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Osteoclasts: What Do They Do and How Do They Do It?

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As Americans live longer, degenerative skeletal diseases, such as osteoporosis, become increasingly prevalent. Regardless of cause, osteoporosis reflects a relative enhancement of osteoclast activity. Thus, this unique bone resorptive cell is a prominent therapeutic target. A number of key observations provide insights into the mechanisms by which precursors commit to the osteoclast phenotype and how the mature cell degrades bone. The osteoclast is a member of the monocyte/macrophage family that differentiates under the aegis of two critical cytokines, namely RANK ligand and M-CSF. Tumor necrosis factor (TNF)-α also promotes osteoclastogenesis, particularly in states of inflammatory osteolysis such as that attending rheumatoid arthritis. Once differentiated, the osteoclast forms an intimate relationship with the bone surface via the αvβ3 integrin, which transmits matrix-derived, cytoskeleton-organizing, signals. These integrin-transmitted signals include activation of the associated proteins, c-src, syk, Vav3, and Rho GTPases. The organized cytoskeleton generates an isolated microenvironment between the cell's plasma membrane and the bone surface in which matrix mineral is mobilized by the acidic milieu and organic matrix is degraded by the lysosomal protease, cathepsin K. This review focuses on these and other molecules that mediate osteoclast differentiation or function and thus serve as candidate anti-osteoporosis therapeutic targets.   (Am J Pathol 2007, 170:427–435; DOI: 10.2353/ajpath.2007.060834)

Skeletal mass and structure dictate the life style of many Americans. Because 50% of women reaching 65 years of age will experience an osteoporotic fracture, skeletal health has a profound financial and social impact. Despite its static reputation, bone is an ever-changing organ that is remodeled by the continuous activities of osteoclasts and osteoblasts. Because osteoclasts are culprits in many diseases of systemic and local bone loss, their activity is essential for the process of bone remodeling that replaces effete, brittle bone with new.

The osteoclast, which is the sole bone-resorbing cell, is a unique polykaryon whose activity, in the context of the osteoblast, dictates skeletal mass. All forms of acquired osteoporosis reflect increased osteoclast function relative to that of the osteoblast. Thus, pharmacological arrest of the osteoclast is a mainstay of treating systemic bone loss as attends menopause and as occurs locally, as in the periarticular osteolysis of rheumatoid arthritis and skeletal metastasis.

Much of what we know about the osteoclast is derivative of observations made in osteopetrotic animals and patients. Osteopetrosis is, by definition, increased bone mass attributable to arrested bone resorption. Although virtually all forms of osteopetrosis are genetically based, the disease may be induced in children treated with bisphosphonates, which promote osteoclast apoptosis.[1]

The osteopetrotic spectrum reflects either failed normal recruitment of osteoclasts or resorptive dysfunction of the differentiated cells. The subset of osteopetrosis that is caused by arrested osteoclastogenesis can be further subdivided into osteoclast-autonomous and nonautonomous forms.[2] Osteoclast-autonomous osteopetroses are those in which the molecular defect is present in the osteoclast or its precursor. Osteoclast nonautonomous forms represent those in which the molecular defect is present in cells that support osteoclast precursor differentiation or function of the mature resorptive cell.
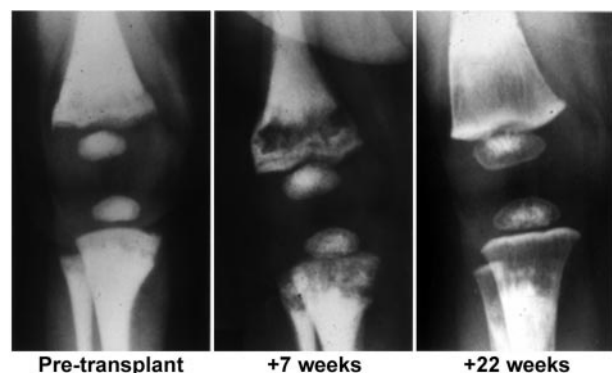
Figure 1. First cure of patient with malignant osteopetrosis. The patient, a 3-month-old female, received a marrow transplant from her HLA/MLC compatible brother. Dramatic resolution of the sclerotic bone was evident within 7 weeks. The patient is well 27 years later. The presence of Y-chromosomes in her osteoclasts after transplant established the cell's hematopoietic ontogeny in man (reprinted with permission from the *N Engl J Med* 1980, 302:701–708).[5]

Thus, only osteoclast-autonomous osteopetrosis is rescued by marrow transplantation, which is the gold standard for establishing that the genetic defect is restricted to osteoclast lineage cells.

