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Osteoclasts have Multiple Roles in Bone in Addition to Bone Resorption

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Abstract

Osteoclasts are the cells that degrade bone to initiate normal bone remodeling and mediate bone loss in pathologic conditions by increasing their resorptive activity. They are derived from precursors in the myeloid/monocyte lineage that circulate in the blood after their formation in the bone marrow. These osteoclast precursors (OCPs) are attracted to sites on bone surfaces destined for resorption and fuse with one another to form the multinucleated cells that resorb calcified matrixes under the influence of osteoblastic cells in bone marrow. Recent studies have identified functions for OCPs and osteoclasts in and around bone other than bone resorption. For example, they regulate the differentiation of osteoblast precursors and the movement of hematopoietic stem cells from the bone marrow to the bloodstream; they participate in immune responses, and secrete cytokines that can affect their own functions and those of other cells in inflammatory and neoplastic processes affecting bone. Here, we review these findings, which define new roles for osteoclasts and OCPs in the growing field of osteoimmunology and in common pathologic conditions in which bone resorption is increased.

Keywords

Osteoclast; cytokine; osteoblast; bone resorption; inflammatory arthritis

INTRODUCTION

Osteoclasts are the cells that degrade (resorb) bone during normal bone remodeling and in pathologic states in which bone resorption is increased. Bone remodeling is the process in which microscopic trenches are formed on the surfaces of bone trabeculae in the spongy bone seen at the ends of long bones and inside vertebrae. Osteoclasts form these trenches by secreting hydrochloric acid and proteases, such as cathepsin K, into an extracellular lyzosomal compartment beneath a ruffled part of their basal cell membrane to dissolve the mineral and matrix components of bone simultaneously (1). Precursors of osteoblasts, the cells that form bone, are recruited to these trenches from the adjacent bone marrow stromal cell population and differentiate into osteoblasts, which lay down new matrix and mineralize it. Bone remodeling can be increased in response to many influences, including mechanical strain, cytokines, hormones, and growth factors. In many pathologic conditions, such as postmenopausal osteoporosis, rheumatoid arthritis, and periodontitis, the amount of bone removed by osteoclasts exceeds that laid down by osteoblasts and bone becomes weakened.

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Osteoclast formation from osteoclast precursors (OCPs) is regulated predominantly by osteoblastic cells during normal bone remodeling (Figure 1). Osteoblastic cells in the bone marrow express two cytokines that are required for OCP differentiation into osteoclasts: macrophage-colony stimulating factor (M-CSF) and receptor activator of NF-κB ligand (RANKL). M-CSF binds to its receptor, c-fms, on OCPs and activates signaling through MAP kinases and ERKs during the early phase of OCP differentiation (2). RANKL binds to its receptor, RANK, on the surface of OCPs activating signaling through NF-κB, c-Fos, phospholipase Cγ (PLCγ) and nuclear factor of activated T cells c1 (NFATc1) to induce differentiation of OCPs into osteoclasts (3). T and B lymphocytes also express RANKL (4,5) and regulate osteoclast formation in pathologic states in which their numbers are increased, such as following sex steroid deficiency after the menopause and in inflamed joints of patients with rheumatoid arthritis.

Osteoclast formation is also regulated by so-called co-stimulatory immune-mediated signaling through receptors, including osteoclast-associated receptor (OSCAR) and triggering receptor expressed in myeloid cells-2 (TREM2) (6). These receptors activate immunoreceptor tyrosine-based activation motifs (ITAMs) in adaptor molecules such as Fc receptor common γ subunit (FcRγ) and DNAX-activating protein 12 (DAP12) in OCPs. This activation, like RANKL/RANK signaling, leads to phosphorylation of PLCγ and calcium-dependent activation of NFATc1 (3). These two pathways were shown recently to be linked directly to one another in OCPs by scaffold proteins (7). The ligand for OSCAR is expressed by osteoblastic cells, but it and the ligand for these other receptors in OCPs have not been identified. Co-stimulatory signaling is required for osteoclast formation during embryonic development and it likely is activated in inflammatory bone diseases.

