Osteoclast Activity and Subtypes as a Function of Physiology and Pathology—Implications for Future Treatments of Osteoporosis

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Osteoclasts have traditionally been associated exclusively with catabolic functions that are a prerequisite for bone resorption. However, emerging data suggest that osteoclasts also carry out functions that are important for optimal bone formation and bone quality. Moreover, recent findings indicate that osteoclasts have different subtypes depending on their location, genotype, and possibly in response to drug intervention.

The aim of the current review is to describe the subtypes of osteoclasts in four different settings: 1) physiological, in relation to turnover of different bone types; 2) pathological, as exemplified by monogenomic disorders; 3) pathological, as identified by different disorders; and 4) in drug-induced situations.

The profiles of these subtypes strongly suggest that these osteoclasts belong to a heterogeneous cell population, namely, a diverse macrophage-associated cell type with bone catabolic and anabolic functions that are dependent on both local and systemic parameters. Further insight into these osteoclast subtypes may be important for understanding cell–cell communication in the bone microenvironment, treatment effects, and ultimately bone quality. **(*Endocrine Reviews* 32: 31–63, 2011)**

I. Introduction

Osteoclasts are multinucleated bone-resorbing cells that are unique in their ability to degrade mineralized matrices, such as bone and calcified cartilage (1). Osteoclasts have for a long time been considered bone-resorbing "machines," yet some years ago it was demonstrated that not all osteoclasts are the same and that careful elucidation of the osteoclast subtype may prove

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Abbreviations: AGE, Advanced glycation end-product; BMD, bone mineral density; CAII, carbonic anhydrase II; ClC-7, chloride channel 7; CT-1, cardiotrophin-1; CTX, C-terminal crosslinked telopeptide of type I collagen; ER, estrogen receptor; GLP, glucagon-like peptide; HRT, hormone replacement therapy; ICTP, carboxyterminal peptide of type I collagen; MMP, matrix metalloproteinase; ONJ, osteonecrosis of the jaw; OPG, osteoprotegerin; RA, rheumatoid arthritis; RANK, receptor activator of nuclear factor κB; RANKL, RANK ligand; SERM, selective estrogen receptor modulator; TRACP, tartrate-resistant acid phosphatase; V-ATPase, vacuolar type ATPase.

beneficial (1–4). As illustrated in Table 1, under normal circumstances, osteoclasts are influenced by a complex combination of systemic hormones and local mediators present in the different bones. Under pathological conditions, such as cessation of estrogen production or inflammatory conditions, additional cytokines are present. Even the bone type and age may influence the phenotype of the osteoclast (2, 5–7). Finally and importantly, different classes of drugs used for treatment of osteoporosis and other diseases also influence the osteoclasts significantly.

The aim of this review is to provide a thorough description of the complex nature of osteoclasts under healthy and diseased states and to describe their modulation by drugs that have been approved for use or are under development. The paper will also emphasize the role of osteoclasts in initiating bone formation, a recently discovered activity of these cells that has gained much attention (3, 8–13).

II. Bone Remodeling

Bone remodeling is required for optimal control of calcium homeostasis and strength of the bones and is essential for the continued maintenance of a healthy skeleton (14). Bone remodeling is performed by three cell types: 1) the osteoclasts, which are the sole cells in the body possessing the ability to degrade both the inorganic calcium matrix and the organic collagen matrix; 2) the osteoblasts, which are the bone-forming cells; and 3) the osteocytes, which appear to regulate the activity of both osteoclasts and osteoblasts (14, 15). In healthy adults, under normal circumstances, bone resorption is always followed by an equal degree of bone formation, a tightly balanced process referred to as coupling (9, 16). The modulation of activities of the cells involved in the remodeling cycle was recently described in detail (15).

Coupling was initially discovered in the 1960s when Frost and co-workers (17, 18) demonstrated that osteoblasts filled the resorption pits created by osteoclasts in more than 97% of the cases (17–21). Since then, coupling has been understood as a coordinated and balanced induction of osteoblastic bone formation in response to prior bone resorption (19). Uncoupling occurs when the balance between resorption and formation is disrupted, which often leads to pathological situations such as osteoporosis or osteopetrosis (3, 9, 14, 22, 23). However, uncoupling also occurs under physiological conditions, *i.e.*, during skeletal growth in children, where bone formation exceeds bone resorption (10).