The pioneering experiments of Donald Walker,[3,4] performed in the 1970s, provided the first insights into the origin of the osteoclast. At that time, there was little information regarding the ontogeny of osteoclasts, and in fact, a popular hypothesis held that the osteoclast and osteoblast enjoyed a common precursor. Walker demonstrated that parabiosis to normal littermates or infusion of wild-type spleen cells cured osteopetrotic mice. Because the cause of osteopetrosis is failure of either osteoclast recruitment or function, Walker's experiments established that the murine resorptive cell's precursor is of hematopoietic origin. The cure of an osteopetrotic infant by marrow transplantation established that the same holds in humans (Figure 1).[5] This transgender transplant allowed donor cells to be tracked and thus established that the osteoclast is of hematopoietic origin. Ultimately, Suda's group[6] demonstrated that the osteoclast precursor is a member of the monocyte/macrophage family, and, although the resorptive cell can be generated from mononuclear phagocytes of various tissue sources, the principal precursor resides in the marrow. This observation laid the foundation for generating osteoclasts *in vitro*, thus providing the opportunity to perform meaningful biochemical and molecular experiments. We now know that the osteoclast precursor circulates and that assumption of the osteoclast phenotype *in vivo*, including multinucleation and the capacity to resorb bone, requires contact with skeletal matrix.

Osteoclastogenic Cytokines

Suda's[6] initial experiments also revealed that generation of osteoclasts in culture requires physical contact of the precursor cells with specific mesenchymal cells such as osteoblasts or marrow stromal cells. Although perplexing at first, this critical observation yielded the discovery of
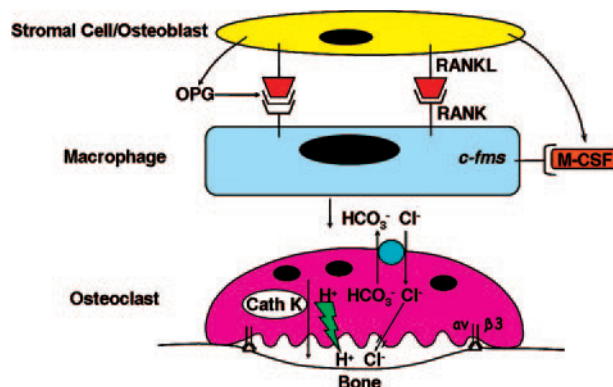
Figure 2. Osteoclast differentiation and function. Osteoblast lineage cells produce the osteoclastogenic cytokines RANKL and M-CSF, which recognize their respective receptors RANK and c-fms on macrophages, principally of marrow origin. OPG, also synthesized by osteoblast lineage cells, is a soluble decoy receptor that binds RANKL, thus preventing its interaction with RANK. RANKL and M-CSF are sufficient to promote the osteoclast phenotype. On contact with bone, the osteoclast polarizes via matrix-derived signals transmitted by the $\alpha v \beta 3$ integrin, enabling the cell to form an isolated microenvironment between itself and the bone surface. The microenvironment is acidified by H^+ATPase-mediated extracellular transport of protons. Intracellular pH is maintained by an electroneutral HCO_3^-/Cl^- exchanger. The Cl^- entering the cell is released into the resorptive microenvironment by an ion channel charge coupled to the H^+ATPase. The acidified microenvironment mobilizes the bone mineral, thereby exposing the organic phase of bone that is degraded by cathepsin K (modified and reprinted with permission from *Science* 2000, 289:1504–1508).

the key osteoclastogenic cytokine, receptor activator of nuclear factor-κB ligand (RANKL).[7,8] RANKL, a member of the TNF superfamily, is a membrane-residing protein on osteoblasts and their precursors that recognizes its receptor, RANK, on marrow macrophages, prompting them to assume the osteoclast phenotype. Like TNF, RANKL is a homotrimer but contains four unique surface loops that distinguish it from other TNF family cytokines.[9] Mutagenesis of selected residues in these RANKL loops modulates RANK's capacity to promote osteoclastogenesis. These studies prompted development of structure-based inhibitory peptides that arrest bone resorption and are thus therapeutic candidates (Figure 2).[10] Although RANKL, in physiological circumstances, is principally expressed by mesenchymal cells of osteoblast lineage, the osteoclastogenic cytokine is produced in abundance, by T lymphocytes in states of skeletal inflammation such as rheumatoid arthritis.[11] In this circumstance, RANKL may be cleaved from the cell membrane and then interact with RANK as a soluble ligand.

