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Science in Medicine

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Dallas Jones,[1,2] Laurie H. Glimcher,[1,2,3] and Antonios O. Aliprantis[1,2]

[1]Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts, USA. [2]Department of Medicine, Division of Rheumatology, Allergy and Immunology, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA. [3]Ragon Institute of Massachusetts General Hospital, Harvard, and MIT, Boston, Massachusetts, USA.

Over the past decade and a half, the biomedical community has uncovered a previously unappreciated reciprocal relationship between cells of the immune and skeletal systems. Work in this field, which has been termed "osteoimmunology," has resulted in the development of clinical therapeutics for seemingly disparate diseases linked by the common themes of inflammation and bone remodeling. Here, the important concepts and discoveries in osteoimmunology are discussed in the context of the diseases bridging these two organ systems, including arthritis, osteoporosis, cancer, and infection, and the targeted treatments used by clinicians to combat them.

Introduction

Advances in both medicine and basic science have elevated expectations that treatments for complex diseases will promptly emerge. However, the findings generated from these various disciplines have further emphasized the multifactorial nature of many diseases, which has hampered the progress toward remediation (1). This is especially true for diseases of the skeletal system that can manifest in individuals of any age, sex, or socioeconomic status as a result of genetic as well as environmental components (2). In a normal physiological state, the skeletal system provides support, mobility, protection for vital organs, and a mineral reservoir for calcium and phosphate. To achieve and adapt to these functions, the skeleton exists in a dynamic equilibrium characterized by continuous osteoclast-mediated bone resorption and osteoblast-mediated bone deposition (3). This biological process has been termed bone "remodeling" and occurs in coupled fashion with osteoblasts producing the key osteoclast differentiation factors and osteoclasts promoting bone formation via osteoblastic mediators they produce and liberate as they degrade bone. Osteoclasts differentiate from myeloid precursors in response to the cytokine RANKL and adhere to the bone surface where they secrete acid and proteolytic enzymes to degrade the inorganic and organic constituents of bone, respectively. Following the resorptive phase, mesenchymal-derived osteoblasts migrate to the eroded area and initiate new bone formation through the secretion of an extracellular matrix consisting of both collagenous and noncollagenous proteins. It has become evident that both innate and adaptive immune cells exert profound effects on osteoclasts and osteoblasts through a panoply of cell-surface and secreted mediators (4). Moreover, an appreciation of the reciprocal influence of bone cells on immunity is emerging. Herein, the intersection of the immune and skeletal systems is discussed within the context of commonly encountered skeletal diseases. This review does not aim to provide a complete overview of osteoimmunology (for comprehensive review, see ref. 4) but rather to expose the reader to the key concepts, controversies, and molecules in this emerging field as it relates to the major afflictions of the skeleton. Table 1 summarizes the clinical therapeutics mentioned in this review, and Figures 1 and 2 depict the molecules involved in crosstalk between immune and bone cells in the context of the diseases discussed here.

Inflammatory arthritis — dysregulation of bone remodeling RANKL and IL-17 as key mediators

The identification of RANKL and its intimate relationship to inflammation and bone destruction in RA launched the field of osteoimmunology over a decade ago. RANKL was originally cloned independently of its role in skeletal physiology as an immediate early gene induced in T cells after activation of T cell receptor signaling (5, 6). It was not until the RANKL-knockout mouse was generated and mutations were identified in human osteopetrotic patients that its function as the master regulator of osteoclastogenesis was realized (7–9). RANKL is a TNF family cytokine that can be produced by a number of different cell types including osteoblasts and fibroblasts in addition to T cells. Activation of the RANKL receptor (RANK) on mononuclear osteoclast precursors initiates a cascade of transcriptional changes culminating in multinucleated giant cell formation through cell fusion and the expression of the machinery needed for bone resorption including molecules needed for attachment to bone, acid secretion, and proteolysis. Interestingly, many of the transcription factors important for osteoclast differentiation are key regulators of immune responses, such as NF-κB and nuclear factor of activated T cells c1 (NFATc1); this finding was an early insight into the intimate relationship of skeletal biology and immunology. Indeed, genetic absence of *Nfatc1* in mice abrogates osteoclast differentiation in vivo and in vitro (10–12). The signaling and transcriptional mediators shared among immune cells and osteoclasts is an interesting molecular aspect of osteoimmunology that has been reviewed elsewhere (13). In addition to its effects on osteoclast precursors, RANKL has important roles in regulating immune processes such as lymph node organogenesis and self tolerance (14).