Osteoclast formation and activity are limited by a variety of cytokines, and in particular by osteoprotegerin (OPG), the cognate inhibitor of RANKL, which binds to RANKL and prevents its interaction with RANK (reviewed in (8)). Osteoblastic cells and B cells are induced to express OPG in response to many of the influences that induce RANKL expression to limit bone degradation, and the RANKL/OPG ratio is a major determinant of bone resorption and bone mass. However, osteoclasts and OCPs can also negatively regulate their formation directly in response to RANKL. For example, while RANKL activation of c-Fos induces NFATc1 activation in OCPs, it also induces secretion of interferon β by OCPs. Binding of interferon β to its receptor on OCPs (9) leads to degradation of tumor necrosis factor receptor associated protein 6 (TRAF6). RANK does not have intrinsic kinase activity. TRAF6 is an adaptor molecule that mediates RANKL/RANK-induced activation of a variety of signaling pathways in OCPs (reviewed in (8)) and it also functions as an E3 ligase (10) which promotes its own degradation to negatively limit osteoclast formation (9).

The identification of these cytokines and the signaling pathways they activate has greatly increased our understanding of how osteoclast formation and bone resorption are regulated by osteoblastic cells. However, an unexpected twist or two has been added to the relationship between osteoclastic and other cells in bone marrow recently. As a result, it is now recognized that osteoclastic cells not only resorb bone; they also regulate the function of other cell types, such as osteoblastic cells, they secrete cytokines, regulate hematopoietic stem egression from bone marrow, and function as immune cells in inflammatory bone diseases. Many of the drugs used to treat common bone diseases inhibit osteoclast formation or activity. These new findings suggest that inhibition of some osteoclast functions could have unexpected detrimental or beneficial effects on cells in bone and around bone.

OSTEOCLASTIC INTERACTIONS WITH OSTEOBLASTIC CELLS

Osteoblastic regulation of osteoclast formation has been considered to be a one-way regulatory relationship between these two cells types until recently, with osteoblastic cells having a dominant role. Like osteocytes within bone, osteoblastic cells also have extensive dendritic processes extending from their cell bodies into the marrow space (Figure 1). In this way they are able to interact with many hematopoietic cells within the bone marrow cavity, including OCPs, and with bone lining cells and osteocytes within bone (11). Thus, there is an extensive communication network among various types of osteoblastic cells that extends from the marrow to deep inside bone (Figure 1).

Osteoblastic cells in the marrow space fulfill their osteoclast regulatory role through up-regulation of RANKL expression on the surface of these cytoplasmic processes, which interact with similar types of extensions from the surfaces of OCPs and osteoclasts. Recent studies indicate that osteoclastic cells are not passive slaves in this interaction and that they can directly regulate osteoblastic cells (12). There is also direct contact between these cell types not only through RANKL/RANK interaction, but also through ephrinB2, a ligand expressed by OCPs, and its receptor, EphB4, on osteoblastic cells. Ephrin/Eph signaling facilitates neuronal axon pathfinding and arterial-venous link up during embryonic development, processes in which cell processes extend over relatively large distances (reviewed in (13)). Zhao et al. (14) found unexpectedly that during interactions between osteoclastic and osteoblastic cells, so-called reverse signaling through the ephrinB2 ligand back into OCPs inhibited OCP differentiation. This inhibition occurs through a mechanism that down-regulates c-Fos activation of NFATc1. A more surprising finding in Zhao et al.'s studies was that signaling through the EphB4 receptor in osteoblastic cells promoted osteoblast differentiation from precursors. The stimulatory pathway in osteoblastic cells has not yet been identified nor is it known exactly where in bone remodeling units these interactions take place. It is not known if they occur near the cutting edges of remodeling units where osteoclasts are actively resorbing and to where OCPs are actively recruited to replenish the pool of osteoclasts, or in the centers of these units where osteoblastic precursors differentiate into matrix-forming osteoblasts. Whatever the mechanisms involved, these findings raise the possibility that stimulation of the Ephrin/Eph signaling could lead to increased bone mass in individuals with diseases, such as osteoporosis and rheumatoid arthritis in which there is a generalized increase in bone resorption and a decrease in bone formation.