RANKL activity is negatively regulated in the circulation by osteoprotegerin (OPG), which competes with RANK as a soluble decoy receptor.[12] In fact, the discovery of RANKL as the key osteoclastogenic cytokine followed on the observation that mice overexpressing OPG develop osteopetrosis. OPG, like RANKL, is produced by osteoblast lineage cells,[13] and disturbance of the OPG/RANKL ratio seems to dictate the rate of bone resorption in a number of pathological states.[14] Furthermore, homozygous deletion of the OPG gene, *TNFRSF11B*, causes juvenile Paget's disease.[15]

TNF-α promotes osteoclastogenesis in conditions such as inflammatory osteolysis[16] and interestingly, postmenopausal osteoporosis.[17] The proinflammatory cyto-

kine enjoys a potent synergistic relationship with RANKL, but whether TNF-α, alone, prompts osteoclast differentiation is controversial. Although Lam and colleagues[18] report that TNF-α is incapable of inducing osteoclast precursors to differentiate unless attended by permissive levels of, or primed by, RANKL, Kim and colleagues[19] found that the inflammatory cytokine is capable of osteoclastogenesis in the absence of RANK signaling *in vitro* if attended by transforming growth factor (TGF)-β. Although the latter *in vitro* observation is provocative, the failure of TNF-α to induce meaningful osteoclast formation in RANK-deficient mice calls into question the biological relevance of TGF-β as a substitute for RANKL.[20] On the other hand, there is reasonable evidence that the capacity of TNF-α to activate the fully differentiated osteoclast may occur independently of RANK signaling.[21,22]

The unique osteoclastogenic properties of RANKL reflect structural components that dictate its capacity to uniquely occupy RANK,[9] which activates TRAF6, probably an essential step in osteoclast differentiation. In fact, competition for TRAF6 by the LIM domain-only protein FHL2 reduces TRAF6/RANK association and osteoclastogenesis.[23] Although other receptors such as interleukin (IL)-1R1, CD40, and Toll-like receptor also recruit TRAF6, they do not do so as abundantly as RANK, which may explain their failure to induce osteoclast differentiation alone.[24,25]

On the other hand, the role of TRAF6 in osteoclastogenesis is controversial. Two laboratories independently generated TRAF6$^{-/-}$ mice, and both strains are osteopetrotic. In one case, osteoclasts are abundant but dysfunctional because of failure to organize their cytoskeleton.[26] In contrast, the other TRAF6$^{-/-}$ strain is devoid of osteoclasts.[27] The fact that a cell-permeable peptide, based on the crystal structure of the RANK sequence recognizing TRAF6, arrests osteoclastogenesis *in vitro*[28] supports the concept that the adaptor molecule is essential for osteoclast differentiation.

RANKL promotes osteoclastogenesis by stimulating a variety of transcription factors and all three families of MAP kinases.[2] The key genomic osteoclastogenic event is activation of an AP-1/NFATc1 transcription complex.[29,30] RANKL generates this complex by inducing expression of the c-Fos family[31] and promoting nuclear translocation of Jun proteins.[32] NFATc1, in turn, is dephosphorylated by calcineurin, which also promotes its nuclear translocation. Importantly, deletion or inactivation of c-Fos,[33] c-Jun,[32] or NFATc1[34] results in failed osteoclast differentiation and severe osteopetrosis. We find that in keeping with its inability to promote osteoclastogenesis on its own, TNF-α is an inefficient activator of NFATc1 (W. Zou, unpublished data). RANKL also promotes bone resorption by inducing the mature osteoclast to generate a complex composed of its receptor, TRAF6, and c-Src, which the cytokine specifically recruits to lipid rafts in the plasma membrane.[35] This event requires organization of fibrillar actin and is mediated via the phosphoinositide-3-kinase (PI3-K)/AKT pathway.

TNF-α, which is expressed as both a membrane-residing and soluble molecule, is probably the key cytokine mediating the periarticular bone loss of rheumatoid arthritis.[16] It promotes osteoclast formation and activation in the inflamed joint by stimulating RANKL production by marrow stromal cells and by directly stimulating differentiation of osteoclast precursors.[18,36,37] TNF-α and RANKL are synergistic, and minimal levels of one markedly enhance the osteoclastogenic capacity of the other.[18]

TNF-α targets two membrane receptors, but its osteoclastogenic properties are mediated by TNF receptor type 1 (p55r). Although controversial, we find TNF receptor type 2 (p75r) is actually anti-osteoclastogenic. Thus, mice bearing only p55r generate substantially more osteoclasts in response to the cytokine than do those expressing only p75r.[38] In keeping with this observation, soluble TNF-α, which preferentially activates p55r has potent osteoclastogenic properties, whereas those of the membrane-associated cytokine, which recognizes p75r, are negligible.[38] Likewise, lipopolysaccharide, which is central to the alveolar bone loss attending periodontal inflammation, mediates its osteoclastogenic effects via p55r.[39]