Hypogonadal osteoporosis is usually caused by a decrease or loss of sex steroid production, which results in accelerated osteoclastogenesis and bone resorption (24) that cannot be completely countered by an increase in bone formation. This results in low bone mass, in deterioration of the microarchitecture of the skeleton, and often in fractures (25). Osteoporotic fractures are associated with increased morbidity and mortality and give rise to a significant public health problem (24).

Osteopetrosis, on the other hand, is a rare, inherited disease in various species including man, which was originally identified by Albers-Schönberg in 1904 (26). In the majority of cases, it is caused by defective resorption by the osteoclasts, resulting in high bone mass with poor bone quality and increased fracture frequency due to defective bone remodeling (1, 26, 27). However, recent studies also characterized patients with osteopetrosis due to dysfunctional osteoclastogenesis either directly affecting the osteoclast precursors or indirectly through the osteoblasts, and thus the phenotype was caused by the absence of osteoclasts, rather than inactivity of these cells (28–31). Interestingly, the studies of osteopetrotic patients have indicated that the presence of osteoclasts, but not their activity, is essential for bone formation, indicating that some aspects of the coupling principle should be revised (1, 3).

Because hypogonadal osteoporosis is associated with increased numbers and activity of osteoclasts (16), most treatments developed so far, such as bisphosphonates and hormone replacement therapy (HRT)/selective estrogen receptor modulators (SERMs), have focused on eliminating or reducing the number of osteoclasts and thereby reducing bone resorption (32). These treatments are associated with secondary decreases in bone formation due to the coupled nature of the bone remodeling process, which naturally limits their efficacy (3, 24). However, as seen in the osteopetrotic syndromes, there are indications that bone resorption and bone formation can be dissociated, and from recent studies it appears that the osteoclast itself, whether it is a physiological, pathological, or drug-induced subtype, is highly important for a secondary effect on bone formation (1, 3).

In this review, we describe differences in osteoclast activity and subtypes in relation to physiology, pathophysiology, and medication, with special attention to coupling in the bone remodeling process. Ultimately, this review highlights potential directions for new treatment modalities.

III. The Classical Osteoclast

Osteoclastogenesis is a complex process requiring both the correct extracellular stimuli and the correct cellular molecules to interact without impediment (22, 33). Osteoclasts arise from hematopoietic stem cells that, in the presence of

TABLE 1. A simplified summary of osteoclast phenotypes as a function of physiology, pathology, and drugs, also indicating areas of osteoclast biology that are not well-understood

	Osteoclast no. 		Resorptive process			Bone formation 
		Bone resorption	Acid	Cat K	MMP	
Classical osteoclast	Normal	Normal	+++	+++	+/−	Balanced
Physiology						
Targeted	Recruitment to specific areas increased	Increased	++	?	?	Balanced
Stochastic	Not clear	Not clear	+	?	?	Not clear
Night	Normal	Increased	++	++	+/−	Minor up-regulation
Day	Normal	Decreased	+	?	?	Minor down-regulation
Chondroclast	Normal	Normal	+	+	++	Balanced
Endochondral	Normal	Normal	+	+	−	Balanced
Intramembraneous	Normal	Normal	+	−	+	Not balanced, opposite side of bone than resorption
Trabecular	Normal	Normal	++	++	−	Balanced
Cortical	Normal	Normal	++	++	−	Balanced
Pathology						
Osteoporosis	Increased	Increased		+ + ++	−	Increased, but less than resorption
Age	Increased	Increased	++	++	?	Decreased
OC-rich OP	Greatly increased	Decreased	−	−	−	Increased according to increased OC number
OC-poor OP	No osteoclasts	Decreased	−	−	−	Decreased?
Pycnodysostosis	Unchanged/increased osteoclast size	Decreased	+	−	+++	Not clear
Paget's	Greatly increased at local sites	Increased at local sites	++	+	+++	Increased locally, but does not compensate resorption
RA	Greatly increased at local sites	Not known	+	+	+++	Not known
Lytic metastases	Greatly increased at local sites	Increased at local sites	+	+	+++	Increased locally, but does not compensate resorption
Drug-induced						
BPs	Reduced	Decreased	−	−	?	Decreased secondary to resorption
HRT/SERMs	Reduced	Decreased	−	−	?	Decreased secondary to resorption
Calcitonin	Unchanged	Decreased	−	−	?	Not changed or minor decrease
PTH	Increased/unchanged	Decreased	++	++	?	Increased, but only temporarily
Strontium ranelate	Unchanged	Decreased?	−	−	?	Increased
GCs	Unchanged/increased	Unchanged/increased	+	+	?	Decreased strongly
Denosumab	Greatly reduced	Decreased	−	−	−	Decreased secondary to resorption
Cat K inhibitors	Unchanged	Decreased	+	−	+++	Decreased secondary to resorption
GLP-2	Unchanged	Decreased	−	−	?	Not changed, but long term effects are not known
Acidification inhibitors	Increased	Decreased	−	−	−	Increased, but so far only in animal models