Ephrin/Eph signaling has been shown more recently to be involved in other aspects of bone cell functions. Microarray analysis revealed that ephrinB2 mRNA expression was up-regulated in the mouse marrow stromal cell line, Kusa 4b10, after treatment with PTH(1-34) or PTHrP (1-141). This was confirmed by quantitative real time PCR in vitro and in RNA samples taken from metaphyseal bones of mice after PTH treatment (15). EphrinB2 protein expression was increased in both osteoclasts and osteoblasts as shown by Western blotting and immunostaining of femoral sections from PTH-treated mice. Blockade of ephrinB2/EphB4 interaction inhibited mineralization of Kusa 4b10 cells. These findings suggest that PTH or PTHrP could systemically upregulate ephrinB2 expression in osteoblasts to promote their differentiation and bone formation through EphB4 and thus contribute to the anabolic action of PTH or PTHrP (15). Thus, PTHrP might regulate osteoblast functions through ephrinB2 in a paracrine or autocrine manner, given PTHrP's known role to act in such a manner in osteoblasts (16).

Ephrin-b1 is a ligand for eph tyrosine kinase receptors and signaling through these is crucial for the epithelial mesenchymal interactions regulating cranial and oral morphogenesis. Over 20 mutations have been described in *EFNB1*, the gene encoding ephrin-b1, in patients with

craniofrontonasal syndrome, an X-linked disorder whose main clinical manifestations include coronal craniosynostosis, frontonasal dysplasia, and digital defects (17). Dysfunction of Ephrin-b1/eph signaling may be responsible for premature fusion of the calvarial bones and limb deformity, a relatively common birth defect seen in patients with craniofrontonasal syndrome (18).

An additional unexpected twist to the growing complex functions of OCPs was provided by Lee et al. (19). They used comparative mRNA expression profiling to study differences between osteoclasts and macrophages and identified a cDNA fragment in OCP mRNA that encodes a protein (Atp6v0d2), which is almost identical to a subunit of v-ATPase. Atp6v0d2 is a component of the V-type H^+ ATP6i proton pump complex in osteoclasts that secretes H^+ (20). H^+ form HCl in remodeling sites along with Cl^- flow passively through the osteoclast chloride channel, ClC-7, and in this way the mineral in bone matrix is dissolved. Atp6v0d2 is highly expressed in the cytoplasmic membrane of osteoclasts around the so-called the sealing zone, which forms a tight junction between the cell and the bone and surround the part of the cell with ruffled borders under which resorption takes place. It is not expressed by osteoblasts. Lee et al. generated Atp6v0d2-deficient mice to explore its function in osteoclasts. They found that the mice had small osteoclasts due to defective fusion of osteoclast precursors and reduced bone resorption. This resulted in osteopetrosis, a congenital disorder of bone modeling in which marrow cavities are filled or partly filled with unresorbed bone and cartilage matrix because of defective osteoclast formation or activity. Unexpectedly, these mice also had increased numbers of osteoblasts associated with enhanced bone formation. The OCP fusion defect was not due to reduced expression of DC-STAMP, which is required in OCPs for precursor fusion (21). Atp6v0d2 is not expressed by osteoblasts, and the mechanism whereby it inhibits bone formation is not known. Intuitively, it would make sense for osteoclasts to have a mechanism to inhibit osteoblastic bone formation at sites where they are resorbing bone. Secretion of an inhibitory protein by them through the function of Atp6v0d2 is a possible mechanism. How or if the function of this sub-unit of the protein pump is linked to its positive role in OCP fusion remains to be determined. These findings and those of Zhao et al. raise the possibility that a single therapeutic agent could inhibit osteoclast activity and increase bone formation, presumably by different mechanisms.

