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Osteoimmunology: Interactions of the Bone and Immune System

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Bone and the immune system are both complex tissues that respectively regulate the skeleton and the body's response to invading pathogens. It has now become clear that these organ systems often interact in their function. This is particularly true for the development of immune cells in the bone marrow and for the function of bone cells in health and disease. Because these two disciplines developed independently, investigators in each don't always fully appreciate the significance that the other system has on the function of the tissue they are studying. This review is meant to provide a broad overview of the many ways that bone and immune cells interact so that a better understanding of the role that each plays in the development and function of the other can develop. It is hoped that an appreciation of the interactions of these two organ systems will lead to better therapeutics for diseases that affect either or both. (*Endocrine Reviews* 29: 403–440, 2008)

I. Introduction

BONE IS A COMPLEX organ with multiple functions. It provides structural integrity for the body, it is the site of hematopoiesis, and it is a storehouse for calcium and

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Abbreviations: AIRE, Autoimmune regulator; BMP, bone morphogenic protein; BMSC, bone marrow stromal cells; BSAP, B-cell specific activation activation protein; CD40L, CD40 ligand; Ebf, early B cell factor; Eph, erythropoietin-producing hepatocyte kinase; FasL, Fas ligand; FcRγ, Fc receptor γ; G-CSF, granulocyte colony-stimulating factor; GCV, ganciclovir; GM-CSF, granulocyte M-CSF; HSC, hematopoietic stem cell(s); IFN, interferon; IgH, Ig heavy; ITAM, immunoreceptor tyrosine-based activation motif; LIF, leukemia inhibitory factor; LPS, lipopolysaccharide; M-CSF, macrophage colony-stimulating factor; MITF, microphthalmia-associated transcription factor; MK, megakaryocyte(s); MMP, matrix metalloproteinase; MyD88, myeloid differentiation factor 88; NF-κB, nuclear factor κB; OCL, osteoclast-like cell(s); OPG, osteoprotegerin; OSCAR, osteoclast-associated receptor; OVX, ovariectomy; Pax, paired box; PI3K, phosphatidylinositide-3-kinase; PIR-A, paired Ig-like receptor A; PLC, phospholipase-C; RANK, receptor activator of NF-κB; RANKL, RANK ligand; ROS, reactive oxygen species; SCF, stem cell factor; SIRP β1, signal regulatory protein β1; TCR, T-cell receptor; T_H, T-helper; TLR, Toll-like receptor; TPO, thrombopoietin; TRAF, TNF receptor-associated factor; TRAIL, TNF-related apoptosis inducing ligand; TREM-2, triggering receptor expressed by myeloid cells-2; TRIF, Toll/IL-1 receptor domain-containing adaptor inducing interferon-β; XHIM, X-liked hyper IgM.

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phosphorus (1). Likewise, the immune system is complex and provides organisms with protection from invading pathogens (2). Multiple overlapping and interacting mechanisms have evolved to regulate both systems. Interactions between bone and immune cells are now well described. It has become apparent that to explain the phenotype of many *in vivo* models with abnormal bone metabolism, one can no longer view either system in isolation. Rather, to understand their function, they must be viewed as a single integrated system. Examples of recently identified interactions of bone and immune cells include the findings: 1) that cells related to osteoblasts, which form bone, are critical regulators of the hematopoietic stem cell (HSC) niche from which all blood and immune cells derive; and 2) that osteoclasts, which are the cells that resorb bone, appear to share a common origin with the myeloid precursor cells that also gives rise to macrophages and myeloid dendritic cells. It has also been shown *in vitro* that cells that are relatively far along in their differentiation toward antigen-presenting dendritic cells retain the ability to form mature bone-resorbing osteoclasts (3). Finally, over the last 30 yr, it has become well established that multiple soluble mediators of immune cell function including cytokines, chemokines, and growth factors also regulate osteoblast and osteoclast activity (4). It is likely that immune cells and cytokines are critically responsible for the changes in bone turnover and bone mass that occur in postmenopausal osteoporosis and inflammatory conditions such as rheumatoid arthritis, periodontal disease, or inflammatory bowel disease.

The regulation of bone by hematopoietic and immune cells serves a variety of functions. It is likely that developing hematopoietic cells regulate bone turnover and maintain the marrow cavity by interacting with osteoblasts and osteoclasts during normal bone development (5). Conversely, during inflammatory states either locally produced or circulating cytokines, which are the products of activated immune cells, mediate increased bone turnover and the bone pathology in diseases such as rheumatoid arthritis and inflammatory bowel disease. We are only beginning to understand the breadth of bone and immune cell interactions, and this review is by no means complete. However, by appreciating the interactions of these two systems, it is hoped that future research into this area will develop in the context of the synergies between them so that the mechanisms underlying bone and immune cell function in both health and disease can be better understood.

II. The Origins of Bone Cells

A. Osteoclasts

Osteoclasts are multinucleated giant cells that form from the fusion of mononuclear precursor cells. Mature osteoclasts are unique in their capacity to efficiently resorb bone and contain a variety of specific cell structures that facilitate this process (1). The origin of the osteoclast precursor cell has been well studied. Initial work demonstrated that osteoclasts share many characteristics with macrophages (6). Although, osteoclasts and macrophages appear to express some common antigens (7), there are also clear differences in the expression of surface antigens that separate these two cell types (8, 9). Mononuclear cells, which can differentiate into osteoclast-like cells (OCL) in a variety of *in vitro* culture systems, are present in the bone marrow and the peripheral blood (10, 11).

The availability of multiple antibodies recognizing cell surface molecules, which are expressed on hematopoietic cells (12–15), has allowed the identification of bone marrow peripheral blood and spleen cell populations that can form OCL *in vitro*. Studies from multiple laboratories have identified several candidate populations with the ability to form OCL in coculture with stromal cells, when cultured alone in liquid medium or when cultured in methylcellulose. In experiments performed before the identification of receptor activator of nuclear factor (NF)-κB ligand (RANKL), which is the critical cytokine that regulates osteoclast formation (16), investigators relied on coculture of various fractions of cells (generally from bone marrow) with stromal or osteoblastic cells (17). In these assays, cells were stimulated to induce osteoclastogenesis by treatment with a stimulator of resorption like 1,25 OH_2 vitamin D_3 or PTH. Interestingly, these assays require cell-cell interaction between osteoblastic and osteoclastic cells because OCL did not form in these cultures if the two cell types were separated by a membrane (18). The majority of these early studies focused on myeloid lineage cells. They demonstrated that rodent cells expressing mature macrophage markers, which were isolated from the bone marrow or spleen, gave rise to OCL when they were cocultured with bone marrow stromal cells (BMSC) (19). Muguruma and Lee (20) identified an osteoclast progenitor population in murine bone marrow that was negative for mature markers of B lymphocytes (CD45R/B220), granulocytes (Gr-1), macrophages (CD11b/Mac-1), and erythroid cells (Ter-119). This population did not express Sca-1, which is a marker that is found on HSC but was positive for the progenitor marker CD117/c-kit (20). These cells could progress to tartrate-resistant acid phosphatase-expressing mononuclear cells when they were cultured in semisolid media and OCL when they were cultured with 1,25 OH_2 vitamin D_3-treated-ST2 stromal cells. However, the cells in this fraction were considered multipotential because they were also able to differentiate into granulocytes, macrophages, and erythroid cells. Interestingly, when the c-kit low population was separated, it also could generate osteoclasts, but in a more restricted fashion. Tsurukai *et al.* (9) isolated cells from coculture of murine bone marrow hematopoietic cells and osteoblastic cells by passage through a Sephadex column and found that the population that was enriched for osteoclast precursors expressed monocytic markers but not markers of B or T lymphocytes. Using a coculture assay with ST2 cells, Hayashi *et al.* (21) found that osteoclast precursors were in the c-kit-positive (+) fraction and that expression of c-fms, the macrophage colony-stimulating factor (M-CSF) receptor, inhibited the efficiency of c-kit-positive cells forming OCL in culture.

Arai *et al.* (22) used both coculture with ST2 stromal cells and direct stimulation with RANKL and M-CSF, as well as antibodies against c-fms and the monocytic marker CD11b/Mac-1, to demonstrate that murine bone marrow cell populations expressing c-kit formed OCL in culture. These au-

thors concluded that a population of murine bone marrow cells with the phenotype c-kit$^+$, c-fms$^+$ CD11b^{lo} contained a multipotential progenitor cell population that gave rise to osteoclasts with high frequency. This population did not express RANK (the receptor for RANKL) when it was isolated from bone marrow but did after it was cultured with M-CSF. Interestingly, these precursors were not completely restricted to osteoclastogenesis because in methylcellulose cultures they generated macrophages and mononuclear tartrate-resistant acid phosphatase-positive cells. Microglia, the specialized phagocytic cells in the central nervous system, also appear to arise from a precursor cell that can give rise to osteoclasts (23). We have found that the osteoclast precursor cells in murine bone marrow are negative for CD3 and CD45R, negative or low for CD11b, and positive for expression of c-fms (24). Expression of c-kit further separated this population of murine bone marrow into two populations: 1) cells that rapidly formed OCL *in vitro* when cultured with M-CSF and RANKL (c-kit high cells); and 2) cells that formed OCL more slowly *in vitro* (c-kit low to negative cells). Expression of CD11b in this population of osteoclast precursors occurred transiently during *in vitro* culture. Initially, we found the population that most efficiently formed osteoclasts to be negative to low for this antigen. However, culture with M-CSF and RANKL induced mononuclear osteoclast precursor cells to transiently express high levels of CD11b. Expression of this antigen was lost in multinucleated cells (24).

The relationship of osteoclasts to dendritic cells, which present antigen to T lymphocytes as part of the adaptive immune response (25), is now also established. Both human and murine cells, expressing early markers of the myeloid dendritic cell lineage, can differentiate into osteoclasts *in vitro* (23, 26, 27). In addition, it appears that dendritic cells, which are relatively late in their lineage development, retain the ability to form osteoclasts *in vitro*. Alnaeeli *et al.* (3) showed that murine bone marrow cells, which were treated *in vitro* with cytokines so that they could present antigen to T lymphocytes, formed OCL in culture when they were treated with M-CSF and RANKL. However, Speziani *et al.* (28) found that neither mature myeloid dendritic cells generated *in vitro* nor plasmacytoid dendritic cells generated *in vivo* formed OCL in culture.

The ability of a common progenitor cell to differentiate into macrophages, osteoclasts, and myeloid dendritic cells has been proposed for some time (26, 27). However, only recently has it been demonstrated that a myeloid murine bone marrow cell can be isolated to the purity of single cell clones and retain the capacity to differentiate into macrophages and dendritic cells (29). We now have good evidence that this macrophage/myeloid dendritic cell precursor (29) can also differentiate into an OCL *in vitro* (J. Lorenzo, unpublished data). Hence, it appears that a common precursor cell exists for macrophages, myeloid dendritic cells, and osteoclasts. Commitment of the common macrophage/myeloid dendritic cell/osteoclast precursor to the osteoclast lineage occurs relatively quickly (within 24 h) after treatment of these cells with RANKL (30).

Expression of the myeloid-specific antigen CD11b has been used by a number of investigators to identify a circulating osteoclast precursor cell (31–34). The number of these cells in the circulation is regulated by the inflammatory state of the organism and in particular by TNFα. Most recently, Yao *et al.* (34) demonstrated that expression of CD11b and Gr-1 could be used to identify this circulating osteoclast precursor population. In humans, expression of CD14 and the lack of expression of CD16 have been used to identify osteoclast precursor cells in peripheral blood (35, 36). In addition to CD14, osteoclast precursors in human peripheral blood also express receptor activator of NF-κB (RANK) (37). Migration and adhesion of human CD14-positive monocytes to sites of inflammation from the peripheral circulation may be mediated through activation of microvascular endothelial cells by proinflammatory cytokines (38)

One interesting aspect of osteoclastogenesis is that cells with a cell surface phenotype that is similar to that of osteoclast precursor cells in bone marrow can be identified in the spleen. However, osteoclastogenesis does not occur in the spleen under any known condition. One possible explanation for this paradox is that the population of cells found in the spleen, despite having a similar phenotype to cells found in the bone marrow, are missing crucial elements that prevent their forming osteoclasts in splenic tissues. However, this hypothesis seems improbable because multiple investigators have established the *in vitro* osteoclastogenic potential of splenocytes. Another possibility is that the microenvironment in the spleen does not allow the production of osteoclasts either because it lacks critical signaling molecules or because it produces inhibitory signals. Miyamoto *et al.* (39) proposed that to complete osteoclastogenesis an adherent condition, which is defined by the expression of specific molecules, is required. This would ensure the correct interactions between osteoclast progenitors and supporting cells that express the correspondent ligands. Osteoblast lineage cells in the bone marrow might produce these signals, whereas the spleen or other nonbone tissues would not.

The latter hypothesis is supported by the recent findings that late osteoclast differentiation and activation require a novel combination of costimulatory molecules, which act in concert with M-CSF and RANKL to complete osteoclastogenesis (40). These molecules involve proteins containing an immunoreceptor tyrosine-based activation motif (ITAM) domain. They are found in adapter molecules like DAP12 and the Fc receptor γ (Fc$R\gamma$). The search for receptors associated with these ITAM adapters in myeloid cells has identified at least two candidates that associate with Fc$R\gamma$ [osteoclast-associated receptor (OSCAR) and paired Ig-like receptor A (PIR-A)] and two that associate to DAP12 [the triggering receptor expressed by myeloid cells-2 (TREM-2) and the signal regulatory protein β1 (SIRP β1)] (41). The ligands for these receptors are currently unknown.

Fusion of osteoclast precursor cells into mature resorbing osteoclasts is a regulated process (42). Recently, expression of CD200 and CD200R on osteoclasts was found to influence this fusion process because osteoclast number was decreased and bone mass was increased in CD200-deficient mice (43). CD200-deficient mice also had a normal number of osteoclast precursor cells but a decreased rate of osteoclastogenesis *in vitro*.

Although the myeloid origin of osteoclasts is well established, it has been proposed that cells of the B-lymphoid

lineage can also give rise to osteoclast progenitors. Several groups have suggested the existence of bipotential progenitors for B lymphocytes and macrophages in bone marrow, which have the ability to differentiate into osteoclasts (44–46). We have found that paired box (Pax) 5 −/− mice, which have a block in B lymphocyte development at the pro-B cell stage, have an increased number of osteoclasts in their bones and decreased bone mass (47). However, the osteoclast precursor cell population in Pax5 −/− mice is myeloid in origin. In previous work (48), we found that OCL formed in cultures of murine bone marrow cells that express the B lymphocyte marker CD45R. These studies relied on populations of CD45R-positive murine bone marrow cells that were separated by fluorescence-activated cell sorting to a purity of 98–99%. However, in more recent work (24), we found that purification of the CD45R-positive murine bone marrow population by a second round of fluorescence-activated cell sorting to a purity more than 99.9% essentially eliminated the ability of purified CD45R population to form OCL *in vitro*. Hence, it appears that the OCL, which form in cultures of CD45R-positive cells, require the presence of a contaminating non-CD45R-expressing population of cells to form OCL. We are aware of no other studies of the osteoclastic potential of CD45R-expressing murine bone marrow cells that purified their populations to the degree that we have now done. However, we suspect that additional rounds of purification of other CD45R-positive populations, which have been proposed to contain osteoclast precursors, will likely also demonstrate them to be contaminated with small amounts of non-CD45R-expressing osteoclast precursors. We believe that these contaminating cells are critical for OCL formation in these cultures and may represent osteoclast precursor cells with a high proliferative capacity.

In humans it was recently demonstrated that the nuclei of myeloma cells, which are malignant cells of B lymphocyte origin, can be identified in osteoclasts. It was further proposed that this may be a mechanism for the increased osteoclastic activity seen in this condition (49). However, it has not been demonstrated that nonmalignant B lymphocyte lineage cells integrate into osteoclasts *in vivo* in humans.

B. Osteoblasts

Osteoblasts are derived from a mesenchymal progenitor cell that is multipotential and also can differentiate into marrow stromal cells and adipocytes (50). The signals that regulate the decision of mesenchymal progenitor cells to form osteoblasts are incompletely understood. However, a number of critical paracrine signals and cell autonomous transcription factors have been identified. These include the transcription factors Runx2 and osterix, which when absent prevent osteoblast formation, and the bone morphogenic protein (BMP) family (51–53), which initiates the signals for osteoblast differentiation. Most recently, it was found that Wnt signaling pathways are involved in the decision of the mesenchymal progenitor cell to become either an adipocyte or an osteoblast (54–58).

As matrix calcifies under the influence of the osteoblast-produced enzyme, bone-specific alkaline phosphatase, a portion of the osteoblasts 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, which regulate bone turnover, to cells at the bone surface. Osteocytes interconnect with each other and the cells at the bone surface via cellular projections, which are termed dendritic processes. These reside in channels in the mineralized bone, named canaliculi (59, 60). Most recently much interest has been generated by the discovery that the relatively osteocyte-specific protein sclerostin is an important regulator of the Wnt signaling pathway in osteoblast lineage cells (61).

III. Role of Osteoblasts in Hematopoiesis

In mammals during the early stages of gestation, hematopoiesis takes place in the yolk sac and then in the fetal liver. Eventually, it migrates to the bone marrow where, unless disturbed, it remains throughout the rest of life. Several investigators have documented the close proximity and/or attachment of hematopoietic cells to bone matrix and/or bone cells. These studies showed that multipotential HSC in the bone marrow were located adjacent to the endosteal surfaces of bone. They also demonstrated that cells closest to the bone surface were more proliferative than those that were farther away from the endosteum (62–67). Electron micrographs from Deldar *et al.* (68) found that granulocytes and reticular cells were either in close juxtaposition or in contact with endosteal bone-lining cells. There is a relatively high frequency of pre-B and terminal deoxynucleotidyl transferase-positive (TdT+) cells near the endosteal bone surface, and this frequency declines in cells that are closer to the center of the bone marrow cavity (69). HSC differentiation was also demonstrated to occur in close proximity to endosteal osteoblasts (70). Cheng *et al.* (71) observed, when isolating BMSC (a source of osteoblast progenitors) from bone marrow, that complexes existed, which were composed of BMSC and megakaryocytes (MK). This result implied that there was a physical association between mesenchymal and hematopoietic cells. It was also found that long-term HSC were attached to spindle-shaped osteoblast-like cells on bone surfaces, which expressed N-cadherin but not CD45 (72). However, this result is controversial because other investigators do not find HSC to express N-cadherin (73). These authors also demonstrated that the majority of HSC associate with sinusoidal vessels in the bone marrow and that only a minority of HSC are in close proximity to endosteal cells (73). Other authors have found that interaction of the chemokine CXCL12 on support cells with its receptor CXCR4 on HSC is critical for maintenance of HSC in the bone marrow (74). Expression of CXCL12 on support cells is found on cells in both the vasculature and the endosteum (75). The interactions of support and hematopoietic cells lead to the concept of the hematopoietic niche, which is a specialized structure that supports HSC and facilitates their replication and differentiation (Fig. 1).

It is clear that even the use of the most vigorous methods to expel bone marrow from mouse long bones leaves many cells adherent to endosteal surfaces. In the hematopoietic literature, these cells are often referred to as osteoblasts.

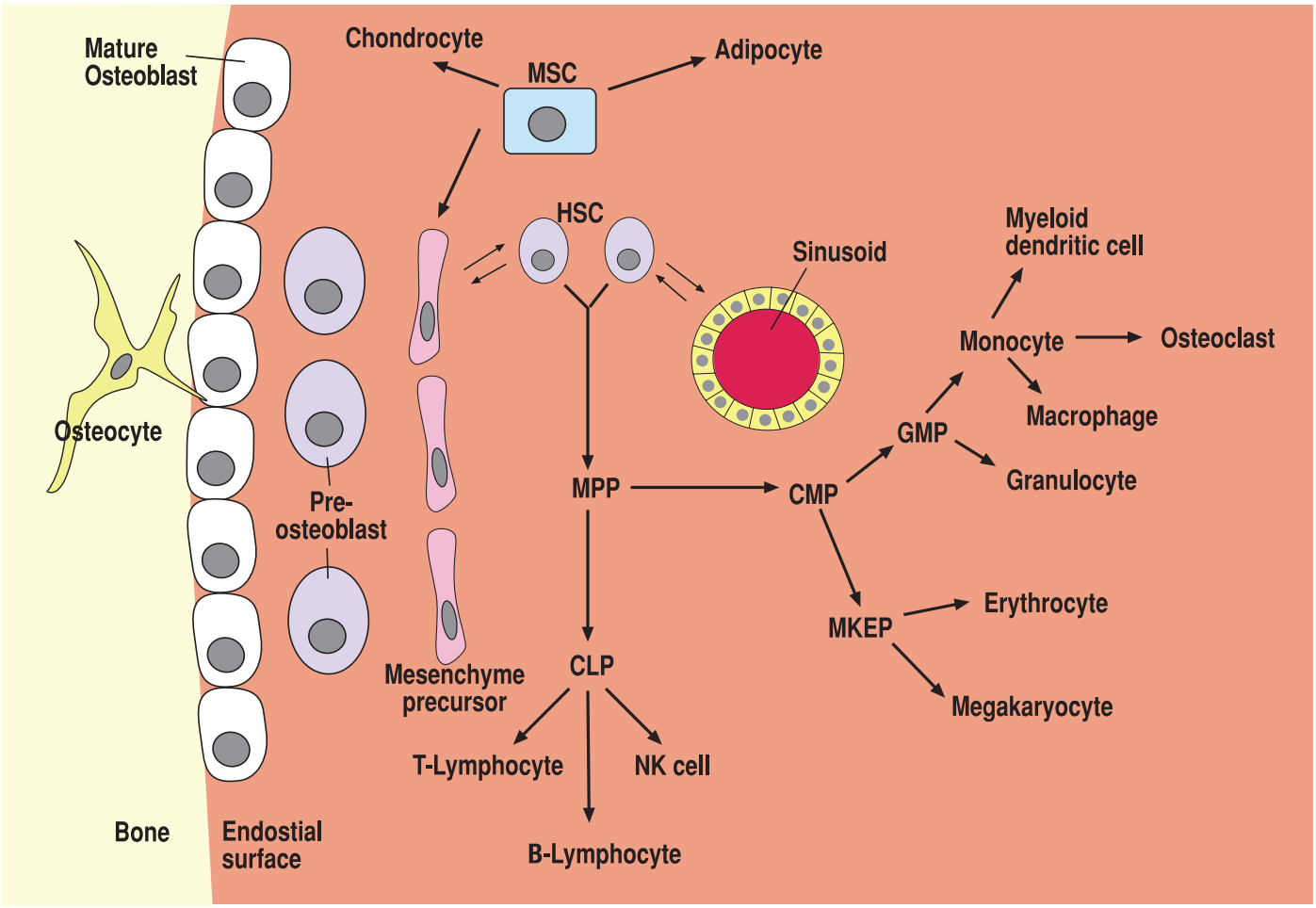

FIG. 1. Scheme for the interactions of osteoblasts with hematopoiesis. HSCs reside in the bone marrow adjacent to either osteoblast lineage cells or sinusoids. Both of these likely produce signals that control HSC replication and differentiation. HSC can remain dormant or replicate to either self-renew or differentiate into multipotential progenitor (MPP) cells. MPP can differentiate into either common lymphoid progenitors (CLP), which have the capacity to differentiate into precursors for T-lymphocytes, B-lymphocytes or natural killer (NK) cells; or MPP can become common myeloid precursor (CMP) cells, which are the precursor cells for all other hematopoietic lineages. CMP can differentiate into either granulocyte-macrophage progenitor (GMP) cells or megakaryocyte-erythroid progenitor (MKEP) cells. In turn, MKEP can differentiate into either erythrocytes or megakaryocytes. GMP can differentiate into monocytes or granulocytes. Bone marrow monocytes are precursors for myeloid dendritic cells, macrophages, and osteoclasts. Osteoblasts derive from a mesenchymal precursor cell (MSC) that is multipotential and can also differentiate into chondrocytes and adipocytes. Like the hematopoietic system, differentiation of MSC toward the osteoblast lineage involves multiple intermediates including mesenchyme precursors, preosteoblasts, and mature (matrix producing) osteoblasts. Finally, some mature osteoblasts appear to differentiate further into osteocytes, which are encased in the mineralized matrix of bone. [Derived from Ref. 578.]

Although this population predominantly contains cells of the osteoblast lineage, adherent cells of hematopoietic origin, such as osteoclasts and macrophages, are also present. It is also possible that bone-lining cells support HSC in the niche. Bone-lining cells are members of the osteoblast lineage and are thought to be mature cells that are different from osteocytes. Both bone-lining cells and osteocytes are believed to be more mature than matrix-producing osteoblasts. It has been recently demonstrated that osteoclasts are important for the release of hematopoietic progenitors from the niche (76). Mice injected with lipopolysaccharide (LPS) have increased numbers of osteoclasts on the endosteum and increased numbers of hematopoietic colony-forming cells in the peripheral blood, as a result of a marked mobilization of HSC from the bone marrow (76). Mice treated with RANKL also had an increased number of osteoclasts on their bone surfaces and increased levels of circulating colony-forming progenitors including Lin-Sca-1+c-kit+ progenitor cells (76). Conversely, mice treated with calcitonin, an inhibitor of osteoclast formation, or mice with defective osteoclasts had a reduced number of circulating progenitors (76).

Primary osteoblast lineage cells, which are established from humans and/or mice, have been shown to synthesize granulocyte colony-stimulating factor (G-CSF), granulocyte M-CSF (GM-CSF), M-CSF, IL-1, IL-6, lymphotoxin, TGFβ, TNFα, leukemia inhibitory factor (LIF), and stem cell factor (SCF) or c-kit ligand (77–89). Importantly, all of these cytokines have been demonstrated to play a role in hematopoiesis (90, 91) and many are also involved in osteoclast development.

One of the first definitive findings regarding the role for osteoblast lineage cells in hematopoiesis was the demonstration that when CD34+ hematopoietic progenitors were cul-

tured on a monolayer of osteoblast lineage cells for 2 wk, there was an 8-fold increase in hematopoietic cell number (89). It was then demonstrated that human osteoblast lineage cells constitutively produce membrane-bound G-CSF and that osteoblast lineage cell-bound G-CSF was responsible for approximately 55% of the increase in hematopoietic cell number (89). Assessment of the morphology of hematopoietic progenitors, which were cultured with osteoblast lineage cells, showed that the osteoblast lineage cells were able to support the survival of immature hematopoietic cells such as the long-term culture-initiating cells (92). In addition, osteoblast lineage cells were able to support the survival and, to a smaller extent, the proliferation of early myeloid progenitors (89). Interestingly, the coculture of CD34+ bone marrow cells with osteoblast lineage cells did not alter the secretion of G-CSF, GM-CSF, or TGFβ-1 by the osteoblast lineage cells (93). However, the coculture of CD34+ bone marrow cells with osteoblast lineage cells minimally elevated LIF secretion and markedly elevated osteoblast lineage cell IL-6 production (93). Although the exact mechanism(s) responsible for the increase in osteoblast lineage cell-synthesized IL-6 remains to be determined, it appears to be regulated by unknown factor(s), which are secreted by CD34+ cells. Two likely candidates, IL-1β and TNFα, were found not to be responsible (93). Transgenic mice with a constitutively active PTH/PTHrP receptor, whose expression is restricted to osteoblast lineage cells, had increased trabeculae and trabecular osteoblasts (94). Bone marrow from these transgenic mice had an increased number of Lin-Sca-1+cKit+ HSC compared with controls (94). The increase in HSC was due to the increased ability of the stromal cells from the transgenic mice to support HSC growth and differentiation. Injection of wild-type mice with PTH also produced an increase in these HSC (94). These data support the idea that cells of the osteoblast lineage are important regulators of the bone marrow hematopoietic niche.

Recently, significant insights have been generated concerning the molecular mechanisms regulating osteoblast lineage cell-hematopoietic cell interactions (72, 94, 95). Long term HSC were shown to be adjacent to osteoblast-lineage cells, and their number was increased by approximately 2.3-fold in mice upon deletion of the bone morphogenetic protein receptor 1A. Significantly, bone morphogenetic protein receptor 1A-deficient mice also had a similar increase in osteoblast-lineage cell number (72). Similarly, it was demonstrated that expansion of the osteoblast-lineage cell population in bone by stimulation of the PTH/PTHrP receptor increased the number of HSC in bone marrow (94). This effect appeared to be mediated by Jagged-1-Notch-1 signaling because Jagged-1 levels were increased in mice with osteoblast-targeted activation of the PTH/PTHrP receptor. In addition, the increase in the number of HSC in cultures of cells from transgenic mice with osteoblast-lineage cell-targeted activation of the PTH/PTHrP receptor was abrogated by inhibitors of Notch signaling. It has also been shown that PTH directly stimulates production of Jagged-1 by osteoblast-lineage cells (96). In a converse experiment, it was found that targeted destruction of osteoblast-lineage cells in mice led to a decrease in HSC in bone marrow (95). Interactions of HSC and osteoblast lineage cells appear to be mediated by interactions of Tie2 on HSC and angiopoietin-1 on osteoblast lineage cells. This signaling system appears to inhibit cell division in HSC, while maintaining their capacity for self-renewal (97, 98).

Annexin II also appears to be involved in osteoblast-lineage cell-HSC interaction (99). Osteoblasts express this protein, which appears to mediate HSC adhesion, and HSC number in the marrow of annexin II-deficient mice was significantly reduced. Production of IL-10 by osteoblasts has also been shown recently to promote the self-renewal of HSC in the bone marrow (100).

Erythropoietin-producing hepatocyte kinases (Ephs) are small receptor tyrosine kinases that function to regulate a variety of cellular systems including immune and bone cells (101, 102). The Eph family has 15 members and is separated into Eph A and Eph B subgroups. The ligands of Ephs are called ephrins. Both Ephs and ephrins are cell surface molecules, and both mediate cellular responses. In bone, ephrinB and EphB receptors control skeletal patterning in the developing organism (103). Mice lacking ephrinB1 have defects in rib, joint, and digit development (104). It is now known that osteoclasts express ephrinB2, whereas osteoblasts express EphB receptors, particularly EphB4 (105). Zhao *et al.* (105) demonstrated that activation of ephrinB2 in osteoclasts by EphB4 on osteoblasts had bidirectional effects, which resulted in inhibition of osteoclastogenesis and increased osteoblast differentiation. Inhibition of osteoclastogenesis by ephrinB2 signaling was mediated by decreases in Fos and NFATc1, whereas enhanced osteoblast differentiation required RhoA inactivation.

IV. Role of Osteoblasts in Bone Marrow Cell Transplantation

Cells of the osteoblast lineage facilitate bone marrow transplantation. Specifically, it has been shown that transplantation of donor bone (containing BMSC and/or osteoblast lineage cells) or osteoblast lineage cells isolated from mouse long bones, along with HSC or bone marrow cells, promoted hematopoietic engraftment (106–108). Indeed, this combination allowed for successful transplantation where HSC or bone marrow cells alone failed (106, 107). As another example, a transgenic mouse model was studied. This mouse expressed the herpes virus thymidine kinase gene under the control of the rat collagen α1 type I promoter. The transgene conferred lineage-specific expression of thymidine kinase in developing and mature osteoblast lineage cells and allowed for the conditional ablation of these cells after treatment of the transgenic mice with ganciclovir (GCV) (95). After GCV treatment, these mice had marked changes in bone formation leading to a progressive bone loss. Importantly, treated animals also lost lymphoid, erythroid, and myeloid progenitors in the bone marrow, followed by decreases in the number of HSC. After withdrawal of GCV, osteoblasts reappeared in the bone, and medullary hematopoiesis was reestablished. Because PTH is able to regulate HSC number *in vivo*, PTH has therapeutic potential to enhance bone marrow transplantation. Pharmacological use of PTH increased the number of HSC that were mobilized into the peripheral blood, protected stem cells from repeated exposure to cytotoxic chemother-

apy, and expanded stem cells in transplant recipients (36, 109).

V. B Lymphocyte Differentiation

B lymphopoiesis is a highly ordered process proceeding from progenitor cells in the fetal liver to development in the bone marrow and to mature B cells in the secondary lymphoid organs. The mature B cell terminally differentiates into Ig-secreting plasma cells after activation (110). B cell development is organized around the assembly of a functional B cell receptor through a process of gene rearrangement called V(D)J recombination (111). The bone marrow B cell developmental pathway can be divided into several distinct stages, based on the recombination status of the immunoglobulin genes and the expression of surface antigens (112–114). The earliest characterized committed B cell progenitor (pre-pro-B) expresses the cell surface markers CD45R and AAr.1 and has its Ig heavy (IgH) chain locus in the germ-line configuration (not rearranged) (42, 115). Subsequent differentiation generates pro-B cells that harbor rearranged IgH D and J genes and express the surrogate light chains λ5 and VpreB and signaling adapters Igβ and Igα (116, 117). As the cells mature, rearrangements occur initially in V gene segments of the IgH chain gene and then in the Ig light chain genes, a process that culminates in a functional surface antigen receptor (111, 118). The molecular dissection of the B cell differentiation pathway has been greatly facilitated by the identification of transcription factors, which are critical for this process. These include PU.1, Ikarous, E2A, Ebf-1, and Pax5, which are required for developmental transitions during B lymphopoiesis. Loss of these specific factors precludes the cells from continued maturation and results in a developmental block of cells at the latest stage of differentiation before the arrest.