TNF is produced and targeted by a variety of cells in the inflamed joint. Osteoclast precursor and marrow stromal cells each express p55r.[40] Although both cell types are central to pathogenesis of inflammatory osteolysis, the greater contribution, in states of moderate inflammation, is made by stromal cells, which produce the osteoclastogenic cytokines, RANKL, M-CSF, and IL-1 when exposed to TNF-α. As the inflammatory process becomes more aggressive, TNF-α may promote osteoclast formation by directly stimulating the cell's precursors in the absence of stromal cells responsive to the cytokine.[18,36,37]

IL-1, enhances osteoclastogenesis only in the presence of permissive levels of RANKL.[40] IL-1 also mediates a substantial component of TNF-α's osteoclastogenic effect in both marrow stromal cells and osteoclast precursors and does so in a p38 MAP kinase-dependent manner.[40] The intimate relationship between TNF-α and IL-1 is reflected by the fact that optimal arrest of inflammatory osteoclastogenesis and bone destruction requires blockade of both.[41]

Macrophage colony stimulating factor (M-CSF), which like RANKL is produced by marrow stromal cells, is essential for macrophage survival and proliferation as well as regulating osteoclastogenesis. The pivotal role of M-CSF in osteoclast recruitment is reflected by the *op/op* mouse, which lacks functional M-CSF and has osteoclast-deficient osteopetrosis.[42] In fact, generation of pure populations of osteoclasts *in vitro* is achieved by culturing marrow macrophages in the presence of only RANKL and M-CSF.

A major component of pathological bone loss as occurs in inflammatory osteolysis, reflects enhanced expression of RANKL and M-CSF induced by excess of local cytokines, particularly TNF-α.[40] Interestingly, TNF-α also promotes c-fms production, and the osteolysis of the inflamed joint is completely arrested by blocking the M-CSF receptor.[37,43] In this regard, osteoclastogenesis may be pathologically increased by hypersensitivity to M-CSF. Such a scenario exists in mice lacking SHIP1, a lipid phosphatase that dephosphorylates phosphatidyl-

inositol 3,4,5-triphosphate and thus, inactivates AKT.[44] $SHIP^{-/-}$ osteoclasts are enlarged and aggressively resorb bone prompting an osteoporotic phenotype *in vivo*.

The sole M-CSF receptor, c-fms, is a tyrosine kinase that autophosphorylates on occupancy, thereby activating ERK1/2 and PI3-K/AKT. This signaling pathway promotes osteoclast precursor proliferation and survival of the differentiating and differentiated osteoclast.[45] Prolonged ERK activation by M-CSF prompts its nuclear translocation where it induces c-Fos and probably NFATc1 expression.[45]

Using a chimeric receptor approach, we have established that c-fms activation involves phosphorylation of Y807, which enhances the receptor's kinase activity, leading to autophosphorylation of Y559, Y697, and Y721.[46] These phosphorylated tyrosine residues serve as c-fms docking sites for the SH2 domains of a series of downstream signaling molecules. Characterization of the role of individual tyrosine residues in the c-fms cytoplasmic domain was established in authentic osteoclasts, which express the wild-type M-CSF receptor. Like c-fms, the erythropoietin (Epo) receptor dimerizes on occupancy. Hence, our strategy involved transduction of marrow macrophages with a plasmid coding for the external domain of the Epo receptor linked to the transmembrane and cytoplasmic domains of c-fms. Stimulation with Epo is as effective as M-CSF in the osteoclastogenic process in these transductants, permitting meaningful evaluation of c-fms tyrosine mutations on authentic osteoclast differentiation and activation.[42]

Osteoclast Formation and Function

The capacity to generate osteoclasts *in vitro* and to physiologically confirm the significance of candidate osteoclast-regulating molecules by their genetic deletion *in vivo* has yielded insights into the mechanisms of osteoclast differentiation and cellular resorption of bone. The most successful strategy has been to determine whether genetically manipulated mice have a bone phenotype, principally osteopetrosis in states of osteoclast loss of function and osteoporosis when resorptive activity is increased. This approach permitted identification of a number of essential regulators of osteoclast formation and function. For example, the discovery of osteoclast-deficient osteopetrosis in mice lacking PU.1 confirmed that the ETS domain transcription factor, which is essential for initial macrophage differentiation, mediates the earliest known event in osteoclastogenesis.[47]

