The Osteoclast: Friend or Foe?

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Abstract

Bone is a dynamic organ constantly remodeled to support calcium homeostasis and structural needs. The osteoclast is the cell responsible for removing both the organic and inorganic components of bone. It is derived from hematopoietic progenitors in the macrophage lineage and differentiates in response to the tumor necrosis factor family cytokine receptor activator of NFκB ligand. αvβ3 integrin mediates cell adhesion necessary for polarization and formation of an isolated, acidified resorptive microenvironment. Defects in osteoclast function, whether genetic or iatrogenic, may increase bone mass but lead to poor bone quality and a high fracture risk. Pathological stimulation of osteoclast formation and resorption occurs in postmenopausal osteoporosis, inflammatory arthritis, and metastasis of tumors to bone. In these diseases, osteoclast activity causes bone loss that leads to pain, deformity, and fracture. Thus, osteoclasts are critical for normal bone function, but their activity must be controlled.
INTRODUCTION

Pathologists are often called upon to diagnose neoplastic and inflammatory conditions in bone, and in this context we focus on cells extraneous to the bony matrix, such as metastatic carcinoma. In fact, the bone loss that occurs in these conditions, and in those more common states of systemic bone loss such as osteoporosis, is not the product of these extrinsic cells but reflects an imbalance of the activities of osteoclasts and osteoblasts in which bone resorption supersedes formation. Insights gained into the mechanism of physiological bone resorption are the bases for the development of drugs that arrest bone loss in diseases such as osteoporosis and tumor osteolysis.

Osteoclasts and osteoblasts not only regulate bone mass but do so in concert as each governs the appearance and activity of the other. The regulatory function of the osteoclast on bone remodeling has been long appreciated in the context of skeletal remodeling, an asynchronous event occurring at all times throughout the endocortical and trabecular skeleton. Bone remodeling is initiated by osteoclasts appearing at a nascent site on the surface of cortical or trabecular bone (Figure 1a). Whether initiation of remodeling at a particular location is a stochastic event or reflects alterations in bone matrix such as microdamage repair is controversial, although arrest of remodeling results in poor matrix quality owing presumably to failure to replace effete bone. Following degradation of bone to form a resorption (Howship’s) lacuna approximately 50 μm in depth, marrow mesenchymal cells appear within the resorption cavity and undergo osteoblast differentiation. In young individuals, osteoblasts completely replace the resorbed bone within the remodeling packet and skeletal mass is retained. With age, and particularly following loss of ovarian function, more bone is removed by remodeling osteoclasts than subsequently replaced by osteoblasts, thus accounting for the progressive bone loss. The fact that osteoclast activity precedes that of osteoblasts in the remodeling process suggests that products released from the resorptive cell, per se, or from the degraded matrix, attract osteoblast precursors to the remodeling site (1). Although there is little information regarding osteoclast-produced molecules that attract osteoblasts, bone matrix is rich in growth factors such as TGF-β and insulin-like growth factor, which are potent inducers of bone formation and are released by osteoclastic bone resorption. Additionally, the osteoclast is rich in cytokines, whose release may theoretically recruit osteoblasts to remodeling sites. Identification of the so-called coupling factor, by which osteoclasts communicate with osteoblasts, remains among the most challenging and important tasks of bone biologists.

Although when viewed in its entirety the osteoclast is a large, dramatic-appearing polynuclear, the cell may be difficult to identify in standard histological sections, as sectioning may yield fragments containing a single nucleus. Therefore, H&E staining invariably underestimates osteoclast number. However, the resorptive cell is rich in tartrate-resistant acid phosphatase (TRAP), which, in the context of bone, is a specific and sensitive osteoclast marker, permitting accurate assessment of the cell’s abundance (Figure 1b and 1c). Despite claims to the contrary (2), the osteoclast is the unique bone-resorptive cell and the ability to generate pure populations has provided the opportunity to identify molecular events mediating hard tissue degradation.

MECHANISMS OF OSTEOCLAST DIFFERENTIATION

Prior to the 1980s, the origin of the osteoclast was controversial. In fact, a major hypothesis held that bone-resorbing and bone-forming cells were derived from a common proximal precursor (3). Two series of critical experiments, however, established the precise origin of osteoclasts. In the first instance, Walker demonstrated that curing mice with
osteopetrosis was achieved by parabiosis to normal animals (4) or infusion with wild-type spleen cells or bone marrow (5), indicating that the osteoclast is of hematopoietic origin. In the early 1980s, a female infant was cured of osteopetrosis using marrow derived from a male sibling (6). The ability to follow the Y chromosome confirmed that the osteoclast is, in fact, of hematopoietic origin and its ontogeny differs from that of the osteoblast. Membership of the osteoclast in the monocyte/macrophage family was established by Suda’s group in 1988, when they demonstrated that the resorptive polykaryon can be generated in culture from these mononuclear hematopoietic cells (7).

A puzzling aspect of Suda’s report, however, was the fact that osteoclasts could not be generated from marrow macrophages in culture unless they were in contact with

Osteopetrosis: disorder of dense but brittle bone caused by defects in osteoclast formation or function

Figure 1
Bone remodeling cycle. (a) 1. Osteoclast (OC) precursors fuse to become mature OCs and attach to bone. 2. OCs resorb bone, forming a resorption (Howship’s) lacuna. 3. When elicited by OC activity, bone marrow stromal cells become active osteoblasts (OBs), which secrete bone matrix known as osteoid. 4. When the lacuna has been filled, OBs become quiescent bone-lining cells and osteoid undergoes calcification to become mature bone. (b) The histochemical stain tartrate-resistant acid phosphatase (TRAP) specifically identifies OCs. The opposite side of this trabeculum shows a row of OBs. (c) High-power view of a TRAP-stained OC on bone demonstrates cellular polarization. The resorptive surface, the ruffled border (between arrows), is apposed to the bone, whereas the nuclei are located away from the bone surface.
Figure 2
Mechanisms of osteoclast (OC) differentiation. (a) OC differentiation requires direct contact between OC precursors and osteoblasts or their stromal cell precursors. These supporting cells express membrane-bound forms of the two key osteoclastogenic cytokines, receptor activator of NFκB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF). OC precursors express receptors for RANK and M-CSF. Osteoprotegerin is a soluble decoy receptor for RANKL. (b) Major signaling pathways activated by three key osteoclastogenic receptors: RANK, immunoreceptor tyrosine-based activation motifs (ITAMs), and c-Fms.

