regulate the fate of pro-B cells and what the immunological consequences of this regulation might be.

Autoimmunity, Bone, and TRANCE: The Birth of Osteoimmunology

TRANCE expression on T lymphocytes is induced upon T cell receptor engagement and depends on Ca^{2+} mobilization (54, 109). Initial experiments demonstrated that activated T lymphocytes, or even supernatants from activated T lymphocyte cultures, could support osteoclastogenesis in vitro (110). It was subsequently observed that mice lacking CTLA-4, in which T lymphocytes are systemically activated, exhibit an osteoporotic phenotype associated with increased OC numbers. Transfer of *ctla-4*$^{-/-}$ T lymphocytes into *rag2*$^{-/-}$ mice leads to decreased bone density over time, which can be prevented by OPG treatment. This finding indicated that activated T cells can disrupt bone homeostasis by modulating TRANCE expression (110), although whether T cell–derived TRANCE per se is responsible for aberrant bone metabolism is unclear. In a complementary study, transgenic overexpression of TRANCE restricted to T or T/B lymphocytes was sufficient to partially correct the osteopetrotic phenotype observed in TRANCE-deficient mice (66; Y. Choi & N. Kim, unpublished data). Together, these data definitively showed the ability of lymphocytes, by expression of TRANCE, to regulate bone homeostasis in vivo and confirmed a bona fide interplay between the adaptive immune system and bone metabolism, giving birth to the field of osteoimmunology (**Figure 4**).

In human arthritis, inflammation of the synovial joints is accompanied by bone and cartilage destruction. Various animal models have been established for the study of arthritis, and the role of TRANCE in their pathogenesis has been investigated. Treating adjuvant-induced arthritis in Lewis rats with OPG had no discernible effect on inflammation but

prevented bone loss and cartilage destruction (110). These experiments could not resolve whether preservation of cartilage was an indirect benefit of inhibiting bone erosion or was due to independent mechanisms. A subsequent study demonstrated that bone loss and cartilage destruction were independent in an arthritis model induced by transfer of serum from K/BxN transgenic mice, where T cell activity is not required for onset of disease (111). When K/BxN serum was transferred into TRANCE-deficient mice, inflammation and cartilage destruction were comparable to control recipients, but bone erosion was greatly reduced (111). These findings reinforced the notion that TRANCE per se mediates induction of bone destruction by OCs in animal models of autoimmune arthritis. Examination of the cellular constituents of synovial fluid collected from human arthritis patients revealed that all local T lymphocytes expressed TRANCE, establishing the clinical relevance of the connection between arthritis and immunologically derived TRANCE (110). Recently, investigators have demonstrated that TRANCE, in combination with M-CSF (macrophage colony stimulating factor), can induce transdifferentiation of immature DCs to the OC lineage and that this process is significantly enhanced by RA synovial fluid, potentially identifying another mechanism for disease-related bone destruction (112).

Periodontitis, induced by infection with various subgingival bacteria, is a major cause of tooth loss and is associated with increased risk for heart failure and stroke (113, 114). To examine the etiology of the disease, peripheral blood lymphocytes (PBL) from patients with localized juvenile periodontitis (LJP) were transferred into *rag2*$^{-/-}$ mice, which were then orally inoculated with the Gram-negative bacterium *Actinobacillus actinomycetemcomitans* (114). LJP was recapitulated in the recipient animals and was accompanied by accumulation of OCs at the alveolar sockets (114). Treatment with OPG inhibited the OC infiltration and bone damage (114). In vitro stimulation of PBL showed that TRANCE was induced on CD4^{+} T lymphocytes activated with *A. actinomycetemcomitans* antigens and that disease was attenuated when the same cells were specifically depleted from recipient mice (114). This study demonstrated the importance of CD4^{+} T lymphocytes in the pathogenesis of periodontitis, specifically with regard to disease-related bone destruction.

Bone loss has long been recognized as an extraintestinal complication of inflammatory disorders of the gut, such as Crohn's disease and ulcerative colitis (115). One recent study found that patients with these diseases have elevated levels of serum OPG, which derive from the site of inflammation and inversely correlate with severity of bone loss (116), whereas another study found that Crohn's disease patients have elevated levels of both OPG and soluble TRANCE (117). Mechanistic insight into this link is provided by a study demonstrating that OPG treatment of mice suffering from IL-2-deficiency-induced ulcerative colitis results not only in reduced osteopenia, but also in mitigation of colitis owing to reduced colonic DC survival (118).