Three transcription factors (PU.1, Ebf-1, and Pax5), which act early in B cell differentiation, surprisingly also have profound effects on bone cell development. Because these proteins function in close temporal sequence during B cell development, it might be expected that loss of their function would result in similar bone phenotypes. However, with the exception of being runted and lacking B cells, deletion of these transcription factors in mice produces animals with strikingly different bone phenotypes.

A. PU.1

PU.1, a member of the ETS domain transcription factors, regulates the proliferation and differentiation of B cell and macrophage lineage progenitors (119, 120). The commitment of early progenitors to the B cell lineage depends on a low level/activity of PU.1 in cells. In contrast, macrophage (osteoclasts) lineage commitment depends on high level/activity of PU.1 (121). PU.1-deficient (−/−) mice have no B cells and fail to develop either osteoclasts or macrophages (120). This observation was one of the first to definitively show that osteoclasts are members of the macrophage lineage. PU.1 regulates the lineage fate of these progenitors by directly controlling expression of the IL-7 receptor and c-fms genes (122, 123).

B. Early B cell factor (Ebf)

Ebf-1 is the founding member of a small multigene family of helix-loop-helix proteins that are evolutionarily conserved and have defined roles in cellular differentiation and function. This factor was cloned both from *Saccharomyces cerevisiae* in experiments aimed at identifying the olfactory-restricted olfactory marker protein-1 promoter (124) and by biochemical purification of a factor interacting with the B lymphocyte restricted mb-1 promoter (125). It was named Olf-1, or early B cell factor (Ebf), which in turn led to its current designation as O/E-1. Mice express at least three additional members of this family, Ebf-2 (mMot1/O/E-3), Ebf-3 (O/E-2), and Ebf-4 (126–128). Isolation of the Ebf homolog Collier from *Drosophila* demonstrated the existence of a new family of evolutionarily conserved proteins Collier/Olf/EBF. Mouse Ebf-1, -2, -3, and -4 bind DNA as homo- or heterodimers (129). Ebf1 gene expression is required for B cell fate specification, whereas Pax5, which is regulated by Ebf1, is required for the differentiation of B lymphocyte lineage cells (130, 131). Mice deficient in either of these transcription factors have arrested B cell differentiation at very early stages of B lymphopoiesis (Hardy A and B) (130).

Ebf proteins are involved in both embryonic and adult development of the nervous system. Ebf-1 and -3 expression is seen in Purkinje cells and the cerebellum, and all four Ebf proteins are expressed at high levels in olfactory epithelium (132). Interestingly, OAZ is an Ebf-interacting protein involved in BMP signaling (110). OAZ interacts with Smad1 where Smads and O/E proteins compete for OAZ. Thus, it may be that Ebf proteins regulate BMP signaling. Additional support for this idea comes from the observation that Ebf-1 potentates activation of the B cell-specific gene mb-1 (CD79a) by Pax5 (133). Importantly, Runx1 and Ebf-1 synergized to activate mb-1. This may be important because Runx1, a runt homology domain transcription factor, which is required for hematopoiesis during embryonic development, is expressed in cartilaginous anlagen in the embryo, resting zone chondrocytes, and suture lines of the calvaria (134). Runx1 continues to be expressed in these tissues in adult mice, but not in mature cartilage or mineralized bone. Our preliminary data confirm the original report that Ebf-1-deficient (−/−) mice are growth retarded (130). This is most likely due to the skeletal phenotype, which appears to result from a cell autonomous role of Ebf-1 on osteoblasts. Ebf-1, like all of the known Ebf genes, is highly expressed in adipocytes (130, 135). Analysis of the preadipocyte cell lines, 3T3 L1, indicates that these genes are expressed strongly in undifferentiated cells and their expression increases with differentiation (135). Overexpression of Ebf-1 enhances terminal adipocyte differentiation in preadipocyte cell lines and induces adipogenesis in multipotential cells. The fact that Ebf genes are expressed throughout adipocyte differentiation raises the possibility that they are key regulators of the pathway. However, the exact mechanism by which Ebf-1 stimulates adipogenesis *in vitro* or *in vivo* remains to be elucidated. Ebf1 mRNA is expressed in osteoblasts at all stages of differentiation and also in adipocytes (136). Tibiae and femora from Ebf-1−/− mice had a striking increase in all bone formation parameters examined, including the number of osteoblasts, osteoid vol-

ume, and bone formation rate (136). Serum osteocalcin, a marker of bone formation, was significantly elevated in mutant mice. The number of osteoclasts in bone were normal in younger (4 wk old) Ebf1−/− mice but increased in older (12 wk old) Ebf1−/− mice. This correlated well with *in vitro* osteoclast development from bone marrow cells. In addition to increased osteoblastogenesis, Ebf1−/− mice had a dramatic increase in adipocyte number in the bone marrow. Increased adiposity was also seen histologically in the liver but not in the spleen of these mice (136). Thus Ebf1−/− mice appear to be a new model of lipodystrophy. EBF1 is a rare example of a transcription factor that regulates both the osteoblast and adipocyte lineages similarly.

It is possible that the loss of B cells could account for the changes in bone that are seen in these mutants. However, this seems unlikely because RAG-1- or μMT-heavy chain-deficient mice, which also lack most B cells, do not have a similar bone phenotype (47). It has been reported recently that B cell-deficient mice (μMT heavy chain-deficient) are osteopenic due to increased bone resorption caused by a decrease of B cell-secreted osteoprotegerin (OPG) (137). In contrast, Ebf1−/− mice, which lack all but the very earliest population of pro-B cells, have increased bone mass and increased osteoclasts.

C. Pax5

Pax5 is a member of the multigene family that encodes the Pax transcription factors. This highly conserved motif was originally identified in *Drosophila* (138). At present, nine paired box containing genes (Pax1–Pax9) have been isolated in mammals (131, 139). Three Pax gene-deficient conditions have been studied, and all exhibit developmental mutations. The Pax1 gene is mutated in different forms of undulated, which have skeletal changes in the vertebra (140). It is unknown whether any of these mutant mice express an altered bone phenotype. Human disorders have also been associated with mutations of Pax genes. Pax3 is mutated in Waardenburg's syndrome, which causes deafness, and Pax6 is altered in aniridia and in Peter's anomaly (141, 142). All of these mutations suggest the importance of Pax proteins in the specialization, proliferation, and migration of progenitor cells.

The Pax5 gene codes for the transcription factor B-cell specific activation protein (BSAP) (143). During embryogenesis, Pax5 is transiently expressed in the mesencephalon and spinal cord in a pattern that is different from other Pax genes (143). Later in development, expression moves to the fetal liver where it correlates with the onset of B lymphopoiesis. Within the hematopoietic system, BSAP is expressed exclusively in the B lymphocyte lineage cells, extending from pro-B cells to mature B cells, but it is not found in terminally differentiated plasma cells (143, 144). Testis is the only other tissue in the adult mouse that expresses BSAP.

Loss of Pax5 results in an unanticipated massive decrease in trabecular bone in both the tibia and femur of 15-d-old mice (47). Bone volume (tibia) was reduced by 67%, and osteoid volume was reduced by 55%. Observed increases in bone resorption may be accounted for, at least in part, by a greater than 100% increase in the number of osteoclasts in Pax5-deficient (−/−) bone. These data demonstrate a marked increase in the number of osteoclasts in Pax5−/− mice and suggest that they are functional. The number of osteoblasts in the mutant mice was reduced, although not significantly. These results imply that osteopenia in Pax5−/− mice was due, in large part, to an increase in osteoclasts. However, we cannot rule out the possibility that a delay in the development of osteoblasts contributes to the bone phenotype. In fact, a delay in osteoblast development may be responsible, at least in part, for the runting of these mice. Therefore, we propose that loss of Pax5 causes a bone phenotype by deregulating certain genes that enhance osteoclastogenesis and delay formation.

VI. Role of Megakaryocytes in Bone Turnover

Similar to their role in hematopoiesis, cells of the osteoblast lineage support megakaryopoiesis. Studies by Ahmed *et al.* (145) demonstrated that culture of CD34+ cells on osteoblast lineage cells resulted in expansion of CD34+ and CD34+CD41+ (early MK) cells. When various combinations of cytokines were added to the cultures, it was determined that SCF, IL-3, IL-11, and thrombopoietin (TPO) were most effective in increasing CD34+CD41+ and CD41+ (late MK) cell number. Similarly, it was determined that BMSC were able to support MK differentiation and platelet formation (71). In other experiments, it was shown that culture of human MK on BMSC, which express SCF, resulted in adhesion of the MK to the BMSC and proliferation of the MK through SCF-c-kit interaction (146). Separation of the MK from the BMSC by a cell-impermeable membrane blocked proliferation, indicating that a cell-cell interaction was required.

MK arise from pluripotential hematopoietic progenitor cells that pass through a series of identifiable stages of differentiation that culminate in the production of terminally differentiated MK and the release of platelets. As with B cell differentiation, the molecular dissection of the MK differentiation pathway has been greatly facilitated by the identification of transcription factors that are required for the successful advance of cells from stage to stage. Loss of these specific factors precludes the cells from continued maturation and results in the accumulation of cells at the latest stage of differentiation, before the arrest. The selective loss of two different transcription factors, GATA-1 and NF-E2, which were originally thought to be required exclusively for erythroid lineage development, has now been shown to play a critical role in MK differentiation. GATA-1 knockdown mice and NF-E2-deficient mice exhibit a phenotype characterized by marked megakaryocytosis and thrombocytopenia (147, 148).

The GATA family of zinc-finger transcription factors in vertebrates is presently composed of six members, GATA-1 through GATA-6. GATA is a single polypeptide chain with DNA binding activity in the C-terminal zinc finger (149). GATA-1 is almost solely restricted to hematopoietic lineage cells and is a critical factor for erythroid cell development. GATA-1 is expressed in MK, multipotential hematopoietic progenitors, and mast cells (150). In GATA-1-deficient mice, MK number is increased approximately 10-fold in the bone

marrow and spleen of adult mice, and platelet numbers in the peripheral blood are markedly reduced (15% of normal) (151). It has been documented that MK from GATA-1-deficient mice express lower levels of TGFβ-1, platelet-derived growth factor, and vascular endothelial growth factor than do wild-type control MK (152). TGFβ-1 levels are increased in the spleen and bone (including bone marrow) of GATA-1-deficient mice but not in the plasma. The animals develop myelofibrosis after 1 yr of age (152, 153), which is preceded by a high bone mass phenotype (detected after 3–4 months), which is associated with a greater than 3-fold increase in bone volume and bone formation indices (154).

NF-E2 is a heterodimeric nuclear protein comprised of two polypeptide chains, a hematopoietic-specific 45-kDa subunit and a widely expressed p18 subunit. Both proteins belong to the basic leucine zipper family of transcription factors (155, 156). Expression of p45 is restricted to erythroid precursors, MK, mast cells, and multipotential progenitors. Mice lacking p45 NF-E2 exhibit profound thrombocytopenia, which results from a maturational arrest of MK and a lack of platelets in the peripheral blood (148). MK number is increased 2- to 5-fold in the bone marrow and spleen of adult p45NF-E2-deficient mice. These mice respond to exogenous TPO with a marked increase in bone marrow cell proliferation but no detectable increase in platelet production. Although MK number is markedly elevated in p45 NF-E2-deficient mice, TPO levels are normal (148, 157, 158) Interestingly, these mice also develop a high bone mass phenotype with up to a 5-fold increase in bone volume and bone formation parameters (154, 159).

The strikingly similar bone phenotype, along with the elevated osteoblast number and MK number in both NF-E2 and GATA-1-deficient animal models, led us to examine the potential interaction between osteoblast lineage cells and MK. We showed that when osteoblast lineage cells were cocultured with MK, osteoblast proliferation was increased 3- to 6-fold by a mechanism that required direct cell-to-cell contact (154). Miao *et al.* (160) also demonstrated that direct cell-to-cell contact of BMSC with MK enhanced osteoblastogenesis. In other studies, MK have also been reported to stimulate the differentiation of osteoblasts as defined by enhanced expression of procollagen (161). Thus, MK acts to stimulate both osteoblast proliferation and differentiation *in vitro*.

MK may also play a role in osteoclastogenesis as documented by the expression of OPG and RANKL in MK (161–166). The fact that MK expresses RANKL suggests that they may be an additional vector for osteoclast induction, particularly during inflammatory responses.

In contrast, MK expression of OPG suggests that MK may also play a role in inhibiting osteoclastogenesis. Recent data by our laboratory demonstrated that, *in vitro*, MK and MK conditioned media (CM) inhibited osteoclast development by up to 10-fold (98). We examined MK CM for known inhibitors of osteoclastogenesis and could demonstrate by ELISA that low levels of OPG were present (167). However, Chagraoui *et al.* (164) did not find OPG in MK CM, suggesting that the OPG, if secreted, was not detectable because it was bound to MK-expressed RANKL (161, 164, 165). Importantly, in our work, the addition of anti-OPG antibody failed to neutralize the ability of MK CM to inhibit osteoclast formation, suggesting that MK-secreted OPG was not responsible for the inhibition of osteoclast development.

We confirmed that OPG was not responsible for the MK-mediated inhibition of osteoclast development by testing MK derived from OPG-deficient ($-/-$) mice. These experiments demonstrated that MK from OPG$-/-$ and control mice inhibited osteoclast formation equivalently. Finally, using tandem mass spectrophotometry, we demonstrated that there exists a factor or factors in MK CM that inhibit osteoclast development, and while the identity of this inhibitory factor remains to be determined, it was not any of the major factors known to inhibit osteoclast formation including OPG, IL-4, IL-10, IL-12, IL-13, IL-18, interferon γ (IFN-γ), TGFβ, GM-CSF, osteoclast inhibitory lectin, calcitonin, amylin, or calcitonin-gene-related peptide (167).

Taken together, these data suggest that MK play a dual role in regulating skeletal mass. They secrete a factor(s) that inhibits osteoclast formation while directly stimulating osteoblast proliferation and differentiation. Both of these MK-mediated actions may contribute to the osteosclerosis seen in GATA-1-deficient and NF-E2-deficient mice.

VII. Cytokines and Local Immune Cell Factors as Regulators of Bone Cell Functions

A. Receptor activator of nuclear factor-κB ligand (RANKL), receptor activator of nuclear factor-κB (RANK), and osteoprotegerin (OPG)

Characterization of the functions of RANKL and its receptors (RANK and OPG) (Fig. 2) have contributed significantly to the emergence of osteoimmunology, specifically with respect to examination of the interplay between active immunity and maintenance of bone homeostasis (16, 168, 169). Because there are a number of recent reviews on the diverse physiological function of the RANKL-RANK-OPG axis (169–171), we will focus here on its role in the context of osteoimmunology.

The discovery of RANKL, a TNF superfamily member that has potent activity as a stimulator of both the formation of osteoclasts from precursor cells and bone-resorbing activity in mature osteoclasts, clarified our understanding of how stromal and osteoblastic cells regulate bone resorption (172, 173). RANKL is the critical cytokine that directs the terminal differentiation of osteoclast precursor cells and stimulates and maintains resorption activity in mature cells. Importantly, this activity occurs *in vitro* in the absence of BMSC (172–174).

In vivo RANKL-deficient mice have significant osteopetrosis and no osteoclasts, but a normal number of monocyte/macrophages (175). These mice also exhibit failed tooth eruption, which is 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 osteoclasts (175, 176). Marrow stromal and osteoblastic cells produce RANKL, and regulation of its mRNA expression in murine marrow cell cultures correlates with activation of osteoclastogenesis (177). Many well-known osteotropic factors, including cyto-

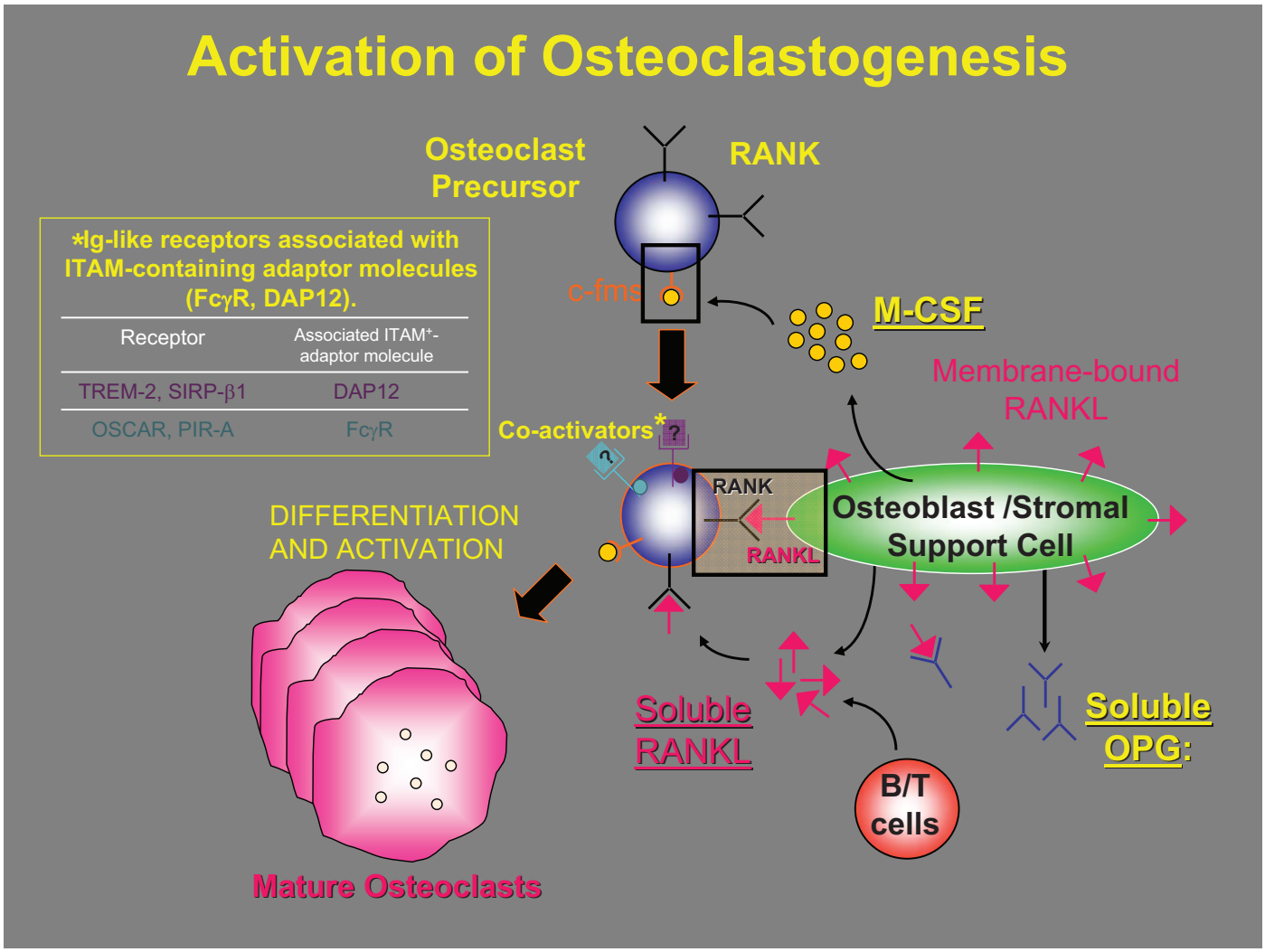

FIG. 2. Activation of osteoclastogenesis. Osteoclast precursor cells replicate and are induced to express RANK when stimulated by the binding of M-CSF to its receptor c-fms. In states in which osteoclastogenesis is stimulated, osteoblast or stromal support cells express relatively more RANKL than OPG. This facilitates the binding of RANKL to RANK, which is the critical signal for the differentiation of mature osteoclasts from precursor cells. However, the formation of mature osteoclasts is significantly enhanced by costimulatory molecules on osteoclast precursor cells. It is critical that the ITAM proteins DAP12 and FcγR on the surface of osteoclast precursor cells interact with their respective Ig-like receptors (TREM-2 and SIRP-β1 with DAP12; OSCAR and PIR-A with FcγR) for costimulation to occur. In addition, in inflammatory states and, possibly, in normal physiology, B and T lymphocytes also produce RANKL, which can influence osteoclastogenesis.

kines and hormones, are now believed to exert their primary osteoclastogenic activity by inducing RANKL expression in osteoblast lineage cells (16, 170). Conversely, the shedding of membrane-bound RANKL appears to be a mechanism for inhibiting osteoblast-mediated osteoclast formation by removing RANKL from the osteoblast surface. The process appears mediated by expression of matrix metalloproteinase (MMP) 14 (178) because osteoclasts were increased in mice deficient in this enzyme.

OPG is a novel secreted TNF receptor superfamily member (TNFRSF-11B) and a potent inhibitor of osteoclast formation that acts as a decoy receptor for RANKL (173, 174, 179). It was initially identified as a soluble factor that was capable of inhibiting osteoclastogenesis *in vitro* (179, 180) and inducing osteopetrosis when transgenically overexpressed in mice (179). In marrow, it is produced by a variety of cells, including stromal cells, B lymphocytes, and dendritic cells (181). In addition to RANKL, OPG also binds the TNF-like ligand TRAIL (TNF-related apoptosis inducing ligand) (182). Mice that lack OPG were shown to have severe osteoporosis, increased numbers of osteoclasts, and arterial calcification (183, 184). The latter finding highlights a potential genetic link between osteoporosis and vascular calcification (170). Overexpression of OPG in transgenic mice caused osteopetrosis, decreased osteoclast numbers, and extramedullary hematopoiesis (179).

The biologically active receptor for RANKL is RANK. Like OPG, RANK is a TNF receptor superfamily member (TNFRSF-11A). It was first identified on dendritic cells (185), but it is also present on osteoclast precursors and mature osteoclasts (186). RANK expression at the RNA level is detected in a variety of cell types and tissues (185). RANK-deficient mice were demonstrated to phenocopy the defect in osteoclast development that was observed in the RANKL-knock-

out mouse, confirming the exclusive specificity of RANKL for osteoclast-expressed RANK (186). In humans, gain-of-function mutations in RANK were found to be associated with familial expansile osteolysis and expansile skeletal hyperphosphatasia (17–21, 187–191) .

Although OCL can form *in vitro* in the absence of RANK or TNF receptor-associated factor (TRAF) 6 signaling when exposed to a cocktail of cytokines and growth factors (192–194), the significance of this *in vitro* finding is questionable because osteoclasts are not detected in RANK-deficient animals (186, 195). More likely, additional cytokines and growth factors produced at sites of inflammation or physiologically during bone turnover act as cofactors that enhance or modulate the response of osteoclasts and their precursors to RANKL-RANK stimulation (196–198).

B. RANKL-RANK mediated signals for osteoclast differentiation

Efforts aimed at elucidating the signaling mechanisms involved in RANKL-mediated osteoclastogenesis have been informative (16, 170, 199). RANK signal transduction is mediated by adapter proteins called TRAFs (174, 200–203). Of the six known TRAFs, RANK interacts with TRAF1, -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 (200–203). Genetic experiments show that TRAF6-deficient mice have severe osteopetrosis, implying that the key signals sent through RANK in osteoclast precursors are mediated by the adapter molecule TRAF6 (204, 205) (Y. Choi, unpublished data).

Downstream of TRAF6, RANKL signaling in osteoclasts has been shown to activate phosphatidylinositide-3-kinase (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 RANKL signaling has been recently reviewed elsewhere (16, 168–171, 206–208). In addition to the signaling pathways mentioned above, RANKL stimulation also triggers reactive oxygen species (ROS) production (209). ROS, like superoxide anions, hydroxyl radicals, and H_2O_2, have been associated with many cellular responses, including metabolic bone diseases found in aged osteoporotic women (210). Recent reports suggest that ROS act as a key second messenger during osteoclastogenesis (209), such that RANKL stimulation induces the production of ROS in osteoclast precursors via the small GTPase Rac1 and the ROS-inducing factor nicotinamide adenine dinucleotide phosphate oxidase 1. It is not clear how ROS cross-regulates the signaling pathways necessary for osteoclast differentiation, although one interesting hypothesis is that ROS may potentiate MAPK activation by inactivating protein tyrosine phosphatase activity in a manner similar to mechanisms recently described in B cells (211).

C. Costimulation in RANKL-induced osteoclast differentiation

The formation and activation of osteoclasts are processes that are tightly regulated by osteoblast lineage cells, which provide at least two known essential factors for osteoclastogenesis, RANKL and M-CSF (Fig. 2). In addition, stromal cells produce various osteotropic factors that influence osteoclastogenesis. These factors can be divided into two groups: those that influence the activity of osteoblasts (*e.g.*, TNFα, which induces RANKL on osteoblasts), and those that affect the osteoclast precursors or osteoclasts *per se*. A series of experiments showed that M-CSF and RANKL together appear to be sufficient to induce the differentiation of bone marrow precursors, spleen cells, or blood monocytes to become mature osteoclasts *in vitro*. However, the expression of M-CSF, RANKL, and their receptors is not limited to bone cells. For example, M-CSF and RANKL are important cytokines for the activity/viability of macrophages and dendritic cells. Despite this pleiotropy, osteoclasts 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 powerful inhibitors of osteoclastogenesis in soft tissues that are 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 (212). Hence, our hypothesis proposed that osteoclast differentiation is controlled not only by two "essential factors," M-CSF and RANKL (analogous to major histocompatibility complex/antigen complexes interacting with T-cell receptor(TCR)/CD4 or TCR/CD8), but also by other "non-essential but critical costimulatory molecules" (analogous to B7 family proteins interacting with CD28) (213). Because the *in vivo* concentrations of M-CSF and RANKL produced by osteoblasts in response to bone-resorbing hormones are likely to be much lower than that used in *in vitro* experiments, costimulatory molecules are likely to influence physiological differentiation of osteoclasts in a manner analogous to T cell activation, whereby signals from the costimulatory receptor CD28 amplify requisite signals from the TCR complex (212, 213). In addition, as with T cells, the requirement for a particular set of costimulatory factors/receptors for osteoclasts should vary depending on the microenvironment. Cells expressing ligands for costimulatory receptors expressed on osteoclasts also vary, but those interacting with osteoclasts themselves, such as osteoblasts, most often provide costimulation (analogous to dendritic cell providing B7 family proteins or TNF family proteins like 4–1BBL to T cells) (212). The signals resulting from the interaction of costimulatory factors and their receptors on osteoclast 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 osteoclast differentiation and activation.

In support of the costimulation hypothesis, we have identified a novel cell surface receptor, OSCAR, which is preferentially expressed on osteoclasts, and have shown that in addition to normal RANKL-RANK signaling, interaction of OSCAR with its putative ligand (OSCAR-L) is important for osteoblast-induced osteoclast differentiation (213). Moreover, it appears that OSCAR-L expression is most prevalent on osteoblastic cells (213). Therefore, the OSCAR receptor/

ligand pairing could be characterized as a putative costimulation receptor/factor for efficient osteoclast differentiation and may provide bone-specific costimulation required for the differentiation of osteoclasts in conjunction with the essential factors M-CSF and RANKL. This signaling combination may provide a mechanistic explanation of why osteoclasts are found only on bone surfaces *in vivo*.

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 (41, 214). For osteoclast development *in vivo*, it appears that some surface receptors on osteoclast 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 (41, 214). Hence, whereas a single deficiency for either DAP12 or FcRγ results in only minor osteoclast defects, double deficiency results in severe osteopetrosis (41, 214). Additional analysis of mutant mice suggests that these receptors activate calcineurin via Syk and phospholipase-C (PLC)γ (41, 214, 215). More signaling proteins have been identified in lymphocytes that bridge Syk (or ZAP-70) and PLCγ, and lead to Ca^{2+} activation (216, 217). Indeed, Gab2 and PLCγ2 have recently been shown to be critical for generation of functional osteoclasts (218, 219). In addition, after the implication that Tec family kinases are likely to be involved in ITAM-mediated signaling (220), we have obtained data that Brutun's tyrosine kinase-deficient cells from X-linked immunodeficiency mice have defects in the multinucleation of preosteoclasts (Y. Choi, unpublished data). It will not be surprising if additional molecules (or family members) that were previously implicated in the ITAM-mediated signaling in immunocytes (*e.g.*, lymphocytes or monocytes) are identified as playing an equivalent role in osteoclast differentiation.

However, it is important to point out that the osteopetrosis observed in the mice with defects in the costimulation pathway (*e.g.*, DAP12/FcRγ double-deficient mice) is much less severe than that in RANKL or RANK knockout mice, and that, in contrast to RANKL or RANK knockout mice, these animals exhibit significant numbers of osteoclasts. This is consistent with the hypothesis that costimulatory receptors for osteoclast differentiation are not essential and that multiple redundancies probably exist (213).

Sustained Ca^{2+} mobilization is necessary for osteoclast differentiation because NFATc1 activation is absolutely required for the process (221). The NFAT family of transcription factors was originally identified as a set of regulators of gene transcription in activated T cells (222). Recently, it was found that RANK signaling induces expression of the NFAT family member NFATc1 (NFAT2) and that this factor is critical for osteoclast development because NFATc1-deficient precursor cells exhibit an absolute failure to differentiate into osteoclasts (221). Like other NFAT family members, the induction and activation of NFATc1 relies 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 points to the existence of an autonomic feedback loop. This likely triggers NFATc1 induction through a TRAF6 and c-fos-mediated mechanism that is initiated by RANKL/RANK stimulation (223). Thus, Ca^{2+} signaling via costimulatory receptors on preosteoclasts is critical for amplification of NFATc1 activity to a level sufficient for osteoclast differentiation. Interestingly, NFATc1, in conjunction with microphthalmia-associated transcription factor (MITF) and PU.1, transactivates OSCAR expression during RANKL-induced osteoclast differentiation (Y. Choi, unpublished data). This suggests that there is a positive feedback circuit from RANKL to NFATc1 via costimulatory receptors such as OSCAR during osteoclast differentiation, which ensures a high level of NFATc1 activity. Recent data suggest that costimulatory receptors also activate another transcription factor, cAMP response element-binding protein, via CaMKIV, that cooperates with NFATc1 to activate osteoclast-specific genes (224).

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 for osteoclastogenesis. However, we still do not fully understand why this system evolved and whether there exists a state in osteoclast development that mimics anergy-(induced tolerance) in lymphocytes.

D. Macrophage colony-stimulating factor

In addition to RANKL, M-CSF is important for normal osteoclast formation (Fig. 2). This cytokine was originally identified as a regulator of macrophage formation (225); however, it was subsequently shown that a spontaneous mouse mutant (*op/op*) with a phenotype of absent osteoclasts and defective macrophage/monocyte formation was deficient in M-CSF (226–228). Injection of M-CSF into *op/op* mice corrected the defect in osteoclast formation and bone resorption (229), as did expression of the protein specifically in osteoblastic cells (230).

Stimulators of bone resorption were shown to increase the production of M-CSF in bone (231–233), and multiple transcripts of M-CSF are produced by alternative splicing (234, 235). The membrane-bound form of M-CSF is regulated by stimulators of resorption and facilitates the differentiation of osteoclasts from precursor cells (232, 236). This may be significant because in marrow cultures soluble M-CSF inhibited OCL formation that was stimulated by 1,25-dihydroxyvitamin D_3 (237, 238).

The role of M-CSF in regulating osteoclast apoptosis has also been examined. Addition of M-CSF to mature osteoclast cultures prolongs their survival (239, 240). This response may be important for the development of the osteopetrotic phenotype in *op/op* mice because transgenic expression in myeloid cells of Bcl-2, which blocks apoptosis, partially reversed the defects in osteoclast and macrophage development in these animals (241). The effects of M-CSF on osteoclasts has been linked to activation of a Na/HCO_3 cotransporter (242). M-CSF also is a potent stimulator of RANK expression in osteoclast precursor cells (22), and it is critical for the expansion of the osteoclast precursor pool size (24).

E. Additional colony stimulating factors

Like M-CSF, GM-CSF and IL-3 affect osteoclast differentiation (238, 243, 244). Both appear to inhibit RANKL-medi-

ated osteoclastogenesis (245, 246). In contrast, these factors enhance the expansion of osteoclast precursor cells (247, 248). It is now known that these CSF drive a common myeloid precursor cell toward lineages other than osteoclasts (3, 245). Both also inhibit expression of TNF receptors on myeloid precursor cells (249). IL-3 also inhibits osteoblast differentiation, which may be one mechanism for how multiple myeloma influences bone because this malignancy can produce IL-3 (250).

G-CSF decreases bone mass in rodents when injected systemically (251, 252), and this response appeared to result from increased osteoclast formation and decreased osteoblast function. G-CSF also mobilizes hematopoietic precursor cells from bone marrow into the circulation (253) and increases the number of circulating osteoclast precursor cells (254), which is likely related to its ability to increase osteoclast resorptive activity. In mice, overexpression of G-CSF inhibited the ability of osteoblasts to respond to bone morphogenetic protein (255). In addition, mice overexpressing G-CSF had increased bone resorption, which was not increased with ovariectomy, as occurred in wild-type mice (256).

F. Interleukin-1

There are two separate IL-1 gene products, IL-1α and IL-1β, which have identical activities (257). IL-1 is a potent peptide stimulator of *in vitro* bone resorption (258), and it also has potent *in vivo* effects (259). Its effects on resorption appear to be both direct on osteoclasts (260) and indirect through its ability to stimulate RANKL production (261). In addition, both RANKL- and 1,25-dihydroxyvitamin D_3-stimulated osteoclast formation *in vitro* are mediated, in part, by effects on IL-1 (197, 262). IL-1 enhances the activity of RANKL to stimulate osteoclastogenesis (263) and also increases prostaglandin synthesis in bone (258, 264), which may account for some of its resorptive activity because prostaglandins are also potent resorption stimuli (265). Direct stimulation of osteoclastogenesis by IL-1 in mixed murine stromal and hematopoietic cell cultures is dependent on RANKL expression in the stromal/osteoblastic cells but not TNF (266).