osteoblast-lineage cells. This observation suggested that a key osteoclastogenic molecule resides on the surface of osteoblasts and their precursors (Figure 2a). This osteoblast-produced molecule, receptor activator of NFκB ligand (RANKL), was identified when its inhibitor, a circulating decoy receptor subsequently named osteoprotegerin (OPG), was found to arrest osteoclast formation and induce osteopetrosis in transgenic mice (8). RANKL, a member of the tumor necrosis factor (TNF) superfamily, is expressed on the surface of osteoblast-lineage cells, and functions by interacting with its receptor RANK on osteoclast precursors (9, 10). In keeping with its pivotal role in osteoclast differentiation, RANKL-deleted mice, like those overexpressing OPG, develop osteoclast-deficient
osteopetrosis, whereas RANKL transgensics experience a hyper-resorptive state, leading to
osteoporosis. RANKL also targets the mature osteoclast, stimulating its resorptive activity,
an event that appears to involve cytoskeletal organization (11).

TNFα also promotes osteoclast differentiation, via its Type 1 or p55 receptor (12). Fur-
thermore, TNFα and RANKL enjoy a synergistic relationship, as a minimal amount of one markedly enhances the osteoclastogenic properties of the other (13, 14). Although controversy exists as to whether TNFα alone induces the osteoclast phenotype, there is little question this inflammatory cytokine re-
quires constitutive levels of RANKL in vivo as it is virtually ineffective in RANK−/− mice (15). This unique capacity of RANKL, but not TNFα, by itself to promote the osteoclast phenotype is at first instance curious, as their activated receptors share many of the same downstream signals, such as activation of NFκB and AP-1 transcription factors as well as MAP kinases, all of which are critical to osteoclast differentiation (Figure 2b). It appears, however, that the fundamental osteoclastogenic properties of occupied RANK relate to its ability to bind TRAF6. Whereas both receptors bind TRAF2 and -5, which are also important for osteoclast differentiation (16, 17), ligand-occupied RANK, but not the p55 TNF receptor, recruits TRAF6. In addition to stimulating MAPK, PI3K, and NFκB pathways, this adaptor protein activates calcineurin, which dephosphorylates the transcrip-
tion factor NFAT1, thus promoting its nuclear translocation. Nuclear NFAT1 partners
with the AP-1 proteins c-Fos and c-Jun, whose expression and activation, respectively, are also promoted by TRAF6 (18). These three transcription factors in concert induce the NFAT2 promoter. NFAT2, again in association with c-Fos/c-Jun, transactivates osteoclastogenic genes (19). In keeping with their critical roles in osteoclastogenesis, loss of function NFAT (20), c-Jun (19), and c-Fos (21) mutant mice each exhibit arrested osteoclasmogenesis and osteopetrosis. Another transcripton factor activated by RANK, but not p55 TNF receptor, signaling is MiTF, which is also required for the differentiation of mature osteoclasts.

An additional signal critical in osteoclast differentiation is that emanating from the im-
munonuclease tyrosine-based activation mo-
tif (ITAM)-bearing receptors DAP12 and FcRγ (22, 23). These receptors are known as
costimulators and act in a similar manner to similiar ITAM-bearing molecules in B and T
lymphocytes. DAP12 and FcRγ are activated by cell-cell contact, with incompletely de-
defined ligands expressed on osteoclast precursors and osteoblasts, respectively. The signal-
ing pathway downstream of ITAMs includes Syk, PLCγ2, and NFAT (22–24). The discovery
of the role of ITAMs in osteoclast differentiation has strengthened the concept of os-
teoimmunology, coined by Arron & Choi (25) to describe the study of the interrelationships
between bone and the immune system. In fact, many key molecules in the osteoclastogenic
pathway, including RANK, RANKL, PLCγ2, NFAT, and NFκB, are critical for lymph node
and immune cell differentiation as well.

Macrophage colony-stimulating factor (M-CSF) is the second cytokine required to
generate the resorptive cell in vitro, as it promotes precursor proliferation and survival of
all members of the osteoclast lineage, exerting its effects through the tyrosine kinase recep-
tor c-Fms. In fact, pure populations of osteoclasts are generated in vitro by culturing mar-
row macrophages with only RANKL and M-
CSF. M-CSF is produced by a variety of cells,
particularly endothelium, but in the context
of osteoclastogenesis, those of the osteoblast
lineage appear to be the major source. Its ex-
pression is stimulated by TNFα, and thus
M-CSF plays a critical role in the bone loss
of inflammatory osteolysis (see below) (26).
The essential role of M-CSF in the osteo-
clastogenic process was first established by
the op/op mouse, which bears a mutation of the Csf1 gene coding for M-CSF, and is char-
acterized by osteoclast-deficient osteopetrosis (27). Critical signals downstream of c-Fms

RANKL: receptor activator of NFκB ligand
OOPG: osteoprotegerin
TNF: tumor necrosis factor
ITAM: immunoreceptor tyrosine-based activation motif
M-CSF: macrophage
   colony-stimulating factor
Polarization: a process of cytoskeletal reorganization that allows the osteoclast to form a sealing zone and resorb bone.

OSTEOCLAST POLARIZATION AND THE CYTOSKELETON

The mature osteoclast is rich in mitochondria and is dramatically polarized when attached to bone, with its nuclei localized toward the antiresorptive surface (Figure 3a and 3b). The bone-residing osteoclast also contains a unique, highly convoluted ruffled membrane, which is the resorptive organelle. This structure is formed upon contact with bone by directed transport of acidified cytoplasmic vesicles toward the bone-apposed plasma membrane. The ruffled membrane is surrounded by a structure rich in fibrillar actin known as the actin ring or sealing zone, which is juxtaposed to the bone surface. The actin ring creates an isolated extracellular microenvironment, enclosed by the cell and the bone surface, in which organic and inorganic components of bone matrix are degraded.