RA is a female-predominant autoimmune disease affecting approximately 1% of the population that results in chronic inflammation in the lining, or synovium, of peripheral diarthrodial joints (reviewed in ref. 15). Autoreactive T cells may initiate the inflam-

Table 1
Clinical therapeutics targeting osteoimmunology pathways mentioned in this review

| Class	Examples	Indications[A]	Mechanism in the context of osteoimmunology	Refs.
Bisphosphonates	Alendronate, Risedronate, Ibandronate, Pamidronate, Zoledronate	Osteoporosis, cancer-associated osteolysis	Inhibit bone resorption by osteoclasts; may cause ONJ through inhibition of bone remodeling	64, 86
RANKL antagonists	Denosumab	Osteoporosis, cancer-associated osteolysis, RA	Blocks bone resorption by preventing RANKL mediated osteoclastogenesis	20, 127
TNF-α antagonists	Etanercept, Infliximab, Adalimumab	RA	Reduces inflammation; limits bone erosion by decreasing RANKL-mediated osteoclastogenesis	18, 20
IL-1 antagonists	Anakinra	RA	Reduces inflammation; limits bone erosion by decreasing RANKL-mediated osteoclastogenesis	18, 20
IL-6 antagonists	Tocilizumab	RA	Reduces inflammation; limits bone erosion by decreasing RANKL-mediated osteoclastogenesis	19, 20
IL-17 antagonists	LY2439821, AIN457	RA	Reduces inflammation; limits bone erosion by decreasing RANKL-mediated osteoclastogenesis	36, 37
IL-12/IL-23 antagonists	Ustekinumab	Psoriasis, psoriatic arthritis	Reduces inflammation; limits bone erosion by decreasing RANKL-mediated osteoclastogenesis	38
Costimulation blockers	Abatacept	RA	Disrupts costimulation of T cells by APCs; may directly inhibit osteoclast formation	18, 20, 46
PTH agonists	Teriparatide	Osteoporosis	Increases bone formation by stimulating the production of anabolic Wnt proteins from T cells	79
Estrogens	Conjugated estrogen, Estradiol	Postmenopausal osteoporosis	Reduces bone resorption; Pathway that may involve suppression of T cell derived pro-osteoclastic cytokines	67–69

[A]Indications listed here are not necessarily approved by the FDA.

matory response, which is then perpetuated by autoantibody-producing B cells and innate immune cells including macrophages that release proinflammatory cytokines such as TNF-α, IL-1, and IL-6. Alternatively, disease initiation may arise from dysregulated innate immunity in response to environmental triggering of pattern recognition receptors (PRR) such as TLRs, leading to proinflammatory cytokines and activation of adaptive immunity. In either case, ultimately the normally thin synovial lining composed of fibroblast- and macrophage-like cells undergoes intense proliferation and angiogenesis to form a hyperplastic structure called the pannus. The pannus invades the joint space and destroys the cartilage through inflammatory cytokines that are directly toxic to chondrocytes and by degradative enzymes liberated from synoviocytes (16). Where the pannus tissue encroaches on periarticular bone, erosions occur through coupling of inflammation to osteoclast formation. Damage to articular cartilage and periarticular bone results in irreversible joint dysfunction and reduces quality of life (17). Knowledge of these basic mechanisms of RA pathogenesis has led to clinical approval of targeted biologic therapeutics that (a) block proinflammatory cytokines, (b) interrupt T cell costimulation, and (c) deplete B cells (18, 19).

Osteoclasts form where pannus tissue interfaces with periarticular bone and mediate bone erosions (reviewed in ref. 20). Data supporting this conclusion stem from studies where inflammatory arthritis was induced in rodents in either a genetically osteoclast-deficient background or concomitant with systemic administration of osteoprotegerin (OPG), a decoy receptor and inhibitor of RANKL (21–23). In each case, the rodents developed similar levels of inflammation, but demonstrated dramatically reduced periarticular bone destruction. Similar results have been reported in rodent models using high-potency bisphosphonates, such as zoledronic acid, which inhibit osteoclast function (24, 25). In humans, clinical trials of high potency bisphosphonates as well as denosumab, a monoclonal blocking antibody to RANKL approved for osteoporosis treatment, also strongly support the osteoclast as the primary mediator of bone erosion in RA (26, 27).