OSTEOCYTES REGULATE OSTEOCLASTS

Osteocytes are derived from bone forming osteoblasts that become embedded in unmineralized bone matrix (osteoid) while they are laying it down on bone surfaces during bone modeling and remodeling. They are the most abundant osteoblastic cells, comprising >90 % of bone cells. Osteocytes have extensive dendritic processes that extend widely within calcified matrix inside microscopic canaliculi that are filled with interstitial fluid. They are considered to play important roles in mineral homeostasis by regulating the movement of ions and cations into and from the matrix around them into the interstitial fluid. Osteocyte dendritic processes interact with those of other osteocytes and with stromal cells within the adjacent bone marrow (Reviewed in (22)) and thus osteocytes could orchestrate the functions of the cells in this network and respond to signals from them. These processes could detect changes in the pressure within the canaliculi in response to mechanical stress and other influences. They get damaged when bones break or when microfractures occur in the trabecular or cortical components of bone. Previous studies have suggested that as osteocytes undergo apoptosis they may send signals to osteoblastic cells in the marrow to induce osteoclast formation (23) and that viable osteocytes can send signals to inhibit osteoclast activation (24).

More definitive evidence that osteocytes regulate osteoclast formation and activation was provided Tatsumi et al. (11). They devised to a strategy to kill osteocytes in bone by generating transgenic mice expressing the receptor for diphtheria toxin (DT) specifically in osteocytes and then injecting the mice with DT. Two days after the injections, they observed that RANKL mRNA levels increased in bone marrow cells from the mice. Six days later, they found that 70–80% of osteocyte lacunae in the bones of the mice were empty as a result of DT-induced osteocyte apoptosis. The authors concluded that as these osteocytes were undergoing apoptosis they sent signals through their dendritic processes to osteoblastic cells in the bone marrow to induce increased RANKL expression by them and promote osteoclast differentiation. The mice had significant bone loss 40 days after the DT injections due to increased osteoclast activity. Osteoblasts subsequently replaced the lost bone, indicating that the DT did not adversely affect osteoblast precursors in bone marrow.

To further investigate the function of osteocytes the Tatsumi et al. used a tail suspension model of disuse-induced bone loss. Control mice lost bone in their hind limbs following tail suspension through increased osteoclastic bone resorption. In contrast, transgenic mice expressing the DT receptor and injected with DT 1 day before tail suspension did not lose bone. Presumably these mice did not lose bone because the DT induced osteocyte apoptosis or at least prevented unloading-induced signals being sent from osteocytes to osteoblasts to stimulate osteoclast formation. Exactly how this unloading-induced signaling occurs remains to be determined, but these findings support the proposed regulatory role for osteocytes in the regulation of bone resorption and bone volume (22).

OSTEOCLASTS AS IMMUNE CELLS

Numerous types of cells, including T and B lymphocytes, macrophages and dendritic cells participate in immune responses and have been recognized as immune cells for many years. OCPs are formed in the bone marrow from myeloid precursors, which can also give rise to macrophages and dendritic cells when OCPs are cultured with M-CSF or GM-CSF plus IL-4 (25). It is still not clear at exactly which stage OCPs lose their potential for differentiation along multiple pathways or when “trans-differentiation” occurs in vivo. Like macrophages, OCPs express Fcγ receptors (6,26). Their involvement in immune responses and their origin from circulating monocytes has been recognized for many years (27), although their relationship to macrophages has been controversial (28). However, their roles as immune cells or immune response modulators has become clearer more recently (3,29). OCPs enter the bloodstream and circulate like other hematopoietic cells. They can be detected in the blood and spleens of mice and in the bloodstream of humans using antibodies to CD11b, c-kit, Gr-1, c-fms (the receptor for c-Fos) and RANK (30) (31) (32). Their numbers are increased in the blood of humans (30) and mice (30) (33) with various forms of inflammatory arthritis in which TNF production is increased. Transgenic mice over-expressing TNF (TNF-Tg) have high serum TNF levels and develop a form of inflammatory erosive arthritis similar to rheumatoid arthritis. Treatment of these mice with anti-TNF therapy reversed the increase in OCP numbers in their blood, suggesting that TNF induces OCP egression from the blood.