Mice lacking the p50 and p52 nuclear factor (NF)-κB subunits also fail to generate osteoclasts and are osteopetrotic.[48] NF-κB is activated in osteoclast precursors by IKK via the classical (canonical) and alternative pathways. The β isoform of IKK induces the classic pathway by phosphorylating the cytosolic NF-κB binding proteins, IκBs, thus targeting them for proteosomal degradation thereby mobilizing NF-κB's transcriptional activity. Importantly, administration of nondegradable IκB peptides or those inhibiting NEMO-mediated IKK activation, prevents the bone destructive complications of inflammatory arthritis in mice.[49–52]

The role of IKKα in basal and pathological osteoclastogenesis is less clear than that of the β isoform. IKKα modulates the alternative NF-κB pathway and mice lacking NF-κB-inducing kinase (NIK), are resistant to RANKL-stimulated osteoclastogenesis and the bone destruction attending inflammatory arthritis.[53] On the other hand, mice bearing an IKKα-inactivating mutation are indistinguishable from wild type as regards lipopolysaccharide-induced osteoclastogenesis and periarticular osteolysis.[54]
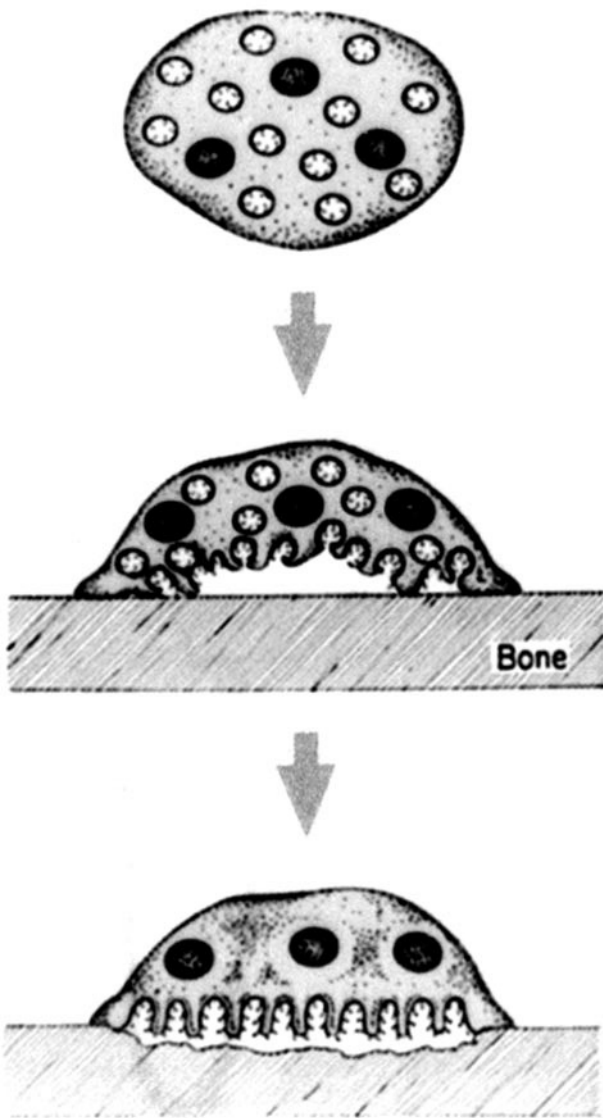
Once differentiated, the capacity of the mature osteoclast to resorb bone depends on its ability to synthesize and mobilize a series of electrolytes and degradative enzymes. Hence, the resorbing osteoclast must create an isolated microenvironment between itself and the bone surface into which it secretes protons via an electrogenic $H^+ATPase$ (proton pump).[55,56] In fact, mutations of the $H^+ATPase$ is the most common known cause of osteopetrosis in man.[57] The potential intracellular alkalinity induced by the massive proton transport is prevented by electroneutral chloride/bicarbonate exchanger.[58] The Cl^- that enters the cell in exchange for HCO_3^-, is transported into the resorptive microenvironment via a channel, charge coupled to the $H^+ATPase$, thus generating HCl, which produces an ambient pH approximating 4.5.[59] The acidity within the degradative space mobilizes the mineral phase exposing the organic matrix of bone, which is subsequently degraded by the collagenolytic lysosomal protease cathepsin K.[60,61] Inactivating mutations of the Cl^- channel also cause human osteopetrosis,[62] whereas the sclerosing bone disease pyknodysostosis reflects failure to produce functional cathepsin K.[61]