The source of pro-osteoclastogenic RANKL in RA has been an intense area of research, and the activated T cell has emerged as a primary player through direct and indirect mechanisms (reviewed in ref. 28). A seminal paper published in 1999 was the first to link RANKL expression on T cells to osteoclastogenesis (23). In this study, TCR ligation was shown to induce expression of both membrane-bound and secreted RANKL, which supported osteoclastogenesis in vitro. Two additional studies confirmed the ability of activated human T cells to support in vitro osteoclastogenesis (29, 30). In contrast, a similarly timed publication suggested that T cells exert antiosteoclastogenic activity through the ability of IFN-γ, a product of the Th1 subset, to promote degradation of a key signal transduction molecule downstream of the RANKL receptor (31). Consistent with this observation, while IFN-γ–positive T cells were shown to promote osteoclast differentiation in vitro via RANKL, their full osteoclastogenic potential was only realized when IFN-γ activity was blocked (32). However, in vivo the effects of IFN-γ on osteoclast differentiation may be context dependent, promoting bone loss in the setting of infection and estrogen withdrawal, as discussed in the sections below (33). Concomitant with these early
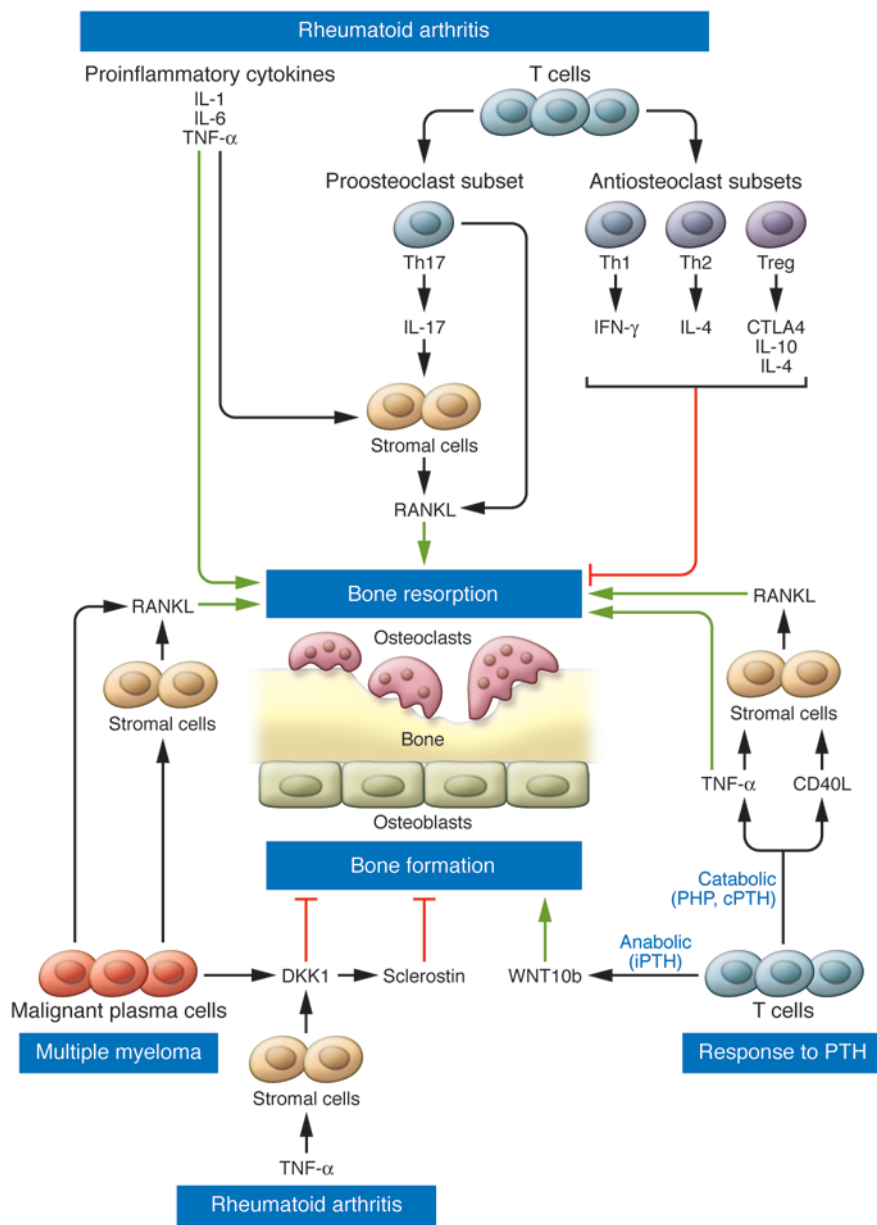
Figure 1
Schematic of the immune system's influence on skeletal remodeling. The effect of growth factors, cytokines, and cell-surface molecules generated by lymphoid populations on osteoclast-mediated bone resorption and osteoblast-mediated bone formation are depicted for physiologic and pathologic conditions. Red lines represent the repressive effects, and green arrows represent the stimulatory effects that the specific factor has on the target bone cell population. Stromal cells reflect various mesenchymal-derived cells such as osteoblast-lineage cells and synovial fibroblasts. iPTH, intermittent PTH; cPTH, continuous PTH.

studies, Kotake et al. made the important observation that IL-17 was capable of driving osteoclast differentiation by inducing RANKL expression on osteoblasts (34). In addition, cells expressing both CD4 and IL-17 were identified in RA synovium. This study not only implicated a new effector cytokine and T cell subset in this pathway (Th17 cells), but also an additional mechanism through which T cells could effect bone destruction: the induction of RANKL on mesenchymal-derived cells. Taken together, these early reports suggested that T cells influence osteoclast formation, but clarification of the details was needed.