OCPs express CXCR4, the receptor for stromal cell-derived growth factor (SDF-1). SDF-1 regulates the movement of hematopoietic cells from bone marrow into and from the bloodstream in a dose-dependent manner (reviewed in (34)). Expression levels of SDF-1 by bone marrow stromal cells are reduced in TNF-Tg mice (35) and we believe that this is one of the mechanisms whereby TNF promotes OCP egression from the marrow into the bloodstream. Another mechanism may be related to TNF increasing the expression of c-Fms by OCPs thereby increasing the bone marrow OCP pool (36). SDF-1 and TNF levels are increased in the joints of TNF-Tg mice and of patients with inflammatory arthritis. Thus,

OCPs could be attracted to sites of inflammation in and around affected bones where SDF-1 concentrations are increased (34). Why SDF-1 levels are decreased in bone marrow of these mice and increased in their joints in response to increased TNF production remains unexplained, but may be TNF concentration dependent.

OCPs not only respond to TNF, they and osteoclasts also secrete TNF and other cytokines, such as IL-6 and IL-1 (37,38). Secretion of these cytokines is increased in response to TNF (39). Thus, TNF could induce an auto-amplifying cycle at sites of inflammation in and around bones to enhance osteoclast formation (34) directly through autocrine and indirectly through paracrine mechanisms. These mechanisms could enhance TNF's established action to increase osteoclast formation indirectly by promoting RANKL expression by accessory cells, such as T cells and synoviocytes (8).

TNF expressed by OCPs could also increase the activity of osteoclasts by a mechanism involving activation of c-Fos in OCPs (34). c-Fos is activated downstream of NF-κB in OCPs in response to RANKL and TNF to regulate osteoclast differentiation (34). We found that when we over-expressed c-Fos in OCPs and treated the cells with TNF, the osteoclasts derived from these cells had increased resorptive activity (34). Thus, at sites in bone where TNF levels are increased, TNF could increase bone resorption by a number of mechanisms: indirectly by increasing RANKL expression by accessory cells and directly by increasing not only osteoclast formation, but also osteoclastic resorptive activity.

Although TNF mediates bone loss in a variety of pathologic states, like RANKL it can also directly limit osteoclast formation. However, the inhibitory mechanisms involved are different from those described earlier and activated by RANKL. Both RANKL and TNF activate the canonical NF-κB pathway in OCPs to induce osteoclast formation directly by inducing the phosphorylation and subsequent degradation of inhibitory kappa kinase β (IKKβ) (reviewed in (40)). Consequently, NF-κB p65/p50 dimers are released from IKKβ and translocate to the nucleus where they induce expression of osteoclastogenic genes. RANKL, but not TNF, also activates the non-canonical or alternative NF-κB pathway in OCPs. This pathway is activated by phosphorylation and degradation of another inhibitory NF-κB protein, p100, which binds to the NF-κB protein, RelB, and prevents it translocating to nuclei. Upon activation of the alternative pathway, p100 is processed in the proteasome and a p52 fragment of it is released. P52 binds to RelB and p52/RelB dimers can then translocate to the nucleus to induce gene expression. RANKL induces more osteoclasts than TNF *in vitro* (34), but the reason for this has been unexplained. We found that TNF, but not RANKL up-regulates NF-κB p100 expression in OCPs (41) and hypothesized that p100 might limit osteoclast formation by TNF. We treated OCPs from NF-κB p100-deficient mice with TNF and found that it induced similar numbers of osteoclasts from these as RANKL, suggesting that induction of p100 expression limits TNF-mediated osteoclast formation. Up-regulation of NF-κB p100 expression or prevention of its degradation may be a mechanism to limit excessive bone resorption in a variety of bone diseases. Recently, we have found that osteoclasts can increase their activity through another autocrine mechanism in response to RANKL. For example, they increase their secretion of VEGF-C, a member of the VEGF family of angiogenic proteins in response to RANKL and TNF. VEGF-C in turn increases the resorptive activity of osteoclasts directly in vitro, although it does not increase the formation of osteoclasts from OCPs (35). Osteoclasts in inflamed joints of TNF-transgenic mice have increased expression of VEGF-C (42), supporting our in vitro findings. A major function of VEGF-C is to promote the formation of lymphatic channels. We have found that the numbers and size of lymphatic vessels around affected joints in these TNF-Tg mice is increased (42,43). This increase could enhance the immune responses around these joints by permitting an increase in the rate of flow of lymph and other inflammatory mediators from affected joints. It will be important to determine if inhibition of osteoclasts has beneficial or