Although the precise extracellular molecules inducing osteoclast polarization are incompletely understood, they are likely mediated via the αvβ3 integrin (28, 29). Integrins are α/β transmembrane heterodimers existing in a basal (low binding affinity) or an activated (high binding affinity) state. Once activated, they can transmit a range of intracellular signaling pathways. Their activation can be mediated directly by the ligand itself or indirectly by signaling downstream of growth factor receptors. The osteoclast and placenta are particularly rich in αvβ3, although the integrin is expressed in other cells—particularly endothelium—in states of inflammation and neovascularization. This integrin recognizes the amino acid motif Arg-Gly-Asp (RGD), a component of bone matrix proteins such as osteopontin and bone sialoprotein. The β3 subunit is also expressed in platelets, but in association with the αIIb subunit. In fact, mutations of β3 prompt the human bleeding dyscrasia known as Glanzmann’s thrombasthenia. Mice with a deleted β3 integrin subunit develop enhanced bone mass owing to osteoclast dysfunction (30). Whether the same occurs in humans is less certain. Although Glanzmann’s disease has been associated with osteopetrosis in one reported case (31), most Glanzmann’s patients have relatively normal bone mass, perhaps related to the compensatory expression of β1 integrin chains (32).

αvβ3 expression is characteristic of marrow macrophages as they become osteoclasts. Noncommitted osteoclast precursors, in the form of marrow macrophages, do not express αvβ3, instead bearing the closely related integrin αvβ5 (30). As the cell commits to the osteoclast but not mature macrophage, phenotype αvβ5 is replaced by αvβ3. Osteoclast motility, adhesion to bone matrix, and polarization of the resorptive machinery all require αvβ3. Osteoclasts of β3−/− mice fail to form actin rings and have attenuated ruffled membranes, eventuating in diminished resorptive activity and increased bone mass (30). Absence of αvβ3 also prevents ovariectomy-induced bone loss (33). In contrast, absence of β5 exerts an opposite effect, namely, stimulated bone resorption and acceleration of postmenopausal osteoporosis (34).

In most cells, integrins mediate matrix contact via stable structures called focal adhesions, which also contain signaling and cytoskeletal molecules, leading to the formation of stress fibers. Mammalian osteoclasts organize their fibrillar actin into sealing zones in lieu of stress fibers and form podosomes instead of focal adhesions (29). Podosomes, formed as the osteoclast attaches to bone, consist of a core of F-actin surrounded by a rosette-like structure containing αvβ3 and cytoskeletal proteins, including α-actinin and vinculin (Figure 3c). Podosomes are thought to fuse to form the sealing zone, in which a ring of actin is surrounded by αvβ3 (Figure 3d). Osteoclastic bone resorption is a cyclical phenomenon in which the cell migrates to a resorptive site, degrades the

include the PI3K/Akt pathway, critical for cell survival, and the MAPK ERK, which is required for precursor survival and proliferation (Figure 2b).
Figure 3

Osteoclast polarization. (a) Electron micrograph shows the highly convoluted ruffled border adjacent to the bone surface. The cytoplasm is also rich in mitochondria. A nucleus is visible toward the basolateral surface of the cell. (b) Schematic diagram of an osteoclast polarized on bone. The ruffled border is surrounded by the sealing zone, where αvβ3 integrin mediates attachment to the bone matrix. (c) The initial adhesive structure is the podosome, in which filamentous actin (green) is associated with cytoskeletal proteins such as α-actinin and vinculin. The cytoskeletal core is surrounded by αvβ3 attached to bone. Podosomes fuse to form the sealing zone. (d) Confocal image of an osteoclast on bone, labeled with FITC-phalloidin (green) to identify actin. Anti-αvβ3 antibody (and TRITC-conjugated secondary antibody, red) demonstrates the ring-like sealing zone surrounded by αvβ3.

underlying matrix, then detaches and re-initiates the cycle. In motile osteoclasts, the sealing zone is disassembled and nonpodosomal integrins move to lamellipodia at the cell’s leading edge. Optimal bone resorption depends upon these cycles of attachment, sealing zone formation, and migration, and the integrin is critical for all phases of the process.
The ability to effectively express exogenous proteins in authentic osteoclasts and their precursors has yielded insights into how αvβ3 functions in these cells (35). Deletion of the β3 cytoplasmic domain arrests the osteoclast, reflecting the importance of intracellular events to integrin function. Mutation of serine 752 to proline in the β3 cytoplasmic domain also disrupts integrin function and occurs in a subset of patients with Glanzmann’s disease (35). Re-expression of wild-type β3 in β3−/− mice restores the osteoporotic response to ovariectomy, but expression of β3 lacking its cytoplasmic domain, or with the Ser752Pro mutation, does not have this effect in vivo (33).

A number of β3 cytoplasmic domain-associated signaling molecules that mediate the integrin’s capacity to organize the osteoclast cytoskeleton have been identified (Figure 4). c-Src is particularly important in this regard. The importance of this proto-oncogene in osteoclast function first came to light in 1991 when Soriano et al. (36) found that severe osteopetrosis is the dominant phenotype of the c-Src−/− mouse. Importantly, the mutant mice contain abundant, albeit dysfunctional, osteoclasts that lack ruffled membranes and actin rings (37). Subsequent studies demonstrated that c-Src regulates osteoclasts via its role as both a kinase and an adaptor molecule (38, 39). We find that c-Src, in its inactive state, constitutively associates with the β3 cytoplasmic domain of the unliganded integrin. αvβ3 activation recruits Syk, another tyrosine kinase, to the β3 tail, where it is phosphorylated by the now active c-Src (28). Recruitment and activation of Syk depends upon its association with the ITAM proteins DAP12 and FcRγ (28).