In addition to arthritis, periodontal disease, and inflammatory bowel disease, pathologic bone loss is observed in patients suffering from other autoimmune diseases (diabetes mellitus and lupus erythematosus), chronic viral infections (HIV), allergic diseases (asthma), and metastatic breast and lung cancers (49, 50, 119). The contribution to pathogeneses by osteoimmunologic factors merits further investigation and may provide viable therapeutic options for alleviating painful sequelae associated with a variety of conditions.

Although autoimmunity is, in some cases, associated with bone loss, each productive T cell response does not have such a deleterious outcome. T cells also secrete cytokines, such as IFN-γ, IL-4, and TGF-β, that inhibit the pro-osteoclastogenic effects of TRANCE (48–50) (**Figure 4**). The role of the Th1 cytokine IFN-γ, in particular, appears to be

TLR: Toll-like receptor

crucial in preventing T lymphocyte–mediated osteoclastogenesis (120). TGF-β is characterized as both an osteotropic and immunosuppressive cytokine. Although the largest repository of latent TGF-β is in bone, its role in OC formation is complex and insufficiently understood (14). TGF-β downmodulates TRANCE expression in OBs, thereby negatively impacting their ability to mediate osteoclastogenesis in culture (121). However, TGF-β has also been shown to potentiate TRANCE expression in activated T lymphocytes (109) and enhance osteoclastogenesis in cultures supplemented with soluble TRANCE (121). Additional studies are necessary to determine whether TGF-β uses multiple regulatory mechanisms, and if so, what disparate purposes they might serve. Given the variety of T lymphocyte–associated cytokines with osteotropic function, it will also be useful to clarify the correlation between Th1/Th2 cytokine polarization and any attendant osteoimmunologic bone destruction.

TOLL-LIKE RECEPTORS, INFLAMMATION, AND BONE METABOLISM

Toll-like receptors (TLRs) are members of an ancient receptor family that shares homology with IL-1R and are critical activators of the innate immune response (122). They are most highly expressed on APCs such as DCs, macrophages, and B cells, but some members are expressed on a diverse array of tissues. Ligation of these receptors by conserved microbial molecules or endogenous danger factors results in the upregulation of costimulatory molecules and the elaboration of inflammatory cytokines in preparation for an adaptive immune response. TLR signaling is mediated by the adaptors MyD88, TRAF6, and TRIF, which activate various downstream signaling pathways, including IKK-NF-κB, MAPK, and IRF (122).

Because macrophages and DCs share a common progenitor with OCs, it is not surprising that TLR expression is also detected on bone cells (123–125). Direct signaling of various TLRs (including TLR4) on OC precursors inhibits TRANCE-mediated osteoclastogenesis (123). The data that microbial products inhibit OC differentiation via TLRs are counterintuitive because bacterial infection can cause inflammatory bone diseases such as periodontitis, osteomyelitis, and bacterial arthritis (126). Bone mineral density is reduced in such diseases because of excessive bone resorption by OCs. In addition, lipopolysaccharide may be a potent stimulator of bone loss by causing an increase in the number of OCs in mice. Moreover, TLR activation can enhance OB-mediated OC differentiation by inducing TRANCE and TNF-α on OBs (124, 125, 127).

The basis for the apparent discrepancy between TLR stimulation as a potent negative regulator of osteoclastogenesis and the association of bacterial infection with excessive bone resorption by OCs remains unclear. As described earlier, alveolar bone destruction in periodontitis caused by infection of Gram-negative bacteria is mediated by enhanced osteoclastogenesis owing to T cell responses and subsequent upregulation of TRANCE (114). In the same study, bacterial infection of immunodeficient (SCID) mice did not lead to significant levels of alveolar bone loss, suggesting that bacterial products do not have a direct role in osteoclastogenesis because SCID mice have no known defect in OC precursors or OBs (114). Therefore, bone loss associated with bacterial infection is likely an indirect outcome of exacerbated T cell responses.