IL-1 is produced in bone (267), and its activity is present in bone marrow serum (268, 269). A natural inhibitor of IL-1, IL-1 receptor antagonist, is an analog of IL-1 that binds but does not activate the biologically important type I IL-1 receptors (270–272).

There are two known receptors for IL-1: type I and type II (273). All known biological responses to IL-1 appear to be mediated exclusively through the type I receptor (274). IL-1 receptor type I requires interaction with a second protein, IL-1 receptor accessory protein, to generate postreceptor signals (275–277). Signaling through type I receptors involves activation of specific TRAFs and NF-κB (278, 279). IL-1 receptor type II is a decoy receptor that prevents activation of type I receptors (280). One recent report found a decrease in the bone mass of mice that were deficient in the bioactive type I IL-1 receptor (281); however, this has not been our experience (282).

Expression of myeloid differentiation factor 88 (MyD88) but not Toll/IL-1 receptor domain-containing adapter inducing IFN-β (TRIF) was necessary for IL-1 to stimulate RANKL production in osteoblasts and prolong the survival of osteoclasts (283). Survival of osteoclasts by treatment with IL-1 appears to require PI3K/AKT and ERK (284).

G. Tumor necrosis factor

Like IL-1, TNF represents a family of two related polypeptides (α and β) that are the products of separate genes (285–289). TNFα and TNFβ have similar biological activities and are both potent stimulators of bone resorption (258, 290, 291).

In vivo administration of TNFα was shown to increase the serum calcium of mice (291) and to stimulate new osteoclast formation and bone resorption (292). Like IL-1, TNF also enhances the formation of OCL in bone marrow culture (291). The ability of TNF to stimulate osteoclast formation in mixed stromal cell/osteoclast precursor cell cultures was dependent on the production of IL-1 (293). In addition, TNF-induced osteolysis was found to be dependent on M-CSF production (294).

TNF was shown to directly stimulate osteoclast formation in an *in vitro* culture system by a mechanism that was independent of RANK because it occurred in cells from RANK-deficient mice (192, 193, 295). The significance of this *in vitro* finding is questionable, however, because *in vivo* administration of TNF to RANK-deficient mice caused only an occasional osteoclast to form (195).

Like IL-1, TNF binds to two cell surface receptors, TNF receptor 1 or p55 and TNF receptor 2 or p75 (296). In contrast to IL-1, however, both receptors transmit biological responses. Mice deficient in TNF receptor 1 and TNF receptor 2 have been produced (297–299). These animals appear healthy and are not reported to have an abnormal bone phenotype. TNF may also regulate c-fms expression in osteoclast precursor cells (34)

TNF also appears to regulate the abundance of osteoclast precursor cells in the bone marrow by increasing expression of c-fms, the receptor for M-CSF (300). It also enhances RANK signaling mechanisms, which activate osteoclasts and their precursor cells (196), and it enhances expression of the costimulatory molecule PIR-A leading to activation of NFATc1 (301).

H. Additional TNF superfamily members

Fas ligand (FasL), which binds its receptor Fas on responsive cells, regulates apoptosis and other cellular processes in multiple cell types (302). In osteoblasts, FasL inhibits differentiation through a caspase 8-mediated mechanism (303). In osteoclasts, addition of FasL to cultures of osteoclast precursor cells, which were also treated with M-CSF and RANKL, increased osteoclast formation. Osteoclast precursors and mature osteoclasts express Fas and FasL (304). Expression of Fas was up-regulated by RANKL treatment in the RAW 264.7 osteoclast precursor cell line and treatment of mature osteoclasts with Fas-induced apoptosis (305). However, in contrast to their similar effects on osteoclastogenesis in cultures of precursor cells, there appears to be counterregulatory roles of RANKL and FasL on mature osteoclast apoptosis because at high concentrations, RANKL inhibited the ability of FasL

to induce this response (306). The effect of FasL deficiency on bone mass is controversial. One group has found that this index is decreased in FasL-deficient mice (305), whereas another found it to be increased (307). However, the significance of studying bone mass in Fas- or FasL-deficient mice is probably minimal because these models have a generalized lymphoproliferative disorder, which activates a wide variety of immune responses affecting bone and makes interpreting the results of these studies difficult. Most recently, it was shown that estrogen receptor α in osteoclasts regulates FasL production by these cells, which, in turn, mediates bone loss induced by estrogen withdrawal in mice (308).

TRAIL is another TNF-superfamily member that has a wide variety of activities. Treatment of osteoclasts with TRAIL induced apoptosis (309), and this effect may be mediated through up-regulation of the death receptor DR5 (310). In bone, injection of TRAIL for 8 d in 4-wk-old mice induced an increase in bone mass. *In vitro* this effect was associated with an increase in the cyclin-dependent kinase inhibitor, $p27^{Kip1}$, through effects of TRAIL on the ubiquitin-proteosome pathway (311). TRAIL may also be a factor in the effects of myeloma on osteoblasts (312).

CD40 ligand (CD40L) is involved in the differentiation of naive T lymphocytes into T-helper (T_H) 1 effector cells (313). In humans, deficiency of CD40L causes X-liked hyper IgM (XHIM) syndrome. Bones of XHIM patients develop spontaneous fractures and are osteopenic (314). Activated T lymphocytes from XHIM patients have normal RANKL and deficient IFN-γ production, which may contribute to decreased bone mass in these patients (314). In addition, expression of CD40L in rheumatoid arthritis synovial cells induced RANKL expression in these cells and enhanced their ability to stimulate osteoclastogenesis, which suggests that this mechanism is involved in the effects of rheumatoid arthritis on bone (315).

I. Interleukin-6

IL-6, like IL-1 and TNF, has a wide variety of activities related to immune cell function and to the replication and differentiation of a number of cell types (316, 317). Osteoblastic cells (both rodent and human) produce IL-6 and IL-6 receptors (318, 319). Another source of IL-6 in the bone microenvironment is BMSC, which can produce IL-6 after they are stimulated with IL-1 and TNF (320). The receptor for IL-6 is composed of two parts: a specific IL-6 binding protein (IL-6 receptor), which can be either membrane-bound or soluble, and gp130, an activator protein that is common to a number of cytokine receptors (321). Soluble IL-6 receptor binds IL-6, and this complex can then activate cells that contain the gp130 signal peptide (321, 322). The shedding of IL-6 receptor from osteoblasts is stimulated by IL-1 and TNFα (323).

The ability of IL-6 to stimulate bone resorption *in vitro* is variable and depends on the assay system that is used (319, 324–326). It appears that a major effect of IL-6 is to regulate osteoclast progenitor cell differentiation into mature osteoclasts (327, 328). IL-6 also directly stimulates both RANKL and OPG mRNA production in bone (329), and it enhances production of prostaglandins (330). There is also one publication that suggests that IL-6 can stimulate osteoclastogenesis *in vitro* by a RANKL-independent mechanism (331). IL-6 appears to mediate some of the increased resorption and bone pathology that is seen in the clinical syndromes of Paget's disease (185), hypercalcemia of malignancy (332), fibrous dysplasia (333), giant cell tumors of bone (334), and Gorham-Stout disease (335). There have been conflicting data on the role of IL-6 in PTH-mediated responses in bone because some investigators have found it critical (336) whereas others have not (337).

J. Additional interleukin-6 family members

IL-6 is a member of a group of cytokines that share the gp130 activator protein in their receptor complex (338, 339). Each family member utilizes unique ligand receptors to generate specific binding. Signal transduction through these receptors utilizes the JAK/STAT (Janus kinase/signal transduction and activators of transcription) pathway (321).

1. Interleukin-11

IL-11 is produced by bone cells in response to a variety of resorptive stimuli (340). It stimulates osteoclast formation in murine bone marrow cultures (341) and bone resorption in a variety of *in vitro* assays (342, 343). Interestingly, it has no effect on isolated mature osteoclasts. In mice deficient in the specific IL-11 receptor, trabecular bone mass is increased, and this appears to result from decreased bone turnover, which is associated with decreased *in vitro* osteoclast formation and resorption (344).

2. Leukemia inhibitory factor

LIF is produced by bone cells in response to a number of resorption stimuli (86, 345, 346). The effects of LIF on bone resorption are variable. In a number of *in vitro* model systems, LIF stimulated resorption by a prostaglandin-dependent mechanism (347), whereas in other *in vitro* assays, it had inhibitory effects (348, 349). In neonatal murine calvaria cultures, LIF stimulated both RANKL and OPG (329).

Local injections of LIF *in vivo* were shown to increase both resorption and formation parameters, as well as the thickness of the treated bones (350). In mice that lacked the specific LIF receptor and hence, could not respond to LIF, bone volume was reduced, and osteoclast number was increased 6-fold (351).

3. Oncostatin M

Oncostatin M was demonstrated to stimulate multinuclear cell formation in murine and human bone marrow cultures (322, 352). These cells appeared to be macrophage polykaryons, however, and not osteoclasts (352). In contrast, oncostatin M inhibited OCL formation that was stimulated by 1,25-dihydroxyvitamin D_3 in human marrow cultures (352), and it decreased bone resorption rates in fetal mouse long bone cultures (353). *In vivo*, overexpression of oncostatin M in transgenic mice induced a phenotype of osteopetrosis (354). Hence, it appears that oncostatin M is an inhibitor of osteoclast formation and bone resorption (355).

The role of all IL-6 family members in osteoclast formation has to be examined in the light of data demonstrating that mice lacking the gp130 activator protein have an increased number of osteoclasts in their bones compared with normal

animals (356). Because gp130 is an activator of signal transduction for all members of the IL-6 family, this result argues that at least some IL-6 family members have a predominantly inhibitory effect on osteoclast formation and bone resorption. Available data implicate oncostatin M (353) and possibly LIF (348, 349) in this role.

K. Interleukin-7

IL-7 is a cytokine that has diverse effects on the hematopoietic and immunological systems (357) and is best known for its nonredundant role in supporting B and T lymphopoiesis. Studies have demonstrated that IL-7 also plays an important role in the regulation of bone homeostasis (358, 359). However, the precise nature of how IL-7 affects osteoclasts and osteoblasts is controversial, because it has a variety of actions in different target cells. Systemic administration of IL-7 up-regulated osteoclast formation in human peripheral blood cells by increasing osteoclastogenic cytokine production in T cells (360). Significantly, IL-7 did not induce bone resorption and bone loss in T cell-deficient nude mice *in vivo* (361). In addition, treatment of mice with a neutralizing anti-IL-7 antibody inhibited ovariectomy-induced proliferation of early T cell precursors in the thymus, demonstrating that ovariectomy up-regulates T cell development through IL-7. This latter effect may be a mechanism by which IL-7 regulates ovariectomy-induced bone loss (362). However, the interpretation of results from *in vivo* IL-7 treatment studies is complicated by secondary effects of IL-7, which result from the production of bone-resorbing cytokines by T cells in response to activation by this cytokine (360, 361).

In contrast with previously reported studies (358, 360, 361), we found differential effects of IL-7 on osteoclastogenesis (363). IL-7 inhibited osteoclast formation in murine bone marrow cells that were cultured for 5 d with M-CSF and RANKL (363). In IL-7-deficient mice, osteoclast number was markedly increased and trabecular bone mass was decreased compared with wild-type controls (364). In addition, we found that trabecular bone loss after ovariectomy was similar in wild-type and IL-7-deficient mice (364). Curiously, IL-7 mRNA levels in bone increase with ovariectomy, and this effect may be linked to alterations in osteoblast function with estrogen withdrawal (359, 365). Addition of IL-7 to the medium of newborn murine calvaria cultures inhibited bone formation, as did injection of IL-7 above the calvaria of mice *in vivo* (359). When IL-7 was overexpressed locally by osteoblasts, trabecular bone mass was increased compared with wild-type mice (366). Furthermore, targeted overexpression of IL-7 in IL-7-deficient mice rescued the osteoporotic bone phenotype of the IL-7-deficient mice (367). These studies indicated that the actions of IL-7 on bone cells are dependent on whether IL-7 is delivered systemically or locally.

L. Interleukin-8 and other chemokines

Recruitment and homing of myeloid cells often occur under the direction of chemokines and their receptors. This superfamily of relatively small proteins induce interactions through cognate G protein-coupled receptors to initiate cytoskeletal rearrangement, adhesion, and directional migration (368, 369). Chemokines can be divided into four branches, depending on the spacing and sequence motifs of their first cysteine (C) residues. These are CXC, CC, C, and CX_3C, where X is any other amino acid (370, 371). The majority of chemokine receptor interactions occur through the CC and CXC chemokines, which are referred to as major, whereas C and CX_3C chemokines are referred to as minor.

Many cells produce chemokines that bind specific G protein-coupled receptors. IL-8, a CXC chemokine, is produced by osteoclasts (372) and stimulates osteoclastogenesis and bone resorption by a mechanism that is reported to be independent of the RANKL pathway (373–375). IL-8 may also be produced by certain cancers and stimulate lytic bone lesions in metastatic disease (373–375). Effects of IL-8 on bone may be in part mediated by up-regulation of nitric oxide synthase expression in osteoclasts (376).

CCL3 (macrophage inflammatory protein-1 α) is a direct stimulator of osteoclastogenesis that is expressed in bone and bone marrow cells (377–380). This response is proposed to be independent of RANK activation (381). CCL3 is also a mediator of the osteolytic activity of multiple myeloma (382–384). Activation of osteoclastogenesis by CCL3 is mediated by the receptors CCR1 and CCR5 (385). Interestingly, CCL3 and IL-8 stimulate motility but suppress resorption in mature osteoclasts (386).

CCL9 (macrophage inflammatory peptide γ) and its receptor, CCR1, are also an important chemokine ligand receptor interaction that regulates osteoclasts (387). Injection of M-CSF to induce osteoclastogenesis and bone resorption in osteopetrotic tl/tl rats, which lack M-CSF, caused a rapid (within 2 d) up-regulation of CCR1 as well as its ligand CCL9 in the bones of tl/tl mice and a rapid increase in osteoclastogenesis (388). Furthermore, antibodies to CCL9 ameliorated the ability of M-CSF injections to stimulate osteoclastogenesis in this model.

RANKL appears to be a major inducer of CCL9 and CCR1 in osteoclasts (389), and induction of CCR1 by RANKL is dependent on NFATc1 expression (390). CCL9 and other chemokines that bind CCR1 (CCL3, CCL5, and CCL7) are produced by osteoclasts, osteoblasts, and their precursors in bone. In addition, expression of these chemokines in differentiating osteoblasts is induced by proinflammatory cytokines (IL-1 and TNF) (391). Additional chemokine receptors that are produced on osteoclasts include CCR3, CCR5, and CX_3CR1 (385, 387).

Inhibition of CCR1 expression with small interfering RNA or by blocking NFATc1 activation with cyclosporin A inhibited migration of RAW 264.7 cells (a model for osteoclast precursors) and murine bone marrow cells in Boyden chambers (390). Furthermore, inhibition of CCR1 signaling with a mutated form of CCL5, which blocks the binding of CCR1 to its ligands, prevented OCL formation in murine bone marrow cultures (390). In addition, antibody neutralization of CCL9 inhibited RANKL-induced osteoclastogenesis by 60–70% in murine bone marrow cultures (389).

CXCL12 (stromal cell derived factor-1) and its receptor CXCR4 are involved in a variety of cellular processes including hematopoietic cell homeostasis and immune re-

sponses (392). Osteoclast precursor cells express CXCR4 (393), and expression of this receptor is down-regulated by differentiation of these cells toward the osteoclast lineage (394, 395). Treatment of the cell line RAW 264.7 with CXCL12 induced expression of MMP9, which may be a mechanism for the migration of precursor cells toward bone (393). In human osteoclast precursor cells, CXCL12 stimulated migration and enhanced osteoclastogenesis in response to RANKL and M-CSF (393, 394). Expression of CXCL12 is up-regulated in osteoclasts when they differentiate on a calcium phosphate matrix (394). Production of CXCL12 may also be involved in the recruitment of precursor cells, which form giant cell tumors of bone (396), and in the increased osteolysis that is seen in multiple myeloma (397).

CCL2 (monocyte chemoattractant protein-1) is a potent chemokine for monocytes and a variety of other immune cells. Its receptor is CCR2, which is expressed at high levels on monocytes (398). In bone with an induced inflammatory lesion, CCL2 was expressed at high levels in osteoblasts (399). Induction of CCL2 in these lesions was mediated by proinflammatory cytokines (400). CCL2 also may be involved in tooth eruption because it is expressed by dental follicle cells (401–403). Among the factors that stimulate CCL2 in the dental follicle are PTHrP (404), platelet-derived growth factor-BB, and fibroblast growth factor-2 (405). However, CCL2 is not critical for tooth eruption because there were only minor changes in the temporal pattern of this process in CCL2-deficient mice (406). CCL2 is induced by RANKL in mononuclear precursor cells (407) and enhances OCL formation in these cells (408). However, cells induced by treatment of cultures with CCL2 alone, while multinucleated and calcitonin receptor positive, did not resorb bone unless they were also exposed to RANKL (408). Most recently, it was shown that treatment of osteoblasts with PTH increased CCL2 expression and enhanced the fusion of preosteoclasts (409).

M. Interleukin-10

IL-10 is produced by activated T and B lymphocytes (410). It is a direct inhibitor of osteoclastogenesis (411, 412) and osteoblastogenesis (413). This effect is associated with increased tyrosine phosphorylation of a variety of proteins in osteoclast precursor cells (414). The direct effects of IL-10 on RANKL-stimulated osteoclastogenesis are associated with decreases in NFATc1 expression and reduced translocation of this transcription factor into the nucleus (415) as well as suppressed c-Fos and c-Jun expression (416). Administration of IL-10 may have utility as a mechanism to control wear-induced osteolysis (417). In the dental follicle cells, which function to regulate tooth eruption, treatment *in vitro* with IL-10 inhibited RANKL production and enhanced OPG (418). Hence, there appears to also be an indirect effect of IL-10 on osteoclastogenesis that is mediated by its ability to regulate RANKL and OPG production.

Treatment of bone marrow cell cultures with IL-10 suppressed the production of osteoblastic proteins and prevented the onset of mineralization (413). IL-10 also inhibited the formation of OCL in bone marrow cultures without affecting macrophage formation or the resorptive activity of mature osteoclasts (419). This effect appears to involve the production of novel phosphotyrosine proteins in osteoclast precursor cells (414). IL-10 also stimulates a novel inducible nitric oxide synthase (376).

4-1BB is an inducible T cell costimulatory molecule that interacts with 4-1BB ligand. Treatment of RANKL-stimulated osteoclast precursor cells *in vitro* with 4-1BB ligand enhanced IL-10 production. In addition, expression of IL-10 was greater in RANKL-stimulated wild-type osteoclast precursor cell cultures than in cultured cells from 4-1BB-deficient mice (420). These results imply that some effects of IL-10 on osteoclasts may be mediated through interactions of 4-1BB with 4-1BB ligand.

N. Interleukin-12

IL-12 is a cytokine that is produced by myeloid and other cell types. It induces T_H1 differentiation in T lymphocytes and the subsequent expression of IFN-γ (421). IL-12 has an inhibitory effect on osteoclastogenesis. However, the mechanism by which this effect occurs *in vitro* is controversial. Some authors have demonstrated direct inhibitory effects of IL-12 on RANKL-stimulated osteoclastogenesis in purified primary osteoclast precursors and RAW 264.7 cells (422). This effect was associated with inhibition of the expression of NFATc1 in the osteoclast precursor cells. Interestingly, the inhibitory effects of IL-12 on osteoclastogenesis were absent in cells that were pretreated with RANKL (422). In contrast, others have found that the inhibitory effects of IL-12 on osteoclastogenesis are indirect. One group demonstrated that the inhibitory effects of IL-12 are mediated by T lymphocytes and do not involve production of IFN-γ (423). A second group disputes this result and found inhibition of osteoclastogenesis by IL-12 in cells from T lymphocyte depleted cultures and cells from T lymphocyte-deficient nude mice (424). The latter authors also demonstrated that antibodies to IFN-γ blocked some of the inhibitory effect of IL-12 on RANKL-stimulated osteoclast formation.

O. Interleukin-15

IL-15, like IL-7, is a member of the IL-2 superfamily and shares many activities with IL-2 including the ability to stimulate lymphocytes. It has been shown to enhance osteoclast progenitor cell number in culture (425). Production of IL-15 by T lymphocytes has been linked to the increased osteoclastogenesis and bone destruction seen in the bone lesions of rheumatoid arthritis (426).

P. Interleukin-17 and interleukin-23

IL-17 is a family of related cytokines that are unique and contain at least six members (A–F) (427). IL-17E is also called IL-25 (428). These cytokines are central for the development of the adaptive immune response and the products of a subset of CD4 T lymphocytes with a unique cytokine expression profile, termed T_H17. This is in contrast to the more established T lymphocyte cytokine-expressing subsets T_H1 and T_H2.

IL-17A was initially identified as a stimulator of osteoclastogenesis in mixed cultures of mouse hematopoietic cells and

osteoblasts (429). It stimulated osteoclastogenesis by inducing prostaglandin synthesis and RANKL. Production of IL-17A in rheumatoid arthritis appears involved in the production of activated osteoclasts and bone destruction in involved joints (429–431). Effects of IL-17 on osteoclastogenesis and bone resorption are enhanced by TNFα, which is also produced in the inflamed joints of patients with rheumatoid arthritis (432). Inhibition of IL-17A in an antigen-induced arthritis model inhibited the joint and bone destruction that is typically seen and decreased production of RANKL, IL-1β, and TNFα in the involved lesions (433).

IL-23 is an IL-12-related cytokine composed of one subunit of p40, which it shares with IL-12, and one subunit of p19, which is unique (434). It is critical for the differentiation of the T_H17 subset of T lymphocytes along with TGFβ and IL-6 (435). IL-23 appears most important for expanding the population of T_H17 T lymphocytes. This is a subset of T lymphocytes that produce RANKL and have a high osteoclastogenic potential that is mediated by their production of IL-17 (436). Using a LPS-induced model of inflammatory bone destruction, Sato *et al.* (436) found markedly decreased loss of bone in mice that were deficient in either IL-17 or IL-23. Hence, production of both IL-23 and IL-17 is involved in the bone loss in this model. These authors also demonstrated IL-23 mRNA expression in the synovial tissue of involved joints from patients with rheumatoid arthritis, which suggests that similar mechanisms are involved in the bone loss that occurs in this condition in humans.

Q. Interleukin-18

IL-18 is similar to IL-1 in its structure and is a member of the IL-1 superfamily (437). IL-18 synergizes with IL-12 to induce IFN-γ production (438), and its levels are increased at sites of inflammation such as rheumatoid arthritis (439). It is expressed by osteoblastic cells and inhibits osteoclast formation through a variety of mechanisms. These include its ability to stimulate GM-CSF (100), which is produced by T cells in response to IL-18 treatment (440). It also stimulates IFN-γ production *in vivo* in bone (441), and its inhibitory effects on osteoclastogenesis and bone resorption are enhanced by cotreatment with IL-12 (442). Finally, it has been shown to increase production of OPG (443). In IL-18 overexpressing transgenic mice, osteoclasts were decreased; although, curiously, so was bone mass. These results indicate that there also may be effects of IL-18 on bone growth (441). Interestingly, IL-18 has been shown to indirectly stimulate osteoclastogenesis through its effects on T lymphocytes (444). IL-18 is also a mitogen for osteoblastic cells *in vitro* (445).

R. Interferons

IFN-γ is a type II IFN with a wide variety of biological activities. *In vitro*, IFN-γ has inhibitory actions on bone resorption (446, 447). These appear to be direct and are mediated by its effects on osteoclast progenitor cells. IFN-γ inhibits the ability of 1,25-dihydroxyvitamin D_3, PTH, and IL-1 to stimulate the formation of OCL in cultures of human bone marrow (448). IFN-γ also inhibits RANK signaling by accelerating the degradation of TRAF6 through activation of the ubiquitin/proteasome system (449); however, it does not directly inhibit resorption in mature osteoclasts (450). IFN-γ is also reported to have stimulatory effects on resorption through its ability to stimulate RANKL and TNFα production in T lymphocytes (451). It is an inhibitor of osteoblast proliferation (445, 452, 453) and has variable effects on osteoblast differentiation (452, 454, 455).

The effects of IFN-γ on bone *in vivo* are variable because both inhibitory and stimulatory effects have been reported. In mice with collagen-induced arthritis, loss of the IFN-γ receptor leads to increased bone destruction (456, 457). Similarly, in mice that are injected with bacterial endotoxin over their calvaria, loss of IFN-γ receptor resulted in an enhanced resorptive response (449).

In contrast in rats, ip injection of IFN-γ for 8 d induced osteopenia (458). In patients who have osteopetrosis, because they produce defective osteoclasts, administration of IFN-γ stimulated bone resorption and appeared to partially reverse the disease. The latter effects are possibly due to the ability of IFN-γ to stimulate osteoclast superoxide synthesis (459, 460), osteoclast formation *in vivo* (461), or a generalized immune response (462).

Type I IFNs (IFN-α and IFN-β) are typically produced in response to invading pathogens (463). Mice deficient in the IFN-α/β receptor component IFNAR1 have a reduction in trabecular bone mass and an increase in osteoclasts (464). RANKL induces IFN-β in osteoclasts, and IFN-β, in turn, inhibits RANKL-mediated osteoclastogenesis by decreasing c-fos expression (464). IFN-α has also been shown to inhibit bone resorption *in vitro* although its mechanism of action is not as well studied as that of IFN-γ and -β (465). *In vivo*, IFN-α had no effect on bone turnover (466).

S. Additional cytokines

IL-4 and IL-13 are members of a group of locally acting factors that have been termed "inhibitory cytokines." The effects of IL-4 and IL-13 seem related and appear to affect both osteoblasts and osteoclasts. Transgenic mice that overexpress IL-4 had a phenotype of osteoporosis (467). This effect may result from both an inhibition of osteoclast formation and activity (468, 469) and an inhibition of bone formation (470). IL-13 and IL-4 inhibited IL-1-stimulated bone resorption by decreasing the production of prostaglandins and the activity of cyclooxygenase-2 (471). IL-4 and IL-13 have also been demonstrated to induce cell migration (chemotaxis) in osteoblastic cells (472). IL-4 and IL-13 influence the ability of osteoblasts to regulate osteoclast formation and activity through their ability to increase OPG and inhibit RANKL production (473, 474). The direct inhibitory actions of IL-4 on osteoclast precursor cell maturation into osteoclasts are stronger than that of IL-13 and involve effects on STAT6, NF-κB, peroxisome proliferator-activated receptor γ1, MAPK signaling, Ca^{2+} signaling, NFATc1, and c-Fos (474–479) .

Macrophage migration inhibitory factor (MIF) was initially identified as an activity in conditioned medium from activated T lymphocytes that inhibited macrophage migration in capillary tube assays (480). Once purified and cloned

(481), it became available for functional studies and was shown to have a variety of activities. In addition to T lymphocytes, it is produced by pituitary cells and activated macrophages. Mice that overexpress MIF globally were found to have high turnover osteoporosis (482). In contrast, MIF-deficient mice failed to lose bone mass or increase osteoblast or osteoclast number in bone with ovariectomy (483). Hence, MIF appears to be another mediator of the effects of estrogen withdrawal on bone. Estrogen down-regulates MIF expression in activated macrophages (484), and a similar response may occur in bone or bone marrow to mediate some of the effects of ovariectomy on bone mass. MIF is made by osteoblasts (485), and its production by these cells was up-regulated by a variety of growth factors including TGFβ, FGF-2, IGF-II, and fetal calf serum (486). *In vitro*, MIF increased MMP9 and MMP13 expression in osteoblasts (487) and inhibited RANKL-stimulated osteoclastogenesis (488).

VIII. Regulation of Osteoblasts by Immune Cells and Cytokines

A variety of cytokines are known to regulate osteoblastic cells. TNFα inhibits the differentiation of osteoblasts (489–492). IL-1, TNFα, and IFN-γ inhibits collagen synthesis in osteoblasts (452, 455, 493–495). IL-4 and IL-13 suppress prostaglandin synthesis in bone and are reported to be chemoattractants for osteoblasts (471, 472). IL-4 has been shown to act as a direct stimulator of proliferation and inhibitor of differentiation in an osteoblastic cell line (496). Similarly, IL-4-overexpressing mice exhibited a decrease in bone formation and decreased differentiated osteoblasts on their bone surface (467). The role of cytokines in osteoblast apoptosis has also been studied. TNFα is potently proapoptotic for osteoblasts (497), possibly through induction of the Fas-FasL system (498). Activated T lymphocytes also produce products that drive differentiation of human BMSC toward an osteoblastic phenotype (499). B7-H3 is an Ig superfamily member that is expressed on the surface of antigen-presenting cells. Recently, B7-H3 was found to be expressed on developing osteoblasts, and its expression was increased during cell maturation (500). Furthermore, B7-H3-deficient mice had decreased cortical bone mineral density compared with littermate controls (500).

IX. Role of Osteoclasts in Regulating Osteoblasts

It is generally believed that, in addition to their *bona fide* function as mediators of bone resorption, osteoclasts can influence osteoblast differentiation and function through a process termed "coupling" (501–504). It has been postulated that, during remodeling of adult bone, bone formation occurs via the coupling process in response to bone resorption. Failure of such coupling was suggested to cause unbalanced bone remodeling, resulting in osteopetrosis or osteoporosis (502, 503). In support of the coupling hypothesis, a variety of osteopetrotic mouse models with defective osteoclast formation or function demonstrate decreased bone formation. For example, c-Fos and RANKL-deficient mice, which lack osteoclasts, also have reduced bone formation although these mice have no known osteoblast-intrinsic defects (175, 213). In addition to these genetically altered mice, clinical trials also support the concept of coupling. Humans who are treated with bisphosphonates to inhibit bone resorption in combination with daily PTH injections, which increase bone mass, have a diminished anabolic response to PTH compared with patients given daily PTH without bisphosphonates (505). One interpretation of these data is that anabolic regimens of PTH require osteoclasts (and the coupling factors produced by them) to increase bone formation. Not all osteopetrotic mice demonstrate altered bone formation. For example, blockade of chloride channel-7 prevents bone resorption in ovariectomized rats, whereas bone formation in this rodent model is unaltered (506). In addition, mice lacking c-Src also show osteopetrosis with increased bone formation (507, 508). However, interpretation of this model is more complicated because c-Src also has a role in osteoblasts and its absence causes increased osteoblast differentiation *in vitro* (509).

Many of the correlative studies supporting the notion of coupling have previously been extensively reviewed (503, 504). However, we know of no direct evidence supporting the hypothesis that osteoclasts *per se* trigger enhanced bone formation during remodeling. Thus, we would like to discuss several outstanding issues that need to be clarified to validate our understand of coupling. For example, it is critical to clarify genetically whether coupling does indeed require bone resorption by mature osteoclasts and, hence, the release of byproducts from bone matrix (502, 503). Although it cannot be mutually excluded, it is possible that coupling simply requires the presence of mature osteoclasts because their encoded gene products are sufficient to carry out the coupling mission.

It is unknown whether coupling requires contact between osteoclasts and osteoblasts. Many soluble factors (*e.g.*, IGF-I) have been implicated in the coupling process (502, 503), but these hypotheses still require genetic confirmation. As mentioned above, a membrane-associated factor (EphrinB2), which is produced by osteoclast-lineage cells independent of their resorptive action, has been suggested to influence bone formation by osteoblasts (105). However, the question remains whether EphrinB2-expressing mature osteoclast-lineage cells are the cells mediating the coupling of bone formation and resorption because the recruitment of bone-forming cells occurs after resorption is completed. Along this line, it is necessary to first define, molecularly, the differentiation status of the tentatively identified osteoclasts and osteoblasts in the coupling process. For example, one may have to define what the differentiation state of osteoblast lineage cells that potentially interact with osteoclasts is. Are they bone matrix-producing cells or RANKL-expressing cells? Conversely, it needs to be determined whether the osteoclasts that are involved in the coupling process are bone-resorbing multinucleated cells or osteoclast lineage cells that are committed but not necessarily fully differentiated. Our recent data show that an increased number of gene-mutated mononuclear osteoclasts can induce increased bone formation *in vivo* (510). This result suggests that even mononuclear osteoclast lineage cells can potentially interact with osteoblast lineage cells to regulate coupling.

In summary, the coupling hypothesis needs further veri-

fication because it is of great importance both physiologically and clinically to identify the factor(s) involved in this phenomenon and, more importantly, to show whether osteoclasts do indeed influence bone formation during bone remodeling.

X. Role of the Immune System in Bone Disease: the Birth of Osteoimmunology

Production of proinflammatory cytokines by immune cells and the subsequent induction of RANKL-mediated osteoclast formation and bone resorption have been linked to human diseases. Perhaps the most extensive studies have been on the role of cytokines in the development of the osteolytic lesions observed in rheumatoid arthritis and other inflammatory joint diseases (Fig. 3).