Hall and Ridley’s pioneering studies established the critical role the Rho-family GTPases play in cytoskeletal organization in stromal cells (40, 41), and the same holds in the context of the osteoclast. At least two such GTPases, namely Rac and Rho, are activated downstream of αvβ3 (29). However, Rho and Rac appear to be activated by distinct mechanisms and likely have distinct molecular roles in the osteoclast cytoskeleton. Whereas the pathway upstream of Rho activation in osteoclasts has not been defined, that leading to Rac activation has been delineated. All GTPases transit from their inactive, GDP-bound form to their activated, GTP-associated state under the influence of guanine nucleotide exchange factors (GEFs). Vavs are Rac-activating GEFs, and the osteoclast expresses a unique isoform of the molecule, namely Vav3 (38). The essential role Vav3 plays in organizing the osteoclast cytoskeleton is illustrated by the failure of Vav3−/− osteoclasts to spread and form actin rings. Confirming Vav3’s participation in the αvβ3/Rac signaling pathway, occupancy of the integrin fails to activate Rac in the absence of this GEF. Syk, following recruitment to the activated αvβ3/c-Src complex, acts as the Vav3 kinase (Figure 4) (28). Phosphorylated Vav3

**Figure 4**
Collaboration between αvβ3 and c-Fms in the osteoclast. c-Src is constitutively bound to the αvβ3 cytoplasmic tail. Upon integrin ligation, c-Src becomes activated (phosphorylated) and then Syk is phosphorylated and recruited to the integrin in an immunoreceptor tyrosine-based activation motif (ITAM)-dependent manner. Activation of c-Fms by macrophage colony-stimulating factor leads to phosphorylation of Vav3 and activation of Rac and enhances osteoclast migration and bone resorption when αvβ3 is also activated.
activates Rac, leading in turn to the organization of the osteoclast cytoskeleton. Importantly, the osteoclasts generated from αvβ3- (30), c-Src-, Syk- (28), and Vav3-deficient mice (42) are qualitatively similar in that they fail to spread or effectively resorb bone. The role of the Rho pathway has not yet been studied using genetic models lacking particular components in osteoclasts. In vitro inhibitor studies suggest that the GTPase modulates sealing zone formation via its effector mDIA2 and the cytosolic histone deacetylase HDAC6 (43).

The osteoclast cytoskeleton is also controlled by interaction between αvβ3 and M-CSF pathways. M-CSF, via its receptor c-Fms, activates the integrin by targeting its cytoplasmic domain, which alters the conformation of its extracellular ligand-binding region (29). As a consequence of integrin activation, OCs stimulated with M-CSF undergo rapid cytoskeletal reorganization, leading to the formation of membrane extensions known as lamellipodia and the acquisition of a motile phenotype, mediated by the activation of Vav3/Rac pathway (44). Thus, although high levels of M-CSF induce β3−/− osteoclasts to form morphologically normal actin rings, the growth factor cannot induce cell migration in the absence of the integrin. Interestingly, Choi’s laboratory recently established that the absence of a particular isoform of the osteoclast H+ATPase engenders resorptive dysfunction in mice by a mechanism independent of acidification (51).

Because of the magnitude of extracellular acidification required to degrade bone, the osteoclast is probably humans’ major proton-transporting cell. Intra-osteoclastic pH is maintained by an energy-independent Cl−/HCO3− exchanger on the cell’s antiresorptive surface (52). Electroneutrality, in turn, is conserved by a ruffled membrane-residing electroneutral Cl− channel, ClC-7, which is charge-coupled to H+ATPase (53). Hence, acidification of the extracellular resorptive microenvironment represents production of HCl within that space.

This balanced sequence of ion transport mediating acidification of the resorptive microenvironment is sufficient to mobilize the mineral phase, which occurs prior to degradation of bone organic matrix that consists largely of type 1 collagen. Although there is evidence that neutral matrix metalloproteinases participate in bone resorption (54), the pH of the resorptive space indicates that...
Mechanisms of bone resorption by osteoclasts. Acidification is initiated by carbonic anhydrase, generating $\text{H}^+$ and $\text{HCO}_3^-$ ions. $\text{H}^+$ is transported out of the cell and into the resorption lacuna by an electronegenic proton pump ($\text{H}^+\text{ATPase}$) located only in the ruffled border. This resorptive organelle is formed by the transport of vesicles containing the pump to the bone-apposed surface of the cell following $\alpha\beta_3$-mediated cell attachment. Intracellular pH is maintained by a $\text{Cl}^-/\text{HCO}_3^-$ exchanger at the antiresorptive surface. Electroneutrality is maintained by a chloride channel (ClC-7) located in the ruffled membrane that transports $\text{Cl}^-$ into the resorptive lacuna. The organic bone matrix, composed largely of type I collagen, is degraded by cathepsin K, an acid protease secreted into the resorptive space.

the principal enzyme is an acidic protease. In fact, bone collagen degradation is under the aegis of the lysosomal enzyme, cathepsin K, which, like the ion-transporting molecules, polarizes to the ruffled membrane upon osteoclast/bone attachment (55, 56). The resorptive space of cathepsin K–deficient mice contains naked collagen fibers from which bound hydroxyapatite has been removed, establishing that acidification, per se, is sufficient to mobilize bone mineral.

**DISORDERS OF DIMINISHED RESORPTION**

**Osteopetrosis**

Albers-Schoenberg first described osteopetrosis a century ago, and it still bears his name (57). All patients have marble-appearing bone with radiographic loss of distinction between cortex and trabeculae (58). Because resorption is dysfunctional, so is skeletal modeling, resulting in abnormally shaped bones. The family of osteopetrotic diseases is heterogeneous, ranging from an infantile malignant form inherited as an autosomal recessive to a relatively benign phenotype transmitted as an autosomal dominant. Most recessive forms of the disease, which occurs in approximately 1 in 300,000 births, are uniformly fatal unless treated, with patients dying of the consequences of marrow failure or neurological deficits due to brain and cranial nerve compression. Whereas patients with autosomal dominant osteopetrosis typically enjoy normal longevity, an intermediate autosomal
A recessive phenotype exists characterized by life to adulthood, but with significant complications such as cerebral calcification (59).