Similar to macrophages or DCs, OC precursors also produce proinflammatory cytokines, such as TNF-α, in response to various TLR ligands (123). Moreover, although TLR stimulation inhibits OC differentiation, OC precursors treated with TLR ligands still retain high levels of phagocytic activity, which is a major host-defense mechanism for the clearance of bacterial infection. Therefore, the net outcome of TLR stimulation in OC precursors is likely the enhancement of

immune responses toward achieving bacterial clearance. This enhancement of immune responses can be achieved by promoting cytokine production from precursor cells and by inhibiting their differentiation into nonphagocytic, nonimmune cells, such as mature OCs. Thus, interaction of these microbial products with TLRs on OC precursors appears to favor the role of OC precursors as part of the proinflammatory system by inhibiting their differentiation into mature OCs and by promoting the production of inflammatory cytokines. However, because these cells can differentiate into mature OCs if TLR ligands are removed (123), after a microbial infection is cleared the presence of residual, activated T cells can lead to the differentiation of phagocytic precursors into mature, bone-resorbing OCs. In addition, TNF-α produced by OC precursors upon TLR stimulation can enhance osteoclastic bone resorption.

TLRs are thus likely to regulate the balance of immune responses and bone metabolism during acute attacks of vertebrate hosts by various microbes. However, physiologic in vivo stimulation of TLRs, which are expressed on various cells, may result in different effects on bone metabolism depending on the nature of the given immune responses. In addition, ongoing stimulation of TLRs by commensal bacteria might affect bone metabolism. In support of this idea, recent data show that mice deficient in mediators of the TLR/IL-1R signaling pathway (MyD88 or IRAK-M) exhibit an altered bone metabolism, although it is not clear whether the defects are due to the signals from TLRs or IL-1R (128, 129).

AN OSTEOIMMUNOLOGICAL SIGNALING NETWORK AND GENE REGULATORY MECHANISM

In addition to interplay between cellular constituents of the immune system and bone, there are also numerous parallels between the signaling networks used by the cells of each system. There are cases both of common, shared pathways and of analogous signaling mechanisms, which activate specialized gene targets via system-specific mediators. In this section, we touch briefly on the better characterized shared pathways (58–60, 64, 80) and focus on some of the novel osteoimmunologic signaling mechanisms that have recently been identified (**Figure 5**).

Costimulation

The formation and activation of OCs are processes tightly regulated by OBs, which provide at least two known essential factors for osteoclastogenesis, TRANCE and M-CSF. In addition, stromal cells produce various osteotropic factors that influence OB-induced osteoclastogenesis of bone marrow precursors. These factors can be divided into two groups: those that influence activity of OBs (e.g., TNF-α that induces TRANCE expression in OBs), and those that affect the OC precursors or OCs per se. A series of experiments showed that M-CSF and TRANCE together appear to be sufficient to induce the differentiation of bone marrow precursors, spleen cells, or blood monocytes to become mature OCs in vitro. However, the expression of M-CSF, TRANCE, and their receptors is not limited to bone cells. For example, M-CSF and TRANCE are important cytokines for the activity/viability of macrophages and DCs. Despite this pleiotropy, OCs are not found in soft tissues, raising the question of why the same set of signaling receptors leads to different functional outcomes in different anatomical environments. One possibility is the existence of costimulatory molecule(s) present only in bone. Alternatively, there could be a powerful inhibitor of osteoclastogenesis in soft tissues that is not found in bone.

To address this question, we proposed the hypothesis a few years ago that there exists a mechanism in preosteoclasts analogous to the costimulation requirement for T cell activation (130). Hence, our hypothesis proposed that OC differentiation is controlled