Osteoclast Cytoskeleton

The osteoclast enjoys a unique cytoskeleton that enables it to polarize on bone and thus degrade mineralized matrix. Certainly, the two most dramatic features of the osteoclast cytoskeleton are its ruffled membrane and actin rings, both of which are formed when the cell contacts bone. The ruffled membrane is the product of intracellular acidified vesicles transiting, probably via microtubules, to the bone-apposed plasma membrane[63] into which they insert under the aegis of the small GTPase Rab3D.[64] The product of this event is delivery of the $H^+ATPase$ into the plasma membrane, which greatly increases its surface extent, yielding a villous-like structure unique to the osteoclast. It is the cell's resorptive organelle and appears only during the process of bone degradation (Figure 3). Unlike most other cells, osteoclasts do not organize their fibrillar actin into stress fibers, but instead form actin rings or sealing zones on contact with bone. The actin ring is a circumferential structure that surrounds the ruffled membrane and isolates the acidified resorptive microenvironment from the general extracellular space.[65]

The fact that skeletal degradation requires physical intimacy between the osteoclast and bone indicates that molecules mediating cell/matrix recognition and attach-
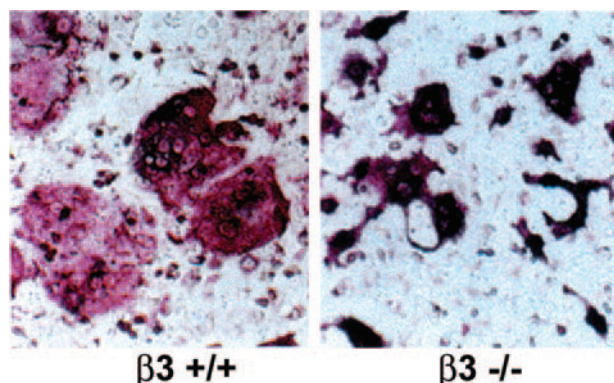
tected.[69] Thus, αvβ3 presents as a candidate anti-resorptive therapeutic target and in fact, small molecule inhibitors of the integrin are in clinical trial for treatment of osteoporosis.[70–72]

The αv family of integrins recognizes the amino acid motif Arg-Gly-Asp (RGD), resident in a number of bone matrix proteins such as osteopontin and bone sialoprotein. Occupancy by these ligands activates the integrin by changing its conformation.[73] This event, known as outside-in signaling, induces a number of intracellular events, one of the most prominent being organization of the actin cytoskeleton.

αvβ3 is also modulated by an inside-out mechanism that is stimulated by intracellular events, such as those stimulated by M-CSF occupancy of its receptor c-fms.[45] C-fms autophosphorylation of Tyr697 activates the integrin by signals that alter the conformation of its cytoplasmic domain.[45] In fact, αvβ3 and c-fms enjoy a collaborative relationship during osteoclastogenesis. This relationship is illustrated by the capacity of high-dose M-CSF to rescue the retarded osteoclast differentiation, in a c-Fos- and ERK1/2-dependent manner that occurs on β3 integrin subunit deletion.[45] ERK seems to regulate the osteoclast by two distinct pathways. Short-term activation of the MAP kinase stimulates proliferation of the resorptive cell's precursors whereas prolonged ERK activation prompts its nuclear translocation where it induces expression of early immediate genes, such as c-Fos, essential to osteoclast differentiation.[45] The paradox of arrested osteoclast differentiation of αvβ3-deficient precursors *in vitro* in face of a 3.5-fold increase *in vivo* of mature osteoclasts in mice lacking the integrin may be explained by the abundant M-CSF present in the marrow of the mutant animals.[45,66] Although exposure of αvβ3-deficient osteoclasts to high-dose M-CSF rescues osteoclastogenesis and cytoskeletal organization, the integrin is necessary for the cell's capacity to resorb bone.[45]

Because αvβ3 is the principal integrin expressed by osteoclasts and competitive ligands arrest bone resorption *in vitro*,[70] we deleted the β3 integrin subunit in mice.[66] Mice lacking αvβ3 generate osteoclasts incapable of optimal resorptive activity as their ruffled membranes and actin rings are abnormal *in vivo*.[66] The deranged cytoskeleton of the mutant osteoclasts is also manifest by failure of the cell to spread *in vitro*[66] (Figure 4). In consequence, β3$^{-/-}$ mice progressively increase bone mass with age. Interestingly, αvβ3 also regulates osteoclast longevity. The unoccupied integrin transmits a positive death signal mediated via caspase 8, and, therefore, resorptive cells lacking αvβ3 actually survive longer than wild type.[74]

The osteoclast functions in a cyclical manner, first migrating to a bone resorptive site to which it attaches. It degrades the underlying bone, detaches, and reinitiates the cycle. During matrix attachment, αvβ3 is predominantly in its inactive conformation and resident in podosomes, which in turn reside in the actin ring.[65] Podosomes are dynamic, adhesive dot-like structures consisting of an actin core surrounded by the integrin and associated cytoskeletal proteins such as vinculin, α-actinin, and talin. Thus, the signals mediating matrix