Regardless of clinical manifestation, the bones of patients with classical osteopetrosis exhibit similar features that reflect the failure to resorb the primary spongiosa. This region of the distal growth plate consists of bars of hypertrophic cartilage surrounded by bone, which, in normal circumstances, is resorbed and replaced by secondary spongiosa or metaphyseal bone. Because of osteoclast dysfunction, the primary spongiosa is not resorbed in osteopetrotic patients, resulting in its accumulation in the trabecular space. Thus, the diagnostic and unique feature of osteopetrosis is the accumulation of cartilaginous bars surrounded by bone throughout the marrow cavity (Figure 6a). This abnormal skeletal material results in structurally compromised bone, explaining the predisposition to fracture experienced by patients with all forms of the disease (60). However, the accumulation of nonresorbed primary spongiosa may not, in all circumstances, account for the marrow failure exhibited by patients with malignant osteopetrosis, as the same morphological event occurs in those with the benign phenotype. Two other marrow-obliterating events often occur in autosomal recessive osteopetrosis, which may explain its attendant marrow failure. First, secondary hyperparathyroidism typically occurs in this condition and leads to marrow fibrosis (Figure 6b) (61). Second, despite osteoclast dysfunction, patients with

![Figure 6](https://example.com/figure6.png)

**Figure 6**
Histologic features of osteopetrosis. *(a)* H&E-stained section of primary spongiosa in a child with osteopetrosis demonstrates retained cartilage (*purple*) and surrounding bone (*pink*). *(b)* Masson trichrome–stained section shows numerous osteoclasts (*arrow*) and very fibrotic marrow with few hematopoietic cells. *(c)* Masson trichrome–stained section shows marrow space filled by osteoclasts.
the disorder often have increased numbers of osteoclasts, which may be so abundant as to obliterate the marrow space (Figure 6).

The great majority of currently characterized forms of human osteopetrosis involve dysfunctional ion-transporting proteins, leading to failure to acidify the resorptive microenvironment (58). For example, patients with a lack of carbonic anhydrase II (CAII) activity, inherited as an autosomal recessive trait, develop osteopetrosis with mild (59) to severe (62) clinical abnormalities. In addition to their skeletal manifestations, CA-deficient patients suffer from cerebral calcification and renal tubular acidosis. Three mutations of the CAII gene account for 90% of all those affected (63). Although CAII-deficient osteopetrosis may be cured by marrow transplantation, renal tubular acidosis and cerebral calcification still progress (62).

The most common cause of severe autosomal recessive osteopetrosis is, however, due to mutation of the gene coding the α3 (116 kDa) subunit of the osteoclast H⁺ATPase (64). Osteoclasts in children with H⁺ATPase-deficient osteopetrosis are histologically normal, but ultrastructurally they may lack ruffled membranes, consistent with the failure of acidifying vesicles to insert in the bone-apposed plasma membrane (65). Two missense mutations account for all defects in the nine unrelated families (66) and appear to affect only the osteoclast, as marrow transplantation is curative. The ability to prevent osteopetrosis in α3-subunit-deficient mice by unmatched hematopoietic stem cell transplantation in utero holds promise for similar success in carrier families (67).

Mutation of the chloride channel gene CLCN7 is the second most common cause of human osteopetrosis with a broad spectrum of inheritance and clinical manifestations, and may be autosomal recessive or dominant (68–70). Surprisingly, severe or intermediate osteopetrosis may develop in children heterozygous for CLCN7 mutations. Many, if not all, cases previously described as type II autosomal dominant osteopetrosis probably reflect chloride channel dysfunction (69–71). This type of benign osteopetrosis is characterized by distinct vertebral and pelvic radiographs as well as predisposition to fracture (72, 73). A specific CLCN7 polymorphism may contribute to genetic regulation of bone mineral density and, hence, predisposition to postmenopausal osteoporosis (74).

Mutation of the cathepsin K gene in humans also promotes osteoclast dysfunction and causes a sclerotic bone disease distinct from osteopetrosis. This cathepsin K–deficient disorder, pyknodysostosis, is manifest by diffuse skeletal sclerosis, short stature and distinct cranial-facial abnormalities, and partial dissolution of the terminal phalanges of the hands and feet (75, 76). Like those with osteopetrosis, pyknodysostotic individuals have a predisposition to fracture.

DRUG-INDUCED OSTEOCLAST SUPPRESSION

Glucocorticoids

Antiresorptive medications are the most common means of treating osteoporosis, and most disorders of osteoclast arrest are induced iatrogenically. Glucocorticoid (GC)-induced bone loss is second in frequency only to attending menopause. In its chronic form, it is a disorder of attenuated bone formation due to arrested remodeling (77). GCs block osteoclast precursor proliferation and disrupt the organization of the cytoskeleton by inhibiting Vav3 and Rac, which decreases bone resorption. This dampening of osteoclast activity affects the remodeling sequence, translating into poor bone formation and diminution in bone quality. The reduction of the bone-suppressive effect of GCs in mice deleted of the GC receptor only in osteoclasts and their precursors confirms the role of inhibited remodeling in GC-induced osteoporosis. It likely also contributes to the avascular necrosis often experienced by patients chronically administered these drugs (78).
Bisphosphonates

Bisphosphonates are drugs that have had a profound impact on the treatment of osteoporosis and other disorders of accelerated resorption (79). Bisphosphonates comprise two general chemical and functional groups depending upon whether or not they are nitrogen (N) containing. Both families of bisphosphonates are avidly incorporated into the skeleton and are mobilized from bone and taken up by osteoclasts. N-free bisphosphonates are metabolized to generate toxic ATP analogs, thus directly promoting apoptosis. The N-containing compounds also ultimately cause osteoclast apoptosis, probably because of arrest of isoprenylated protein-regulated signaling pathways. However, their principal mode of action appears to be to block geranylgeranylation of small GTPases, an event required for cytoskeletal organization and bone resorption.