detrimental effects on this and other recently discovered functions of OCPs and osteoclasts in inflammatory arthritis and other conditions associated with increased cytokine-induced osteoclast formation and activity and to fully investigate the function of VEFG-C produced by osteoclasts.

EFFECTS OF OSTEOCLAST INTERACTION WITH BONE MATRIX

In studies examining the effects of cytokines on c-Fos over-expressing OCPs, we made the unexpected observation that OCP interaction with bone matrix increased osteoclast formation. We cultured c-Fos expressing OCPs on bone slices in the presence of M-CSF and found that they differentiated spontaneously into osteoclasts in the absence of any other cytokines, but not when these cells were cultured on plastic (38). To determine if this osteoclast formation occurred in response to a factor secreted by c-Fos-expressing OCPs, we cultured OCPs on bone slices in the upper chamber of a transwell culture system. Surprisingly, we found that GFP-expressing OCPs in control cultures on bone slices in the upper chambers induced osteoclast formation from c-Fos-expressing OCPs cultured on plastic in the lower chamber. We used IL-1 receptor and TNF antagonists to determine if IL-1 or TNF was responsible for this osteoclast formation and found that the former, but not the latter was inhibitory, indicating that IL-1 was released by the OCPs in response to their interaction with bone matrix.

Like TNF, IL-1 induces RANKL expression by accessory cells, but unlike TNF it does not induce osteoclast formation by itself. Previous studies have shown that IL-1 is a powerful stimulator of bone resorption in vivo (44). It activates osteoclasts (45), prolongs their survival (45) in vitro, and can induce osteoclast formation from c-Fos-expressing OCPs in the absence of RANKL (38). Osteoclast interaction with bone matrix enhances osteoclast survival as a result of integrin-induced signaling and this increases bone resorption (46). IL-1 produced by OCPs bound to bone matrix could enhance this effect on resorption, particularly if osteoclast expression of c-Fos is also increased, for example in response to TNF and RANKL at sites of inflammation in bone.

IL-1 production by OCPs could have further clinical significance. For example, osteoclasts cultured on bone slices are more resistant to the apoptosis inducing effects of bisphosphonates, the most commonly prescribed drugs for the prevention of bone loss. Higher concentrations of bisphosphonates are required to induce apoptosis of osteoclasts cultured on bone compared cells cultured on plastic (47). This enhanced survival may be due in part to integrin-mediated signaling from bone matrix (46), but it could also result from the effects of IL-1 released by OCPs and osteoclasts. Bisphosphonates are less effective inhibitors of bone resorption around the joints of patients with inflammatory arthritis than in the spine and hips of patients with osteoporosis (48,49). This could be due in part to the survival and activation enhancing effects of IL-1 released in affected joints by osteoclasts and other cells and also to TNF-mediated up-regulation of the anti-apoptotic protein, Bcl-xL, in osteoclasts (50).