RANKL expression on T lymphocytes is induced upon T cell receptor engagement and depends on Ca^{+2} mobilization (208, 511). Initial experiments demonstrated that activated T lymphocytes, or even supernatants from activated T lymphocyte cultures, were capable of supporting osteoclastogenesis *in vitro* (512). It was subsequently observed that mice lacking CTLA4 ($ctla4^{-/-}$), in which T lymphocytes are systemically activated, exhibit an osteoporotic phenotype associated with increased osteoclast numbers. Transfer of $ctla4^{-/-}$ T lymphocytes into $rag2^{-/-}$ mice, which lack lymphocytes, 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 RANKL expression (512), although it is not clear whether T cell-derived RANKL *per se* is responsible for the aberrant bone metabolism in this model. In a complementary study, transgenic overexpression of RANKL restricted to T or T/B lymphocytes was sufficient to partially correct the osteopetrotic phenotype observed in RANKL-deficient mice (213) (Y. Choi, unpublished data). Together these data definitively showed the ability of lymphocytes to regulate bone homeostasis *in vivo* through expression of RANKL and confirmed a *bona fide* interplay between the adaptive immune system and bone metabolism.

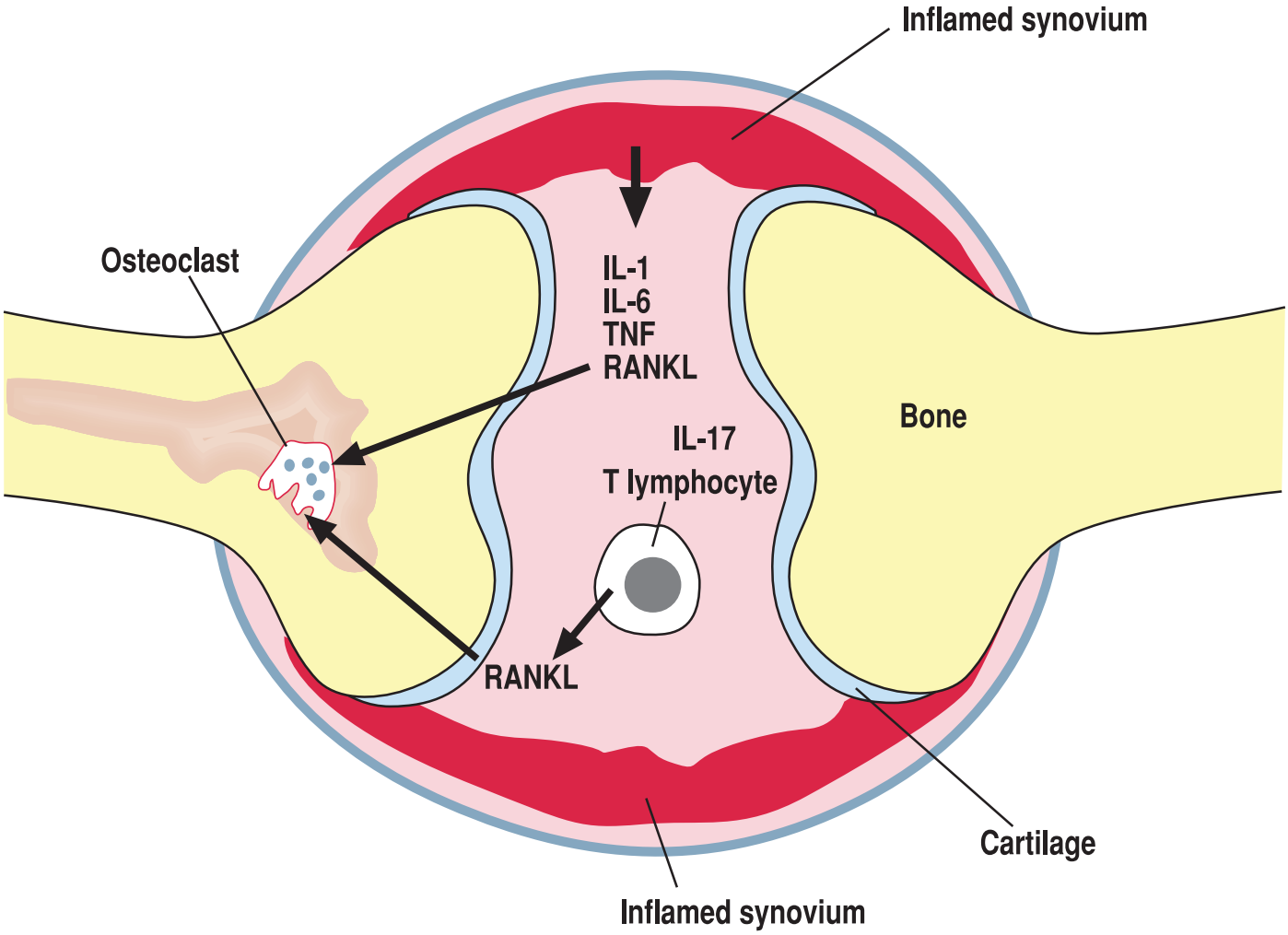

FIG. 3. Regulation of osteoclastogenesis in inflammation. In inflammatory states such as inflammatory arthritis, local production of proinflammatory cytokines (IL-1, IL-6, and TNF) as well as RANKL by inflamed tissues such as the synovium leads to stimulation of osteoclastogenesis and bone destruction. In addition, IL-17-producing T_H17 T lymphocytes stimulate local production of RANKL by inflamed tissues and produce RANKL themselves, which enhances resorptive destruction of bone at sites adjacent to the inflammation.

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 RANKL 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 (512). 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 the onset of disease (513). When K/BxN serum was transferred into RANKL-deficient mice, inflammation and cartilage destruction were comparable to control recipients, but bone erosion was greatly reduced (513). These findings reinforced the notion that RANKL *per se* mediates induction of bone destruction by osteoclasts 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 RANKL, establishing the clinical relevance of the connection between arthritis and immunologically derived RANKL (512). Recently, it has been demonstrated that RANKL, in combination with M-CSF, can induce transdifferentiation of immature dendritic cells to the osteoclast lineage and that this process is significantly enhanced by rheumatoid arthritis synovial fluid, potentially identifying another mechanism for disease-related bone destruction (27).

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 (514, 515). To examine the etiology of the disease, peripheral blood leukocytes from patients with localized juvenile periodontitis were transferred into *rag2*$^{-/-}$ mice, which were then orally inoculated with the Gram-negative bacterium *Actinobacillus actinomycetemcomitans* (515). Localized juvenile periodontitis was recapitulated in the recipient animals and was accompanied by accumulation of osteoclasts at the alveolar sockets (515). It was demonstrated that treatment with OPG inhibited the osteoclast infiltration and bone damage (515). *In vitro* stimulation of peripheral blood lymphocytes showed that RANKL was induced on CD4$^+$ T lymphocytes that were activated with *A. actinomycetemcomitans* antigens and that disease was attenuated when the same cells were specifically depleted from recipient mice (515). 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 bowel disorders, like Crohn's disease and ulcerative colitis (516). 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 (517), whereas another study found that Crohn's disease patients have elevated levels of both OPG and soluble RANKL (518). 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 mitigation of colitis due to reduced colonic dendritic cell survival (519).

In addition to arthritis, periodontal disease, and inflammatory bowel disorders, pathological 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 (520–522). The contribution to pathogeneses by osteoimmunological factors merits further investigation and may provide viable therapeutic options for alleviating painful sequella associated with a variety of conditions.

Although autoimmunity is, in some cases, associated with bone loss, all T cell responses do not necessarily have such a deleterious outcome. T Cells also secrete cytokines, like IFN-γ, IL-4, and TGFβ, that have been shown to inhibit the proosteoclastogenic effects of RANKL (520, 521, 523). In particular, the role of the T_H1 cytokine, IFN-γ, appears to be crucial in preventing T lymphocyte-mediated osteoclastogenesis (449). TGFβ is characterized as both an osteotropic and immunosuppressive cytokine. Although the largest repository of latent TGFβ is in bone, its role in osteoclast formation is complex and insufficiently understood (168). TGFβ down-modulates RANKL expression in osteoblasts, thereby negatively impacting their ability to mediate osteoclastogenesis in culture (524). However, TGFβ has also been shown to potentiate RANKL expression in activated T lymphocytes (208) and enhance osteoclastogenesis in cultures supplemented with soluble RANKL (524). Additional studies will be necessary to determine whether TGFβ utilizes multiple regulatory mechanisms, and if so, what disparate purposes they might serve. If most of the cytokines produced by activated T cells are antiosteoclastogenic, the question becomes how T cells induce bone loss by activating osteoclasts in, for example, inflammatory joints. The answer is recently provided by the discovery of T_H17 cells (Fig. 3), which produce IL-17 and have been shown to be critical mediators of many inflammatory autoimmune diseases such as multiple sclerosis or rheumatoid arthritis (525). T_H17 cells do not produce large amounts of IFN-γ or IL-4 that are antiosteoclastogenic, rather they produce IL-17 (525). Recent data show that IL-17-producing T_H17 cells can induce monocytes to become osteoclasts via RANKL. In addition, IL-17 can further increase the level of RANKL in stromal cells, thereby enhancing overall osteoclastogenesis in the pathogenic joint (436). Given the variety of T lymphocyte-associated cytokines with osteotropic function, it will also be useful to clarify the correlation between $T_H1/T_H2/T_H17$ cytokine polarization and any attendant osteoimmunological bone destruction.

XI. Role of Immune Cells in Estrogen-Withdrawal-Induced Bone Loss

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. Production of cytokines is likely involved

because in mice deficient in the receptor for IL-1 (526), TNFα (527), and IL-6 (528), estrogen withdrawal induced by ovariectomy did not cause bone loss. Production of IL-1 (529), IL-1 receptor (530), TNFα (531), M-CSF (532), IL-6, and IL-6 receptor (533–535) is regulated by estrogen in bone or hematopoietic cells, which may link cytokines to estrogen withdrawal-induced bone loss. Production of IL-7 has also been linked to the bone loss that occurs with estrogen deficiency (359). However, this result is controversial because trabecular bone loss after ovariectomy was found to be similar in wild-type and IL-7-deficient mice (364). Responses of osteoclast precursor cells to RANKL are inhibited by estrogen, and this effect is mediated, in part, by a down-regulation of JNK activation in these cells (536, 537).

T cells have also been proposed to influence the rapid bone loss that occurs after acute estrogen deficiency. This response was postulated to be mediated by enhanced TNFα production (528, 538). 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, which lack T lymphocytes, did not lose bone mass after OVX. This result suggested that T cells are critical for this response (528, 538). However, this hypothesis is controversial because similar experiments using nude rats (539) and nude RAG2- or TCR-α-deficient mice (all of which lack functional T lymphocytes) demonstrated that OVX-induced trabecular bone loss in these models was equivalent to that seen in wild-type mice (540). Curiously, loss of cortical bone with OVX was different between T cell-deficient and wild-type models and dependent on the bone that was examined (540). These results suggest that there may be compartmental and bone-specific effects of T cell depletion on OVX-induced bone loss. Additional experiments will be required to determine how T cells are involved in this response of bone. These studies will likely require mutant mouse models that are deficient in specific immunoregulatory molecules to mechanistically examine the causes of OVX-induced bone loss.

Partially purified populations of B lymphocytes from murine bone marrow are reported to form osteoclasts *in vitro* when they were treated with M-CSF and RANKL (44, 46, 48, 541). In addition, production of osteoclastogenic activity in these populations was increased after ovariectomy. However, when isolated to very high purity, purified B lymphocytes failed to differentiate into osteoclasts *in vitro* (24). These results demonstrate that the osteoclastogenic potential of B lymphocyte populations in murine bone marrow is dependent on contaminating cells. Most recently, we and others have found that trabecular bone loss after ovariectomy was similar in wild-type mice and mice that were deficient in the majority of their B lymphocytes (364, 540, 542).

XII. Modulation of Immunity by the RANKL-RANK-OPG Axis

The significance of RANKL-RANK-OPG signaling in regulating the immune system continues to emerge. Initial studies of RANKL- and RANK-deficient mice demonstrated the importance of these signals for secondary lymphoid organ development because these animals display a lack of peripheral lymph nodes and abnormalities in B cell follicle formation and marginal zone integrity in the spleen (171, 199). In this section, however, we will focus on the role that RANKL-RANK plays in shaping the immune response in the adult immune system.

To date, most reported data indicate that RANKL modulates immunity through dendritic cells. Dendritic cells are the most potent professional antigen-presenting cells and are required to initiate T cell-mediated immunity *in vivo* (543). Dendritic cells differentiate from the hematopoietic monocyte/macrophage progenitor cell lineage and, as close relatives of osteoclasts, can be generated *in vitro* by treating a common precursor cell with GM-CSF. Such treatment has been shown to suppress c-Fos and Fra-1 (26, 544), which are key transcription factors for osteoclast differentiation. These results highlight a mechanism of developmental divergence between these two cell types. Upon receipt of inflammatory or activating stimuli, dendritic cells home to the T cell areas of the lymph nodes to activate antigen-specific T cells. Productive activation relies on numerous dendritic cell-specific factors, including alteration of the chemokine receptor repertoire, up-regulation of costimulatory molecules, and cytokine production. These modifications are induced by exogenous inflammatory stimuli, as well as signals transmitted by the TNF family members TNFα and CD40L.

RANKL signaling has also been implicated in dendritic cell function, particularly with regard to regulation of dendritic cell survival. Activated dendritic cells are relatively short-lived cells, with a half-life as low as 1–2 d upon arrival in the lymph node (545), yet *in situ* imaging studies suggest that individual T dendritic cell couplings may last 37 h or longer (546–548). RANKL-prolonged dendritic cell survival is attributed to up-regulation of the antiapoptotic protein Bcl-xL (549), through a pathway requiring the NF-κB components p50 and c-Rel (550). Treatment of dendritic cells with RANKL also activates the antiapoptotic serine/threonine kinase, Akt/PKB, through recruitment of PI3K by TRAF6 and Cbl-b to RANK, in a mechanism dependent on the kinase activity of c-Src (201, 551). RANKL-prolonged dendritic cell survival also has *in vivo* relevance because pretreatment of peptide-pulsed dendritic cells with RANKL before sc injection into recipient mice results in significantly elevated dendritic cell persistence in draining lymph nodes and enhanced T_h-1 cytokine production and T cell memory formation (552). Dendritic cell vectors intended for use in immunotherapy have been shown to persist longer when pretreated with RANKL (553), and enforced autocrine RANKL-RANK, but not CD40L-CD40, signaling on dendritic cells has been shown to enhance antitumor immunity (554). $Opg^{-/-}$ dendritic cells potentiate *in vitro* mixed lymphocyte reactions, despite CD86, MHCII, and antigen presentation levels identical to syngeneic $opg^{+/-}$ dendritic cells (555).

Blockade of RANKL signaling *in vivo* results in a slightly reduced $CD4^+$ T cell response to lymphocytic choriomeningitis virus infection, although the response is severely inhibited in the absence of CD40 signaling (556). These experiments highlight the requirement for TNF family member signaling in the generation of antiviral immunity, as well as the degree to which the functions of RANK-RANKL and CD40-CD40L interactions overlap. However, physiological

signaling through RANK is more limited in scope than CD40, in that treatment of immature dendritic cells with RANKL cannot initiate activation, and RANKL signaling does not complement the *cd40*$^{-/-}$ defect in germinal center formation and B cell affinity maturation (549, 555). This disconnect is likely not explained by intrinsic signaling differences because RANK and CD40 activate the same set of signaling cascades, but instead by differential expression patterns and kinetics. For example, on T cells CD40L is rapidly and transiently expressed and is limited only to the CD4$^+$ T cell subset (557). In contrast, RANKL is expressed on both CD4$^+$ and CD8$^+$ T cells (552) and is capable of binding both its functional (RANK) and decoy (OPG) receptors. These interactions are also likely to succeed CD40-CD40L signaling because CD40L is a key inducer of RANK and OPG expression by dendritic cells (181). The physiological role of CD40-CD40L *vs.* RANK-RANKL signaling in dendritic cell function may, therefore, depend on the phase of the immune response. CD40-CD40L signaling may be more prominent during the initiation and effector phases, when many cellular components of the immune system are strongly activated. By contrast, RANK-RANKL signaling may be more important during the waning phases, to ensure that T memory formation is established, and then to wind down remaining T dendritic cell interactions, possibly through OPG interference with RANKL signaling. The severe phenotype of RANKL and RANK-deficient mice has thus far not allowed a thorough examination of the role of RANKL in memory T cell formation.

Evidence also suggests that RANKL may be important for survival of interstitial dendritic cells engaged in antigen surveillance during the interim period separating immune responses. Human CD34$^+$ immature dendritic cells have been shown to express both RANK and RANKL and are therefore capable of providing an autocrine survival signal. Peripheral maturation of these dendritic cells leads to a down-regulation of RANKL, suggesting a requirement for an independent source of RANKL to validate dendritic cell activation (558).

RANKL may also be involved in actively inducing tolerance. RANKL signaling has been directly implicated in the induction of oral tolerance in mice. Feeding low-dose ovalbumin to mice concomitant with iv RANKL treatment produced T cells that were refractory to rechallenge and correlates with *in vitro* production of the suppressive cytokine IL-10 by mucosal dendritic cells (559). Another study has demonstrated that RANKL-mediated signaling is required to prevent the onset of autoimmune disease in a TNFα-inducible mouse model of diabetes and that blockade of RANKL-RANK interactions parallel a diminution of CD4$^+$CD25$^+$ regulatory lymphocytes, which are necessary to prevent cytotoxic T lymphocyte-mediated islet cell destruction (560). In a recent study of murine cardiac allograft tolerance, RANKL-RANK signaling was shown to be important for the generation of regulatory T cells via intratracheal delivery of alloantigen (561). It remains unclear, however, whether RANKL directly triggers T lymphocyte suppression or, alternatively, acts through dendritic cell intermediaries. RANKL has also been shown to be induced preferentially among key costimulatory molecules on T cells activated by tolerogenic dendritic cells (562). In addition to systemic action of RANKL, a recent report suggests a potential role of RANKL-RANK interactions in UV-induced immunosuppression in the skin. In the study, Loser *et al.* showed that UV-activated keratinocytes, by expressing RANKL, activated nearby Langerhans cells, which in turn preferentially expanded regulatory T cells (563).

In addition to modulating T cell tolerance via dendritic cells, RANKL may mediate its action during thymic selection. Although earlier studies using RANKL or RANK null mice did not reveal any significant defect in thymocyte development, recent studies nonetheless indicate that the RANKL-RANK interaction and its signaling molecule TRAF6 are required for the development of autoimmune regulator (AIRE)-expressing medullary thymic epithelial cells (564, 565). AIRE-expressing medullary thymic epithelial cells play a critical role in preventing autoimmune diseases by expressing tissue-restricted antigens and thus deleting potentially self-reactive thymocytes during development (566–568). Whether the RANKL-RANK interaction is continuously required for the maintenance of AIRE-positive thymic epithelia cells during adult life is an important question because interference with this pathway may limit the therapeutic use of anti-RANKL blockers to treat various chronic bone diseases like osteoporosis. Further studies are necessary to yield molecular insights into the generation and maintenance of T lymphocyte tolerance that critically requires the interaction of RANKL-RANK.

Study of the role of RANKL-RANK-OPG signaling in the immune system continues to emerge, although progress is slower than that produced by the extensive analysis of this pathway in bone (169, 206). Future studies will provide more insight into how much the RANKL axis controls immune responses during normal homeostasis, infection, or inflammatory challenges. The outcome of these studies will have a major impact on the feasibility of using anti-RANKL therapy to treat chronic diseases of bone such as osteoporosis.

XIII. Toll-Like Receptors, Inflammation, and Osteoimmunology

Toll-like receptors (TLRs) are members of an ancient receptor family that share homology with IL-1R and are critical activators of the innate immune response (569). They are most highly expressed on antigen-presenting cells like dendritic cells, 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 up-regulation of costimulatory molecules and the elaboration of inflammatory cytokines in preparation for an adaptive immune response. TLR signaling is mediated by the adapters MyD88, TRAF6, and TRIF, which activate various downstream signaling pathways, including inhibitory κB kinase-NF-κB, MAPK, and IFN regulatory factor-1 (569).

Because macrophages and dendritic cells share a common progenitor with osteoclasts, it is not surprising that TLR expression is also detected on bone cells (74, 570, 571). Direct signaling of various TLRs (including TLR4) on osteoclast

precursors inhibits RANKL-mediated osteoclastogenesis (74). The data that microbial products inhibit osteoclast differentiation via TLRs is counterintuitive because bacterial infection can cause inflammatory bone diseases such as periodontitis, osteomyelitis, and bacterial arthritis (572). Bone mineral density is reduced in such diseases because of excessive bone resorption by osteoclasts. In addition, LPS has been suggested to be a potent stimulator of bone loss by causing an increase in the number of osteoclasts in mice. Moreover, TLR activation can enhance osteoblast-mediated osteoclast differentiation by inducing RANKL and TNFα on osteoblasts (570, 571, 573). Our recent data suggest that TLR inhibits RANKL-induced osteoclast differentiation in part by inducing the expression of type I IFNs. IFN-β receptor-deficient monocytes are resistant to TLR-mediated suppression during RANKL-induced osteoclast differentiation (Y. Choi, unpublished data). A negative feedback regulatory mechanism via type I IFN has been previously described (464). Activation of the *fos* gene by RANKL leads to up-regulation of IFN-β, which mediates a feedback mechanism blocking further c-Fos-dependent activity (464). As such, it was shown that mice deficient for the IFN-α/β receptor (IFNAR1) suffer from an osteoporotic phenotype that is characterized by an increase in osteoclasts (464). Promoter characterization showed that RANKL-mediated up-regulation of IFN-β utilizes activator protein-1 binding sites, and that c-fos-deficient osteoclast precursors are incapable of inducing IFN-β production (464). To facilitate osteoclast development, therefore, osteoclast precursors need to up-regulate the cytokine signaling regulator suppressors of cytokine signaling 3 to inhibit IFN-mediated suppression (483, 574, 575) . Additional studies are needed to determine whether type 1 IFN production or its action is different when osteoblast lineage cells are present with osteoclast precursors during bacterial infection.

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 osteoclasts remains unclear. As described earlier, alveolar bone destruction in periodontitis caused by infection of Gram-negative bacteria is mediated by enhanced osteoclastogenesis, which results in T cell activation and subsequent up-regulation of RANKL (515). In the same study, bacterial infection of severe combined immunodeficient mice did not lead to significant levels of alveolar bone loss. This result implies that bacterial products do not have a direct role in osteoclastogenesis because severe combined immunodeficiency mice have no known defect in osteoclast precursors or osteoblasts (515). Therefore, it is likely that bone loss associated with bacterial infection may be an indirect outcome of exacerbated T cell responses.

Similar to macrophages or dendritic cells, osteoclast precursors also produce proinflammatory cytokines, such as TNFα, in response to various TLR ligands (74). Moreover, whereas TLR stimulation inhibits osteoclast differentiation, osteoclast 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 osteoclast 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 osteoclasts. Thus, interaction of these microbial products with TLRs on osteoclast precursors appears to favor the role of osteoclast precursors as part of the proinflammatory system by inhibiting their differentiation into mature osteoclasts and promoting the production of inflammatory cytokines. However, because these cells can differentiate into mature osteoclasts if TLR ligands are removed (74), it appears that 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 osteoclasts. In addition, TNFα produced by osteoclast precursors upon TLR stimulation can enhance osteoclastic bone resorption.

Conversely, the RANKL axis may regulate the inflammatory action of TLR stimulation. For example, a recent report suggests that LPS-induced production of proinflammatory cytokines via TLR4 was reduced in OPG-deficient mice, whereas it was increased in RANKL null mice, which demonstrated increased lethality after LPS injection. Moreover, if mice were pretreated with RANKL, they were somewhat protected from LPS-induced death (576). These results suggest that RANKL may suppress cytokine responsiveness to LPS (or other TLR ligands) *in vivo*.

TLRs are thus likely to regulate the balance of immune responses and bone metabolism during acute attacks of vertebrate hosts by various microbes. However, physiological *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 IL-1 receptor-associated kinase-M) exhibit an altered bone metabolism, although it is not clear whether the defects are due to the signals from TLRs or IL-1R (283, 577).

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References

1. **Raisz LG, Kream BE, Lorenzo JA, Larsen PR, Kronenberg HM, Melmed S, Polonsky KS** 2002 Metabolic bone disease. In: Davies

TF, Larsen PR, Kronenberg HM, eds. Williams textbook of endocrinology. Philadelphia: W.B. Saunders; 1373
2. **Rosenberg HF, Gallin JI, Paul WE** 1999 Inflammation. In: Paul WE, ed. Fundamental immunology. Philadelphia: Lippincott-Raven; 1051
3. **Alnaeeli M, Penninger JM, Teng YT** 2006 Immune interactions with CD4+ T cells promote the development of functional osteoclasts from murine CD11c+ dendritic cells. J Immunol 177:3314–3326
4. **Horowitz MC, Lorenzo J, Bilezikian J, Raisz L, Rodan G** 1996 Local regulators of bone: IL-1, TNF, and lymphotoxin, interferon γ, IL-8, IL-10, IL-4, the LIF/IL-6 family and additional cytokines. In: Bilezikian JP, Raisz LG, Rodan GA, eds. Principals of bone biology, 2nd ed. San Diego: Academic Press; 961–977
5. **Raisz LG** 1981 What marrow does to bone. N Engl J Med 304: 1485–1486
6. **Athanasou NA, Heryet A, Quinn J, Gatter KC, Mason DY, McGee JO** 1986 Osteoclasts contain macrophage and megakaryocyte antigens. J Pathol 150:239–246
7. **Sminia T, Dijkstra CD** 1986 The origin of osteoclasts: an immunohistochemical study on macrophages and osteoclasts in embryonic rat bone. Calcif Tissue Int 39:263–266
8. **Kukita T, Roodman GD** 1989 Development of a monoclonal antibody to osteoclasts formed *in vitro* which recognizes mononuclear osteoclast precursors in the marrow. Endocrinology 125:630–637
9. **Tsurukai T, Takahashi N, Jimi E, Nakamura I, Udagawa N, Nogimori K, Tamura M, Suda T** 1998 Isolation and characterization of osteoclast precursors that differentiate into osteoclasts on calvarial cells within a short period of time. J Cell Physiol 177:26–35
10. **Fujikawa Y, Quinn JMW, Sabokbar A, McGee JO, Athanasou NA** 1996 The human osteoclast precursor circulates in the monocyte fraction. Endocrinology 137:4058–4060
11. **Quinn JMW, Sabokbar A, Athanasou NA** 1996 Cells of the mononuclear phagocyte series differentiate into osteoclastic lacunar bone resorbing cells. J Pathol 179:106–111
12. **Akashi K, Traver D, Miyamoto T, Weissman IL** 2000 A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature 404:193–197
13. **Kondo M, Weissman IL, Akashi K** 1997 Identification of clonogenic common lymphoid progenitors in mouse bone marrow. Cell 91:661–672
14. **Spangrude GJ, Heimfeld S, Weissman IL** 1988 Purification and characterization of mouse hematopoietic stem cells. Science [Erratum (1989) 244:1030] 241:58–62
15. **Uchida N, Weissman IL** 1992 Searching for hematopoietic stem cells: evidence that Thy-1.1lo Lin- Sca-1+ cells are the only stem cells in C57BL/Ka-Thy-1.1 bone marrow. J Exp Med 175:175–184
16. **Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT, Martin TJ** 1999 Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. Endocr Rev 20:345–357
17. **Suda T, Takahashi N, Martin TJ** 1995 Modulation of osteoclast differentiation: update 1995. Endocr Rev 13:66–80
18. **Takahashi N, Akatsu T, Udagawa N, Sasaki T, Yamaguchi A, Moseley JM, Martin TJ, Suda T** 1988 Osteoblastic cells are involved in osteoclast formation. Endocrinology 123:2600–2602
19. **Cecchini MG, Hofstetter W, Halasy J, Wetterwald A, Felix R** 1997 Role of CSF-1 in bone and bone marrow development. Mol Reprod Dev 46:75–83
20. **Muguruma Y, Lee MY** 1998 Isolation and characterization of murine clonogenic osteoclast progenitors by cell surface phenotype analysis. Blood 91:1272–1279
21. **Hayashi S, Miyamoto A, Yamane T, Kataoka H, Ogawa M, Sugawara S, Nishikawa S, Nishikawa S, Sudo T, Yamazaki H, Kunisada T** 1997 Osteoclast precursors in bone marrow and peritoneal cavity. J Cell Physiol 170:241–247
22. **Arai F, Miyamoto T, Ohneda O, Inada T, Sudo T, Brasel K, Miyata T, Anderson DM, Suda T** 1999 Commitment and differentiation of osteoclast precursor cells by the sequential expression of c-Fms and receptor activator of nuclear factor κB (RANK) receptors. J Exp Med 190:1741–1754
23. **Servet-Delprat C, Arnaud S, Jurdic P, Nataf S, Grasset MF, Soulas C, Domenget C, Destaing O, Rivollier A, Perret M, Dumontel C, Hanau D, Gilmore GL, Belin MF, Rabourdin-Combe C, Mouchiroud G** 2002 Flt3+ macrophage precursors commit sequentially to osteoclasts, dendritic cells and microglia. BMC Immunol 3:15
24. **Jacquin C, Gran DE, Lee SK, Lorenzo JA, Aguila HL** 2006 Identification of multiple osteoclast precursor populations in murine bone marrow. J Bone Miner Res 21:67–77
25. **Reis e Sousa C** 2006 Dendritic cells in a mature age. Nat Rev Immunol 6:476–483
26. **Miyamoto T, Ohneda O, Arai F, Iwamoto K, Okada S, Takagi K, Anderson DM, Suda T** 2001 Bifurcation of osteoclasts and dendritic cells from common progenitors. Blood 98:2544–2454
27. **Rivollier A, Mazzorana M, Tebib J, Piperno M, Aitsiselmi T, Rabourdin-Combe C, Jurdic P, Servet-Delprat C** 2004 Immature dendritic cell transdifferentiation into osteoclasts: a novel pathway sustained by the rheumatoid arthritis microenvironment. Blood 104:4029–4037
28. **Speziani C, Rivollier A, Gallois A, Coury F, Mazzorana M, Azocar O, Flacher M, Bella C, Tebib J, Jurdic P, Rabourdin-Combe C, Delprat C** 2007 Murine dendritic cell transdifferentiation into osteoclasts is differentially regulated by innate and adaptive cytokines. Eur J Immunol 37:747–757
29. **Fogg DK, Sibon C, Miled C, Jung S, Aucouturier P, Littman DR, Cumano A, Geissmann F** 2006 A clonogenic bone marrow progenitor specific for macrophages and dendritic cells. Science 311: 83–87
30. **Mochizuki A, Takami M, Kawawa T, Suzumoto R, Sasaki T, Shiba A, Tsukasaki H, Zhao B, Yasuhara R, Suzawa T, Miyamoto Y, Choi Y, Kamijo R** 2006 Identification and characterization of the precursors committed to osteoclasts induced by TNF-related activation-induced cytokine/receptor activator of NF-κ B ligand. J Immunol 177:4360–4368
31. **De Klerck B, Carpentier I, Lories RJ, Habraken Y, Piette J, Carmeliet G, Beyaert R, Billiau A, Matthys P** 2004 Enhanced osteoclast development in collagen-induced arthritis in interferon-γ receptor knock-out mice as related to increased splenic CD11b+ myelopoiesis. Arthritis Res Ther 6:R220—R231
32. **Li P, Schwarz EM, O'Keefe RJ, Ma L, Boyce BF, Xing L** 2004 RANK signaling is not required for TNFα-mediated increase in CD11(hi) osteoclast precursors but is essential for mature osteoclast formation in TNFα-mediated inflammatory arthritis. J Bone Miner Res 19:207–213
33. **Li P, Schwarz EM, O'Keefe RJ, Ma L, Looney RJ, Ritchlin CT, Boyce BF, Xing L** 2004 Systemic tumor necrosis factor α mediates an increase in peripheral CD11b high osteoclast precursors in tumor necrosis factor α-transgenic mice. Arthritis Rheum 50:265–276
34. **Yao Z, Li P, Zhang Q, Schwarz EM, Keng P, Arbini A, Boyce BF, Xing L** 2006 Tnf increases circulating osteoclast precursor numbers by promoting their proliferation and differentiation in the bone marrow through up-regulation of c-fms expression. J Biol Chem. 281:11846–11855
35. **Komano Y, Nanki T, Hayashida K, Taniguchi K, Miyasaka N** 2006 Identification of a human peripheral blood monocyte subset that differentiates into osteoclasts. Arthritis Res Ther 8:R152
36. **Sorensen MG, Henriksen K, Schaller S, Henriksen DB, Nielsen FC, Dziegiel MH, Karsdal MA** 2007 Characterization of osteoclasts derived from CD14+ monocytes isolated from peripheral blood. J Bone Miner Res 25:36–45
37. **Atkins GJ, Kostakis P, Vincent C, Farrugia AN, Houchins JP, Findlay DM, Evdokiou A, Zannettino AC** 2006 RANK expression as a cell surface marker of human osteoclast precursors in peripheral blood, bone marrow, and giant cell tumors of bone. J Bone Miner Res 21:1339–1349
38. **Kindle L, Rothe L, Kriss M, Osdoby P, Collin-Osdoby P** 2006 Human microvascular endothelial cell activation by IL-1 and TNF-α stimulates the adhesion and transendothelial migration of circulating human CD14(+) monocytes that develop with RANKL into functional osteoclasts. J Bone Miner Res 21:193–206
39. **Miyamoto T, Arai F, Ohneda O, Takagi K, Anderson DM, Suda T** 2000 An adherent condition is required for formation of multinuclear osteoclasts in the presence of macrophage colony-stimulating factor and receptor activator of nuclear factor κB ligand. Blood 96:4335–4343