Figure 3. Formation of the osteoclast ruffled membrane. The unattached osteoclast contains numerous acidified vesicles bearing H$^+$ATPases (proton pumps) illustrated as spikes. On attachment to bone, matrix-derived signals polarize the acidified vesicles to the bone-apposed plasma membrane into which they insert under the aegis of Rab3D. Insertion of the vesicles into the plasma membrane greatly increases its complexity and delivers the H$^+$ATPases to the resorptive microenvironment.

ment must be involved. Cell/matrix recognition is mediated by integrins. These α/β heterodimers consist of long extracellular and relatively short intracellular domains that function not only to attach cells to extracellular matrix but also to transmit matrix-derived signals to the cell's interior. We have discovered that the αv family of integrins are differentially expressed by osteoclasts during their maturation and that two members, namely αvβ3 and αvβ5, are functional in these cells. αvβ5, but not αvβ3 appears on marrow macrophages maintained in the sole presence of M-CSF.[66] With exposure to RANKL and assumption of the osteoclast phenotype, αvβ5 disappears to be replaced by αvβ3.[67] Interestingly, αvβ5 deficiency accelerates bone loss in the estrogenopryvic[68] state whereas oophorectomized animals lacking αvβ3 are pro-

Figure 4. $\alpha v \beta 3$ integrin-deficient osteoclasts have an abnormal cytoskeleton. Both wild-type ($\beta 3^{+/+}$) and $\beta 3^{-/-}$ osteoclasts contain tartrate-resistant acid phosphatase (red reaction product) and are multinucleated. Whereas wild-type osteoclasts spread in culture, those lacking $\alpha v \beta 3$ fail to do so, manifesting a deranged cytoskeleton (reprinted with permission from the *J Clin Invest* 2000, 105:433–440).[66]

attachment probably do not require activated $\alpha v \beta 3$. When bound to a ligand, $\alpha v \beta 3$ leaves the podosome and moves to lamellipodia, which mediate osteoclast motility. During bone resorption, the integrin is found in the ruffled membrane.

Localization of $\alpha v \beta 3$ to the podosome requires intracellular signals mediated via the integrin's cytoplasmic domain.[65] For example, occupancy of c-fms promotes inside-out signaling of the integrin in a process uniquely requiring Ser752 in the $\beta 3$ cytoplasmic tail, thereby altering the conformation of the $\alpha v \beta 3$ external domain to the activated state, which is required for growth factor-stimulated resorption.[65,75] Given their transience and dot-like architecture, it is unlikely that $\alpha v \beta 3$-bearing podosomes are the structures isolating the osteoclast-resorptive microenvironment from the general extracellular space. Intracellular transmission of matrix-derived signals, which organize the cell's cytoskeleton, would be a more likely role for the integrin.

M-CSF and $\alpha v \beta 3$ collaboratively induce cytoskeletal organization by transiting Rho family proteins, RhoA and Rac, from their inactive GDP-bound to their active GTP-bound states.[45] This observation suggests that molecules that regulate Rho family GTPases may mediate integrin activation. In fact, Vav3, a Rac-specific guanine nucleotide exchange factor (GEF) in osteoclasts, is essential for organizing the cell's cytoskeleton and its bone resorptive activity.[76] In consequence, Vav3-deficient osteoclasts fail to activate Rac in response to M-CSF or $\alpha v \beta 3$ occupancy. These mutant osteoclasts resemble those lacking $\alpha v \beta 3$. Moreover, Vav3-deficient mice have increased skeletal mass and are protected from bone loss induced by systemic resorption stimuli such as RANKL and parathyroid hormone.

In 1991, Soriano and colleagues[77] made the surprising observation that the dominant phenotype of the c-src knockout mouse is osteopetrosis, subsequently shown to reflect failure of the mutant osteoclasts to organize their cytoskeleton. C-src regulates the osteoclast cytoskeleton both as an adaptor protein and tyrosine kinase.[78,79] In fact both roles of c-src are necessary for $\alpha v \beta 3$ to function
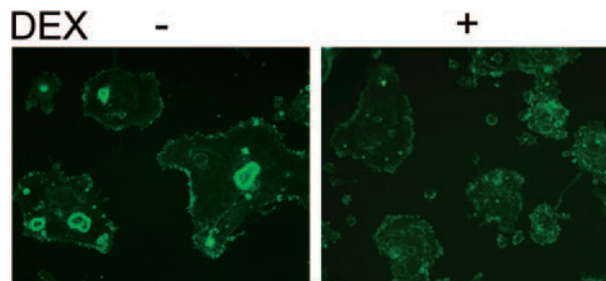
Figure 5. Glucocorticoids disrupt the osteoclast cytoskeleton. Osteoclasts, generated on dentin in the presence and absence of dexamethasone (DEX), were stained with FITC-phalloidin to visualize the actin cytoskeleton. The well-demarcated actin rings present in naïve osteoclasts are disrupted by the glucocorticoid (reprinted with permission from the *J Clin Invest* 2006, 116:2152–2160).[84]