OSTEOCLASTS AS REGULATORS OF HEMATOPOIETIC CELL FUNCTION

Hematopoietic cells in bone marrow are derived from hematopoietic stem cell (HSCs), some of which leave the marrow and circulate in the blood. Osteoblastic cells in the marrow and on endosteal surfaces of bones in mice support HSCs through signals that maintain self-renewal potential in undifferentiated stem cells (Reviewed in (51)). HSCs appear to reside at the endosteal surfaces in niches from where they leave the marrow and enter the blood. Osteoblastic regulation of the egression of hematopoietic stem and progenitor cells from the bone marrow into the bloodstream is mediated in part by PTH (52) through a cyclic AMP-Jagged-1-dependent mechanism (53).

Recent studies by have identified an unexpected role for osteoclasts in this process. While studying HSC mobilization, Kollet et al (54) noted that it was associated with increased numbers of osteoclasts on bone surfaces. They knew that stress-inducing conditions, such as chemotherapy and inflammation, or treatment of patients with granulocyte colony-stimulating factors (G-CSF), induced massive stem cell mobilization from the marrow. This was associated with activation of proteolytic enzymes, which release adhesions between stem cells and their bone marrow microenvironment (reviewed in (55)). They treated mice with RANKL and found that it induced increased osteoclast formation *in vivo.* This was accompanied by stem and progenitor cell mobilization associated with increased osteoclast expression of MMP-9 and cathepsin K. These enzymes degrade proteins in bone, but they also cleave membrane-bound kit ligand, a growth and adhesion factor for HSCs. RANKL also decreased the expression of osteoblast kit ligand and osteopontin, which also affect stem cell numbers. Kollet et al examined protein tyrosine phosphatase-epsilon (PTP ε)-deficient mice, which have osteoclasts with defective resorbing activity and mild osteopetrosis. They found that RANKL did not mobilize HSCs and progenitor cells from the marrow in these mice, further linking this mobilization to osteoclasts. These data link osteoblasts and HSCs with osteoclasts in normal and pathologic bone remodeling.

SUMMARY

Osteoclasts have been recognized for many years as the cells that resorb bone in response to a large number of influences, most of which act indirectly on osteoclasts and their precursors through cells in the osteoblast lineage. Recent reports indicate that osteoclasts and OCPs have multiple additional functions, which affect the activity of cells in and around bone. These include positive and negative effects on the functions of osteoblastic cells, such as bone matrix formation and mineralization and the egression of hematopoietic stem cells from the marrow into the blood. During normal and pathologic bone remodeling osteoclasts and OCPs also secrete cytokines and other factors, which could have important regulatory roles in these processes. Pharmaceutical companies have responded to the growing need to develop new drugs that can inhibit osteoclast formation and/or activity to prevent the bone loss that occurs after the menopause, with aging and in other pathologic inflammatory processes. These new findings suggest that drugs which profoundly affect osteoclast formation or activity could have unexpected negative or positive effects on bone homeostasis.

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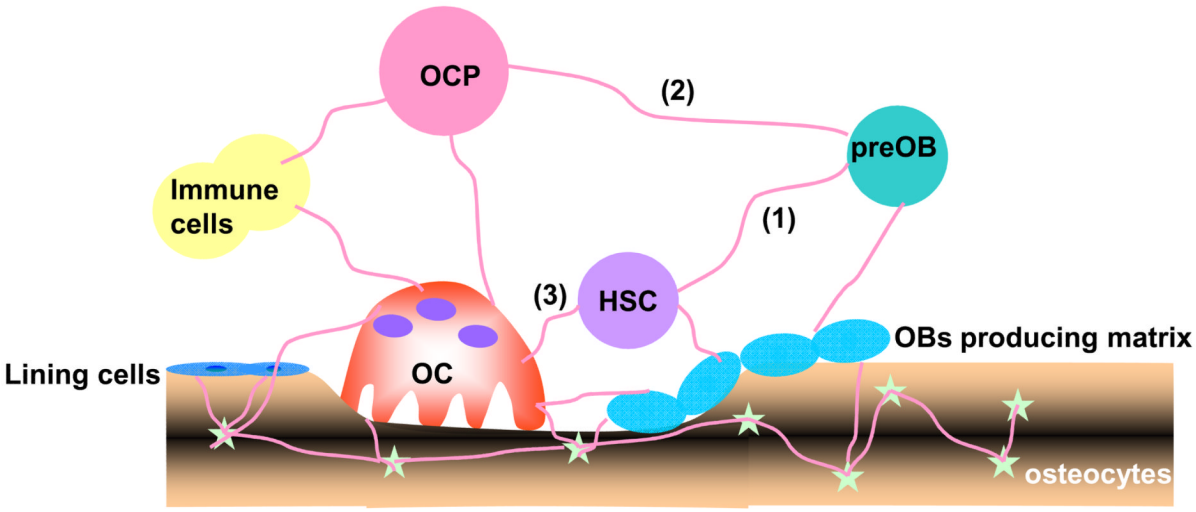