A clearer picture emerged as the ability to manipulate T helper subset differentiation in vitro matured. In a comprehensive study by Sato et al., Th1 and Th2 cells were both shown to inhibit osteoclast formation through their canonical cytokines IFN-γ and IL-4, respectively (35). In contrast, Th17 cells were potent stimulators of osteoclastogenesis through IL-17. This effect was dependent

on the presence of osteoblasts, though Th17 cells expressed membrane-bound RANKL, leading the authors to conclude that T cell–derived RANKL is not sufficient for osteoclast differentiation. A tight correlation was found among the expression of IL-23 subunits (*IL23A* and *IL12B*), the upstream cytokine inducing Th17 differentiation, and *RANKL* in synovial biopsies from RA patients (35). Accordingly, biologic therapies inhibiting the IL-17 pathway by directly targeting this cytokine (LY2439821, AIN457) (36, 37) or blocking IL-23 (Ustekinumab) (38) are in clinical development for inflammatory arthritis.

In addition to T cells, the synovial fibroblast is a source of RANKL in RA. Synovial fibroblasts from RA patients express RANKL and support osteoclastogenesis in vitro (39, 40). In vivo, RANKL is expressed by synovial fibroblasts (41), with especially robust expression near the pannus-bone interface where erosions are found (42). The relative importance of RANKL derived from

T cells, synovial fibroblasts, or osteoblast-like cells in RA has yet to be resolved and awaits definition.

Other mediators. A fourth T cell subset, the antiinflammatory Treg, inhibits osteoclast differentiation and function in vitro and suppresses inflammatory bone erosions in mice (43–45). Tregs negatively influence osteoclastogenesis through two mechanisms. The first involves cell contact and cytotoxic T lymphocyte antigen 4 (CTLA4), a membrane-bound molecule that blocks costimulation by APCs (43). In a T cell–independent model of RA generated by transgenic overexpression of TNF-α in mice, administration of CTLA4 moderately suppressed bone erosion, but had no influence on inflammation (46). The antierosive effect of abatacept, a CTLA4-Ig fusion protein approved for the treatment of RA, indicates a potential clinical relevance of this observation (47). Contact-independent production of cytokines by Tregs that negatively regulate osteoclast differentiation such as IL-4 and IL-10 is a second mechanism (48).

Clinical strategies utilizing inhibitors that target proinflammatory cytokines, such as TNF-α, IL-6, and IL-1, have been approved for the treatment of RA (18, 19). These cytokines, which are produced by synovial fibroblasts and macrophages, promote bone destruction in RA by direct and indirect mechanisms. Indirectly, these cytokines augment RANKL expression in mesenchymal lineage cells (20, 34, 49, 50). They also directly act on osteoclast precursors to drive differentiation in synergy with RANKL (20, 51–53). Given the strong correlation between the activity of these cytokines on both inflammation and bone erosion, it is difficult to resolve which of these cytokines, if any, is the predominant driver of bone erosion in RA. In the inflamed joint, all may contribute.

Uncoupling bone resorption from formation in RA. Compared with physiologic bone turnover, a distinguishing feature of bone erosion in RA is the uncoupling of bone resorption from formation by osteoblasts (54). Two mechanisms underlying this observation have been defined. First, proinflammatory cytokines such as IL-1 and TNF-α directly inhibit osteoblast differentiation and function (20). Second, soluble inhibitors of the Wingless (Wnt) pathway may suppress bone formation. In one recent study of a mouse model of RA, fewer cells expressing late osteoblast markers and reduced bone formation rates were found in bone adjacent to inflamed joints (55). Accordingly, increased levels of Wnt inhibitors were found in the inflamed synovial tissue. Diarra et al. showed that TNF-α upregulates the Wnt inhibitor Dickkopf-1 (DKK1) in synovial fibroblasts and that blocking antibodies to DKK1 could both promote bone formation, generating osteophytes around inflamed joints, and completely prevent bone erosions (56). DKK1 negatively regulates Wnt signaling by binding the Wnt coreceptors low-density lipoprotein receptor-related protein 5 (LRP5) and LRP6 (57). This finding was replicated in 3 models of RA, running the gamut from a T cell–dependent model of arthritis to one driven by transgenic TNF-α overexpression (56). Expression of DKK1 was documented in RA patients; serum levels positively correlated with disease activity and were suppressed by anti-TNF therapy. In contrast, patients with ankylosing spondylitis, another TNF-α–dependent inflammatory arthropathy that unlike RA is characterized by periarticular new bone formation, had low serum DKK1 levels. The ability of DKK1 inhibition to reduce bone erosion and promote bone formation in RA may be related to the observation that Wnt pathway activation increases the expression of OPG and drives osteoblastic differentiation of periosteal cells, respectively (56, 58). In addition, inhibition of

DKK1 can also block TNF-α–induced expression of another Wnt antagonist, Sclerostin, in osteoblasts (59). Future work will define whether activating the Wnt pathway can prevent erosion and promote bone healing in RA.