Bisphosphonates prevent postmenopausal bone loss in many women (80), but there is concern regarding their impact on selective yet undefined patient subpopulations (81). Like GCs, these remodeling-arresting drugs blunt microfracture repair, which might impact bone quality (82). Although presently anecdotal, some bisphosphonate-treated patients suffer unusual long bone fractures (83, 84), which are typically associated with compromised skeletal matrix.

Of more immediate concern, however, is the impact of bisphosphonates on the efficacy of the only available bone anabolic drug, teriparatide (parathyroid hormone 1–34). This agent, when administered intermittently, stimulates osteoblastic bone formation and is presently the most potent anti-osteoporosis drug available (85). Unfortunately, the bone-forming effects of the drug are substantially compromised when administered in conjunction with, or following, bisphosphonates (86). Because teriparatide is carcinogenic in a specific strain of rats, its administration in humans is presently limited to two years (87). Thus, a reasonable future strategy is to cycle the anabolic drug with antiresorptive therapies. Given the blunting effects of bisphosphonates on subsequent stimulated bone formation, shorter-acting resorption inhibitors, which are less likely to arrest remodeling, may be preferable.

DISORDERS OF ACCELERATED RESORPTION

Postmenopausal Osteoporosis

In the first years following menopause, bone turnover is accelerated, with increased activity of both osteoclasts and osteoblasts. Owing to an imbalance in the processes of resorption and synthesis, however, the net effect is bone loss, which is largely trabecular. Thus, the most prevalent sites of fracture in osteoporosis are the wrist, femoral neck, and vertebral bodies, in which the trabecular structure is key to overall bone strength. The primary driver of this process is the loss of estrogen, which acts by binding nuclear estrogen receptors (ERα and ERβ) and by binding to DNA sequences known as estrogen response elements (88). The action of estrogen receptors can be either activating or repressing (89). Additionally, estrogen can act through a membrane receptor to induce so-called nongenomic effects, which may include the induction of osteoclast apoptosis (90).

Estrogen deficiency increases the number of osteoclasts via direct and indirect mechanisms (Figure 7). In vitro, estrogen induces apoptosis of osteoclast lineage cells, although the maturational stage at which its effects are greatest is controversial (91–93). Therefore, osteoclast life span should be increased in the absence of estrogen. Although not established in humans, evidence gathered in the past few years indicates that estrogen deficiency in mice may promote bone resorption via T cell–mediated expression of TNFα and IFNγ. TNFα synergizes with RANKL to induce differentiation of osteoclast precursors (13, 14, 94). Additionally, TNFα acts on bone marrow stromal cells to increase their
Hypothetical model of the immune regulation of postmenopausal osteoporosis. At menopause, decreased levels of estrogen inhibit the production of TGF-β by bone marrow stromal cells, which leads to the upregulation of the cytokine IL-7. IL-7 promotes the proliferation of T cells and their secretion of both IFNγ and TNF. IFNγ acts on antigen presenting cells to increase MHC class II expression and presentation of a variety of antigens, which causes further T cell proliferation and, thus, TNF production. TNF acts on stromal cells to increase levels of RANKL, which leads to osteoclast differentiation and activation. TNF also acts directly on progenitors, synergizing with RANKL for differentiation. Estrogen also directly stimulates osteoclast lineage cells to undergo apoptosis. Low estrogen levels, therefore, enhance osteoclast survival.

Expression of RANKL (26). IFNγ upregulates MHC class II in macrophages and dendritic cells. These may present a variety of self and bacterial antigens (present in all healthy individuals), leading to a generalized increase in T cell activation and a concomitant increase in T cell TNFα secretion (95–98). Although IFNγ has direct inhibitory effects on osteoclastogenesis, its net impact in the context of antigen-mediated T cell activation may be proresorptive (99). Additionally, estrogen directly stimulates TGF-β production, and thus this factor decreases at menopause (100). TGF-β normally represses levels of IFNγ, as well as the cytokine IL-7. The postmenopausal rise in IL-7 stimulates T cell proliferation, promotes antigen presentation via upregulation of IFNγ, and possibly contributes to T cell expression of RANKL (101, 102). This elegant series of experiments suggests that, in the mouse, estrogen deficiency establishes interrelated positive feedback loops leading to immune-mediated osteoclast activation. However, this area remains controversial. Others have failed to find significant differences in the response to ovariectomy in mice deficient in T cells or IL-7 (103, 104). Furthermore, the role of cytokines and T cells in human osteoporosis has yet to be delineated.

Zaidi and colleagues recently reported an alternative means by which TNFα may...
modulate postmenopausal osteoporosis. They find that follicle-stimulating hormone (FSH) is a powerful inducer of osteoclastic bone resorption (105). Osteoclasts and their precursors express the FSH receptor and, despite the fact that mice lacking FSHβ or the FSH receptor are hypogonadal, they have normal bone mass. Circulating FSH increases during menopause, and this hormone directly controls TNFα production by macrophages and neutrophils (106). Thus, FSH may contribute to the increase in serum TNFα in menopausal women, contributing to accelerated bone resorption by osteoclasts.

The primary treatments for osteoporosis are bisphosphonates, which target the osteoclast to reduce bone resorption, and selective estrogen receptor modulators, which have estrogen-like effects on bone and pituitary but not in the uterus or breast. In light of the new data on the role of FSH, selective estrogen receptor modulators may act not only on the osteoclast but, additionally, by decreasing FSH secretion by the pituitary.

Osteoporosis also occurs in aging men, but its progress is slow, paralleling the prolonged continuous drop in free serum testosterone (107). Because estrogen in men is produced by the action of aromatase on androgen, levels of the two sex steroids change in tandem. Although androgens have direct actions on bone cells, including osteoclasts, the relative contributions of androgens and estrogens to bone mass in humans is still unclear. On the basis of studies of men with aromatase deficiency who are estrogen-deficient and usually treated with this hormone, the effects of estrogen on bone resorption appear to dominate (108). Despite the slow decline in bone mass in men, compared with the rapid perimenopausal loss in women, the pathological mechanisms of osteoporosis in both genders are, therefore, similar.