40. **Colonna M** 2003 DAP12 signaling: from immune cells to bone modeling and brain myelination. J Clin Invest 111:313–314
41. **Koga T, Inui M, Inoue K, Kim S, Suematsu A, Kobayashi E, Iwata T, Ohnishi H, Matozaki T, Kodama T, Taniguchi T, Takayanagi H, Takai T** 2004 Costimulatory signals mediated by the ITAM motif cooperate with RANKL for bone homeostasis. Nature 428:758–763
42. **Vignery A** 2000 Osteoclasts and giant cells: macrophage-macrophage fusion mechanism. Int J Exp Pathol 81:291–304
43. **Cui W, Cuartas E, Ke J, Zhang Q, Einarsson HB, Sedgwick JD, Li J, Vignery A** 2007 CD200 and its receptor, CD200R, modulate bone mass via the differentiation of osteoclasts. Proc Natl Acad Sci USA 104:14436–14441
44. **Blin-Wakkach C, Wakkach A, Rochet N, Carle GF** 2004 Characterization of a novel bipotent hematopoietic progenitor population in normal and osteopetrotic mice. J Bone Miner Res 19:1137–1143
45. **Manabe N, Kawaguchi H, Chikuda H, Miyaura C, Inada M, Nagai R, Nabeshima Y, Nakamura K, Sinclair AM, Scheuermann RH, Kuro-o M** 2001 Connection between B lymphocyte and osteoclast differentiation pathways. J Immunol 167:2625–2631
46. **Sato T, Shibata T, Ikeda K, Watanabe K** 2001 Generation of bone-resorbing osteoclasts from B220+ cells: its role in accelerated osteoclastogenesis due to estrogen deficiency. J Bone Miner Res 16: 2215–2221
47. **Horowitz MC, Xi Y, Pflugh DL, Hesslein DG, Schatz DG, Lorenzo JA, Bothwell AL** 2004 Pax5-deficient mice exhibit early onset osteopenia with increased osteoclast progenitors. J Immunol 173: 6583–6591
48. **Katavic V, Grcevic D, Lee SK, Kalinowski J, Jastrzebski S, Dougall W, Anderson D, Puddington L, Aguila HL, Lorenzo JA** 2003 The surface antigen CD45R identifies a population of estrogen-regulated murine marrow cells that contain osteoclast precursors. Bone 32:581–590
49. **Andersen TL, Boissy P, Sondergaard TE, Kupisiewicz K, Plesner T, Rasmussen T, Haaber J, Kolvraa S, Delaisse JM** 2007 Osteoclast nuclei of myeloma patients show chromosome translocations specific for the myeloma cell clone: a new type of cancer-host partnership? J Pathol 211:10–17
50. **Aubin JE** 2001 Regulation of osteoblast formation and function. Rev Endocr Metab Disord 2:81–94
51. **Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G** 1997 Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. Cell 89:747–754
52. **Celeste AJ, Iannazzi JA, Taylor RC, Hewick RM, Rosen V, Wang EA, Wozney JM** 1990 Identification of transforming growth factor β family members present in bone-inductive protein purified from bovine bone. Proc Natl Acad Sci USA 87:9843–9847
53. **Nakashima K, Zhou X, Kunkel G, Zhang Z, Deng JM, Behringer RR, de Crombrugghe B** 2002 The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. Cell 108:17–29
54. **Bennett CN, Longo KA, Wright WS, Suva LJ, Lane TF, Hankenson KD, MacDougald OA** 2005 Regulation of osteoblastogenesis and bone mass by Wnt10b. Proc Natl Acad Sci USA 102:3324–3329
55. **Boyden LM, Mao J, Belsky J, Mitzner L, Farhi A, Mitnick MA, Wu D, Insogna K, Lifton RP** 2002 High bone density due to a mutation in LDL-receptor-related protein 5. N Engl J Med 346:1513–1521
56. **Gong Y, Slee RB, Fukai N, Rawadi G, Roman-Roman S, Reginato AM, Wang H, Cundy T, Glorieux FH, Lev D, Zacharin M, Oexle K, Marcelino J, Suwairi W, Heeger S, Sabatakos G, Apte S, Adkins WN, Allgrove J, Arslan-Kirchner M, Batch JA, Beighton P, Black GC, Boles RG, Boon LM, Borrone C, Brunner HG, Carle GF, Dallapiccola B, De Paepe A, Floege B, Halfhide ML, Hall B, Hennekam RC, Hirose T, Jans A, Juppner H, Kim CA, Keppler-Noreuil K, Kohlschuetter A, LaCombe D, Lambert M, Lemyre E, Letteboer T, Peltonen L, Ramesar RS, Romanengo M, Somer H, Steichen-Gersdorf E, Steinmann B, Sullivan B, Superti-Furga A, Swoboda W, van den Boogaard MJ, Van Hul W, Vikkula M, Votruba M, Zabel B, Garcia T, Baron R, Olsen BR, Warman ML** 2001 LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. Cell 107:513–523
57. **Kato M, Patel MS, Levasseur R, Lobov I, Chang BH, Glass 2nd DA, Hartmann C, Li L, Hwang TH, Brayton CF, Lang RA, Karsenty G, Chan L** 2002 Cbfa1-independent decrease in osteoblast proliferation, osteopenia, and persistent embryonic eye vascularization in mice deficient in Lrp5, a Wnt coreceptor. J Cell Biol 157:303–314
58. **Little RD, Carulli JP, Del Mastro RG, Dupuis J, Osborne M, Folz C, Manning SP, Swain PM, Zhao SC, Eustace B, Lappe MM, Spitzer L, Zweier S, Braunschweiger K, Benchekroun Y, Hu X, Adair R, Chee L, FitzGerald MG, Tulig C, Caruso A, Tzellas N, Bawa A, Franklin B, McGuire S, Nogues X, Gong G, Allen KM, Anisowicz A, Morales AJ, Lomedico PT, Recker SM, Van Eerdewegh P, Recker RR, Johnson ML** 2002 A mutation in the LDL receptor-related protein 5 gene results in the autosomal dominant high-bone-mass trait. Am J Hum Genet 70:11–19
59. **Knothe Tate ML, Adamson JR, Tami AE, Bauer TW** 2004 The osteocyte. Int J Biochem Cell Biol 36:1–8
60. **Klein-Nulend J, Nijweide PJ, Burger EH** 2003 Osteocyte and bone structure. Curr Osteoporos Rep 1:5–10
61. **Baron R, Rawadi G, Roman-Roman S** 2006 Wnt signaling: a key regulator of bone mass. Curr Top Dev Biol 76:103–127
62. **Carsten AL, Bond VP** 1968 Viability of stored bone marrow colony forming units. Nature 219:1082
63. **Gong JK** 1978 Endosteal marrow: a rich source of hematopoietic stem cells. Science 199:1443–1445
64. **Kiel MJ, Yilmaz OH, Iwashita T, Yilmaz OH, Terhorst C, Morrison SJ** 2005 SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. Cell 121:1109–1121
65. **Lord BI, Hendry JH** 1972 The distribution of haemopoietic colony-forming units in the mouse femur, and its modification by x rays. Br J Radiol 45:110–115
66. **Lord BI, Testa NG, Hendry JH** 1975 The relative spatial distributions of CFUs and CFUc in the normal mouse femur. Blood 46:65–72
67. **Shackney SE, Ford SS, Wittig AB** 1975 Kinetic-microarchitectural correlations in the bone marrow of the mouse. Cell Tissue Kinet 8:505–516
68. **Deldar A, Lewis H, Weiss L** 1985 Bone lining cells and hematopoiesis: an electron microscopic study of canine bone marrow. Anat Rec 213:187–201
69. **Hermans MH, Hartsuiker H, Opstelten D** 1989 An in situ study of B-lymphocytopoiesis in rat bone marrow. Topographical arrangement of terminal deoxynucleotidyl transferase-positive cells and pre-B cells. J Immunol 142:67–73
70. **Lord BI** 1990 The architecture of bone marrow cell populations. Int J Cell Cloning 8:317–331
71. **Cheng L, Qasba P, Vanguri P, Thiede MA** 2000 Human mesenchymal stem cells support megakaryocyte and pro-platelet formation from CD34(+) hematopoietic progenitor cells. J Cell Physiol 184:58–69
72. **Zhang J, Niu C, Ye L, Huang H, He X, Tong WG, Ross J, Haug J, Johnson T, Feng JQ, Harris S, Wiedemann LM, Mishina Y, Li L** 2003 Identification of the haematopoietic stem cell niche and control of the niche size. Nature 425:836–841
73. **Kiel MJ, Radice GL, Morrison SJ** 2007 Lack of evidence that hematopoietic stem cells depend on N-cadherin-mediated adhesion to osteoblasts for their maintenance. Cell Stem Cell 1:204–217
74. **Takami M, Kim N, Rho J, Choi Y** 2002 Stimulation by toll-like receptors inhibits osteoclast differentiation. J Immunol 169:1516–1523
75. **Sugiyama T, Kohara H, Noda M, Nagasawa T** 2006 Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity 25:977–988
76. **Kollet O, Dar A, Shivtiel S, Kalinkovich A, Lapid K, Sztainberg Y, Tesio M, Samstein RM, Goichberg P, Spiegel A, Elson A, Lapidot T** 2006 Osteoclasts degrade endosteal components and promote mobilization of hematopoietic progenitor cells. Nat Med 12:657–664
77. **Benayahu D, Horowitz M, Zipori D, Wientroub S** 1992 Hemopoietic functions of marrow-derived osteogenic cells. Calcif Tissue Int 51:195–201
78. **Bilbe G, Roberts E, Birch M, Evans DB** 1996 PCR phenotyping of cytokines, growth factors and their receptors and bone matrix proteins in human osteoblast-like cell lines. Bone 19:437–445
79. **Blair HC, Schlesinger PH** 1990 Purification of a stilbene sensitive

chloride channel and reconstitution of chloride conductivity into phospholipid vesicles. Biochem Biophys Res Commun 171:920–925
80. **Blair HC, Teitelbaum SL, Ghiselli R, Gluck S** 1989 Osteoclastic bone resorption by a polarized vacuolar proton pump. Science 245:855–857
81. **Felix R, Elford PR, Stoerckle C, Cecchini M, Wetterwald A, Trechsel U, Fleisch H, Stadler BM** 1988 Production of hemopoietic growth factors by bone tissue and bone cells in culture. J Bone Miner Res 3:27–36
82. **Gowen M, Chapman K, Littlewood A, Hughes D, Evans D, Russell G** 1990 Production of tumor necrosis factor by human osteoblasts is modulated by other cytokines, but not by osteotropic hormones. Endocrinology 126:1250–1255
83. **Hanazawa S, Ohmori Y, Amano S, Miyoshi T, Kumegawa M, Kitano S** 1985 Spontaneous production of interleukin-1-like cytokine from a mouse osteoblastic cell line (MC3T3–E1). Biochem Biophys Res Commun 131:774–779
84. **Horowitz MC, Coleman DL, Flood PM, Kupper TS, Jilka RL** 1989 Parathyroid hormone and lipopolysaccharide induce murine osteoblast-like cells to secrete a cytokine indistinguishable from granulocyte-macrophage colony-stimulating factor. J Clin Invest 83: 149–157
85. **Keeting PE, Rifas L, Harris SA, Colvard DS, Spelsberg TC, Peck WA, Riggs BL** 1991 Evidence for interleukin-1 β production by cultured normal human osteoblast-like cells. J Bone Miner Res 6:827–833
86. **Marusic A, Kalinowski JF, Jastrzebski S, Lorenzo JA** 1993 Production of leukemia inhibitory factor mRNA and protein by malignant and immortalized bone cells. J Bone Miner Res 8:617–624
87. **Ohsaki Y, Takahashi S, Scarcez T, Demulder A, Nishihara T, Williams R, Roodman GD** 1992 Evidence for an autocrine/paracrine role for interleukin-6 in bone resorption by giant cells from giant cell tumors of bone. Endocrinology 131:2229–2234
88. **Ohtsuki T, Suzu S, Hatake K, Nagata N, Miura Y, Motoyoshi K** 1993 A proteoglycan form of macrophage colony-stimulating factor that binds to bone-derived collagens and can be extracted from bone matrix. Biochem Biophys Res Commun 190:215–222
89. **Taichman RS, Emerson SG** 1994 Human osteoblasts support hematopoiesis through the production of granulocyte colony-stimulating factor. J Exp Med 179:1677–1682
90. **Hassan HT, Zander A** 1996 Stem cell factor as a survival and growth factor in human normal and malignant hematopoiesis. Acta Haematol 95:257–262
91. **Horowitz M, Lorenzo JA** 2002 Local regualtors of bone: IL-1, TNF, lymphotozin, interferon-γ, IL-8, IL-10, IL-4, the LIF/IL-6 family, and additional cytokines. In: Bilezikian JP, Raisz LG, Rodan GA, eds. Principles of bone biology, 2nd ed. New York: Academic Press; 961–977
92. **Taichman RS, Reilly MJ, Emerson SG** 1996 Human osteoblasts support human hematopoietic progenitor cells in vitro bone marrow cultures. Blood 87:518–524
93. **Taichman RS, Reilly MJ, Verma RS, Emerson SG** 1997 Augmented production of interleukin-6 by normal human osteoblasts in response to CD34+ hematopoietic bone marrow cells in vitro. Blood 89:1165–1172
94. **Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, Martin RP, Schipani E, Divieti P, Bringhurst FR, Milner LA, Kronenberg HM, Scadden DT** 2003 Osteoblastic cells regulate the haematopoietic stem cell niche. Nature 425:841–846
95. **Visnjic D, Kalajzic Z, Rowe DW, Katavic V, Lorenzo J, Aguila HL** 2004 Hematopoiesis is severely altered in mice with an induced osteoblast deficiency. Blood 103:3258–3264
96. **Weber JM, Forsythe SR, Christianson CA, Frisch BJ, Gigliotti BJ, Jordan CT, Milner LA, Guzman ML, Calvi LM** 2006 Parathyroid hormone stimulates expression of the Notch ligand Jagged1 in osteoblastic cells. Bone 39:485–486
97. **Arai F, Hirao A, Ohmura M, Sato H, Matsuoka S, Takubo K, Ito K, Koh GY, Suda T** 2004 Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. Cell 118:149–161
98. **Moore KA, Lemischka IR** 2004 "Tie-ing" down the hematopoietic niche. Cell 118:139–140
99. **Jung Y, Wang J, Song J, Shiozawa Y, Wang J, Havens A, Wang Z, Sun YX, Emerson SG, Krebsbach PH, Taichman RS** 2007 Annexin II expressed by osteoblasts and endothelial cells regulates stem cell adhesion, homing, and engraftment following transplantation. Blood 110:82–90
100. **Kang YJ, Yang SJ, Park G, Cho B, Min CK, Kim TY, Lee JS, Oh IH** 2007 A novel function of interleukin-10 promoting self-renewal of hematopoietic stem cells. Stem Cells 25:1814–1822
101. **Mundy GR, Elefteriou F** 2006 Boning up on ephrin signaling. Cell. 126:441–443
102. **Wu J, Luo H** 2005 Recent advances on T-cell regulation by receptor tyrosine kinases. Curr Opin Hematol 12:292–297
103. **Compagni A, Logan M, Klein R, Adams RH** 2003 Control of skeletal patterning by ephrinB1-EphB interactions. Dev Cell 5:217–230
104. **Twigg SR, Kan R, Babbs C, Bochukova EG, Robertson SP, Wall SA, Morriss-Kay GM, Wilkie AO** 2004 Mutations of ephrin-B1 (EFNB1), a marker of tissue boundary formation, cause craniofrontonasal syndrome. Proc Natl Acad Sci USA 101:8652–8657
105. **Zhao C, Irie N, Takada Y, Shimoda K, Miyamoto T, Nishiwaki T, Suda T, Matsuo K** 2006 Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. Cell Metab 4:111–121
106. **El-Badri NS, Wang BY, Cherry, Good RA** 1998 Osteoblasts promote engraftment of allogeneic hematopoietic stem cells. Exp Hematol 26:110–116
107. **Hisha H, Nishino T, Kawamura M, Adachi S, Ikehara S** 1995 Successful bone marrow transplantation by bone grafts in chimeric-resistant combination. Exp Hematol 23:347–352
108. **Nakagawa T, Nagata N, Hosaka N, Ogawa R, Nakamura K, Ikehara S** 1993 Prevention of autoimmune inflammatory polyarthritis in male New Zealand black/KN mice by transplantation of bone marrow cells plus bone (stromal cells). Arthritis Rheum 36:263–268
109. **Adams GB, Martin RP, Alley IR, Chabner KT, Cohen KS, Calvi LM, Kronenberg HM, Scadden DT** 2007 Therapeutic targeting of a stem cell niche. Nat Biotechnol 25:238–243
110. **Ghia P, ten Boekel E, Rolink AG, Melchers F** 1998 B-cell development: a comparison between mouse and man. Immunol Today 19:480–485
111. **Hesslein DG, Schatz DG** 2001 Factors and forces controlling V(D)J recombination. Adv Immunol 78:169–232
112. **Hardy RR, Carmack CE, Shinton SA, Kemp JD, Hayakawa K** 1991 Resolution and characterization of pro-B and pre-pro-B cell stages in normal mouse bone marrow. J Exp Med 173:1213–1225
113. **Li YS, Hayakawa K, Hardy RR** 1993 The regulated expression of B lineage associated genes during B cell differentiation in bone marrow and fetal liver. J Exp Med 178:951–960
114. **Hardy RR, Hayakawa K** 2001 B Cell development pathways. Annu Rev Immunol 19:595–621
115. **Li YS, Wasserman R, Hayakawa K, Hardy RR** 1996 Identification of the earliest B lineage stage in mouse bone marrow. Immunity 5:527–535
116. **Melchers F, Karasuyama H, Haasner D, Bauer S, Kudo A, Sakaguchi N, Jameson B, Rolink A** 1993 The surrogate light chain in B-cell development. Immunol Today 14:60–68
117. **Rolink A, Haasner D, Melchers F, Andersson J** 1996 The surrogate light chain in mouse B-cell development. Int Rev Immunol 13:341–356
118. **Nemazee D** 2000 Receptor editing in B cells. Adv Immunol 74: 89–126
119. **Anderson KL, Smith KA, Pio F, Torbett BE, Maki RA** 1998 Neutrophils deficient in PU.1 do not terminally differentiate or become functionally competent. Blood 92:1576–1585
120. **Tondravi MM, McKercher SR, Anderson K, Erdmann JM, Quiroz M, Maki R, Teitelbaum SL** 1997 Osteopetrosis in mice lacking haematopoietic transcription factor PU.1. Nature 386:81–84
121. **DeKoter RP, Singh H** 2000 Regulation of B lymphocyte and macrophage development by graded expression of PU.1. Science 288: 1439–1441
122. **Scott EW, Simon MC, Anastasi J, Singh H** 1994 Requirement of transcription factor PU.1 in the development of multiple hematopoietic lineages. Science 265:1573–1577
123. **DeKoter RP, Lee HJ, Singh H** 2002 PU.1 regulates expression of the interleukin-7 receptor in lymphoid progenitors. Immunity 16:297–309

124. **Wang MM, Reed RR** 1993 Molecular cloning of the olfactory neuronal transcription factor Olf-1 by genetic selection in yeast. Nature 364:121–126
125. **Hagman J, Belanger C, Travis A, Turck CW, Grosschedl R** 1993 Cloning and functional characterization of early B-cell factor, a regulator of lymphocyte-specific gene expression. Genes Dev 7:760–773
126. **Garel S, Marin F, Mattei MG, Vesque C, Vincent A, Charnay P** 1997 Family of Ebf/Olf-1-related genes potentially involved in neuronal differentiation and regional specification in the central nervous system. Dev Dyn 210:191–205
127. **O'Riordan M, Grosschedl R** 1999 Coordinate regulation of B cell differentiation by the transcription factors EBF and E2A. Immunity 11:21–31
128. **Wang SS, Betz AG, Reed RR** 2002 Cloning of a novel Olf-1/EBF-like gene, O/E-4, by degenerate oligo-based direct selection. Mol Cell Neurosci 20:404–414
129. **Hagman J, Gutch MJ, Lin H, Grosschedl R** 1995 EBF contains a novel zinc coordination motif and multiple dimerization and transcriptional activation domains. EMBO J 14:2907–2916
130. **Lin H, Grosschedl R** 1995 Failure of B-cell differentiation in mice lacking the transcription factor EBF. Nature 376:263–267
131. **Walther C, Guenet JL, Simon D, Deutsch U, Jostes B, Goulding MD, Plachov D, Balling R, Gruss P** 1991 Pax: a murine multigene family of paired box-containing genes. Genomics 11:424–434
132. **Wang SS, Tsai RY, Reed RR** 1997 The characterization of the Olf-1/EBF-like HLH transcription factor family: implications in olfactory gene regulation and neuronal development. J Neurosci 17:4149–4158
133. **Maier H, Ostraat R, Gao H, Fields S, Shinton SA, Medina KL, Ikawa T, Murre C, Singh H, Hardy RR, Hagman J** 2004 Early B cell factor cooperates with Runx1 and mediates epigenetic changes associated with mb-1 transcription. Nat Immunol 5:1069–1077
134. **Lian JB, Balint E, Javed A, Drissi H, Vitti R, Quinlan EJ, Zhang L, Van Wijnen AJ, Stein JL, Speck N, Stein GS** 2003 Runx1/AML1 hematopoietic transcription factor contributes to skeletal development in vivo. J Cell Physiol 196:301–311
135. **Akerblad P, Lind U, Liberg D, Bamberg K, Sigvardsson M** 2002 Early B-cell factor (O/E-1) is a promoter of adipogenesis and involved in control of genes important for terminal adipocyte differentiation. Mol Cell Biol 22:8015–8025
136. **Horowitz MC, Lorenzo JA** 2007 B Lymphocytes and the skeleton. Ann NY Acad Sci 1117:82–93
137. **Li Y, Toraldo G, Li A, Yang X, Zhang H, Qian WP, Weitzmann MN** 2007 B Cells and T cells are critical for the preservation of bone homeostasis and attainment of peak bone mass in vivo. Blood 109:3839–3848
138. **Bopp D, Burri M, Baumgartner S, Frigerio G, Noll M** 1986 Conservation of a large protein domain in the segmentation gene paired and in functionally related genes of *Drosophila*. Cell 47:1033–1040
139. **Burri M, Tromvoukis Y, Bopp D, Frigerio G, Noll M** 1989 Conservation of the paired domain in metazoans and its structure in three isolated human genes. EMBO J 8:1183–1190
140. **Balling R, Deutsch U, Gruss P** 1988 Undulated, a mutation affecting the development of the mouse skeleton, has a point mutation in the paired box of Pax 1. Cell 55:531–535
141. **Baldwin CT, Hoth CF, Amos JA, da-Silva EO, Milunsky A** 1992 An exonic mutation in the HuP2 paired domain gene causes Waardenburg's syndrome. Nature 355:637–638
142. **Ton CC, Hirvonen H, Miwa H, Weil MM, Monaghan P, Jordan T, van Heyningen V, Hastie ND, Meijers-Heijboer H, Drechsler M, Royer-Pokora B, Collins F, Swaroop A, Strong LC, Saunders GF** 1991 Positional cloning and characterization of a paired box- and homeobox-containing gene from the aniridia region. Cell 67:1059–1074
143. **Adams B, Dorfler P, Aguzzi A, Kozmik Z, Urbanek P, Maurer-Fogy I, Busslinger M** 1992 Pax-5 encodes the transcription factor BSAP and is expressed in B lymphocytes, the developing CNS, and adult testis. Genes Dev 6:1589–1607
144. **Urbanek P, Wang ZQ, Fetka I, Wagner EF, Busslinger M** 1994 Complete block of early B cell differentiation and altered patterning of the posterior midbrain in mice lacking Pax5/BSAP. Cell 79:901–912
145. **Ahmed N, Khokher MA, Hassan HT** 1999 Cytokine-induced expansion of human CD34+ stem/progenitor and CD34+CD41+ early megakaryocytic marrow cells cultured on normal osteoblasts. Stem Cells 17:92–99
146. **Avraham H, Scadden DT, Chi S, Broudy VC, Zsebo KM, Groopman JE** 1992 Interaction of human bone marrow fibroblasts with megakaryocytes: role of the c-kit ligand. Blood 80:1679–1684
147. **Shivdasani RA, Fujiwara Y, McDevitt MA, Orkin SH** 1997 A lineage-selective knockout establishes the critical role of transcription factor GATA-1 in megakaryocyte growth and platelet development. EMBO J 16:3965–3973
148. **Shivdasani RA, Rosenblatt MF, Zucker-Franklin D, Jackson CW, Hunt P, Saris CJ, Orkin SH** 1995 Transcription factor NF-E2 is required for platelet formation independent of the actions of thrombopoietin/MGDF in megakaryocyte development. Cell 81:695–704
149. **Tsai SF, Martin DI, Zon LI, D'Andrea AD, Wong GG, Orkin SH** 1989 Cloning of cDNA for the major DNA-binding protein of the erythroid lineage through expression in mammalian cells. Nature 339:446–451
150. **Orkin SH** 1992 GATA-binding transcription factors in hematopoietic cells. Blood 80:575–581
151. **Vyas P, Ault K, Jackson CW, Orkin SH, Shivdasani RA** 1999 Consequences of GATA-1 deficiency in megakaryocytes and platelets. Blood 93:2867–2875
152. **Vannucchi AM, Bianchi L, Cellai C, Paoletti F, Rana RA, Lorenzini R, Migliaccio G, Migliaccio AR** 2002 Development of myelofibrosis in mice genetically impaired for GATA-1 expression (GATA-1(low) mice). Blood 100:1123–1132
153. **Vannucchi AM, Bianchi L, Paoletti F, Pancrazzi A, Torre E, Nishikawa M, Zingariello M, Di Baldassarre A, Rana RA, Lorenzini R, Alfani E, Migliaccio G, Migliaccio AR** 2005 A pathobiologic pathway linking thrombopoietin, GATA-1, and TGF-β1 in the development of myelofibrosis. Blood 105:3493–3501
154. **Kacena MA, Shivdasani RA, Wilson K, Xi Y, Troiano N, Nazarian A, Gundberg CM, Bouxsein ML, Lorenzo JA, Horowitz MC** 2004 Megakaryocyte-osteoblast interaction revealed in mice deficient in transcription factors GATA-1 and NF-E2. J Bone Miner Res 19:652–660
155. **Andrews NC, Erdjument-Bromage H, Davidson MB, Tempst P, Orkin SH** 1993 Erythroid transcription factor NF-E2 is a haematopoietic-specific basic-leucine zipper protein. Nature 362:722–728
156. **Andrews NC, Kotkow KJ, Ney PA, Erdjument-Bromage H, Tempst P, Orkin SH** 1993 The ubiquitous subunit of erythroid transcription factor NF-E2 is a small basic-leucine zipper protein related to the v-maf oncogene. Proc Natl Acad Sci USA 90:11488–11492
157. **Shivdasani RA** 1997 Transcript factors in megakaryocte differentiation and gene expression. In: Kuter DJ, Hunt P, Sheridan W, Zucker-Franklin D, eds. Thrombopoiesis and thrombopoietins, molecular, cellular, preclinical and clinical biology. Totowa, NJ: Humana Press; 189–202
158. **Shivdasani RA, Fielder P, Keller GA, Orkin SH, de Sauvage FJ** 1997 Regulation of the serum concentration of thrombopoietin in thrombocytopenic NF-E2 knockout mice. Blood 90:1821–1827
159. **Kacena MA, Gundberg CM, Nelson T, Horowitz MC** 2005 Loss of the transcription factor p45 NF-E2 results in a developmental arrest of megakaryocyte differentiation and the onset of a high bone mass phenotype. Bone 36:215–223
160. **Miao D, Murant S, Scutt N, Genever P, Scutt A** 2004 Megakaryocyte-bone marrow stromal cell aggregates demonstrate increased colony formation and alkaline phosphatase expression in vitro. Tissue Eng 10:807–817
161. **Bord S, Frith E, Ireland DC, Scott MA, Craig JI, Compston JE** 2005 Megakaryocytes modulate osteoblast synthesis of type-l collagen, osteoprotegerin, and RANKL. Bone 36:812–819
162. **Bord S, Frith E, Ireland DC, Scott MA, Craig JI, Compston JE** 2004 Synthesis of osteoprotegerin and RANKL by megakaryocytes is modulated by oestrogen. Br J Haematol 126:244–251
163. **Bord S, Ireland DC, Beavan SR, Compston JE** 2003 The effects of estrogen on osteoprotegerin, RANKL, and estrogen receptor expression in human osteoblasts. Bone 32:136–141
164. **Chagraoui H, Sabri S, Capron C, Villeval JL, Vainchenker W,**