In summary, innate and adaptive immune cells in the environment of the inflamed joint promote destruction of periarticular bone through a complex network of factors that increase osteoclast activity and uncouple bone resorption from formation. Recent treatment advances in RA interfere with these pathways and preserve periarticular bone.

Osteoimmunology at the interface of therapeutics for osteoporosis

Estrogen, T cells, and bone mass. In healthy individuals, peak bone mass is achieved in early adulthood as a result of robust skeletal growth initiated at the onset of puberty (60). Subsequent to achieving this optimal level of bone mineral density, however, the skeleton in men and women exhibits an age-associated decline in bone mass (61). This progression toward osteoporosis is accelerated in postmenopausal women due to declining levels of estrogen, a hormone that exhibits osteoprotective properties through promoting osteoblast synthetic activity and retarding bone resorption by osteoclasts (62, 63). The realization of estrogen's central role in regulating postmenopausal bone mass has led to the development of multiple analogs clinically approved to treat osteoporosis (64). Interestingly, decreased estrogen levels might also contribute to the age-associated bone loss that is observed in elderly men (65). While estrogen can regulate skeletal remodeling by directly targeting osteoblasts and osteoclasts, it has also been reported to indirectly influence bone mass by targeting the immune system.

Estrogen has long been known to have an impact on lymphocyte physiology. However, the contribution of the adaptive immune system to the onset and progression of postmenopausal osteoporosis is disputed (66). Several studies have demonstrated that estrogen treatment modifies T cell production of cytokines and growth factors that promote bone resorption, including the proosteoclastic cytokines RANKL and TNF-α (67–69). T cells isolated from the bone marrow of postmenopausal women were shown to have elevated levels of RANKL when compared with those of either premenopausal women or postmenopausal women receiving hormone-replacement therapy. In contrast to its antiosteoclastogenic activity, described in the preceding section, in vivo IFN-γ is necessary for ovariectomy-induced bone loss in mice and may drive osteoclast activity by promoting antigen-dependent T cell activation (33). Thus, the physiology of T cells may be altered following estrogen depletion and contribute to elevated bone resorption observed in postmenopausal women (70). In vivo analysis of bone loss in various lymphocyte-deficient mice following ovariectomy, a model that mimics the mechanisms through which bone loss occurs in postmenopausal osteoporosis, has led to conflicting findings (67, 71–74). While a number of factors may account for these differences, further studies will be required to conclusively determine what role, if any, the adaptive immune system plays in postmenopausal bone loss.

Parathyroid hormone acts on bone through T cells. Recently, T cells have been demonstrated to have a more definitive role in mediating the bone-remodeling effects of parathyroid hormone (PTH). Under normal physiological conditions, PTH functions as a key regulator of calcium homeostasis. In response to hypocalcemia, PTH is released by the parathyroid gland and raises blood calcium

levels in part through stimulating bone resorption. Calcium that is stored in the skeleton is released into the circulation and suppresses further production of PTH by the parathyroid gland. In certain pathological conditions, such as primary hyperparathyroidism (PHP), PTH is produced at supraphysiological levels, leading to accelerated bone loss and an increased incidence of fracture (75).

Continuous infusion of PTH in rodents also leads to decreased bone mass through augmenting osteoclast activity, thereby mimicking the catabolic effects of PTH observed in PHP. A recent analysis of several strains of genetically modified mice revealed that T cells play a role in promoting increased bone resorption observed with continuous PTH administration (76, 77). These studies showed that PTH signals through PTH receptor 1 on bone marrow T cells to augment production of TNF-α (77). While TNF-α exerts pro-osteoclastic activity by targeting osteoclast progenitors, the production of TNF-α by T cells was surprisingly shown to induce the upregulation of CD40 on the cell surface of the bone marrow stromal cells. The CD40 ligand–expressing T cells engage and induce signaling through CD40 on the stromal cells, which results in augmented RANKL production and downregulated OPG by this population, thus providing an environment conducive to osteoclastogenesis (76).

While continuous infusion of PTH leads to reduced bone mass through elevated bone resorption, paradoxically, intermittent administration of PTH through daily injections produces an anabolic effect that promotes bone formation through stimulation of osteoblast proliferation and function (78). Because of this latter effect, intermittent PTH infusion is the only FDA-approved anabolic modality for the treatment of osteoporosis (79). Using a series of T cell–deficient mice, it was demonstrated that the anabolic effects of PTH are blunted in the absence of this lymphocyte population. Thus, like their role in the catabolic effects of PTH, T cells appear to have a role in the anabolic effects of PTH (76). A series of additional experiments found that the CD8+ T cell population within the bone marrow is responsible for enhancing the bone anabolic effects of PTH through the production of Wnt10b. As mentioned above, the combination of both human and mouse genetics has demonstrated the central role that Wnt signaling has in regulating bone mass accrual (80). Therefore, compounds that target this pathway could provide a valuable therapeutic approach to preventing bone loss through promoting anabolic bone formation. While PTH can also promote bone formation through mechanisms that are T cell independent, the contribution of T cells in promoting the anabolic effects of PTH underscores the importance of gaining a greater understanding of the function that the adaptive immune system has in regulating skeletal remodeling to identify additional therapeutic targets.