In addition to its association with hypogonadism, osteoporosis is prevalent in other endocrinopathies, such as hyperparathyroidism (109). Many studies have focused on the direct role of active thyroid hormone on bone cells, via its nuclear receptor (110), demonstrating a hormone-specific response by osteoblasts. If thyroid hormone is an important stimulus for bone resorption, then mice lacking functional thyroid hormone receptors would be predicted to have increased bone density. Although such mice have abnormal bone growth, they do not have the expected osteosclerosis (111). In contrast, mice lacking the receptor for thyroid stimulating hormone (TSH) are globally osteoporotic, with high bone turnover that mimics human hyperthyroid bone disease (112). The TSH receptor is expressed on both osteoclasts and osteoblasts and has a suppressive effect on each cell type. Thus, TSH may play an important role in the maintenance of bone mass.

### Inflammatory Arthritis

In inflammatory osteolysis, such as occurs in rheumatic and psoriatic arthritis, osteoclasts erode periarticular bone in areas of active synovitis, resulting in joint collapse (Figure 8) (113). Synovial fibroblasts and activated T cells express abundant osteoclastogenic cytokines such as RANKL within the inflammatory pannus (114–116). The pannus is also rich in RANK-expressing monocytes, which differentiate into bone-resorbing osteoclasts (114, 117). Animal experiments demonstrate that blockade of RANKL-mediated osteoclastogenesis with OPG prevents periarticular bone erosion even in the face of severe inflammation (116). Additionally, mice lacking RANKL, which are severely osteopetrotic owing to a complete absence of osteoclasts, are protected from arthritis-induced bone destruction despite unaltered inflammation (118). Thus, RANKL is a critical mediator of bone erosion in the inflammatory milieu.

Models of inflammation-induced skeletal disease yield a number of common regulatory pathways between osteoclasts and immune cells, including NFκB. This family of transcriptional activators is composed of five subunits that form homo- and heterodimers. NFκB exerts its effects via two pathways, each
Bone erosion in rheumatoid arthritis. (a) Hand radiograph from a patient with rheumatoid arthritis, demonstrating bone loss at the metacarpalphalangeal joints (arrows). Figure courtesy of D. Rubin. (b) Tartrate-resistant-acid-phosphatase-stained section from a patient with rheumatoid arthritis taken from the metacarpalphalangeal joint, showing osteoclasts eroding bone adjacent to inflammatory pannus. Figure courtesy of G. Schett.

with distinct regulatory kinases and inhibitors and eventuating in different combinations of subunits (119). The ubiquitous classical pathway is activated by most NFκB-inducing cytokines, including RANKL and TNFα. In this circumstance, IKKβ controls degradation of IκBα, leading to nuclear translocation of primarily p65/p50 dimers. In the alternative pathway, activated only by a subset of cytokines, including RANKL but not TNFα, NIK and IKKα control processing of p100, leading to transactivation by RelB-containing NFκB complexes.

Both classical and alternative NFκB pathways are activated in arthritis, and their blockade ameliorates inflammatory and osteolytic components of the disease. Classical NFκB can be blocked with an IκB superrepressor (inhibiting degradation of the inhibitor) or a NEMO-binding domain peptide (preventing IKKβ activation) (120–122). Both approaches dampen inflammation and the attendant bone erosion in animal models. Additionally, pure populations of precursors treated with these inhibitors fail to form osteoclasts in vitro. Similarly, absence of NIK, the upstream kinase of the alternative pathway, dampens arthritis-associated lymphocyte-dependent inflammation and directly arrests osteoclastic bone resorption (123). Thus, both the classical and alternative NFκB pathways are potential targets to prevent the bone destruction of inflammatory arthritis.

A broad spectrum of inflammatory cytokines is elaborated by lymphocytes, macrophages, and neutrophils in inflammatory arthritis. These include TNFα, IL-1, IL-6, and MCP-1, which enhance osteoclast differentiation and resorptive function (124). TNFα is particularly important in the pathogenesis of inflammatory osteolysis because it synergizes with RANKL to directly promote osteoclast differentiation (13, 14, 94). TNFα also acts on nonhematopoietic cells (stromal and endothelial cells) to induce expression of M-CSF and RANKL (26, 125). In fact, deletion of TNF receptors in stromal cells suppresses osteolysis more profoundly than does elimination of the same receptors in osteoclast precursors (126). Antibody-mediated blockade of c-Fms, the receptor for M-CSF, also prevents periarticular bone erosion (126).

TNFα blockade is a particularly effective means of treating rheumatoid arthritis (127). However, single-agent therapy is not curative, and side effects of anti-TNFα therapy, including immune suppression, limit the drugs'
use in many patients. Animal models suggest that combination therapy, such as blockade of TNFα and IL-1, is more effective than either drug alone (128).

**Osteolytic Bone Metastasis**

Tumors have distinct patterns of metastasis, suggesting that specific factors expressed in the target tissues determine hospitable sites (represent fertile soil). For example, carcinoma of breast, prostate, and lung, as well as multiple myeloma, have a predilection for the skeleton (129). Bone-homing tumors produce many factors that stimulate osteoclast differentiation or activation. These include parathyroid hormone-related protein (PTHrP), RANKL, IL-6, IL-8, and IL-11 (130). Expression of at least two of these factors (PTHrP and IL-11) is controlled by TGF-β, which is made by osteoblasts and stored in the bone matrix in latent form, and activated when mobilized by the osteoclast. Thus, tumors stimulate osteoclasts, and osteoclast activity ultimately causes tumors to release more of these osteoclast-activating factors, generating a vicious cycle of tumor growth and bone destruction (Figure 9). The result is often bone pain, pathological fracture, or hypercalcemia of malignancy. In the latter, the amount of calcium released from the bone exceeds the capacity for renal excretion, and serum calcium levels rise to levels that can be life threatening.