Wendling F 2003 Expression of osteoprotegerin mRNA and protein in murine megakaryocytes. Exp Hematol 31:1081–1088
165. **Kartsogiannis V, Zhou H, Horwood NJ, Thomas RJ, Hards DK, Quinn JM, Niforas P, Ng KW, Martin TJ, Gillespie MT** 1999 Localization of RANKL (receptor activator of NF κB ligand) mRNA and protein in skeletal and extraskeletal tissues. Bone 25:525–534
166. **Pearse RN, Sordillo EM, Yaccoby S, Wong BR, Liau DF, Colman N, Michaeli J, Epstein J, Choi Y** 2001 Multiple myeloma disrupts the TRANCE/ osteoprotegerin cytokine axis to trigger bone destruction and promote tumor progression. Proc Natl Acad Sci USA 98:11581–11586
167. **Kacena MA, Nelson T, Clough ME, Lee SK, Lorenzo JA, Gundberg CM, Horowitz MC** 2006 Megakaryocyte-mediated inhibition of osteoclast development. Bone 39:991–999
168. **Teitelbaum SL** 2000 Bone resorption by osteoclasts. Science 289: 1504–1508
169. **Walsh MC, Kim N, Kadono Y, Rho J, Lee SY, Lorenzo J, Choi Y** 2006 Osteoimmunology: interplay between the immune system and bone metabolism. Annu Rev Immunol 24:33–63
170. **Boyle WJ, Simonet WS, Lacey DL** 2003 Osteoclast differentiation and activation. Nature 423:337–342
171. **Theill LE, Boyle WJ, Penninger JM** 2002 RANK-L and RANK: T cells, bone loss, and mammalian evolution. Annu Rev Immunol 20:795–823
172. **Fuller K, Wong B, Fox S, Choi Y, Chambers TJ** 1998 TRANCE is necessary and sufficient for osteoblast-mediated activation of bone resorption in osteoclasts. J Exp Med 188:997–1001
173. **Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ** 1998 Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell 93:165–176
174. **Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T** 1998 Osteoclast differentiation factor is a ligand for osteoprotegerin osteoclastogenesis-inhibitory factor and is identical to TRANCE/ RANKL. Proc Natl Acad Sci USA 95:3597–3602
175. **Kong YY, Yoshida H, Sarosi I, Tan HL, Timms E, Capparelli C, Morony S, Oliveira-dos-Santos AJ, Van G, Itie A, Khoo W, Wakeham A, Dunstan CR, Lacey DL, Mak TW, Boyle WJ, Penninger JM** 1999 OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. Nature 397:315–323
176. **Kim N, Odgren PR, Kim DK, Marks Jr SC, Choi Y** 2000 Diverse roles of the tumor necrosis factor family member TRANCE in skeletal physiology revealed by TRANCE deficiency and partial rescue by a lymphocyte-expressed TRANCE transgene. Proc Natl Acad Sci USA 97:10905–10910
177. **Lee SK, Lorenzo JA** 1999 Parathyroid hormone stimulates TRANCE and inhibits osteoprotegerin messenger ribonucleic acid expression in murine bone marrow cultures: correlation with osteoclast-like cell formation. Endocrinology 140:3552–3561
178. **Hikita A, Yana I, Wakeyama H, Nakamura M, Kadono Y, Oshima Y, Nakamura K, Seiki M, Tanaka S** 2006 Negative regulation of osteoclastogenesis by ectodomain shedding of receptor activator of NF-κB ligand. J Biol Chem 281:36846–36855
179. **Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS, Luthy R, Nguyen HQ, Wooden S, Bennett L, Boone T, Shimamoto G, DeRose M, Elliott R, Colombero A, Tan HL, Trail G, Sullivan J, Davy E, Bucay N, Renshaw-Gegg L, Hughes TM, Hill D, Pattison W, Campbell P, Sander S, Van G, Tarpley J, Derby P, Lee R, Boyle WJ** 1997 Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell 89:309–319
180. **Tsuda E, Goto M, Mochizuki S, Yano K, Kobayashi F, Morinaga T, Higashio K** 1997 Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. Biochem Biophys Res Commun 234:137–142
181. **Yun TJ, Chaudhary PM, Shu GL, Frazer JK, Ewings MK, Schwartz SM, Pascual V, Hood LE, Clark EA** 1998 OPG/FDCR-1, a TNF receptor family member, is expressed in lymphoid cells and is up-regulated by ligating CD40. J Immunol 161:6113–6121
182. **Emery JG, McDonnell P, Burke MB, Deen KC, Lyn S, Silverman C, Dul E, Appelbaum ER, Eichman C, DiPrinzio R, Dodds RA, James IE, Rosenberg M, Lee JC, Young PR** 1998 Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL. J Biol Chem 273: 14363–14367
183. **Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J, Capparelli C, Scully S, Tan HL, Xu W, Lacey DL, Boyle WJ, Simonet WS** 1998 Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. Genes Dev 12:1260–1268
184. **Mizuno A, Amizuka N, Irie K, Murakami A, Fujise N, Kanno T, Sato Y, Nakagawa N, Yasuda H, Mochizuki S, Gomibuchi T, Yano K, Shima N, Washida N, Tsuda E, Morinaga T, Higashio K, Ozawa H** 1998 Severe osteoporosis in mice lacking osteoclastogenesis inhibitory factor/osteoprotegerin. Biochem Biophy Res Comm 247:610–615
185. **Anderson DM, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBose RF, Cosman D, Galibert L** 1997 A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature 390:175–179
186. **Dougall WC, Glaccum M, Charrier K, Rohrbach K, Brasel K, De Smedt T, Daro E, Smith J, Tometsko ME, Maliszewski CR, Armstrong A, Shen V, Bain S, Cosman D, Anderson D, Morrissey PJ, Peschon JJ, Schuh J** 1999 RANK is essential for osteoclast and lymph node development. Genes Dev 13:2412–2424
187. **Hughes AE, Ralston SH, Marken J, Bell C, MacPherson H, Wallace RG, van Hul W, Whyte MP, Nakatsuka K, Hovy L, Anderson DM** 2000 Mutations in TNFRSF11A, affecting the signal peptide of RANK, cause familial expansile osteolysis. Nat Genet 24:45–48
188. **Whyte MP, Hughes AE** 2002 Expansile skeletal hyperphosphatasia is caused by a 15-base pair tandem duplication in TNFRSF11A encoding RANK and is allelic to familial expansile osteolysis. J Bone Miner Res 17:26–29
189. **Whyte MP, Mumm S** 2004 Heritable disorders of the RANKL/ OPG/RANK signaling pathway. J Musculoskelet Neuronal Interact 4:254–267
190. **Whyte MP, Obrecht SE, Finnegan PM, Jones JL, Podgornik MN, McAlister WH, Mumm S** 2002 Osteoprotegerin deficiency and juvenile Paget's disease. N Engl J Med 347:175–184
191. **Whyte MP, Reinus WR, Podgornik MN, Mills BG** 2002 Familial expansile osteolysis (excessive RANK effect) in a 5-generation American kindred. Medicine (Baltimore) 81:101–121
192. **Kim N, Kadono Y, Takami M, Lee J, Lee SH, Okada F, Kim JH, Kobayashi T, Odgren PR, Nakano H, Yeh WC, Lee SK, Lorenzo JA, Choi Y** 2005 Osteoclast differentiation independent of the TRANCE-RANK-TRAF6 axis. J Exp Med 202:589–595
193. **Kobayashi K, Takahashi N, Jimi E, Udagawa N, Takami M, Kotake S, Nakagawa N, Kinosaki M, Yamaguchi K, Shima N, Yasuda H, Morinaga T, Higashio K, Martin TJ, Suda T** 2000 Tumor necrosis factor α stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. J Exp Med 191:275–286
194. **Kudo O, Fujikawa Y, Itonaga I, Sabokbar A, Torisu T, Athanasou NA** 2002 Proinflammatory cytokine (TNFα/IL-1α) induction of human osteoclast formation. J Pathol 198:220–227
195. **Li J, Sarosi I, Yan XQ, Morony S, Capparelli C, Tan HL, McCabe S, Elliott R, Scully S, Van G, Kaufman S, Juan SC, Sun Y, Tarpley J, Martin L, Christensen K, McCabe J, Kostenuik P, Hsu H, Fletcher F, Dunstan CR, Lacey DL, Boyle WJ** 2000 RANK is the intrinsic hematopoietic cell surface receptor that controls osteoclastogenesis and regulation of bone mass and calcium metabolism. Proc Natl Acad Sci USA 97:1566–1571
196. **Lam J, Takeshita S, Barker JE, Kanagawa O, Ross FP, Teitelbaum SL** 2000 TNF-α induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. J Clin Invest 106:1481–1488
197. **Lee SK, Gardner AE, Kalinowski JF, Jastrzebski SL, Lorenzo JA** 2005 RANKL-stimulated osteoclast-like cell formation in vitro is partially dependent on endogenous interleukin-1 production. Bone 38:678–685
198. **Takita C, Fujikawa Y, Itonaga I, Taira H, Kawashima M, Torisu T** 2005 Infliximab acts directly on human osteoclast precursors and enhances osteoclast formation induced by receptor activator of nuclear factor κB ligand in vitro. Mod Rheumatol 15:97–103

199. **Walsh MC, Choi Y** 2003 Biology of the TRANCE axis. Cytokine Growth Factor Rev 14:251–263
200. **Darnay BG, Haridas V, Ni J, Moore PA, Aggarwal BB** 1998 Characterization of the intracellular domain of receptor activator of NF-κB (RANK). Interaction with tumor necrosis factor receptor-associated factors and activation of NF-κB and c-Jun N-terminal kinase. J Biol Chem 273:20551–20555
201. **Wong BR, Besser D, Kim N, Arron JR, Vologodskaia M, Hanafusa H, Choi Y** 1999 TRANCE, a TNF family member, activates Akt/PKB through a signaling complex involving TRAF6 and c-Src. Mol Cell 4:1041–1049
202. **Wong BR, Josien R, Lee SY, Vologodskaia M, Steinman RM, Choi Y** 1998 The TRAF family of signal transducers mediates NF-κB activation by the TRANCE receptor. J Biol Chem 273:28355–28359
203. **Ye H, Arron JR, Lamothe B, Cirilli M, Kobayashi T, Shevde NK, Segal D, Dzivenu OK, Vologodskaia M, Yim M, Du K, Singh S, Pike JW, Darnay BG, Choi Y, Wu H** 2002 Distinct molecular mechanism for initiating TRAF6 signalling. Nature 418:443–447
204. **Lomaga MA, Yeh WC, Sarosi I, Duncan GS, Furlonger C, Ho A, Morony S, Capparelli C, Van G, Kaufman S, van der Heiden A, Itie A, Wakeham A, Khoo W, Sasaki T, Cao Z, Penninger JM, Paige CJ, Lacey DL, Dunstan CR, Boyle WJ, Goeddel DV, Mak TW** 1999 TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. Genes Dev 13:1015–1024
205. **Naito A, Azuma S, Tanaka S, Miyazaki T, Takaki S, Takatsu K, Nakao K, Nakamura K, Katsuki M, Yamamoto T, Inoue J** 1999 Severe osteopetrosis, defective interleukin-1 signalling and lymph node organogenesis in TRAF6-deficient mice. Genes Cells 4:353–362
206. **Takayanagi H** 2005 Mechanistic insight into osteoclast differentiation in osteoimmunology. J Mol Med 83:170–179
207. **Takayanagi H** 2007 Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. Nat Rev Immunol 7:292–304
208. **Wang R, Zhang L, Zhang X, Moreno J, Celluzzi C, Tondravi M, Shi Y** 2002 Regulation of activation-induced receptor activator of NF-κB ligand (RANKL) expression in T cells. Eur J Immunol 32:1090–1098
209. **Lee NK, Choi YG, Baik JY, Han SY, Jeong DW, Bae YS, Kim N, Lee SY** 2005 A crucial role for reactive oxygen species in RANKL-induced osteoclast differentiation. Blood 106:852–859
210. **Maggio D, Barabani M, Pierandrei M, Polidori MC, Catani M, Mecocci P, Senin U, Pacifici R, Cherubini A** 2003 Marked decrease in plasma antioxidants in aged osteoporotic women: results of a cross-sectional study. J Clin Endocrinol Metab 88:1523–1527
211. **Singh DK, Kumar D, Siddiqui Z, Basu SK, Kumar V, Rao KV** 2005 The strength of receptor signaling is centrally controlled through a cooperative loop between Ca2+ and an oxidant signal. Cell 121:281–293
212. **Allison JP, Krummel MF** 1995 The yin and yang of T cell costimulation. Science 270:932–933
213. **Kim N, Takami M, Rho J, Josien R, Choi Y** 2002 A novel member of the leukocyte receptor complex regulates osteoclast differentiation. J Exp Med 195:201–209
214. **Mocsai A, Humphrey MB, Van Ziffle JA, Hu Y, Burghardt A, Spusta SC, Majumdar S, Lanier LL, Lowell CA, Nakamura MC** 2004 The immunomodulatory adapter proteins DAP12 and Fc receptor γ-chain (FcRγ) regulate development of functional osteoclasts through the Syk tyrosine kinase. Proc Natl Acad Sci USA 101:6158–6163
215. **Faccio R, Teitelbaum SL, Fujikawa K, Chappel J, Zallone A, Tybulewicz VL, Ross FP, Swat W** 2005 Vav3 regulates osteoclast function and bone mass. Nat Med 11:284–290
216. **Rudd CE, Raab M** 2003 Independent CD28 signaling via VAV and SLP-76: a model for in trans costimulation. Immunol Rev 192:32–41
217. **Samelson LE** 2002 Signal transduction mediated by the T cell antigen receptor: the role of adapter proteins. Annu Rev Immunol 20:371–394
218. **Mao D, Epple H, Uthgenannt B, Novack DV, Faccio R** 2006 PLCγ2 regulates osteoclastogenesis via its interaction with ITAM proteins and GAB2. J Clin Invest 116:2869–2879
219. **Wada T, Nakashima T, Oliveira-Dos-Santos AJ, Gasser J, Hara H, Schett G, Penninger JM** 2005 The molecular scaffold Gab2 is a crucial component of RANK signaling and osteoclastogenesis. Nat Med 11:394–399
220. **Colonna M** 2003 TREMs in the immune system and beyond. Nat Rev Immunol 3:445–453
221. **Takayanagi H, Kim S, Koga T, Nishina H, Isshiki M, Yoshida H, Saiura A, Isobe M, Yokochi T, Inoue J, Wagner EF, Mak TW, Kodama T, Taniguchi T** 2002 Induction and activation of the transcription factor NFATc1 (NFAT2) integrate RANKL signaling in terminal differentiation of osteoclasts. Dev Cell 3:889–901
222. **Crabtree GR** 1999 Generic signals and specific outcomes: signaling through Ca2+, calcineurin, and NF-AT. Cell 96:611–614
223. **Asagiri M, Sato K, Usami T, Ochi S, Nishina H, Yoshida H, Morita I, Wagner EF, Mak TW, Serfling E, Takayanagi H** 2005 Autoamplification of NFATc1 expression determines its essential role in bone homeostasis. J Exp Med 202:1261–1269
224. **Sato K, Suematsu A, Nakashima T, Takemoto-Kimura S, Aoki K, Morishita Y, Asahara H, Ohya K, Yamaguchi A, Takai T, Kodama T, Chatila TA, Bito H, Takayanagi H** 2006 Regulation of osteoclast differentiation and function by the CaMK-CREB pathway. Nat Med 12:1410–1416
225. **Roth P, Stanley ER** 1992 The biology of CSF-1 and its receptor. Curr Top Microbiol Immunol 181:141–167
226. **Felix R, Cecchini MG, Hofstetter W, Elford PR, Stutzer A, Fleisch H** 1990 Impairment of macrophage colony-stimulating factor production and lack of resident bone marrow macrophages in the osteopetrotic op/op mouse. J Bone Miner Res 5:781–789
227. **Wiktor-Jedrzejczak W, Bartocci A, Ferrante AW, Jr., Ahmed-Ansari A, Sell KW, Pollard JW, Stanley ER** 1990 Total absence of colony-stimulating factor 1 in the macrophage-deficient osteopetrotic (op/op) mouse. Proc Natl Acad Sci USA 87:4828–4832
228. **Yoshida H, Hayashi S, Kunisada T, Ogawa M, Nishikawa S, Okamura H, Sudo T, Shultz LD, Nisikawa S** 1990 The murine mutation osteopetrosis is in the coding region of the macrophage colony stimulating factor gene. Nature 345:442–444
229. **Felix R, Cecchini MG, Fleisch H** 1990 Macrophage colony stimulating factor restores *in vivo* bone resorption in the op/op osteopetrotic mouse. Endocrinology 127:2592–2594
230. **Abboud SL, Woodruff K, Liu C, Shen V, Ghosh-Choudhury N** 2002 Rescue of the osteopetrotic defect in op/op mice by osteoblast-specific targeting of soluble colony-stimulating factor-1. Endocrinology 143:1942–1949
231. **Felix R, Fleisch H, Elford PR** 1989 Bone-resorbing cytokines enhance release of marophage colony-stimulating activity by the osteoblastic cell MC3T3- E1. Calcif Tissue Int 44:356–360
232. **Rubin J, Fan X, Thornton D, Bryant R, Biskobing D** 1996 Regulation of murine osteoblast macrophage colony-stimulating factor production by 1,25(OH)2D3. Calcif Tissue Int 59:291–294
233. **Weir EC, Horowitz MC, Baron R, Centrella M, Kacinski BM, Insogna KL** 1993 Macrophage colony-stimulating factor release and receptor expression in bone cells. J Bone Miner Res 8:1507–1518
234. **Cerretti DP, Wignall J, Anderson D, Tushinski RJ, Gallis B, Cosman D** 1990 Membrane bound forms of human macrophage colony stimulating factor (M-CSF, CSF-1). Prog Clin Biol Res 352:63–70
235. **Cerretti DP, Wignall J, Anderson D, Tushinski RJ, Gallis BM, Stya M, Gillis S, Urdal DL, Cosman D** 1988 Human macrophage-colony stimulating factor: alternative RNA and protein processing from a single gene. Mol Immunol 25:761–770
236. **Yao GQ, Sun BH, Hammond EE, Spencer EN, Horowitz MC, Insogna KL, Weir EC** 1998 The cell-surface form of colony-stimulating factor-1 is regulated by osteotropic agents and supports formation of multinucleated osteoclast-like cells. J Biol Chem 273:4119–4128
237. **Fan X, Biskobing DM, Fan D, Hofstetter W, Rubin J** 1997 Macrophage colony stimulating factor down-regulates MCSF-receptor expression and entry of progenitors into the osteoclast lineage. J Bone Miner Res 12:1387–1395
238. **Shinar DM, Sato M, Rodan GA** 1990 The effect of hemopoietic growth factors on the generation of osteoclast-like cells in mouse bone marrow cultures. Endocrinology 126:1728–1735
239. **Fuller K, Owens JM, Jagger CJ, Wilson A, Moss R, Chambers TJ** 1993 Macrophage colony-stimulating factor stimulates survival

and chemotactic behavior in isolated osteoclasts. J Exp Med 178: 1733–1744
240. **Jimi E, Shuto T, Koga T** 1995 Macrophage colony-stimulating factor and interleukin-1α maintain the survival of osteoclast-like cells. Endocrinology 136:808–811
241. **Lagasse E, Weissman IL** 1997 Enforced expression of Bcl-2 in monocytes rescues macrophages and partially reverses osteopetrosis in op/op mice. Cell 89:1021–1031
242. **Bouyer P, Sakai H, Itokawa T, Kawano T, Fulton CM, Boron WF, Insogna KL** 2007 Colony-stimulating factor-1 increases osteoclast intracellular pH and promotes survival via the electroneutral Na/HCO3 cotransporter NBCn1. Endocrinology 148:831–840
243. **Lorenzo JA, Sousa SL, Fonseca JM, Hock JM, Medlock ES** 1987 Colony-stimulating factors regulate the development of multinucleated osteoclasts from recently replicated cells in vitro. J Clin Invest 80:160–164
244. **MacDonald BR, Mundy GR, Clark S, Wang EA, Kuehl TJ, Stanley ER, Roodman GD** 1986 Effects of human recombinant CSF-GM and highly purified CSF-1 on the formation of multinucleated cells with osteoclast characteristics in long-term bone marrow cultures. J Bone Miner Res 1:227–233
245. **Khapli SM, Mangashetti LS, Yogesha SD, Wani MR** 2003 IL-3 acts directly on osteoclast precursors and irreversibly inhibits receptor activator of NF-κB ligand-induced osteoclast differentiation by diverting the cells to macrophage lineage. J Immunol 171:142–151
246. **Udagawa N, Horwood NJ, Elliott J, Mackay A, Owens J, Okamura H, Kurimoto M, Chambers TJ, Martin TJ, Gillespie MT** 1997 Interleukin-18 (interferon-γ-inducing factor) is produced by osteoblasts and acts via granulocyte/macrophage colony-stimulating factor and not via interferon-γ to inhibit osteoclast formation. J Exp Med 185:1005–1012
247. **Kurihara N, Chenu C, Miller M, Civin C, Roodman GD** 1990 Identification of committed mononuclear precursors for osteoclast-like cells formed in long term human marrow cultures. Endocrinology 126:2733–2741
248. **Takahashi N, Udagawa N, Akatsu T, Tanaka H, Shionome M, Suda T** 1991 Role of colony-stimulating factors in osteoclast development. J Bone Miner Res 6:977–985
249. **Yogesha SD, Khapli SM, Wani MR** 2005 Interleukin-3 and granulocyte-macrophage colony-stimulating factor inhibits tumor necrosis factor (TNF)-α-induced osteoclast differentiation by down-regulation of expression of TNF receptors 1 and 2. J Biol Chem 280:11759–11769
250. **Ehrlich LA, Chung HY, Ghobrial I, Choi SJ, Morandi F, Colla S, Rizzoli V, Roodman GD, Giuliani N** 2005 IL-3 is a potential inhibitor of osteoblast differentiation in multiple myeloma. Blood 106:1407–1414
251. **Soshi S, Takahashi HE, Tanizawa T, Endo N, Fujimoto R, Murota K** 1996 Effect of recombinant human granulocyte colony-stimulating factor (rh G-CSF) on rat bone: inhibition of bone formation at the endosteal surface of vertebra and tibia. Calcif Tissue Int 58: 337–340
252. **Takamatsu Y, Simmons PJ, Moore RJ, Morris HA, To LB, Levesque JP** 1998 Osteoclast-mediated bone resorption is stimulated during short-term administration of granulocyte colony-stimulating factor but is not responsible for hematopoietic progenitor cell mobilization. Blood 92:3465–3473
253. **Lapidot T, Petit I** 2002 Current understanding of stem cell mobilization: the roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells. Exp Hematol 30:973–981
254. **Purton LE, Lee MY, Torok-Storb B** 1996 Normal human peripheral blood mononuclear cells mobilized with granulocyte colony-stimulating factor have increased osteoclastogenic potential compared to nonmobilized blood. Blood 87:1802–1808
255. **Kuwabara H, Wada T, Oda T, Yoshikawa H, Sawada N, Kokai Y, Ishii S** 2001 Overexpression of the granulocyte colony-stimulating factor gene impairs bone morphogenetic protein responsiveness in mice. Lab Invest 81:1133–1141
256. **Oda T, Wada T, Kuwabara H, Sawada N, Yamashita T, Kokai Y** 2005 Ovariectomy fails to augment bone resorption and marrow B lymphopoiesis in granulocyte colony-stimulating factor transgenic mice. J Orthop Sci 10:70–76
257. **Dinarello CA** 1991 Interleukin-1 and interleukin-1 antagonism. Blood 77:1627–1652
258. **Lorenzo JA, Sousa S, Alander C, Raisz LG, Dinarello CA** 1987 Comparison of the bone-resorbing activity in the supernatants from phytohemagglutinin-stimulated human peripheral blood mononuclear cells with that of cytokines through the use of an antiserum to interleukin 1. Endocrinology 121:1164–1170
259. **Sabatini M, Boyce B, Aufdemorte T, Bonewald L, Mundy GR** 1988 Infusions of recombinant human interleukins 1 α and 1 β cause hypercalcemia in normal mice. Proc Natl Acad Sci USA 85:5235–5239
260. **Jimi E, Nakamura I, Duong LT, Ikebe T, Takahashi N, Rodan GA, Suda T** 1999 Interleukin 1 induces multinucleation and bone-resorbing activity of osteoclasts in the absence of osteoblasts/stromal cells. Exp Cell Res 247:84–93
261. **Hofbauer LC, Lacey DL, Dunstan CR, Spelsberg TC, Riggs BL, Khosla S** 1999 Interleukin-1β and tumor necrosis factor-α, but not interleukin-6, stimulate osteoprotegerin ligand gene expression in human osteoblastic cells. Bone 25:255–259
262. **Lee SK, Kalinowski J, Jastrzebski S, Lorenzo JA** 2002 1,25 (OH)(2) Vitamin D(3)-stimulated osteoclast formation in spleen-osteoblast cocultures is mediated in part by enhanced IL-1α and receptor activator of NF-κB ligand production in osteoblasts. J Immunol 169:2374–2380
263. **Nakamura I, Jimi E** 2006 Regulation of osteoclast differentiation and function by interleukin-1. Vitam Horm 74:357–370
264. **Sato K, Fujii Y, Asano S, Ohtsuki T, Kawakami M, Kasono K, Tsushima T, Shizume K** 1986 Recombinant human interleukin 1 α and β stimulate mouse osteoblast-like cells (MC3T3–E1) to produce macrophage-colony stimulating activity and prostaglandin E2. Biochem Biophy Res Comm 141:285–291
265. **Klein DC, Raisz LG** 1970 Prostaglandins: stimulation of bone resorption in tissue culture. Endocrinology 86:1436–1440
266. **Ma T, Miyanishi K, Suen A, Epstein NJ, Tomita T, Smith RL, Goodman SB** 2004 Human interleukin-1-induced murine osteoclastogenesis is dependent on RANKL, but independent of TNF-α. Cytokine 26:138–144
267. **Lorenzo JA, Sousa SL, Van Den Brink-Webb SE, Korn JH** 1990 Production of both interleukin-1 α and β by newborn mouse calvaria cultures. J Bone Miner Res 5:77–83
268. **Kawaguchi H, Pilbeam CC, Vargas SJ, Morse EE, Lorenzo JA, Raisz LG** 1995 Ovariectomy enhances and estrogen replacement inhibits the activity of bone marrow factors that stimulate prostaglandin production in cultured mouse calvaria. J Clin Invest 96: 539–548
269. **Miyaura C, Kusano K, Masuzawa T, Chaki O, Onoe Y, Aoyagi M, Sasaki T, Tamura T, Koishihara Y, Ohsugi Y, Suda T** 1995 Endogenous bone-resorbing factors in estrogen deficiency: cooperative effects of IL-1 and IL-6. J Bone Miner Res 10:1365–1363
270. **Arend WP, Welgus HG, Thompson C, Eisenberg SP** 1990 Biological properties of recombinant human monocyte-derived interleukin 1 receptor antagonist. J Clin Invest 85:1694–1697
271. **Eisenberg SP, Evans RJ, Arend WP, Verderber E, Brewer MT, Hannum CH, Thompson RC** 1990 Primary structure and functional expression from complementary DNA of a human inteìeukin-1 receptor antagonist. Nature 343:341–346
272. **Hannum CH, Wilcox CJ, Arend WP, Joslin FG, Dripps DJ, Heimdal PL, Armes LG, Sommer A, Eisenberg SP, Thompson RC** 1990 Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. Nature 343:336–340
273. **Dinarello CA** 1993 Blocking interleukin-1 in disease. Blood Purif 11:118–127
274. **Sims JE, Gayle MA, Slack JL, Alderson MR, Bird TA, Giri JG, Colotta F, Re F, Mantovani A, Shanebeck K, Grabstein KH, Dower SK** 1993 Interleukin 1 signaling occurs exclusively via the type I receptor. Proc Natl Acad Sci USA 90:6155–6159
275. **Huang J, Gao X, Li S, Cao Z** 1997 Recruitment of IRAK to the interleukin 1 receptor complex requires interleukin 1 receptor accessory protein. Proc Natl Acad Sci USA 94:12829–12832
276. **Korherr C, Hofmeister R, Wesche H, Falk W** 1997 A critical role for interleukin-1 receptor accessory protein in interleukin-1 signaling. Eur J Immunol 27:262–267
277. **Wesche H, Korherr C, Kracht M, Falk W, Resch K, Martin MU**

1997 The interleukin-1 receptor accessory protein (IL-1RAcP) is essential for IL-1-induced activation of interleukin-1 receptor-associated kinase (IRAK) and stress-activated protein kinases (SAP kinases). J Biol Chem 272:7727–7731
278. **Eder J** 1997 Tumour necrosis factor α and interleukin 1 signalling: do MAPKK kinases connect it all? Trends Pharmacol Sci 18:319–322
279. **Martin MU, Falk W** 1997 The interleukin-1 receptor complex and interleukin-1 signal transduction. Eur Cytokine Netw 8:5–17
280. **Colotta F, Re F, Muzio M, Bertini R, Polentarutti N, Sironi M, Giri JG, Dower SK, Sims JE, Mantovani A** 1993 Interleukin-1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. Science 261:472–475
281. **Bajayo A, Goshen I, Feldman S, Csernus V, Iverfeldt K, Shohami E, Yirmiya R, Bab I** 2005 Central IL-1 receptor signaling regulates bone growth and mass. Proc Natl Acad Sci USA 102:12956–12961
282. **Vargas SJ, Naprta A, Glaccum M, Lee SK, Kalinowski J, Lorenzo JA** 1996 Interleukin-6 expression and histomorphometry of bones from mice deficient for receptors for interleukin-1 or tumor necrosis factor. J Bone Miner Res 11:1736–1740
283. **Sato N, Takahashi N, Suda K, Nakamura M, Yamaki M, Ninomiya T, Kobayashi Y, Takada H, Shibata K, Yamamoto M, Takeda K, Akira S, Noguchi T, Udagawa N** 2004 MyD88 but not TRIF is essential for osteoclastogenesis induced by lipopolysaccharide, diacyl lipopeptide, and IL-1α. J Exp Med 200:601–611
284. **Lee ZH, Lee SE, Kim CW, Lee SH, Kim SW, Kwack K, Walsh K, Kim HH** 2002 IL-1α stimulation of osteoclast survival through the PI 3-kinase/Akt and ERK pathways. J Biochem 131:161–166
285. **Beutler B, Cerami A** 1986 Cachectin and tumour necrosis factor as two sides of the same biological coin. Nature 320:584–588
286. **Beutler B, Cerami A** 1987 Cachectin: more than a tumor necrosis factor. N Engl J Med 316:379–385
287. **Old LJ** 1985 Tumor necrosis factor (TNF). Science 230:630–632
288. **Oliff A** 1988 The role of tumor necrosis factor (cachectin) in cachexia. Cell 54:141–142
289. **Paul NL, Ruddle NH** 1988 Lymphotoxin. Ann Rev Immunol 6:407–438
290. **Bertolini DR, Nedwin GE, Bringman TS, Smith DD, Mundy GR** 1986 Stimulation of bone resorption and inhibition of bone formation in vitro by human tumour necrosis factors. Nature 319:516–518
291. **Tashjian Jr AH, Voelkel EF, Lazzaro M, Goad D, Bosma T, Levine L** 1987 Tumor necrosis factor-α (Cachectin) stimulates bone resorption in mouse calvaria via a prostaglandin-mediated mechanism. Endocrinology 120:2029–2036
292. **Stashenko P, Dewhirst FE, Peros WJ, Kent RL, Ago JM** 1987 Synergistic interactions between interleukin 1, tumor necrosis factor, and lymphotoxin in bone resorption. J Immunol 138:1464–1468
293. **Wei S, Kitaura H, Zhou P, Ross FP, Teitelbaum SL** 2005 IL-1 mediates TNF-induced osteoclastogenesis. J Clin Invest 115:282–290
294. **Kitaura H, Zhou P, Kim HJ, Novack DV, Ross FP, Teitelbaum SL** 2005 M-CSF mediates TNF-induced inflammatory osteolysis. J Clin Invest 115:3418–3427
295. **Azuma Y, Kaji K, Katogi R, Takeshita S, Kudo A** 2000 Tumor necrosis factor-α induces differentiation of and bone resorption by osteoclasts. J Biol Chem 275:4858–4864
296. **Fiers W, Sim E** 1993 Tumor necrosis factor. In: Sim, E, ed. The natural immune system: humoral factors. Oxford, UK: IRL Press at Oxford University Press; 65
297. **Erickson SL, de Sauvage FJ, Kikly K, Carver-Moore K, Pitts-Meek S, Gillett N, Sheehan KC, Schreiber RD, Goeddel DV, Moore MW** 1994 Decreased sensitivity to tumour-necrosis factor but normal T-cell development in TNF receptor-2-deficient mice. Nature 372:560–563 §bauto1§aauto1
298. **Pfeffer K, Matsuyama T, Kündig TM, Wakeham A, Kishihara K, Shahinian A, Wiegmann K, Ohashi PS, Krönke M, Mak TW** 1993 Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to *L. monocytogenes* infection. Cell 73:457–467
299. **Rothe J, Lesslauer W, Lotscher H, Lang Y, Koebel P, Kontgen F, Althage A, Zinkernagel R, Steinmetz M, Bluethmann H** 1993 Mice lacking the tumour necrosis factor receptor 1 are resistant to TNF-mediated toxicity but highly susceptible to infection by *Listeria monocytogenes*. Nature 364:798–802
300. **Yao Z, Li P, Zhang Q, Schwarz EM, Keng P, Arbini A, Boyce BF, Xing L** 2006 Tumor necrosis factor-α increases circulating osteoclast precursor numbers by promoting their proliferation and differentiation in the bone marrow through up-regulation of c-Fms expression. J Biol Chem 281:11846–11855
301. **Ochi S, Shinohara M, Sato K, Gober HJ, Koga T, Kodama T, Takai T, Miyasaka N, Takayanagi H** 2007 Pathological role of osteoclast costimulation in arthritis-induced bone loss. Proc Natl Acad Sci USA 104:11394–11399
302. **Wesche DE, Lomas-Neira JL, Perl M, Chung CS, Ayala A** 2005 Leukocyte apoptosis and its significance in sepsis and shock. J Leukoc Biol 78:325–337
303. **Kovacic N, Lukic IK, Grcevic D, Katavic V, Croucher P, Marusic A** 2007 The Fas/Fas ligand system inhibits differentiation of murine osteoblasts but has a limited role in osteoblast and osteoclast apoptosis. J Immunol 178:3379–3389
304. **Park H, Jung YK, Park OJ, Lee YJ, Choi JY, Choi Y** 2005 Interaction of Fas ligand and Fas expressed on osteoclast precursors increases osteoclastogenesis. J Immunol 175:7193–7201
305. **Wu X, McKenna MA, Feng X, Nagy TR, McDonald JM** 2003 Osteoclast apoptosis: the role of Fas *in vivo* and *in vitro*. Endocrinology 144:5545–5555
306. **Wu X, Pan G, McKenna MA, Zayzafoon M, Xiong WC, McDonald JM** 2005 RANKL regulates Fas expression and Fas-mediated apoptosis in osteoclasts. J Bone Miner Res 20:107–116
307. **Katavic V, Lukic IK, Kovacic N, Grcevic D, Lorenzo JA, Marusic A** 2003 Increased bone mass is a part of the generalized lymphoproliferative disorder phenotype in the mouse. J Immunol 170:1540–1547
308. **Nakamura T, Imai Y, Matsumoto T, Sato S, Takeuchi K, Igarashi K, Harada Y, Azuma Y, Krust A, Yamamoto Y, Nishina H, Takeda S, Takayanagi H, Metzger D, Kanno J, Takaoka K, Martin TJ, Chambon P, Kato S** 2007 Estrogen prevents bone loss via estrogen receptor α and induction of Fas ligand in osteoclasts. Cell 130:811–823
309. **Roux S, Lambert-Comeau P, Saint-Pierre C, Lepine M, Sawan B, Parent JL** 2005 Death receptors, Fas and TRAIL receptors, are involved in human osteoclast apoptosis. Biochem Biophys Res Commun 333:42–50
310. **Colucci S, Brunetti G, Cantatore FP, Oranger A, Mori G, Pignataro P, Tamma R, Grassi FR, Zallone A, Grano M** 2007 The death receptor DR5 is involved in TRAIL-mediated human osteoclast apoptosis. Apoptosis 12:1623–1632
311. **Zauli G, Rimondi E, Stea S, Baruffaldi F, Stebel M, Zerbinati C, Corallini F, Secchiero P** 2007 TRAIL inhibits osteoclastic differentiation by counteracting RANKL-dependent p27(Kip1) accumulation in pre-osteoclast precursors. J Cell Physiol 214:117–125
312. **Tinhofer I, Biedermann R, Krismer M, Crazzolara R, Greil R** 2006 A role of TRAIL in killing osteoblasts by myeloma cells. FASEB J 20:759–761
313. **Loskog A, Totterman TH** 2007 CD40L—a multipotent molecule for tumor therapy. Endocr Metab Immune Disord Drug Targets 7:23–28
314. **Lopez-Granados E, Temmerman ST, Wu L, Reynolds JC, Follmann D, Liu S, Nelson DL, Rauch F, Jain A** 2007 Osteopenia in X-linked hyper-IgM syndrome reveals a regulatory role for CD40 ligand in osteoclastogenesis. Proc Natl Acad Sci USA 104:5056–5061
315. **Lee HY, Jeon HS, Song EK, Han MK, Park SI, Lee SI, Yun HJ, Kim JR, Kim JS, Lee YC, Kim SI, Kim HR, Choi JY, Kang I, Kim HY, Yoo WH** 2006 CD40 ligation of rheumatoid synovial fibroblasts regulates RANKL-medicated osteoclastogenesis: evidence of NF-κB-dependent, CD40-mediated bone destruction in rheumatoid arthritis. Arthritis Rheum 54:1747–1758
316. **Akira S, Hirano T, Taga T, Kishimoto T** 1990 Biology of multifunctional cytokines: IL-6 and related molecules (IL-1 and TNF). FASEB J 4:2860–2867
317. **Hirano T, Akira S, Taga T, Kishimoto T** 1990 Biological and clinical aspects of interleukin 6. Immunol Today 11:443–449
318. **Feyen JH, Elford P, Di Padova FE, Trechsel U** 1989 Interleukin-6 is produced by bone and modulated by parathyroid hormone. J Bone Miner Res 4:633–638
319. **Lowik CW, Van der PG, Bloys H, Hoekman K, Bijvoet OL, Aar-**