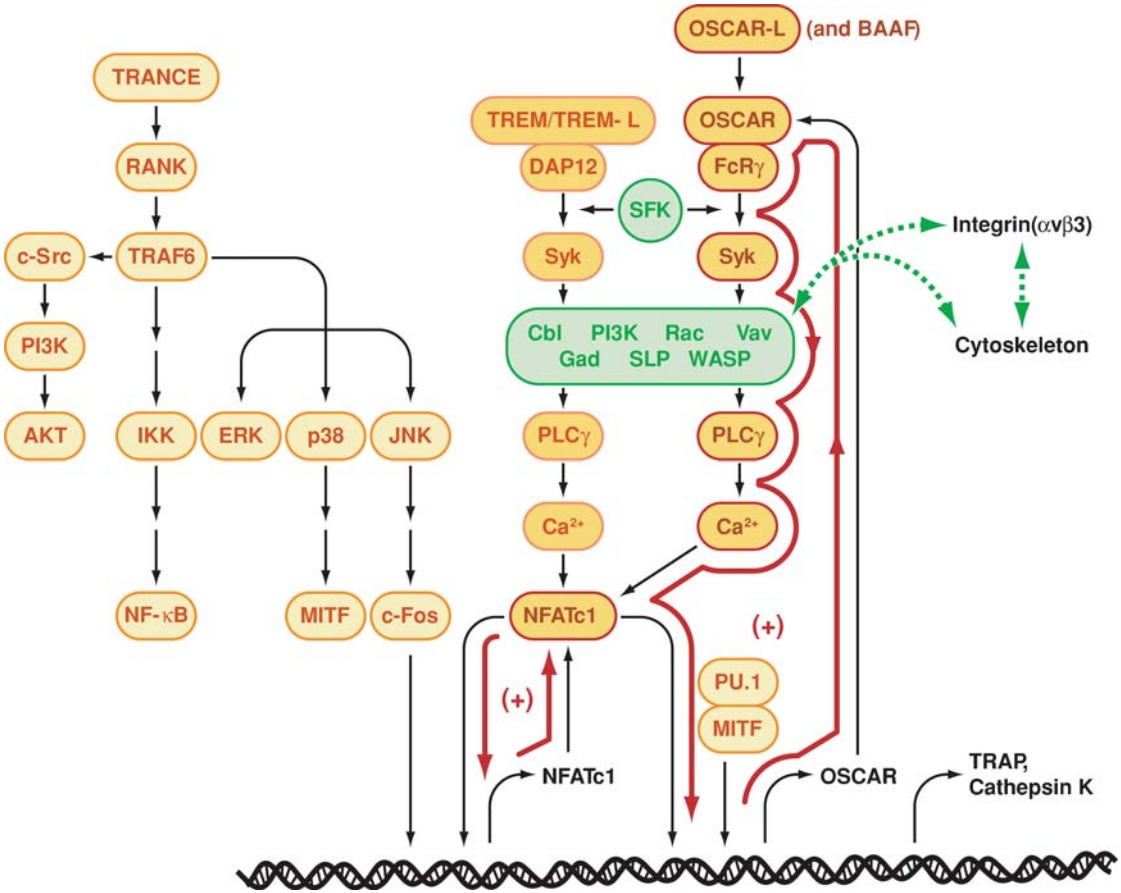

Figure 5

A model for a signaling network necessary for TRANCE-induced OC differentiation and its analog in T cell activation. Factors shaded yellowish have been shown experimentally to be important for OC differentiation. Those shaded green are molecules potentially involved in OC differentiation based on their analogous roles in the TCR signaling network. Src family kinases (SFK) necessary for phosphorylation of ITAMs are not identified. Positive feedback circuits are marked as (+) and shown in red. TREM is constitutively expressed on pOCs (and monocytes), while OSCAR (OC-associated receptor) expression is further upregulated by TRANCE stimulation. The putative ligand for TREM is expressed on pOCs, whereas OSCAR-L is expressed on OBs. The connection to integrin signaling and the cytoskeleton is proposed based on the analogy to T cell signaling requirements. The term BAAF [bone and age associated factor(s)] is proposed to describe putative costimulatory factors for OCs specific to bone. For simplicity, negative regulators of the signaling network are not illustrated but are described in the text.