Cancer and the skeletal system

Lytic bone lesions. In addition to providing mobility and protection for vital organs, the skeleton also serves as an essential environment in which hematopoiesis can occur. The stromal cells that reside within the bone marrow and line the endosteal surfaces provide a niche rich in growth factors, cytokines, and adhesion molecules that sustains the pluripotent state and self renewal of hematopoietic stem cells (81, 82). However, the fertile microenvironment within the bone marrow can also provide a niche for the development and proliferation of certain neoplastic diseases, including those of hematological origin that arise within the marrow as well as metastatic solid tumors (83). Often, the presence of these malignancies

has an adverse effect on the skeleton that commonly manifests as osteolytic lesions causing patients to present with pain, pathologic fractures, hypercalcemia, spinal cord compression, and inexorable decline in mobility and quality of life. A greater understanding of the molecular mechanisms that maintain the balance of bone formation and bone resorption during normal skeletal remodeling has led to the identification of novel therapeutics such as bisphosphonates and denosumab for the treatment of the pathological bone loss associated with these diseases (84–86).

Regulation of bone turnover by myeloma cells. Multiple myeloma (MM) arises from the clonal expansion of malignant plasma cells within the bone marrow and is often associated with adverse skeletal events. Up to 80% of individuals diagnosed with myeloma present with skeletal pathologies over the course of the disease (87). Furthermore, the degree of bone destruction caused by these malignant plasma cells leads to a marked elevation in the incidence of fracture (88). Myelomas are the only skeletal malignancy that are purely lytic and exhibit potent osteolytic capacity through their ability to promote bone resorption by secreting a myriad of cytokines and chemokines as well as altering the RANKL/OPG ratio within the marrow microenvironment to favor osteoclastogenesis (89–92). Given the high degree of osteoclast-mediated bone destruction associated with MM, bisphosphonates are routinely incorporated into treatment regimens. Studies indicate that bisphosphonates are capable of preventing further bone loss in patients with MM and thus decrease both the number of skeletal-related events and the amount of bone pain reported by patients (93, 94). Interestingly, bisphosphonates have also been reported to have antimyeloma effects in part through activating γδ T cells, which possess cytotoxic antitumor properties (95). Therefore, bisphosphonate treatment of individuals with MM may decrease tumor burden as well as reduce the number of osteolytic events. However, given the potential adverse effects of prolonged bisphosphonate use, additional therapies to combat the osteolytic potential of MM are needed. In recent clinical trials, denosumab has been shown to reduce both markers of bone resorption and the number of skeletal-related events in patients with MM, suggesting that this therapy could provide a needed alternative for MM patients (84, 85).

In addition to producing factors that promote bone resorption, MM cells augment the rate of bone destruction by producing factors such as DKK1, which suppress new formation through negatively regulating Wnt signaling (57, 96). DKK1 secreted by these malignant cells binds to the LRP5/6 coreceptors on the surface of osteoprogenitor cells, prohibiting their proliferation and differentiation. Preclinical studies in murine models of MM have found that neutralizing antibodies to DKK1 are capable of increasing bone volume through augmenting osteoblast numbers and serum osteocalcin levels (97). Thus, discovery of novel anabolic agents that promote bone formation through stimulating Wnt signaling could provide an alternative treatment of MM-induced osteolysis.

RANK/RANKL signaling in tumorigenesis and metastasis. As described above, RANK/RANKL signaling is essential for osteoclast formation and bone resorption that occurs during skeletal remodeling. Analysis of *Rank*-null mice also uncovered an unexpected role for this signaling axis in the development of the lactating mammary glands during pregnancy (98). While physiological levels of RANK/RANKL signaling stimulate the proliferation and cell survival of mammary epithelial cells, aberrant RANK/RANKL signaling in these tissues has recently been shown to influence the onset and progression of progestin-mediated breast tumorigenesis (99, 100).