Figure 1. Osteoclasts interact with other cells on bone and within the bone marrow cavity to regulate several mechanisms

1) Like osteocytes within bone, pre-osteoblasts (preOBs) also have extensive dendritic processes extending from their cell bodies to interact with various cell types within the bone marrow cavity, including hematopoietic and immune cells, hematopoietic stem cells (HSCs), osteoclast precursors (OCPs), and other preOBs, in addition to osteocytes within bone.
2) Osteoblasts affect OCP differentiation through RANKL/RANK and ephrinB2/EphB4 signaling pathways; the latter also influences osteoblast differentiation.
3) Osteoclasts regulate HSC mobilization through c-kit and modulate immune cell functions by producing cytokines and other factors.

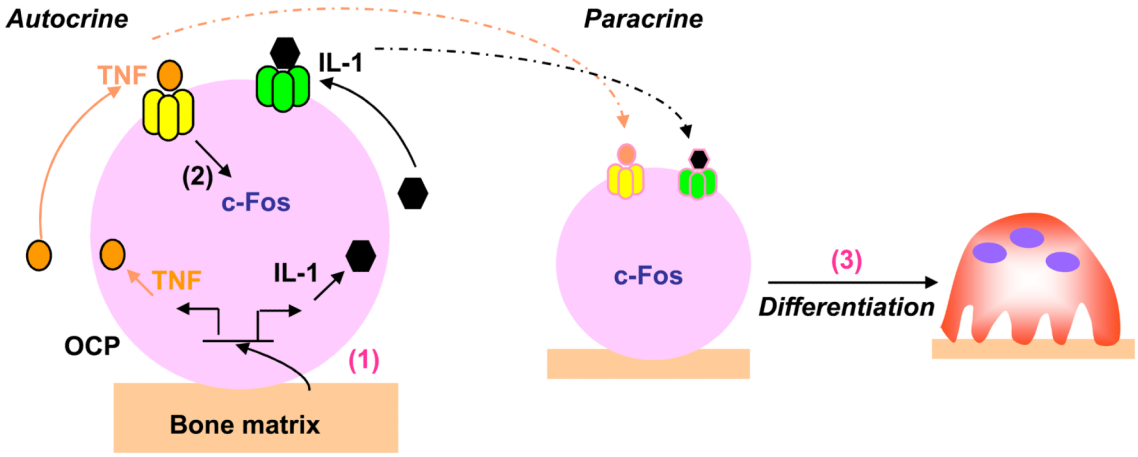

Figure 2. Osteoclast precursors interact with bone matrix to enhance osteoclast formation and function through autocrine and paracrine mechanisms

1) Osteoclast precursors (OCPs) produce and release TNF and IL-1 by interacting with bone matrix.

2) TNF stimulates expression of c-Fos by osteoclast precursors.

3) IL-1 induces differentiation of c-Fos-expressing precursors into mature osteoclasts to resorb bone, which is enhanced by c-Fos expression.