not only by two essential factors, M-CSF and TRANCE (analogous to MHC/antigen complexes interacting with TCR/CD4 or TCR/CD8), but also by other nonessential but critical costimulatory molecules (analogous to B7 family proteins interacting with CD28) (131). Because the in vivo concentrations of M-CSF and TRANCE produced by OBs in response to bone-resorbing hormones is likely to be much lower than is provided in in vitro experiments, costimulatory molecules are likely to influence physiologic differentiation of OCs in a manner analogous to T cell activation, whereby signals from the

costimulatory receptor CD28 complement requisite signals from the TCR complex (130, 131). In addition, as with T cells, the requirement for a particular set of costimulatory factors/receptors for OCs should vary depending on the microenvironment. Cells expressing ligands for costimulatory receptors expressed on OCs also vary, but those interacting with OCs themselves, such as OBs, most often provide costimulation (analogous to DC provision of B7 family proteins or TNF family proteins like 4–1BBL to T cells) (130). The signals resulting from the interaction of costimulatory factors and their receptors on OC precursors determine the efficacy of the signals from the essential osteoclastogenic receptor, RANK (similar to TCR/CD4 or TCR/CD8 for T cells), and the sum of the two will determine the quality of OC differentiation and activation.

In support of our costimulation hypothesis, we have identified a novel cell surface receptor, OSCAR, which is preferentially expressed on OCs, and we have shown that in addition to normal TRANCE-RANK signaling, interaction of OSCAR with its putative ligand (OSCAR-L) is important for OB-induced OC differentiation (131). Moreover, OSCAR-L expression is most prevalent on osteoblastic cells (131). Therefore, the OSCAR receptor/ligand pairing could be characterized as a putative costimulation receptor/factor for efficient OC differentiation, and may provide bone-specific costimulation required for the differentiation of OCs in conjunction with the essential factors M-CSF and TRANCE. This signaling combination may provide a mechanistic explanation of why OCs are found only on bone surfaces in vivo (**Figure 5**).

Although the nature of bone-specific costimulatory molecules, such as OSCAR-L, requires further study, a series of recent experiments have supported our costimulation hypothesis (132, 133). For OC development in vivo, some surface receptors on OC precursors, such as PIR-A, OSCAR, TREM2, and SIRPβ1, associate with ITAM-containing molecules, DAP12 and FcRγ, and provide necessary costimulation and activation of Ca^{2+} signaling (132, 133). Hence, although a single deficiency for either DAP12 or FcRγ results in only minor OC defects, double deficiency results in severe osteopetrosis (132, 133). Additional analysis of mutant mice suggests that these receptors activate calcineurin via Syk and PLCγ (132–134). More signaling proteins have been identified in lymphocytes that bridge Syk (or ZAP-70) and PLCγ, and lead to Ca^{2+} activation (135, 136). Indeed, Gab2 has recently been shown to be critical for generation of functional OCs (137). It will not be surprising if additional molecules (or family members) crucial to T cell signaling are identified as playing an equivalent role in OC differentiation (**Figure 5**).

However, it is important to point out that osteopetrosis in DAP12/FcRγ double-deficient mice is much less severe than that in TRANCE- or RANK-knockout mice, and that, in contrast to TRANCE- or RANK-knockout mice, these animals exhibit significant numbers of OCs. (132, 133). This is consistent with our hypothesis that costimulatory receptors for OC differentiation are not essential and that multiple redundancies probably exist (131).

Sustained Ca^{2+} mobilization is necessary for OC differentiation because NFATc1 activation is absolutely required for the process (138). The NFAT family of transcription factors was originally identified as a set of regulators of gene transcription in activated T cells (139). Recently, researchers found that RANK signaling induces expression of the NFAT family member NFATc1 (NFAT2) and that this factor is critical for OC development, as NFATc1-deficient precursor cells exhibit an absolute failure to differentiate into OCs (138). Like other NFAT family members, the induction and activation of NFATc1 rely on the calcium-regulated phosphatase, calcineurin, thereby explaining negative effects of calcineurin inhibitors like FK506 and cyclosporine on osteoclastogenesis. The ability of NFATc1 to regulate its own expression indicates the existence of an

OSCAR: osteoclast-associated receptor

NFAT: nuclear factor of activated T cells

autonomic feedback loop, although initial triggering of NFATc1 induction is mediated by TRAF6 and c-fos via TRANCE-RANK stimulation (138). Thus, Ca^{2+} signaling via costimulatory receptors on pre-OCs is critical for amplification of NFATc1 activity to a level sufficient for OC differentiation. Interestingly, NFATc1, in conjunction with MITF and PU.1, transactivates OSCAR expression during TRANCE-induced OC differentiation (Y. Choi & N. Kim, unpublished data). This suggests that there is a positive feedback circuit from TRANCE to NFATc1 via costimulatory receptors, such as OSCAR, during OC differentiation, which ensures a high level of NFATc1 activity (**Figure 5**).