Several animal models demonstrate that neoplasia-induced bone destruction is not the direct product of the tumor but of osteoclasts recruited to the malignant site. For example, mice with diminished osteoclast activity, such as those lacking the αvβ3 integrin or c-Src (131), or overexpressing OPG (132), do not develop tumor-associated bone loss upon intracardiac or intratibial injection of cancer cell lines that readily form osteolytic lesions in wild-type animals. Bisphosphonates also block the formation of bone tumors, and osteoclast inhibition prevents spontaneous osteolytic tumors in HTLV-1 Tax transgenic mice (133). In breast cancer patients, treatment with bisphosphonates decreases skeletal events, such as pathological fracture or bone pain, by 30%–40% (134). Although controversial, pharmacological inhibition of osteoclast activity may increase the survival of patients with skeletal metastasis (135).

TGF-β is another potential therapeutic target suggested by the osteoclast-tumor vicious cycle model. Insertion of a dominant-negative TGF-β type II receptor into an osteolytic breast cancer cell line inhibits PTHrP secretion and formation of bone tumors (136, 137). A small molecule inhibitor of TGF-β receptor type I, SD-208, has similar anti-osteolytic activity in this breast cancer model (130).
Most bone metastases due to prostate cancer are radiodense and thus considered osteoblastic. It is therefore curious that many afflicted patients with poor prognosis have extremely high serum levels of markers of bone resorption such as serum N-telopeptide (138–140), suggesting that osteoclast activity is profound even in osteoblastic prostate metastasis. In keeping with this posture, treatment with bisphosphonates reduces morbidity in these individuals.

Macrophage inflammatory protein 1α, which is present at elevated levels in bone marrow plasma and correlates with the presence of osteolytic lesions, may be a key osteoclastogenic factor in multiple myeloma (141). Macrophage inflammatory protein 1α has osteoclastogenic activity independent of RANKL in vitro, but also enhances differentiation in concert with RANKL and IL-6. RANKL is highly expressed in the bone microenvironment in myeloma, and its blockade with OPG inhibits bone destruction in mouse models of the disease (142).

Skeletal tumors usually induce both bone formation and resorption. Myeloma is atypical in this regard, as it is generally a purely osteolytic lesion with a striking paucity of osteoblasts. This absence of bone formation may relate to the overproduction of DKK1, which inhibits the osteoblastogenic Wnt pathway (143). The combination of robust osteoclastic resorption and absent osteoblastic bone formation promotes the pathological fractures frequent in myeloma patients.

Paget’s Disease of Bone

Paget’s disease (PD) is a focal disorder of remodeling characterized by both bone sclerosis and lysis (Figure 10). Both formation and resorption are hyperactive, but the primary disorder arises in the osteoclast (144). Pagetic osteoclasts are numerous, large, and hypernucleated, and their nuclei typically fail to polarize to the cell’s antiresorptive surface. New bone is usually woven and lamellar, and highly vascular and disorganized. It may be significantly deformed, contributing to fracture and pain. Approximately 40% of patients have a first-degree relative with the disease, and transmission appears to be autosomal dominant (145). The pathophysiology of PD is still incompletely understood, but mutation of p62/sequestosome-1 and paramyxovirus infection are compelling candidate mediators. In approximately 30% of familial PD and a few sporadic cases, there is an activating mutation in p62 (146), an adaptor protein important for activating NFκB, p38, and MAPK. Although mutated p62 increases during in vitro osteoclast differentiation in response to
RANKL and TNFα, the full pagetic phenotype is not obtained in vivo using a p62 transgene driven by the TRAP promoter (147). These mice exhibit increased bone resorption without the coordinate stimulated bone formation typical of the disease.

Pagetic osteoclasts contain viral-like inclusions and mRNAs for measles, and several related viruses are present in the majority of PD patients (148). Expression of measles virus nucleocapsid (MVNP) in human osteoclast precursors promotes bone resorption, enhances sensitivity to vitamin D3, and induces IL-6 production, all hallmarks of pagetic osteoclasts (149). Osteoclasts specifically expressing MVNP are increased in number, large, and hyperactive. In keeping with PD being a disorder of accelerated remodeling, the same holds true for the osteoblasts in these conditionally transgenic animals (150). Some of these mice also have focal formation of characteristic pagetoid lesions, with thick trabeculae composed of woven bone. Expression of MVNP in mutant p62 transgenic osteoclasts further increases RANKL-induced NFκB and promotes vitamin D3 hypersensitivity (147), suggesting that these two pathways can act synergistically. Nevertheless, many aspects of PD in humans are not explained by these animal models, including incomplete penetrance of genetic forms, the focal nature of the disease, and its geographic selectivity (144).

CONCLUSIONS

Although osteoclasts make up only a small fraction of bone cells, their activity is critical for maintaining normal skeletal strength and calcium homeostasis. However, pathological changes in the activity of these bone-resorbing cells occur in many diseases and are responsible for significant symptoms. Animal models, and the ability to grow and manipulate these cells in culture, have defined many of the mechanisms whereby these cells differentiate and function in health and disease.

SUMMARY POINTS

1. The osteoclast, derived from hematopoietic progenitors, is responsible for the resorption of organic and inorganic components of bone and is necessary for normal bone homeostasis.
2. Resorption requires αvβ3 integrin-mediated osteoclast migration and attachment to bone matrix, followed by polarization and formation of a sealing zone to create an acidic microenvironment.
3. Defects in components of the resorptive machinery, including CA, H⁺ ATPase, and chloride channel, disrupt bone resorption and cause osteopetrosis.
4. GCs and bisphosphonates disrupt osteoclast function.
5. Estrogen deficiency stimulates osteoclast differentiation and survival, both directly and indirectly, leading to postmenopausal osteoporosis.
6. In arthritic joints, inflammatory cytokines, largely via the NFκB pathway, enhance osteoclastogenesis and cause local osteolysis.
7. Tumors that favor bone as a metastatic site secrete many osteoclastogenic factors, and the resulting bone resorption stimulates tumor cells, leading to a vicious cycle of tumor growth and bone destruction.
FUTURE ISSUES

1. Can effective short-acting specific osteoclast inhibitors be developed?
2. What is the osteoclast-osteoblast coupling factor(s)? How do osteoclasts induce osteoblast activity in vivo?

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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Errata

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