A

B

C
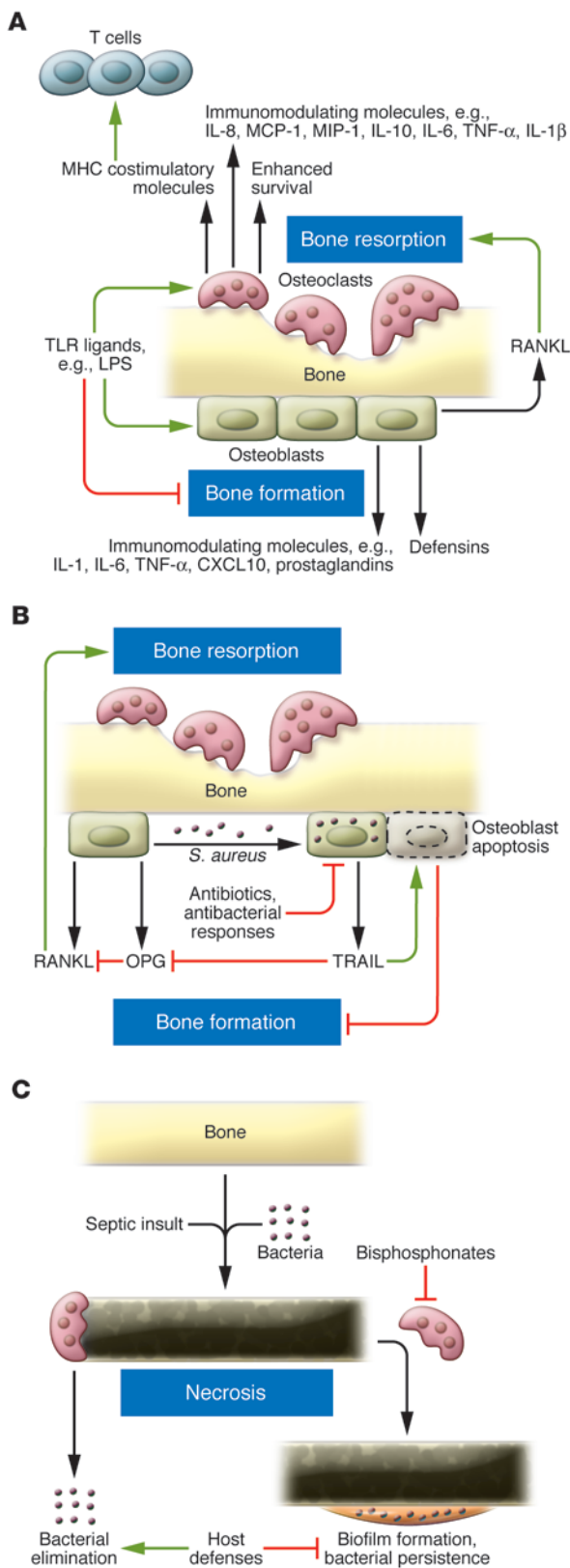
Figure 2
Potential roles of osteoclasts and osteoblasts in host defense against bacterial pathogens. (**A**) TLR ligands have multiple effects on osteoblasts and osteoclasts, eliciting responses that modulate inflammation and immunity, promote bacterial killing, and shift the balance of bone remodeling toward resorption. (**B**) Infection of osteoblasts with *S. aureus* may provide the bacteria with a niche to avoid antibiotics and antibacterial responses and promote bone destruction through TRAIL, which can sequester OPG and induce osteoblast apoptosis. (**C**) A potential mechanism for bisphosphonate ONJ. A septic insult to bone such as a tooth extraction results in necrosis. Normally the necrotic bone is removed by osteoclasts. When osteoclast activity is blocked by bisphosphonates, bacteria produce biofilms on the necrotic tissue allowing them to evade host defense mechanisms.

It was demonstrated that progestin treatment of mice resulted in a robust induction of RANKL within the mammary gland. The upregulation of RANKL in this tissue promoted tumorigenesis by stimulating the proliferation of RANK-expressing mammary epithelial cells and protecting these cells from apoptosis in response to DNA damage. Blocking of RANK/RANKL signaling in mice through either genetic ablation of *Rank* or by treating mice with RANKL-Fc abrogated the onset of mammary tumors. These findings suggest that targeting this pathway with therapies such as denosumab may provide a novel treatment for breast cancer.

Therapies that block RANK/RANKL signaling may also decrease the ability of osteotropic tumors to successfully metastasize to bone. Signaling through RANK on the surface of human epithelial tumor cells as well as melanoma cells has been shown to induce a chemotactic response in these tumor cells. In a murine model of melanoma metastasis, therapeutic treatment of mice with OPG to neutralize RANKL significantly reduced the tumor burden within the bones but not other organs (101). The authors of this paper concluded that RANKL present in the bone microenvironment could function as a "soil factor" allowing for successful engraftment of metastasizing tumors.

Osteoclasts and osteoblasts in host defense against infection

The interaction of bone cells with bacteria. Some of the evolutionary pressure driving the intimate relationship of the immune and skeletal systems likely arose from the need to combat bone infections. Bone infection, or osteomyelitis (OM), arises from either hematogenous spread or direct local extension of bacteria and presents acutely or as chronic infection. In adults, *Staphylococcus aureus* is the most common pathogen in OM (102). Lengthy courses of antibiotics are needed with or without surgical debridement, but antibiotic resistance and bacterial biofilm formation make eradication difficult and recurrences are frequent (103). Areas of bone resorption (osteolysis) surrounded by bone formation (osteosclerosis and periosteal new bone formation) are the radiographic characteristics of OM. The ability of the inflamed environment in OM or chronic periodontal disease to induce bone resorption by osteoclasts has been reviewed elsewhere (104), and many of the mechanisms driving bone loss in RA likely contribute to these conditions as well. Here, we focus on how the osteoclast and osteoblast participate in antibacterial host defense (Figure 2, A–C).