Key to the analogy with lymphocyte costimulation, RANK, like TCR, is still the primary, requisite receptor, the absence of which renders the secondary receptors inconsequential to osteoclastogenesis. One issue that remains to be worked out is a greater understanding of why this system has evolved, and whether there exists a parallel state in OC development that mimics anergy, or induced tolerance, as observed in lymphocytes.

Countervailing Osteoclastogenic Signaling

In addition to OPG (and factors like TGF-β, which induce TRANCE downregulation), there are other negative regulators of RANK signaling in OCs. Although T cells express TRANCE, there is a negative correlation between T lymphocyte activation and signaling through RANK, apparently due to T cell–derived IFN-γ (120). Signaling through the IFN-γR on OCs or OC precursors leads to rapid proteasomal degradation of TRAF6 and to abortive differentiation and function (120). In this way a productive immune response is prevented from having an overlapping, deleterious effect on bone in the surrounding environment.

Another regulatory mechanism involves negative feedback induced by RANK itself. Activation of the *fos* gene by TRANCE leads to upregulation of IFN-β, which mediates a feedback mechanism blocking further c-Fos-dependent activity (140). As such, mice deficient for the IFN-α/β receptor (IFNAR1) suffer from an osteoporotic phenotype characterized by an increase in OCs (140). Promoter characterization showed that TRANCE-mediated upregulation of IFN-β utilizes AP-1-binding sites and that c-Fos-deficient OC precursors are incapable of inducing IFN-β production (140). To facilitate OC development, therefore, OC precursors need to upregulate the cytokine signaling regulator SOCS3 to inhibit IFN-mediated suppression (141–143).

Interferons are cytokines critical to inducing productive immune responses against viruses (IFN-α/β) as well as parasites and bacteria (IFN-γ) (144–146). Type I (IFN-α/β) and type II (IFN-γ) interferons signal through different surface receptors and activate distinct DNA recognition sites [interferon-stimulated response elements (ISRE) and gamma interferon activation sites (GAS), respectively], but they use a common mediator of gene transcription, Stat1. In immune cells, IFN-activated Stat1 induces antiviral and inflammatory gene transcription, although in OC precursors Stat1-dependent mechanisms mitigate osteoclastogenesis. As IFNs are associated with inflammation and active T cell immunity, which is itself associated with enhanced osteoclastogenic factors, it is notable that they are involved in mitigating the effects of T cells on bone erosion. Interestingly, Stat1-deficient mice exhibit increased OB differentiation and bone mass, as Stat1 plays a role in vivo in attenuating the critical OB transcription factor Runx2 (147). It will be interesting to determine whether the bone phenotype in Stat1-deficient mice is consistent under different immunologic conditions, or whether under inflammatory conditions a greater role for Stat1-mediated inhibition of osteoclastogenesis emerges.

Finally, since ITAM-containing molecules provide critical costimulatory signals for OC differentiation, future studies are required

to determine whether ITIM-containing molecules counteract costimulatory signals for OC differentiation via DAP12/FcRγ.

Quantitatively Distinctive Utilization of TRAF6 by TRANCE

It remains puzzling why TRANCE-RANK-TRAF6 signaling is so critical for osteoclastogenesis in vivo. Other immune receptors, including CD40 and IL-1R/TLR, are expressed on OC precursors and use TRAF6 to activate overlapping signaling cascades but do not induce osteoclastogenesis. These observations caused us to question whether qualitative or quantitative differences exist between the TRAF6-mediated signals induced by TRANCE/RANK versus other ligand-receptor pairs.