in the bone resorptive cell. We find c-src constitutively associated with $\alpha v \beta 3$ in osteoclasts but activated on integrin occupancy. Activated $\alpha v \beta 3$ also recruits the tyrosine kinase syk to its cytoplasmic domain, where it is phosphorylated by c-src. Syk, in turn, is a crucial upstream regulator of Vav3. These events occur in the context of the ITAM proteins, Dap12 and FcRγ, which when deleted in tandem arrest terminal osteoclastogenesis because of failed expression of the critical osteoclastogenic transcription factor, NFATc1.[80] Thus, $\alpha v \beta 3$ activation recruits a signaling complex composed of c-src, Syk, ITAM proteins, Vav3, and Rac, which in turn organizes the cell's cytoskeleton thereby promoting bone resorption.

Glucocorticoids and the Osteoclast

Glucocorticoid (GC) therapy is frequently complicated by severe osteoporosis, second in frequency only to that after menopause. The general lack of success in treating steroid-induced bone loss suggests its pathogenesis is incompletely understood. There is little question that GCs suppress bone formation *in vivo*.[81] Surprisingly, however, addition of GCs to osteoprogenitor cells *in vitro* actually increases their bone-forming capacity.[82,83] This paradox raises the possibility that GC-suppression of bone formation *in vivo* reflects, at least in part, targeting of the steroid to intermediary cells, which inhibit the osteoblast.

Bone remodeling is an ever-occurring event characterized by sequential coupling of osteoclasts and osteoblasts. Remodeling units are initiated by the appearance of osteoclasts. After degrading a packet of bone, the resorptive cells are replaced by osteoblasts, which synthesize new bone. The osteoporosis attending GC therapy reflects failure of osteoblasts to restore fully bone previously resorbed in the remodeling site. Thus, by a mechanism yet to be discovered, recruitment of osteoblasts to the remodeling process requires prior osteoclastic activity. This scenario is in keeping with the osteoclast being the intermediary cell by which GCs suppress bone formation. In fact, dexamethasone directly targets the mature osteoclast and specifically deranges its cytoskeleton, an event attended by arrested activation of RhoA, Rac, and Vav3.[84] Steroid-treated resorptive cells do not spread nor do they form actin rings

(Figure 5). This cytoskeletal disruption blunts bone resorption *in vitro* and *in vivo* and, reflecting the remodeling cycle, translates into diminished bone formation.

It seems, therefore, that GCs suppress osteoblast function directly and indirectly via the osteoclast. The inhibited remodeling observed in steroid-treated patients and animals carries implications beyond bone mass. Specifically, the process of remodeling must replace effete bone with new to prevent brittleness. Thus, arrested remodeling in conditions such as chronic renal failure[85] results in qualitatively and structurally compromised bone. The same occurs in some patients treated for many years with resorption-inhibiting bisphosphonates, which dampen remodeling.[86] The retarded bone remodeling characterizing prolonged GC therapy raises the counterintuitive argument that prevention of skeletal complications may actually require some restoration of osteoclast function.

In contrast to its prolonged suppressive effects, short-term GC therapy, which induces extremely rapid skeletal loss, is characterized by transiently increased bone resorption.[87] Why short-term GC therapy stimulates, rather than blunts, osteoclast function is unknown. However, the inflammatory cytokines, often abundant in GC candidate diseases, prevent the cytoskeleton-disruptive effects of the steroid and may therefore enhance resorptive activity in the early stages of treatment.[77] As inflammatory cytokines are suppressed by chronic GC-exposure, the osteoclast-suppressive properties of the steroid become manifest.

Conclusion

The osteoclast is central to skeletal health as regards not only bone mass but also bone quality. The realization that the cell is of hematopoietic origin and subject to cytokine regulation laid the foundation for discovering the intracellular signals that mediate its resorptive capacity. Cytoskeletal organization consequent to integrin and growth factor receptor activation are integral to osteoclast function and offer new anti-resorptive therapeutic targets, possibly avoiding the complications of prolonged suppression of the remodeling process.

Acknowledgments

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