den LA, Papapoulos SE 1989 Parathyroid hormone (PTH) and PTH-like protein (PLP) stimulate interleukin-6 production by osteogenic cells: a possible role of interleukin-6 in osteoclastogeneis. Biochem Biophys Res Commun 162:1546–1552
320. **Girasole G, Jilka RL, Passeri G, Boswell S, Boder G, Williams DC, Manolagas SC** 1992 17α-Estradiol inhibits interleukin-6 production by bone marrow- derived stromal cells and osteoblasts in vitro: a potential mechanism for the antiosteoporotic effect of estrogens. J Clin Invest 89:883–891
321. **Kishimoto T, Taga T, Akira S** 1994 Cytokine signal transduction. Cell 76:253–262
322. **Tamura T, Udagawa N, Takahashi N, Miyaura C, Tanaka S, Yamada Y, Koishihara Y, Ohsugi Y, Kumaki K, Taga T, Kishimoto T, Suda T** 1993 Soluble interleukin-6 receptor triggers osteoclast formation by interleukin 6. Proc Natl Acad Sci USA 90:11924–11928
323. **Franchimont N, Lambert C, Huynen P, Ribbens C, Relic B, Chariot A, Bours V, Piette J, Merville MP, Malaise M** 2005 Interleukin-6 receptor shedding is enhanced by interleukin-1β and tumor necrosis factor α and is partially mediated by tumor necrosis factor α-converting enzyme in osteoblast-like cells. Arthritis Rheum 52: 84–93
324. **Al-Humidan A, Ralston SH, Hughes DE, Chapman K, Aarden L, Russell RGG, Gowen M** 1991 Interleukin-6 does not stimulate bone resorption in neonatal mouse calvariae. J Bone Miner Res 6:3–7
325. **Ishimi Y, Miyaura C, Jin CH, Akatsu T, Abe E, Nakamura Y, Yamaguchi A, Yoshiki S, Matsuda T, Hirano T, Kishimoto T, Suda T** 1990 IL-6 is produced by osteoblasts and induces bone resorption. J Immunol 145:3297–3303
326. **Linkhart TA, Linkhart SG, MacCharles DC, Long DL, Strong DD** 1991 Interleukin-6 messenger RNA expression and interleukin-6 protein secretion in cells isolated from normal human bone: regulation by interleukin-1. J Bone Miner Res 6:1285–1294
327. **Manolagas SC, Jilka RL** 1995 Mechanisms of disease: bone marrow, cytokines, and bone remodeling—emerging insights into the pathophysiology of osteoporosis. N Engl J Med332:305–311
328. **Roodman GD** 1992 Interleukin-6: an osteotropic factor? J Bone Miner Res 7:475–478
329. **Palmqvist P, Persson E, Conaway HH, Lerner UH** 2002 IL-6, Leukemia inhibitory factor, and oncostatin M stimulate bone resorption and regulate the expression of receptor activator of NF-κB ligand, osteoprotegerin, and receptor activator of NF-κB in mouse calvariae. J Immunol 169:3353–3362
330. **Liu XH, Kirschenbaum A, Yao S, Levine AC** 2005 Cross-talk between the interleukin-6 and prostaglandin E(2) signaling systems results in enhancement of osteoclastogenesis through effects on the osteoprotegerin/receptor activator of nuclear factor-κB (RANK) ligand/RANK system. Endocrinology 146:1991–1998
331. **Kudo O, Sabokbar A, Pocock A, Itonaga I, Fujikawa Y, Athanasou NA** 2003 Interleukin-6 and interleukin-11 support human osteoclast formation by a RANKL-independent mechanism. Bone 32: 1–7
332. **Guise TA, Mundy GR** 1998 Cancer and bone. Endocr Rev 19:18–54
333. **Yamamoto T, Ozono K, Kasayama S, Yoh K, Hiroshima K, Takagi M, Matsumoto S, Michigami T, Yamaoka K, Kishimoto T, Okada S** 1996 Increased IL-6-production by cells isolated from the fibrous bone dysplasia tissues in patients with McCune-Albright syndrome. J Clin Invest 98:30–35
334. **Reddy SV, Takahashi S, Dallas M, Williams RE, Neckers L, Roodman GD** 1994 Interleukin-6 antisense deoxyoligonucleotides inhibit bone resorption by giant cells from human giant cell tumors of bone. J Bone Miner Res 9:753–757
335. **Devlin RD, Bone III HG, Roodman GD** 1996 Interleukin-6: a potential mediator of the massive osteolysis in patients with Gorham-Stout disease. J Clin Endocrinol Metab 81:1893–1897
336. **Grey A, Mitnick MA, Masiukiewicz U, Sun BH, Rudikoff S, Jilka RL, Manolagas SC, Insogna K** 1999 A role for interleukin-6 in parathyroid hormone-induced bone resorption *in vivo*. Endocrinology 140:4683–4690
337. **O'Brien CA, Jilka RL, Fu Q, Stewart S, Weinstein RS, Manolagas SC** 2005 IL-6 is not required for parathyroid hormone stimulation of RANKL expression, osteoclast formation, and bone loss in mice. Am J Physiol Endocrinol Metab 289:E784—E793
338. **Manolagas SC, Bellido T, Jilka RL** 1995 New insights into the cellular, biochemical, and molecular basis of postmenopausal and senile osteoporosis: roles of IL-6 and gp130. [Review] Int J Immunopharmacol 17:109–116
339. **Romas E, Udagawa N, Zhou H, Tamura T, Saito M, Taga T, Hilton DJ, Suda T, Ng KW, Martin TJ** 1996 The role of gp130-mediated signals in osteoclast development: regulation of interleukin 11 production by osteoblasts and distribution of its receptor in bone marrow cultures. J Exp Med 183:2581–2591
340. **Elias JA, Tang W, Horowitz MC** 1995 Cytokine and hormonal stimulation of human osteosarcoma interleukin-11 production. Endocrinology 136:489–498
341. **Girasole G, Passeri G, Jilka RL, Manolagas SC** 1994 Interleukin-11: a new cytokine critical for osteoclast development. J Clin Invest 93:1516–1524
342. **Hill PA, Tumber A, Papaioannou S, Meikle MC** 1998 The cellular actions of interleukin-11 on bone resorption *in vitro*. Endocrinology 139:1564–1572
343. **Morinaga Y, Fujita N, Ohishi K, Zhang Y, Tsuruo T** 1998 Suppression of interleukin-11-mediated bone resorption by cyclooxygenases inhibitors. J Cell Physiol 175:247–254
344. **Sims NA, Jenkins BJ, Nakamura A, Quinn JM, Li R, Gillespie MT, Ernst M, Robb L, Martin TJ** 2005 Interleukin-11 receptor signaling is required for normal bone remodeling. J Bone Miner Res 20:1093–1102
345. **Greenfield EM, Horowitz MC, Lavish SA** 1996 Stimulation by parathyroid hormone of interleukin-6 and leukemia inhibitory factor expression in osteoblasts is an immediate-early gene response induced by cAMP signal transduction. J Biol Chem 271:10984–10989
346. **Shiina-Ishimi Y, Abe E, Tanaka H, Suda T** 1986 Synthesis of colony-stimulating factor (CSF) and differentiation inducing factor (D-factor) by osteoblastic cells, clone MC3T3–E1. Biochem Biophys Res Comm 134:400–406
347. **Reid IR, Lowe C, Cornish J, Skinner SJ, Hilton DJ, Willson TA, Gearing DP, Martin TJ** 1990 Leukemia inhibitory factor: a novel bone-active cytokine. Endocinology 126:1416–1420
348. **Lorenzo JA, Sousa SL, Leahy CL** 1990 Leukemia inhibitory factor (LIF) inhibits basal bone resorption in fetal rat long bone cultures. Cytokine 2:266–271
349. **Van Beek E, Van der Wee-Pals L, van de Ruit M, Nijweide P, Papapoulos S, Lowik C** 1993 Leukemia inhibitory factor inhibits osteoclastic resorption, growth, mineralization, and alkaline phosphatase activity in fetal mouse metacarpal bones in culture. J Bone Miner Res 8:191–198
350. **Cornish J, Callon K, King A, Edgar S, Reid IR** 1993 The effect of leukemia inhibitory factor on bone *in vivo*. Endocrinology 132: 1359–1366
351. **Ware CB, Horowitz MC, Renshaw BR, Hunt JS, Liggitt D, Koblar SA, Gliniak BC, McKenna HJ, Papayannopoulou T, Thoma B, Cheng L, Donovan PJ, Peschon JJ, Bartlett PF, Willis CR, Wright BD, Carpenter MK, Davison BL, Gearing DP** 1995 Targeted disruption of the low-affinity leukemia inhibitory factor receptor gene causes placental, skeletal, neural and metabolic defects and results in perinatal death. Development 121:1283–1299
352. **Heymann D, Guicheux J, Gouin F, Cottrel M, Daculsi G** 1998 Oncostatin M stimulates macrophage-polykaryon formation in long-term human bone-marrow cultures. Cytokine 10:98–109
353. **Jay PR, Centrella M, Lorenzo J, Bruce AG, Horowitz MC** 1996 Oncostatin-M: A new bone active cytokine that activates osteoblasts and inhibits bone resorption. Endocrinology 137:1151–1158
354. **Malik N, Haugen HS, Modrell B, Shoyab M, Clegg CH** 1995 Developmental abnormalities in mice transgenic for bovine oncostatin M. Mol Cell Biol 15:2349–2358
355. **Mundy GR** 1996 An OAF by any other name [editorial; comment]. Endocrinology 137:1149–1150
356. **Kawasaki K, Gao YH, Yokose S, Kaji Y, Nakamura T, Suda T, Yoshida K, Taga T, Kishimoto T, Kataoka H, Yuasa T, Norimatsu H, Yamaguchi A** 1997 Osteoclasts are present in gp130-deficient mice. Endocrinology 138:4959–4965
357. **Namen AE, Lupton S, Hjerrild K, Wignall J, Mochizuki DY, Schmierer A, Mosley B, March CJ, Urdal D, Gillis S, Cosman D,**

Goodwin RG 1988 Stimulation of B-cell progenitors by cloned murine interleukin-7. Nature 333:571–573
358. **Miyaura C, Onoe Y, Inada M, Maki K, Ikuta K, Ito M, Suda T** 1997 Increased B-lymphopoiesis by interleukin 7 induces bone loss in mice with intact ovarian function: similarity to estrogen deficiency. Proc Natl Acad Sci USA 94:9360–9365
359. **Weitzmann MN, Roggia C, Toraldo G, Weitzmann L, Pacifici R** 2002 Increased production of IL-7 uncouples bone formation from bone resorption during estrogen deficiency. J Clin Invest 110:1643–1650
360. **Weitzmann MN, Cenci S, Rifas L, Brown C, Pacifici R** 2000 Interleukin-7 stimulates osteoclast formation by up-regulating the T-cell production of soluble osteoclastogenic cytokines. Blood 96: 1873–1878
361. **Toraldo G, Roggia C, Qian WP, Pacifici R, Weitzmann MN** 2003 IL-7 induces bone loss in vivo by induction of receptor activator of nuclear factor κB ligand and tumor necrosis factor α from T cells. Proc Natl Acad Sci USA 100:125–130
362. **Ryan MR, Shepherd R, Leavey JK, Gao YH, Grassi F, Schnell FJ, Qian WP, Kersh GJ, Weitzmann MN, Pacifici R** 2005 An IL-7-dependent rebound in thymic T cell output contributes to the bone loss induced by estrogen deficiency. Proc Natl Acad Sci USA 102: 16735–16740
363. **Lee SK, Kalinowski JF, Jastrzebski SL, Puddington L, Lorenzo JA** 2003 Interleukin-7 is a direct inhibitor of *in vitro* osteoclastogenesis. Endocrinology 144:3524–3531
364. **Lee SK, Kalinowski JF, Jacquin C, Adams DJ, Gronowicz G, Lorenzo JA** 2006 Interleukin-7 influences osteoclast function in vivo but is not a critical factor in ovariectomy-induced bone loss. J Bone Miner Res 21:695–702
365. **Sato T, Watanabe K, Masuhara M, Hada N, Hakeda Y** 2007 Production of IL-7 is increased in ovariectomized mice, but not RANKL mRNA expression by osteoblasts/stromal cells in bone, and IL-7 enhances generation of osteoclast precursors in vitro. J Bone Miner Res 25:19–27
366. **Lee S, Kalinowski JF, Adams DJ, Aguila HL, Lorenzo JA** 2004 Osteoblast specific overexpression of human interleukin-7 increases femoral trabecular bone mass in female mice and inhibits in vitro osteoclastogenesis. J Bone Miner Res 19:S410
367. **Lee S, Kalinowski J, Adams DJ, Aguila HL, Lorenzo, JA** 2005 Osteoblast specific overexpression of human interleukin-7 rescues the bone phenotype of interleukin-7 deficient female mice. J Bone Miner Res 20:S48
368. **Butcher EC, Williams M, Youngman K, Rott L, Briskin M** 1999 Lymphocyte trafficking and regional immunity. Adv Immunol 72:209–253
369. **Campbell JJ, Butcher EC** 2000 Chemokines in tissue-specific and microenvironment-specific lymphocyte homing. Curr Opin Immunol 12:336–341
370. **Baggiolini M** 2001 Chemokines in pathology and medicine. J Intern Med 250:91–104
371. **Horuk R** 2001 Chemokine receptors. Cytokine Growth Factor Rev 12:313–335
372. **Rothe L, Collin-Osdoby P, Chen Y, Sunyer T, Chaudhary L, Tsay A, Goldring S, Avioli L, Osdoby P** 1998 Human osteoclasts and osteoclast-like cells synthesize and release high basal and inflammatory stimulated levels of the potent chemokine interleukin-8. Endocrinology 139:4353–4363
373. **Bendre MS, Gaddy-Kurten D, Mon-Foote T, Akel NS, Skinner RA, Nicholas RW, Suva LJ** 2002 Expression of interleukin 8 and not parathyroid hormone-related protein by human breast cancer cells correlates with bone metastasis in vivo. Cancer Res 62:5571–5579
374. **Bendre MS, Margulies AG, Walser B, Akel NS, Bhattacharrya S, Skinner RA, Swain F, Ramani V, Mohammad KS, Wessner LL, Martinez A, Guise TA, Chirgwin JM, Gaddy D, Suva LJ** 2005 Tumor-derived interleukin-8 stimulates osteolysis independent of the receptor activator of nuclear factor-κB ligand pathway. Cancer Res 65:11001–11009
375. **Bendre MS, Montague DC, Peery T, Akel NS, Gaddy D, Suva LJ** 2003 Interleukin-8 stimulation of osteoclastogenesis and bone resorption is a mechanism for the increased osteolysis of metastatic bone disease. Bone 33:28–37
376. **Sunyer T, Rothe L, Jiang XS, Osdoby P, Collin-Osdoby P** 1996 Proinflammatory agents, IL-8 and IL-10, upregulate inducible nitric oxide synthase expression and nitric oxide production in avian osteoclast-like cells. J Cell Biochem 60:469–483
377. **Kukita T, Nakao J, Hamada F, Kukita A, Inai T, Kurisu K, Nomiyama H** 1992 Recombinant LD78 protein, a member of the small cytokine family, enhances osteoclast differentiation in rat bone marrow culture system. Bone Miner 19:215–223
378. **Kukita T, Nomiyama H, Ohmoto Y, Kukita A, Shuto T, Hotokebuchi T, Sugioka Y, Miura R, Iijima T** 1997 Macrophage inflammatory protein-1 α (LD78) expressed in human bone marrow: its role in regulation of hematopoiesis and osteoclast recruitment. Lab Invest 76:399–406
379. **Scheven BA, Milne JS, Hunter I, Robins SP** 1999 Macrophage-inflammatory protein-1α regulates preosteoclast differentiation in vitro. Biochem Biophy Res Comm 254:773–778
380. **Watanabe T, Kukita T, Kukita A, Wada N, Toh K, Nagata K, Nomiyama H, Iijima T** 2004 Direct stimulation of osteoclastogenesis by MIP-1α: evidence obtained from studies using RAW264 cell clone highly responsive to RANKL. J Endocrinol 180:193–201
381. **Han JH, Choi SJ, Kurihara N, Koide M, Oba Y, Roodman GD** 2001 Macrophage inflammatory protein-1α is an osteoclastogenic factor in myeloma that is independent of receptor activator of nuclear factor κB ligand. Blood 97:3349–3353
382. **Abe M, Hiura K, Wilde J, Moriyama K, Hashimoto T, Ozaki S, Wakatsuki S, Kosaka M, Kido S, Inoue D, Matsumoto T** 2002 Role for macrophage inflammatory protein (MIP)-1α and MIP-1β in the development of osteolytic lesions in multiple myeloma. Blood 100: 2195–2202
383. **Choi SJ, Cruz JC, Craig F, Chung H, Devlin RD, Roodman GD, Alsina M** 2000 Macrophage inflammatory protein 1-α is a potential osteoclast stimulatory factor in multiple myeloma. Blood 96:671–675
384. **Choi SJ, Oba Y, Gazitt Y, Alsina M, Cruz J, Anderson J, Roodman GD** 2001 Antisense inhibition of macrophage inflammatory protein 1-α blocks bone destruction in a model of myeloma bone disease. J Clin Invest 108:1833–1841
385. **Choi J, Oba Y, Jelinek D, Ehrlich L, Lee W, Roodman D** 2003 Blocking CCR1 or CCR5 inhibits both osteoclast formation and increased α1-integrin expression induced by MIP-1α. Eur J Haematol 70:272–278
386. **Fuller K, Owens JM, Chambers TJ** 1995 Macrophage inflammatory protein-1α and IL-8 stimulate the motility but suppress the resorption of isolated rat osteoclasts. J Immunol 154:6065–6072
387. **Lean JM, Murphy C, Fuller K, Chambers TJ** 2002 CCL9/MIP-1γ and its receptor CCR1 are the major chemokine ligand/receptor species expressed by osteoclasts. J Cell Biochem 87:386–393
388. **Yang M, Mailhot G, MacKay CA, Mason-Savas A, Aubin J, Odgren PR** 2006 Chemokine and chemokine receptor expression during colony stimulating factor-1-induced osteoclast differentiation in the toothless osteopetrotic rat: a key role for CCL9 (MIP-1γ) in osteoclastogenesis in vivo and in vitro. Blood 107:2262–2270
389. **Okamatsu Y, Kim D, Battaglino R, Sasaki H, Spate U, Stashenko P** 2004 MIP-1γ promotes receptor activator of NF-κB ligand-induced osteoclast formation and survival. J Immunol 173:2084–2090
390. **Ishida N, Hayashi K, Hattori A, Yogo K, Kimura T, Takeya T** 2006 CCR1 acts downstream of NFAT2 in osteoclastogenesis and enhances cell migration. J Bone Miner Res 21:48–57
391. **Yu X, Huang Y, Collin-Osdoby P, Osdoby P** 2004 CCR1 chemokines promote the chemotactic recruitment, RANKL development, and motility of osteoclasts and are induced by inflammatory cytokines in osteoblasts. J Bone Miner Res 19:2065–2077
392. **Gronthos S, Zannettino AC** 2007 The role of the chemokine CXCL12 in osteoclastogenesis. Trends Endocrinol Metab 18:108–113
393. **Wright LM, Maloney W, Yu X, Kindle L, Collin-Osdoby P, Osdoby P** 2005 Stromal cell-derived factor-1 binding to its chemokine receptor CXCR4 on precursor cells promotes the chemotactic recruitment, development and survival of human osteoclasts. Bone 36:840–853
394. **Grassi F, Piacentini A, Cristino S, Toneguzzi S, Cavallo C, Facchini A, Lisignoli G** 2003 Human osteoclasts express different CXC chemokines depending on cell culture substrate: molecular and

immunocytochemical evidence of high levels of CXCL10 and CXCL12. Histochem Cell Biol 120:391–400
395. **Yu X, Huang Y, Collin-Osdoby P, Osdoby P** 2003 Stromal cell-derived factor-1 (SDF-1) recruits osteoclast precursors by inducing chemotaxis, matrix metalloproteinase-9 (MMP-9) activity, and collagen transmigration. J Bone Miner Res 18:1404–1418
396. **Liao TS, Yurgelun MB, Chang SS, Zhang HZ, Murakami K, Blaine TA, Parisien MV, Kim W, Winchester RJ, Lee FY** 2005 Recruitment of osteoclast precursors by stromal cell derived factor-1 (SDF-1) in giant cell tumor of bone. J Orthop Res 23:203–209
397. **Zannettino AC, Farrugia AN, Kortesidis A, Manavis J, To LB, Martin SK, Diamond P, Tamamura H, Lapidot T, Fujii N, Gronthos S** 2005 Elevated serum levels of stromal-derived factor-1α are associated with increased osteoclast activity and osteolytic bone disease in multiple myeloma patients. Cancer Res 65: 1700–1709
398. **Rollins BJ** 1997 Chemokines. Blood 90:909–928
399. **Rahimi P, Wang C, Stashenko P, Lee SK, Lorenzo JA, Graves DT** 1995 Monocyte chemoattractant protein-1 expression and monocyte recruitment in osseous inflammation in the mouse. Endocrinology 136:2752–2759
400. **Zhu JF, Valente AJ, Lorenzo JA, Carnes D, Graves DT** 1994 Expression of monocyte chemoattractant protein 1 in human osteoblastic cells stimulated by proinflammatory mediators. J Bone Miner Res 9:1123–1130
401. **Que BG, Wise GE** 1998 Tooth eruption molecules enhance MCP-1 gene expression in the dental follicle of the rat. Dev Dyn 212:346–351
402. **Wise GE, Huang H, Que BG** 1999 Gene expression of potential tooth eruption molecules in the dental follicle of the mouse. Eur J Oral Sci 107:482–486
403. **Wise GE, Que BG, Huang H** 1999 Synthesis and secretion of MCP-1 by dental follicle cells—implications for tooth eruption. J Dent Res 78:1677–1681
404. **Wise GE, Que BG, Huang H, Lumpkin SJ** 2000 Enhancement of gene expression in rat dental follicle cells by parathyroid hormone-related protein. Arch Oral Biol 45:903–909
405. **Bsoul S, Terezhalmy G, Abboud H, Woodruff K, Abboud SL** 2003 PDGF BB and bFGF stimulate DNA synthesis and upregulate CSF-1 and MCP-1 gene expression in dental follicle cells. Arch Oral Biol 48:459–465
406. **Graves DT, Alsulaimani F, Ding Y, Marks Jr SC** 2002 Developmentally regulated monocyte recruitment and bone resorption are modulated by functional deletion of the monocytic chemoattractant protein-1 gene. Bone 31:282–287
407. **Kim MS, Day CJ, Morrison NA** 2005 MCP-1 is induced by RANKL, promotes osteoclast fusion and rescues GM-CSF suppression of osteoclast formation. J Biol Chem 280:16163–16169
408. **Kim MS, Day CJ, Selinger CI, Magno CL, Stephens SJ, Morrison NA** 2006 MCP-1 induced human osteoclast-like cells are TRAP, NFATc1 and calcitonin receptor positive but require RANKL for bone resorption. J Biol Chem 281:1274–1285
409. **Li X, Qin L, Bergenstock M, Bevelock LM, Novack DV, Partridge NC** 2007 Parathyroid hormone stimulates osteoblastic expression of MCP-1 to recruit and increase the fusion of pre/osteoclasts. J Biochem 282:33098–33106
410. **Moore KW, de Waal MR, Coffman RL, O'Garra A** 2001 Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 19: 683–765
411. **Owens JM, Gallagher AC, Chambers TJ** 1996 IL-10 modulates formation of osteoclasts in murine hemopoietic cultures. J Immunol 157:936–940
412. **Xu LX, Kukita T, Kukita A, Otsuka T, Niho Y, Iijima T** 1995 Interleukin-10 selectively inhibits osteoclastogenesis by inhibiting differentiation of osteoclast progenitors into preosteoclast-like cells in rat bone marrow culture system. J Cell Physiol 165:624–629
413. **Van Vlasselaer P, Borremans B, Van Der Heuvel R, Van Gorp U, De Waal Malefyt R** 1993 Interleukin-10 inhibits the osteogenic activity of mouse bone marrow. Blood 82:2361–2370
414. **Hong MH, Williams H, Jin CH, Pike JW** 2000 The inhibitory effect of interleukin-10 on mouse osteoclast formation involves novel tyrosine-phosphorylated proteins. J Bone Miner Res 15:911–918
415. **Evans KE, Fox SW** 2007 Interleukin-10 inhibits osteoclastogenesis by reducing NFATc1 expression and preventing its translocation to the nucleus. BMC Cell Biol 19:4
416. **Mohamed SG, Sugiyama E, Shinoda K, Taki H, Hounoki H, Abdel-Aziz HO, Maruyama M, Kobayashi M, Ogawa H, Miyahara T** 2007 Interleukin-10 inhibits RANKL-mediated expression of NFATc1 in part via suppression of c-Fos and c-Jun in RAW264.7 cells and mouse bone marrow cells. Bone 41:592–602
417. **Carmody EE, Schwarz EM, Puzas JE, Rosier RN, O'Keefe RJ** 2002 Viral interleukin-10 gene inhibition of inflammation, osteoclastogenesis, and bone resorption in response to titanium particles. Arthritis Rheum 46:1298–1308
418. **Liu D, Yao S, Wise GE** 2006 Effect of interleukin-10 on gene expression of osteoclastogenic regulatory molecules in the rat dental follicle. Eur J Oral Sci 114:42–49
419. **Owens J, Chambers TJ** 1995 Differential regulation of osteoclast formation: interleukin 10 (cytokine synthesis inhibitory factor) suppresses formation of osteoclasts but not macrophages in murine bone marrow cultures. J Bone Miner Res 10:S220
420. **Shin HH, Lee JE, Lee EA, Kwon BS, Choi HS** 2006 Enhanced osteoclastogenesis in 4–1BB-deficient mice caused by reduced interleukin-10. J Bone Miner Res 21:1907–1912
421. **Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM** 1993 Development of TH1 CD4+ T cells through IL-12 produced by Listeria-induced macrophages. Science 260:547–549
422. **Amcheslavsky A, Bar-Shavit Z** 2006 Interleukin (IL)-12 mediates the anti-osteoclastogenic activity of CpG-oligodeoxynucleotides. J Cell Physiol 207:244–250
423. **Horwood NJ, Elliott J, Martin TJ, Gillespie MT** 2001 IL-12 alone and in synergy with IL-18 inhibits osteoclast formation in vitro. J Immunol 166:4915–4921
424. **Nagata N, Kitaura H, Yoshida N, Nakayama K** 2003 Inhibition of RANKL-induced osteoclast formation in mouse bone marrow cells by IL-12: involvement of IFN-γ possibly induced from non-T cell population. Bone 33:721–732
425. **Ogata Y, Kukita A, Kukita T, Komine M, Miyahara A, Miyazaki S, Kohashi O** 1999 A novel role of IL-15 in the development of osteoclasts: inability to replace its activity with IL-2. J Immunol 162:2754–2760
426. **Miranda-Carus ME, ito-Miguel M, Balsa A, Cobo-Ibanez T, Perez De AC, Pascual-Salcedo D, Martin-Mola E** 2006 Peripheral blood T lymphocytes from patients with early rheumatoid arthritis express RANKL and interleukin-15 on the cell surface and promote osteoclastogenesis in autologous monocytes. Arthritis Rheum 54: 1151–1164
427. **Weaver CT, Hatton RD, Mangan PR, Harrington LE** 2007 IL-17 family cytokines and the expanding diversity of effector T cell lineages. Annu Rev Immunol 25:821–852
428. **Fort MM, Cheung J, Yen D, Li J, Zurawski SM, Lo S, Menon S, Clifford T, Hunte B, Lesley R, Muchamuel T, Hurst SD, Zurawski G, Leach MW, Gorman DM, Rennick DM** 2001 IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. Immunity 15:985–995
429. **Kotake S, Udagawa N, Takahashi N, Matsuzaki K, Itoh K, Ishiyama S, Saito S, Inoue K, Kamatani N, Gillespie MT, Martin TJ, Suda T** 1999 IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. J Clin Invest 103:1345–1352
430. **Lubberts E, Joosten LAB, Chabaud M, Van den Bersselaar L, Oppers B, Coenen-de Roo CJJ, Richards CD, Miossec P, Van den Berg WB** 2000 IL-4 gene therapy for collagen arthritis suppresses synovial IL-17 and osteoprotegerin ligand and prevents bone erosion. J Clin Invest 105:1697–1710
431. **Lubberts E, Van Den BL, Oppers-Walgreen B, Schwarzenberger P, Coenen-De Roo CJ, Kolls JK, Joosten LA, Van den Berg WB** 2003 IL-17 promotes bone erosion in murine collagen-induced arthritis through loss of the receptor activator of NF-κB ligand/osteoprotegerin balance. J Immunol 170:2655–2662
432. **Van bezooijen RL, Farih-Sips HC, Papapoulos SE, Lowik CW** 1999 Interleukin-17: a new bone acting cytokine in vitro. J Bone Miner Res 14:1513–1521
433. **Koenders MI, Lubberts E, Oppers-Walgreen B, Van Den BL, Helsen MM, Di Padova FE, Boots AM, Gram H, Joosten LA, Van den Berg WB** 2005 Blocking of interleukin-17 during reactivation