The initial response to a bacterial challenge is mediated by innate PRRs, such as TLRs that identify molecular patterns unique to microbes (105). In osteoblasts and osteoclasts, TLR

ligands have diverse effects (reviewed in ref. 106). For example, stimulation of osteoblasts with the TLR4 ligand lipopolysaccharide (LPS) increases the expression of RANKL as well as proinflammatory molecules, including cytokines (IL-1, IL-6, TNF-α), chemokines (CXLC10), and prostaglandins, which could serve to promote bone resorption and incite inflammation (Figure 2A and refs. 102, 106–111). Furthermore, LPS inhibits osteoblast differentiation and mineralization, contributing to the imbalance in bone remodeling seen in OM (112).

Interesting data indicate that osteoblasts can be directly infected with *S. aureus*, classically thought of as an extracellular pathogen (Figure 2B) (113). Though not formally proven, this has been proposed as a mechanism by which pathogens causing OM evade host responses and antibiotics (113). Furthermore, infection of osteoblasts with *S. aureus* promotes production of TNF-related apoptosis-inducing ligand (TRAIL), which can tip the bone remodeling scale toward resorption by inducing osteoblast apoptosis (114, 115) and sequestering OPG, resulting in higher RANKL availability (102, 113). Last, some evidence exists that osteoblasts make antibacterial peptides (116, 117). Documentation of the physiologic relevance of this observation and whether osteoblasts kill bacteria is lacking, but could be clinically important.

Osteoclasts also respond to TLR ligands (Figure 2A). Stimulation of osteoclast precursors with LPS inhibits RANKL-induced differentiation, likely by decreasing RANK levels (118, 119). In contrast, exposure after initiation of the differentiation program augments osteoclastogenesis and promotes survival (106, 118). In vivo, administration of sublethal doses of LPS induces systemic bone loss through an incompletely characterized IFN-γ–dependent pathway (33). Osteoclasts also produce proinflammatory mediators, such as IL-8, MCP-1, and MIP-1, after TLR stimulation (120, 121). A recent study showed that LPS stimulation of human osteoclasts resulted in IL-10, IL-6, TNF-α, and IL-1β production as well as increased expression of MHC and costimulatory molecules important for antigen presentation (122). Accordingly, osteoclasts internalized soluble antigen, processed it, and presented it to T cells to induce proliferative and cytokine responses.

Osteoclast blockade and osteonecrosis of the jaw. In vivo, inhibition of osteoclast activity by bisphosphonates is associated with osteonecrosis of the jaw (ONJ), a condition characterized by devitalized bone with overlying soft tissue defects and concomitant chronic infection. ONJ most commonly occurs after dental trauma in patients treated with high-potency bisphosphonates (123). In a mouse model of OM, inhibition of osteoclasts by either bisphosphonates or excess OPG promoted abscess formation and increased bacterial burden (124). This may be due to failure of osteoclasts to remove necrotic bone resulting in a niche for invading bacteria to establish pathogenic biofilms (Figure 2C). Another recent publication showed that dental extraction in normal mice results in bone necrosis surrounding the extraction socket, followed by angiogenesis and bone remodeling to remove the dead bone. In mice treated with bisphosphonates, angiogenesis and remodeling were blocked and necrotic bone was retained (125). It will be important to follow whether postmarketing surveillance of new antiosteoclast agents, such as denosumab (126), reveals an association of these drugs with ONJ.

Future work will examine the role of individual TLRs and other PRRs in osteoclasts and osteoblasts in models of OM and ONJ. Whether manipulation of host defense pathways in these cells can augment innate bone immunity and help eradicate treatment-resistant infections deserves further exploration.

Concluding remarks and future directions
An understanding of the connection between immune cells and osteoclasts and osteoblasts, and the mediators through which these cells communicate, has led to the development of therapeutics aimed at disrupting this often pathologic interaction. Over the next five to ten years, investigators will better define the clinical utility of new antiresorptive and anabolic agents in the treatment of skeletal diseases. Moreover, whether molecules targeting the Wnt pathway can augment bone formation while simultaneously suppressing resorption in humans will be tested. Last, while the majority of osteoimmunology studies have focused on how immune cells have an impact on skeletal remodeling, it is likely that osteoblasts and osteoclasts reciprocally regulate immune responses. A better understanding of this aspect of the osteoimmunology relationship could pave the way for unique strategies to control inflammation and bone loss in disease.

Acknowledgments

Address correspondence to: Antonios O. Aliprantis, Harvard School of Public Health, 651 Huntington Avenue, FXB 205, Boston, Massachusetts 02115, USA. Phone: 617.432.4867; Fax: 617.432.0084; E-mail: aaliprantis@partners.org.

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