Our recent data show that stimulation via overexpressed wild-type CD40 can induce osteoclastogenesis (148). RANK contains three TRAF6-binding sites on its cytoplasmic tail, whereas CD40 has one. Stimulation through modified CD40 molecules containing additional TRAF6-binding sites in the cytoplasmic tail showed a dose-dependent increase in osteoclastogenesis. Moreover, precursors overexpressing TRAF6 alone differentiate into OCs in the absence of additional signals from TRANCE. Thus, our results suggest that differences in the osteoclastogenic capacity of RANK versus other TRAF6-associated receptors may stem, in part, from a quantitative difference in TRAF6-mediated signals. Similar mechanisms of controlling distinct biological outcomes by growth/differentiation factors, despite their use in overlapping signaling cascades, have been reported (149, 150). For example, although both epidermal growth factor (EGF) and nerve growth factor (NGF) use the same kinase cascade, only NGF can induce neuronal differentiation. When EGF receptor was overexpressed, it induced neuronal differentiation similar to NGF (149). In the case of OC differentiation, any TRAF6-binding receptor might have the potential, but only RANK can meet the threshold to induce osteoclastogenesis. Whether OC differentiation occurs through various TRAF6-utilizing receptors also correlates with the induction and persistence of NFATc1 activation. In normal OC differentiation, TRANCE-RANK interaction, in conjunction with costimulation via ITAM-containing molecules, sets the level of NFATc1, determining whether the differentiation process becomes irreversible. However, this concept also suggests that as long as NFATc1 is induced beyond the threshold level, then OC differentiation can be mediated by factors other than TRANCE-RANK. In support of this idea, we have recently found that RANK-deficient splenic cells can become bone-resorbing OCs in vitro when they are stimulated by a cocktail of cytokines (Y. Choi, N. Kim, Y. Kadono, J. Lorenzo, unpublished data). Whether such an outcome is possible in vivo, specifically under abnormal or pathological conditions, requires further investigation.

CONCLUSIONS

The fields of immunology and bone biology have matured such that key cellular and molecular mechanisms governing the homeostasis of the individual systems are largely understood. However, despite extensive cross-regulation between bone metabolism and the immune system, the mechanisms by which one regulates the other, and the biological implications of such interactions, are poorly understood. We believe that this lack of understanding is due in part to the challenges typically associated with crossing disciplinary boundaries that form naturally during the separate evolutions of fields like modern immunology and bone biology. It is difficult enough for scientists/physicians to keep abreast of advances in multiple fields, but even more so to develop the knowledge base, skills, and materials necessary to address important issues.

Therefore, it will be critical to create an environment conducive to the study of

intersystem crosstalk. Awareness of intersystem crosstalk will no doubt contribute to our understanding of how both bone and the immune system are regulated in a physiologic context, both at the molecular level and at the level of organ systems. Moreover, this endeavor will lead to better treatments for human diseases involving both systems, including various inflammatory and metabolic bone diseases, as well as tumor-induced bone lysis. Many of these pathologic processes are major targets for therapeutic intervention and are being pursued in the absence of solid scientific understanding of the molecular and cellular processes underpinning these interactions. According to the first-ever report by the U.S. Surgeon General on bone health, by 2020 one in two Americans over age 50 will be at risk for fractures from osteoporosis or low bone mass. These secondary health concerns become more prominent as people not only live longer but also expect to remain active in old age. Future preventative treatments for chronic bone-related diseases that are often associated with inflammation and that impact quality of life will require a high degree of specificity, especially if tailored for a segment of the population already suffering from, or vulnerable to, other age-related ailments. We believe these issues place osteoimmunology in a position of unique clinical significance.

SUMMARY POINTS

1. Bone cells are influenced by cytokines and cell surface proteins that are expressed on lymphocytes.
2. Bone cells provide key factors necessary for HSC, B cell differentiation and memory, and T cell memory.
3. Identification of the TRANCE-RANK-OPG axis clearly established the physiological connection between immune responses and bone metabolism, thus confirming the importance of various osteotropic cytokines for bone metabolism.
4. Identification of effector cytokines produced by activated T cells as inhibitors of TRANCE-induced osteoclast differentiation explains why normal T cell responses do not overtly affect normal bone metabolism.
5. Identification of costimulatory receptors and their signaling pathways confirms the molecular parallel between T cell activation and osteoclast differentiation.

FUTURE ISSUES TO BE RESOLVED

1. What is the nature of bone marrow stromal cells and what factors are necessary for their interaction with hematopoietic lineage cells?
2. Why are osteoclasts found only on the surface of bone? What are the ligands for costimulatory receptors found on preosteoclasts?
3. To what extent do normal immune responses affect bone homeostasis? And what are the consequences of bone metabolism to adaptive immune responses?
4. How does the interplay between bone and the immune system change with age?

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