of experimental arthritis prevents joint inflammation and bone erosion by decreasing RANKL and interleukin-1. Am J Pathol 167: 141–149
434. **Kastelein RA, Hunter CA, Cua DJ** 2007 Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. Annu Rev Immunol 25:221–242
435. **Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK** 2006 Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 441:235–238
436. **Sato K, Suematsu A, Okamoto K, Yamaguchi A, Morishita Y, Kadono Y, Tanaka S, Kodama T, Akira S, Iwakura Y, Cua DJ, Takayanagi H** 2006 Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. J Exp Med 203:2673–2682
437. **Orozco A, Gemmell E, Bickel M, Seymour GJ** 2007 Interleukin 18 and periodontal disease. J Dent Res 86:586–593
438. **Okamura H, Tsutsui H, Komatsu T, Yutsudo M, Hakura A, Tanimoto T, Torigoe K, Okura T, Nukada Y, Hattori K, Akita K, Namba M, Tanabe F, Konishi K, Fukuda S, Kurimoto M** 1995 Cloning of a new cytokine that induces IFN-γ production by T cells. Nature 378:88–91
439. **Yamamura M, Kawashima M, Taniai M, Yamauchi H, Tanimoto T, Kurimoto M, Morita Y, Ohmoto Y, Makino H** 2001 Interferon-γ-inducing activity of interleukin-18 in the joint with rheumatoid arthritis. Arthritis Rheum 44:275–285
440. **Horwood NJ, Udagawa N, Elliott J, Grail D, Okamura H, Kurimoto M, Dunn AR, Martin T, Gillespie MT** 1998 Interleukin 18 inhibits osteoclast formation via T cell production of granulocyte macrophage colony-stimulating factor. J Clin Invest 101:595–603
441. **Kawase Y, Hoshino T, Yokota K, Kuzuhara A, Nakamura M, Maeda Y, Nishiwaki E, Zenmyo M, Hiraoka K, Aizawa H, Yoshino K** 2003 Bone malformations in interleukin-18 transgenic mice. J Bone Miner Res 18:975–983
442. **Yamada N, Niwa S, Tsujimura T, Iwasaki T, Sugihara A, Futani H, Hayashi S, Okamura H, Akedo H, Terada N** 2002 Interleukin-18 and interleukin-12 synergistically inhibit osteoclastic bone-resorbing activity. Bone 30:901–908
443. **Makiishi-Shimobayashi C, Tsujimura T, Iwasaki T, Yamada N, Sugihara A, Okamura H, Hayashi SS, Terada N** 2001 Interleukin-18 up-regulates osteoprotegerin expression in stromal/osteoblastic cells. Biochem Biophy Res Comm 281:361–366
444. **Dai SM, Nishioka K, Yudoh K** 2004 Interleukin (IL) 18 stimulates osteoclast formation through synovial T cells in rheumatoid arthritis: comparison with IL1 β and tumour necrosis factor α. Ann Rheum Dis 63:1379–1386
445. **Cornish J, Gillespie MT, Callon KE, Horwood NJ, Moseley JM, Reid IR** 2003 Interleukin-18 is a novel mitogen of osteogenic and chondrogenic cells. Endocrinology 144:1194–1201
446. **Gowen M, Mundy GR** 1986 Actions of recombinant interleukin 1, interleukin 2, and interferon-γ on bone resorption in vitro. J Immunol 136:2478–2482
447. **Peterlik M, Hoffmann O, Swetly P, Klaushofer K, Koller K** 1985 Recombinant γ-interferon inhibits prostaglandin-mediated and parathyroid hormone-induced bone resorption in cultured neonatal mouse calvaria. FEBS Lett 185:287–290
448. **Takahashi N, Mundy GR, Roodman GD** 1986 Recombinant human interferon-γ inhibits formation of human osteoclast-like cells. J Immunol 137:3544–3549
449. **Takayanagi H, Ogasawara K, Hida S, Chiba T, Murata S, Sato K, Takaoka A, Yokochi T, Oda H, Tanaka K, Nakamura K, Taniguchi T** 2000 T-cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-γ. Nature 408:600–605
450. **Hattersley G, Dorey E, Horton MA, Chambers TJ** 1988 Human macrophage colony-stimulating factor inhibits bone resorption by osteoclasts disaggregated from rat bone. J Cell Physiol 137:199–203
451. **Gao Y, Grassi F, Ryan MR, Terauchi M, Page K, Yang X, Weitzmann MN, Pacifici R** 2007 IFN-γ stimulates osteoclast formation and bone loss in vivo via antigen-driven T cell activation. J Clin Invest 117:122–132
452. **Gowen M, MacDonald BR, Russell GG** 1988 Actions of recombinant human γ-interferon and tumor necrosis factor α on the proliferation and osteoblastic characteristics of human trabecular bone cells in vitro. Arthritis Rheum 31:1500–1507
453. **Nanes MS, McKoy WM, Marx SJ** 1989 Inhibitory effects of tumor necrosis factor-α and interferon-γ on deoxyribonucleic acid and collagen synthesis by rat osteosarcoma cells (ROS 17/2.8). Endocrinology 124:339–345
454. **Shen V, Kohler G, Jeffrey JJ, Peck WA** 1988 Bone-resorbing agents promote and interferon-γ inhibits bone cell collagenase production. J Bone Miner Res 3:657–666
455. **Smith DD, Gowen M, Mundy GR** 1987 Effects of interferon-γ and other cytokines on collagen synthesis in fetal rat bone cultures. Endocrinology 120:2494–2499
456. **Manoury-Schwartz B, Chiocchia G, Bessis N, Abehsira-Amar O, Batteux F, Muller S, Huang S, Boissier MC, Fournier C** 1997 High susceptibility to collagen-induced arthritis in mice lacking IFN-γ receptors. J Immunol 158:5501–5506
457. **Vermeire K, Heremans H, Vandeputte M, Huang S, Billiau A, Matthys P** 1997 Accelerated collagen-induced arthritis in IFN-γ receptor-deficient mice. J Immunol 158:5507–5513
458. **Mann GN, Jacobs TW, Buchinsky FJ, Armstrong EC, Li M, Ke HZ, Ma YF, Jee WSS, Epstein S** 1994 Interferon-γ causes loss of bone volume in vivo and fails to ameliorate cyclosporin A-induced osteopenia. Endocrinology 135:1077–1083
459. **Key Jr LL, Ries WL, Rodriguiz RM, Hatcher HC** 1992 Recombinant human interferon γ therapy for osteopetrosis. J Pediatr 121:119–124
460. **Key Jr LL, Rodriguiz RM, Willi SM, Wright NM, Hatcher HC, Eyre DR, Cure JK, Griffin PP, Ries WL** 1995 Long-term treatment of osteopetrosis with recombinant human interferon γ. N Engl J Med 332:1594–1599
461. **Vignery A, Niven-Fairchild T, Shepard MH** 1990 Recombinant murine interferon-γ inhibits the fusion of mouse alveolar macrophages in vitro but stimulates the formation of osteoclast-like cells on implanted syngenic bone particles in mice in vivo. J Bone Miner Res 5:637–644
462. **Schoenborn JR, Wilson CB** 2007 Regulation of interferon-γ during innate and adaptive immune responses. Adv Immunol 96:41–101
463. **Takayanagi H** 2005 Inflammatory bone destruction and osteoimmunology. J Periodontal Res 40:287–293
464. **Takayanagi H, Kim S, Matsuo K, Suzuki H, Suzuki T, Sato K, Yokochi T, Oda H, Nakamura K, Ida N, Wagner EF, Taniguchi T** 2002 RANKL maintains bone homeostasis through c-Fos-dependent induction of interferon-β. Nature 416:744–749
465. **Avnet S, Cenni E, Perut F, Granchi D, Brandi ML, Giunti A, Baldini N** 2007 Interferon-α inhibits in vitro osteoclast differentiation and renal cell carcinoma-induced angiogenesis. Int J Oncol 30:469–476
466. **Goodman GR, Dissanayake IR, Gorodetsky E, Zhou H, Ma YF, Jee WS, Epstein S** 1999 Interferon-α, unlike interferon-γ, does not cause bone loss in the rat. Bone 25:459–463
467. **Lewis DB, Liggitt HD, Effmann EL, Motley ST, Teitelbaum SL, Jepsen KJ, Goldstein SA, Bonadio J, Carpenter J, Perlmutter RM** 1993 Osteoporosis induced in mice by overproduction of interleukin 4. Proc Natl Acad Sci USA 90:11618–11622
468. **Nakano Y, Watanabe K, Morimoto I, Okada Y, Ura K, Sato K, Kasono K, Nakamura T, Eto S** 1994 Interleukin-4 inhibits spontaneous and parathyroid hormone-related protein-stimulated osteoclast formation in mice. J Bone Miner Res 9:1533–1539
469. **Shioi A, Teitelbaum SL, Ross FP, Welgus HG, Suzuki H, Ohara J, Lacey DL** 1991 Interleukin 4 inhibits murine osteoclast formation in vitro. J Cell Biochem 47:272–277
470. **Okada Y, Morimoto I, Ura K, Nakano Y, Tanaka Y, Nishida S, Nakamura T, Eto S** 1998 Short-term treatment of recombinant murine interleukin-4 rapidly inhibits bone formation in normal and ovariectomized mice. Bone 22:361–365
471. **Onoe Y, Miyaura C, Kaminakayashiki T, Nagai Y, Noguchi K, Chen QR, Seo H, Ohta H, Nozawa S, Kudo I, Suda T** 1996 IL-13 and IL-4 inhibit bone resorption by suppressing cyclooxygenase-2-dependent prostaglandin synthesis in osteoblasts. J Immunol 156:758–764
472. **Lind M, Deleuran B, Yssel H, Fink-Eriksen E, Thestrup-Pedersen K** 1995 IL-4 and IL-13, but not IL-10, are chemotactic factors for human osteoblasts. Cytokine 7:78–82
473. **Palmqvist P, Lundberg P, Persson E, Johansson A, Lundgren I, Lie**

A, Conaway HH, Lerner UH 2006 Inhibition of hormone and cytokine-stimulated osteoclastogenesis and bone resorption by interleukin-4 and interleukin-13 is associated with increased osteoprotegerin and decreased RANKL and RANK in a STAT6-dependent pathway. J Biol Chem 281:2414–2429

474. **Yamada A, Takami M, Kawawa T, Yasuhara R, Zhao B, Mochizuki A, Miyamoto Y, Eto T, Yasuda H, Nakamichi Y, Kim N, Katagiri T, Suda T, Kamijo R** 2007 Interleukin-4 inhibition of osteoclast differentiation is stronger than that of interleukin-13 and they are equivalent for induction of osteoprotegerin production from osteoblasts. Immunology 120:573–579
475. **Bendixen AC, Shevde NK, Dienger KM, Willson TM, Funk CD, Pike JW** 2001 IL-4 inhibits osteoclast formation through a direct action on osteoclast precursors via peroxisome proliferator-activated receptor γ 1. Proc Natl Acad Sci USA 98:2443–2448
476. **Kamel Mohamed SG, Sugiyama E, Shinoda K, Hounoki H, Taki H, Maruyama M, Miyahara T, Kobayashi M** 2005 Interleukin-4 inhibits RANKL-induced expression of NFATc1 and c-Fos: a possible mechanism for downregulation of osteoclastogenesis. Biochem Biophy Res Comm 329:839–845
477. **Mangashetti LS, Khapli SM, Wani MR** 2005 IL-4 inhibits bone-resorbing activity of mature osteoclasts by affecting NF-κB and Ca2+ signaling. J Immunol 175:917–925
478. **Moreno JL, Kaczmareck M, Keegan AD, Tondravi M** 2003 IL-4 suppresses both osteoclast development and mature osteoclast function by a STAT6-dependent mechanism: irreversible inhibition of the differentiation program activated by RANKL. Blood 102: 1078–1086
479. **Wei S, Wang MW, Teitelbaum SL, Ross FP** 2001 Interleukin-4 reversibly inhibits osteoclastogenesis via inhibition of NF-κB and MAP kinase signaling. J Biol Chem 277:6622–6630
480. **Baugh JA, Bucala R** 2002 Macrophage migration inhibitory factor. Crit Care Med 30:S27–S35
481. **Weiser WY, Temple PA, Witek-Giannotti JS, Remold HG, Clark SC, David JR** 1989 Molecular cloning of a cDNA encoding a human macrophage migration inhibitory factor. Proc Natl Acad Sci USA 86:7522–7526
482. **Onodera S, Sasaki S, Ohshima S, Amizuka N, Li M, Udagawa N, Irie K, Nishihira J, Koyama Y, Shiraishi A, Tohyama H, Yasuda K** 2006 Transgenic mice overexpressing macrophage migration inhibitory factor (MIF) exhibit high-turnover osteoporosis. J Bone Miner Res 21:876–885
483. **Oshima S, Onodera S, Amizuka N, Li M, Irie K, Watanabe S, Koyama Y, Nishihira J, Yasuda K, Minami A** 2006 Macrophage migration inhibitory factor-deficient mice are resistant to ovariectomy-induced bone loss. FEBS Lett 580:1251–1256
484. **Ashcroft GS, Mills SJ, Lei K, Gibbons L, Jeong MJ, Taniguchi M, Burow M, Horan MA, Wahl SM, Nakayama T** 2003 Estrogen modulates cutaneous wound healing by downregulating macrophage migration inhibitory factor. J Clin Invest 111:1309–1318
485. **Onodera S, Suzuki K, Matsuno T, Kaneda K, Kuriyama T, Nishihira J** 1996 Identification of macrophage migration inhibitory factor in murine neonatal calvariae and osteoblasts. Immunology 89:430–435
486. **Onodera S, Suzuki K, Kaneda K, Fujinaga M, Nishihira J** 1999 Growth factor-induced expression of macrophage migration inhibitory factor in osteoblasts: relevance to the plasminogen activator system. Semin Thromb Hemost 25:563–568
487. **Onodera S, Nishihira J, Iwabuchi K, Koyama Y, Yoshida K, Tanaka S, Minami A** 2002 Macrophage migration inhibitory factor up-regulates matrix metalloproteinase-9 and -13 in rat osteoblasts. Relevance to intracellular signaling pathways. J Biol Chem 277: 7865–7874
488. **Lee S, Jacquin C, Leng L, Bucala R, Kuchel G** 2006 Macrophage migration inhibitory factor is an inhibitor of osteoclastogenesis in vitro and in vivo. J Bone Miner Res 21:S162
489. **Gilbert L, He X, Farmer P, Boden S, Kozlowski M, Rubin J, Nanes MS** 2000 Inhibition of osteoblast differentiation by tumor necrosis factor-α. Endocrinology 141:3956–3964
490. **Gilbert L, He X, Farmer P, Rubin J, Drissi H, Van Wijnen AJ, Lian JB, Stein GB, Nanes MS** 2001 Expression of the osteoblast differentiation factor RUNX2 (Cbfa1/AML3/Pebp2αA) is inhibited by tumor necrosis factor-α. J Biol.Chem 277:2695–2701
491. **Kuno H, Kurian SM, Hendy GN, White J, DeLuca HF, Evans CO, Nanes MS** 1994 Inhibition of 1,25-dihydroxyvitamin D3 stimulated osteocalcin gene transcription by tumor necrosis factor-α: structural determinants within the vitamin D response element. Endocrinology 134:2524–2531
492. **Nanes MS, Rubin J, Titus L, Hendy GN, Catherwood B** 1991 Tumor necrosis factor-α inhibits 1,25-dihydroxyvitamin D3-stimulated bone Gla protein synthesis in rat osteosarcoma cells (ROS 17/2.8) by a pretranslational mechanism. Endocrinology 128:2577–2580
493. **Canalis E** 1986 Interleukin-1 has independent effects on deoxyribonucleic acid and collagen synthesis in cultures of rat calvariae. Endocrinology 118:74–81
494. **Centrella M, McCarthy TL, Canalis E** 1988 Tumor necrosis factor-α inhibits collagen synthesis and alkaline phosphatase activity independently of its effect on deoxyribonucleic acid synthesis in osteoblast-enriched bone cell cultures. Endocrinology 123:1442–1448
495. **Stashenko P, Dewhirst FE, Rooney ML, Desjardins LA, Heeley JD** 1987 Interleukin-1 β is a potent inhibitor of bone formation in vitro. J Bone Miner Res 2:559–565
496. **Ura K, Morimoto I, Watanabe K, Saito K, Yanagihara N, Eto S** 2000 Interleukin (IL)-4 and IL-13 inhibit the differentiation of murine osteoblastic MC3T3–E1 cells. Endocr J 47:293–305
497. **Jilka RL, Weinstein RS, Bellido T, Parfitt AM, Manolagas SC** 1998 Osteoblast programmed cell death (apoptosis): modulation by growth factors and cytokines. J Bone Miner Res 13:793–802
498. **Kawakami A, Eguchi K, Matsuoka N, Tsuboi M, Koji T, Urayama S, Fujiyama K, Kiriyama T, Nakashima T, Nakane PK, Nagataki S** 1997 Fas and Fas ligand interaction is necessary for human osteoblast apoptosis. J Bone Miner Res 12:1637–1647
499. **Rifas L, Arackal S, Weitzmann MN** 2003 Inflammatory T cells rapidly induce differentiation of human bone marrow stromal cells into mature osteoblasts. J Cell Biochem 88:650–659
500. **Suh WK, Wang SX, Jheon AH, Moreno L, Yoshinaga SK, Ganss B, Sodek J, Grynpas MD, Mak TW** 2004 The immune regulatory protein B7–H3 promotes osteoblast differentiation and bone mineralization. Proc Natl Acad Sci USA 101:12969–12973
501. **Frost HM** 1964 Dynamics of bone remodeling. In: Frost HM, ed. Bone biodynamics. New York: Little, Brown Co; 315–333
502. **Martin TJ, Rodan GA** 2001 Coupling of bone resorption and formation during bone remodeling. In: Marcus R, Feldman D, Kelsey J, eds. Osteoporosis. Burlington, MA: Academic Press; 361–372
503. **Martin TJ, Sims NA** 2005 Osteoclast-derived activity in the coupling of bone formation to resorption. Trends Mol Med 11:76–81
504. **Parfitt AM** 1982 The coupling of bone formation to bone resorption: a critical analysis of the concept and of its relevance to the pathogenesis of osteoporosis. Metab Bone Dis Relat Res 4:1–6
505. **Khosla S** 2003 Parathyroid hormone plus alendronate—a combination that does not add up. N Engl J Med 349:1277–1279
506. **Schaller S, Henriksen K, Sveigaard C, Heegaard AM, Helix N, Stahlhut M, Ovejero MC, Johansen JV, Solberg H, Andersen TL, Hougaard D, Berryman M, Shiodt CB, Sorensen BH, Lichtenberg J, Christophersen P, Foged NT, Delaisse JM, Engsig MT, Karsdal MA** 2004 The chloride channel inhibitor NS3736 [corrected] prevents bone resorption in ovariectomized rats without changing bone formation. J Bone Miner Res 19:1144–1153
507. **Boyce BF, Yoneda T, Lowe C, Soriano P, Mundy GR** 1992 Requirement of pp60c-src expression for osteoclasts to form ruffled borders and resorb bone in mice. J Clin Invest 90:1622–1627
508. **Soriano P, Montgomery C, Geske R, Bradley A** 1991 Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. Cell 64:693–702
509. **Marzia M, Sims NA, Voit S, Migliaccio S, Taranta A, Bernardini S, Faraggiana T, Yoneda T, Mundy GR, Boyce BF, Baron R, Teti A** 2000 Decreased c-Src expression enhances osteoblast differentiation and bone formation. J Cell Biol 151:311–320
510. **Lee SH, Rho J, Jeong D, Sul JY, Kim T, Kim N, Kang JS, Miyamoto T, Suda T, Lee SK, Pignolo RJ, Koczon-Jaremko B, Lorenzo J, Choi Y** 2006 v-ATPase V0 subunit d2-deficient mice exhibit impaired osteoclast fusion and increased bone formation. Nat Med 12:1403–1409
511. **Wong BR, Rho J, Arron J, Robinson E, Orlinick J, Chao M, Kalachikov S, Cayani E, Bartlett 3rd FS, Frankel WN, Lee SY, Choi**

Y 1997 TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells. J Biol Chem 272:25190–25194
512. **Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A, Morony S, Capparelli C, Li J, Elliott R, McCabe S, Wong T, Campagnuolo G, Moran E, Bogoch ER, Van G, Nguyen LT, Ohashi PS, Lacey DL, Fish E, Boyle WJ, Penninger JM** 1999 Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. Nature 402:304–309
513. **Pettit AR, Ji H, von Stechow D, Muller R, Goldring SR, Choi Y, Benoist C, Gravallese EM** 2001 TRANCE/RANKL knockout mice are protected from bone erosion in a serum transfer model of arthritis. Am J Pathol 159:1689–1699
514. **Clark WB, Loe H** 1993 Mechanisms of initiation and progression of periodontal disease. Periodontol 2000 2:72–82
515. **Teng YT, Nguyen H, Gao X, Kong YY, Gorczynski RM, Singh B, Ellen RP, Penninger JM** 2000 Functional human T-cell immunity and osteoprotegerin ligand control alveolar bone destruction in periodontal infection. J Clin Invest 106:R59–R67
516. **Lopez I, Buchman AL** 2000 Metabolic bone disease in IBD. Curr Gastroenterol Rep 2:317–322
517. **Moschen AR, Kaser A, Enrich B, Ludwiczek O, Gabriel M, Obrist P, Wolf AM, Tilg H** 2005 The RANKL/OPG system is activated in inflammatory bowel disease and relates to the state of bone loss. Gut 54:479–487
518. **Franchimont N, Reenaers C, Lambert C, Belaiche J, Bours V, Malaise M, Delvenne P, Louis E** 2004 Increased expression of receptor activator of NF-κB ligand (RANKL), its receptor RANK and its decoy receptor osteoprotegerin in the colon of Crohn's disease patients. Clin Exp Immunol 138:491–498
519. **Ashcroft AJ, Cruickshank SM, Croucher PI, Perry MJ, Rollinson S, Lippitt JM, Child JA, Dunstan C, Felsburg PJ, Morgan GJ, Carding SR** 2003 Colonic dendritic cells, intestinal inflammation, and T cell-mediated bone destruction are modulated by recombinant osteoprotegerin. Immunity 19:849–861
520. **Goldring SR, Gravallese EM** 2000 Pathogenesis of bone erosions in rheumatoid arthritis. Curr Opin Rheumatol 12:195–199
521. **Martin TJ, Romas E, Gillespie MT** 1998 Interleukins in the control of osteoclast differentiation. Crit Rev Eukaryot Gene Expr 8:107–123
522. **Mundy GR** 2002 Metastasis to bone: causes, consequences and therapeutic opportunities. Nat Rev Cancer 2:584–593
523. **O'Gradaigh D, Compston JE** 2004 T-cell involvement in osteoclast biology: implications for rheumatoid bone erosion. Rheumatology (Oxford) 43:122–130
524. **Quinn JM, Itoh K, Udagawa N, Hausler K, Yasuda H, Shima N, Mizuno A, Higashio K, Takahashi N, Suda T, Martin TJ, Gillespie MT** 2001 Transforming growth factor β affects osteoclast differentiation via direct and indirect actions. J Bone Miner Res 16:1787–1794
525. **Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM** 2006 Th17: an effector CD4 T cell lineage with regulatory T cell ties. Immunity 24:677–688
526. **Lorenzo JA, Naprta A, Rao Y, Alander C, Glaccum M, Widmer M, Gronowicz G, Kalinowski J, Pilbeam CC** 1998 Mice lacking the type I interleukin-1 receptor do not lose bone mass after ovariectomy. Endocrinology 139:3022–3025
527. **Roggia C, Gao Y, Cenci S, Weitzmann MN, Toraldo G, Isaia G, Pacifici R** 2001 Up-regulation of TNF-producing T cells in the bone marrow: a key mechanism by which estrogen deficiency induces bone loss in vivo. Proc Natl Acad Sci USA 98:13960–13965
528. **Poli V, Balena R, Fattori E, Markatos A, Yamamoto M, Tanaka H, Ciliberto G, Rodan GA, Costantini F** 1994 Interleukin-6 deficient mice are protected from bone loss caused by estrogen depletion. EMBO J 13:1189–1196
529. **Pacifici R, Rifas L, Teitelbaum S, Slatopolsky E, McCracken R, Bergfeld M, Lee W, Avioli LV, Peck WA** 1987 Spontaneous release of interleukin 1 from human blood monocytes reflects bone formation in idiopathic osteoporosis. Proc Natl Acad Sci USA 84:4616–4620
530. **Sunyer T, Lewis J, Collin-Osdoby P, Osdoby P** 1999 Estrogen's bone-protective effects may involve differential IL-1 receptor regulation in human osteoclast-like cells. J Clin Invest 103:1409–1418
531. **Pacifici R, Brown C, Puscheck E, Friedrich E, Slatopolsky E, Maggio D, McCracken R, Avioli LV** 1991 Effect of surgical menopause and estrogen replacement on cytokine release from human blood mononuclear cells. Proc Natl Acad Sci USA 88:5134–5134
532. **Srivastava S, Weitzmann MN, Kimble RB, Rizzo M, Zahner M, Milbrandt J, Ross FP, Pacifici R** 1998 Estrogen blocks M-CSF gene expression and osteoclast formation by regulating phosphorylation of Egr-1 and its interaction with Sp-1. J Clin Invest 102:1850–1859
533. **Bernard-Poenaru O, Roux C, Blanque R, Gardner C, de Vemejoul MC, Cohen-Solal ME** 2001 Bone-resorbing cytokines from peripheral blood mononuclear cells after hormone replacement therapy: a longitudinal study. Osteoporos Int 12:769–776
534. **Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, Boyce B, Broxmeyer H, Manolagas SC** 1992 Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science 257:88–91
535. **Lin SC, Yamate T, Taguchi Y, Borba VC, Girasole G, O'Brien CA, Bellido T, Abe E, Manolagas SC** 1997 Regulation of the gp80 and gp130 subunits of the IL-6 receptor by sex steroids in the murine bone marrow. J Clin Invest. 100:1980–1990
536. **Shevde NK, Bendixen AC, Dienger KM, Pike JW** 2000 Estrogens suppress RANK ligand-induced osteoclast differentiation via a stromal cell independent mechanism involving c-Jun repression. Proc Natl Acad Sci USA 97:7829–7834
537. **Srivastava S, Toraldo G, Weitzmann MN, Cenci S, Ross FP, Pacifici R** 2000 Estrogen decreases osteoclast formation by down-regulating receptor activator of NF-κB ligand (RANKL)-induced JNK activation. J Biol Chem 276:8836–8840
538. **Cenci S, Weitzmann MN, Roggia C, Namba N, Novack D, Woodring J, Pacifici R** 2000 Estrogen deficiency induces bone loss by enhancing T-cell production of TNF-α. J Clin Invest 106:1229–1237
539. **Sass DA, Liss T, Bowman AR, Rucinski B, Popoff SN, Pan Z, Ma YF, Epstein S** 1997 The role of the T-lymphocyte in estrogen deficiency osteopenia. J Bone Miner Res 12:479–486
540. **Lee SK, Kadono Y, Okada F, Jacquin C, Koczon-Jaremko B, Gronowicz G, Adams DJ, Aguila HL, Choi Y, Lorenzo JA** 2006 T lymphocyte-deficient mice lose trabecular bone mass with ovariectomy. J Bone Miner Res 21:1704
541. **Kanematsu M, Sato T, Takai H, Watanabe K, Ikeda K, Yamada Y** 2000 Prostaglandin E 2 induces expression of receptor activator of nuclear factor-κB ligand/osteoprotegrin ligand on pre-B cells: implications for accelerated osteoclastogenesis in estrogen deficiency. J Bone Miner Res 15:1321–1329
542. **Li Y, Li A, Yang X, Weitzmann MN** 2007 Ovariectomy-induced bone loss occurs independently of B cells. J Cell Biochem 100:1370–1375
543. **Steinman RM, Bonifaz L, Fujii S, Liu K, Bonnyay D, Yamazaki S, Pack M, Hawiger D, Iyoda T, Inaba K, Nussenzweig MC** 2005 The innate functions of dendritic cells in peripheral lymphoid tissues. Adv Exp Med Biol 560:83–97
544. **Matsuo K, Owens JM, Tonko M, Elliott C, Chambers TJ, Wagner EF** 2000 Fosl1 is a transcriptional target of c-Fos during osteoclast differentiation. Nat Genet 24:184–187
545. **Kamath AT, Henri S, Battye F, Tough DF, Shortman K** 2002 Developmental kinetics and lifespan of dendritic cells in mouse lymphoid organs. Blood 100:1734–1741
546. **Lindquist RL, Shakhar G, Dudziak D, Wardemann H, Eisenreich T, Dustin ML, Nussenzweig MC** 2004 Visualizing dendritic cell networks in vivo. Nat Immunol 5:1243–1250
547. **Shakhar G, Lindquist RL, Skokos D, Dudziak D, Huang JH, Nussenzweig MC, Dustin ML** 2005 Stable T cell-dendritic cell interactions precede the development of both tolerance and immunity in vivo. Nat Immunol 6:707–714
548. **Stoll S, Delon J, Brotz TM, Germain RN** 2002 Dynamic imaging of T cell-dendritic cell interactions in lymph nodes. Science 296:1873–1876
549. **Wong BR, Josien R, Lee SY, Sauter B, Li HL, Steinman RM, Choi Y** 1997 TRANCE (tumor necrosis factor [TNF]-related activation-induced cytokine), a new TNF family member predominantly expressed in T cells, is a dendritic cell-specific survival factor. J Exp Med 186:2075–2080
550. **Ouaaz F, Arron J, Zheng Y, Choi Y, Beg AA** 2002 Dendritic cell

development and survival require distinct NF-κB subunits. Immunity 16:257–270
551. **Arron JR, Vologodskaia M, Wong BR, Naramura M, Kim N, Gu H, Choi Y** 2001 A positive regulatory role for Cbl family proteins in tumor necrosis factor-related activation-induced cytokine (trance) and CD40L-mediated Akt activation. J Biol Chem 276: 30011–30017
552. **Josien R, Li HL, Ingulli E, Sarma S, Wong BR, Vologodskaia M, Steinman RM, Choi Y** 2000 TRANCE, a tumor necrosis factor family member, enhances the longevity and adjuvant properties of dendritic cells in vivo. J Exp Med 191:495–502
553. **Zhong L, Granelli-Piperno A, Choi Y, Steinman RM** 1999 Recombinant adenovirus is an efficient and non-perturbing genetic vector for human dendritic cells. Eur J Immunol 29:964–972
554. **Wiethe C, Dittmar K, Doan T, Lindenmaier W, Tindle R** 2003 Enhanced effector and memory CTL responses generated by incorporation of receptor activator of NF-κB (RANK)/RANK ligand costimulatory molecules into dendritic cell immunogens expressing a human tumor-specific antigen. J Immunol 171:4121–4130
555. **Yun TJ, Tallquist MD, Aicher A, Rafferty KL, Marshall AJ, Moon JJ, Ewings ME, Mohaupt M, Herring SW, Clark EA** 2001 Osteoprotegerin, a crucial regulator of bone metabolism, also regulates B cell development and function. J Immunol 166:1482–1491
556. **Bachmann MF, Wong BR, Josien R, Steinman RM, Oxenius A, Choi Y** 1999 TRANCE, a tumor necrosis factor family member critical for CD40 ligand-independent T helper cell activation. J Exp Med 189:1025–1031
557. **Roy M, Waldschmidt T, Aruffo A, Ledbetter JA, Noelle RJ** 1993 The regulation of the expression of gp39, the CD40 ligand, on normal and cloned CD4+ T cells. J Immunol 151:2497–2510
558. **Cremer I, Dieu-Nosjean MC, Marechal S, Dezutter-Dambuyant C, Goddard S, Adams D, Winter N, Menetrier-Caux C, Sautes-Fridman C, Fridman WH, Mueller CG** 2002 Long-lived immature dendritic cells mediated by TRANCE-RANK interaction. Blood 100:3646–3655
559. **Williamson E, Bilsborough JM, Viney JL** 2002 Regulation of mucosal dendritic cell function by receptor activator of NF-κB (RANK)/RANK ligand interactions: impact on tolerance induction. J Immunol 169:3606–3612
560. **Green EA, Choi Y, Flavell RA** 2002 Pancreatic lymph node-derived CD4(+)CD25(+) Treg cells: highly potent regulators of diabetes that require TRANCE-RANK signals. Immunity 16:183–191
561. **Aramaki O, Inoue F, Takayama T, Shimazu M, Kitajima M, Ikeda Y, Okumura K, Yagita H, Shirasugi N, Niimi M** 2005 Various costimulatory pathways are essential for induction of regulatory cells by intratracheal delivery of alloantigen. Transplant Proc 37: 1934–1936
562. **Hochweller K, Anderton SM** 2005 Kinetics of costimulatory molecule expression by T cells and dendritic cells during the induction of tolerance versus immunity in vivo. Eur J Immunol 35:1086–1096
563. **Loser K, Mehling A, Loeser S, Apelt J, Kuhn A, Grabbe S, Schwarz T, Penninger JM, Beissert S** 2006 Epidermal RANKL controls regulatory T-cell numbers via activation of dendritic cells. Nat Med 12:1372–1379
564. **Hamazaki Y, Fujita H, Kobayashi T, Choi Y, Scott HS, Matsumoto M, Minato N** 2007 Medullary thymic epithelial cells expressing Aire represent a unique lineage derived from cells expressing claudin. Nat Immunol 8:304–311
565. **Rossi SW, Kim MY, Leibbrandt A, Parnell SM, Jenkinson WE, Glanville SH, McConnell FM, Scott HS, Penninger JM, Jenkinson EJ, Lane PJ, Anderson G** 2007 RANK signals from CD4+3- inducer cells regulate development of Aire-expressing epithelial cells in the thymic medulla. J Exp Med 204:1267–1272
566. **Anderson MS, Venanzi ES, Chen Z, Berzins SP, Benoist C, Mathis D** 2005 The cellular mechanism of Aire control of T cell tolerance. Immunity 23:227–239
567. **Anderson MS, Venanzi ES, Klein L, Chen Z, Berzins SP, Turley SJ, von Boehmer H, Bronson R, Dierich A, Benoist C, Mathis D** 2002 Projection of an immunological self shadow within the thymus by the aire protein. Science 298:1395–1401
568. **Cheng MH, Shum AK, Anderson MS** 2007 What's new in the Aire? Trends Immunol 28:321–327
569. **Takeda K, Akira S** 2004 TLR signaling pathways. Semin Immunol 16:3–9
570. **Kikuchi T, Matsuguchi T, Tsuboi N, Mitani A, Tanaka S, Matsuoka M, Yamamoto G, Hishikawa T, Noguchi T, Yoshikai Y** 2001 Gene expression of osteoclast differentiation factor is induced by lipopolysaccharide in mouse osteoblasts via Toll-like receptors. J Immunol 166:3574–3579
571. **Hayashi S, Yamada T, Tsuneto M, Yamane T, Takahashi M, Shultz LD, Yamazaki H** 2003 Distinct osteoclast precursors in the bone marrow and extramedullary organs characterized by responsiveness to Toll-like receptor ligands and TNF-α. J Immunol 171: 5130–5139
572. **Nair SP, Meghji S, Wilson M, Reddi K, White P, Henderson B** 1996 Bacterially induced bone destruction: mechanisms and misconceptions. Infect Immun 64:2371–2380
573. **Zou W, Schwartz H, Endres S, Hartmann G, Bar-Shavit Z** 2002 CpG oligonucleotides: novel regulators of osteoclast differentiation. FASEB J 16:274–282
574. **Fox SW, Haque SJ, Lovibond AC, Chambers TJ** 2003 The possible role of TGF-β-induced suppressors of cytokine signaling expression in osteoclast/macrophage lineage commitment in vitro. J Immunol 170:3679–3687
575. **Hayashi T, Kaneda T, Toyama Y, Kumegawa M, Hakeda Y** 2002 Regulation of receptor activator of NF-κB ligand-induced osteoclastogenesis by endogenous interferon-β (INF-β) and suppressors of cytokine signaling (SOCS). The possible counteracting role of SOCSs- in IFN-β-inhibited osteoclast formation. J Biol Chem 277:27880–27886
576. **Maruyama K, Takada Y, Ray N, Kishimoto Y, Penninger JM, Yasuda H, Matsuo K** 2006 Receptor activator of NF-κB ligand and osteoprotegerin regulate proinflammatory cytokine production in mice. J Immunol 177:3799–3805
577. **Li H, Cuartas E, Cui W, Choi Y, Crawford TD, Ke HZ, Kobayashi KS, Flavell RA, Vignery A** 2005 IL-1 receptor-associated kinase M is a central regulator of osteoclast differentiation and activation. J Exp Med 201:1169–1177
578. **Yin T, Li L** 2006 The stem cell niches in bone. J Clin Invest 116:1195–1201

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