The table shows the subtype of osteoclasts, the number of osteoclasts, the effect on bone resorption, which part of the resorption machinery that is active/affected, and the effect on bone formation. OC, Osteoclast; OP, osteopetrosis; Cat K, cathepsin K; BPs, bisphosphonates; GCs, glucocorticoids; MMP, matrix metalloproteinase; RA, rheumatoid arthritis; PTH, parathyroid hormone; HRT, hormone replacement therapy; SERMs, selective estrogen receptor modulators; GLP-2, glucagon-like peptide 2.

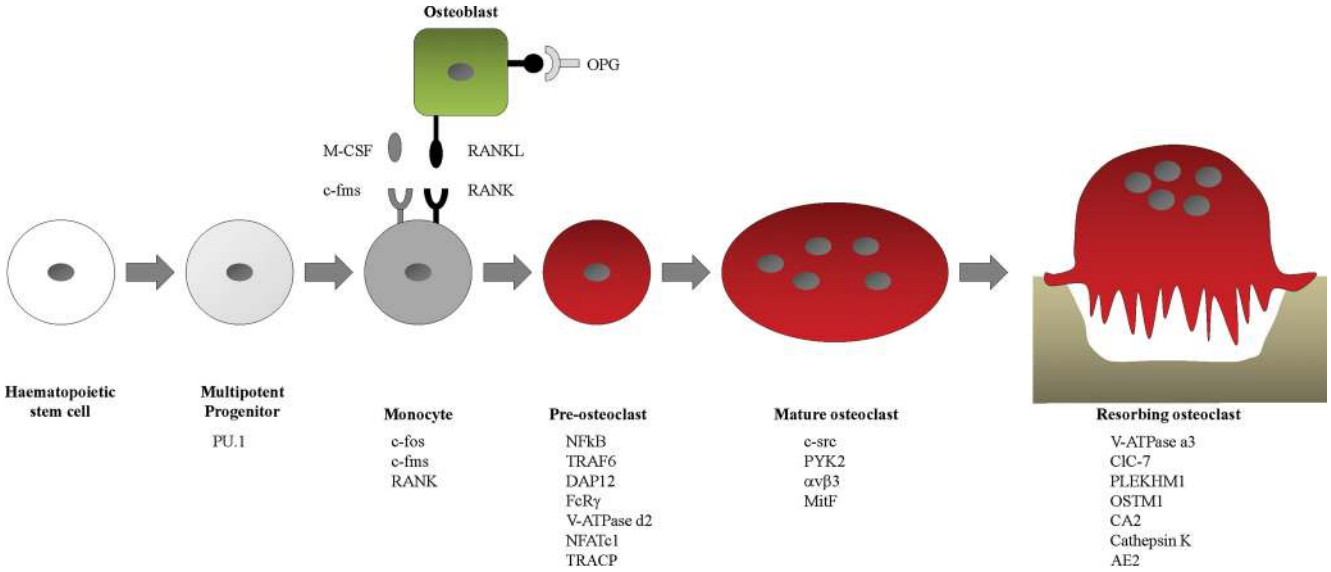


FIG. 1. Schematic illustration of the molecules involved in osteoclastogenesis and function. (See Refs. 1, 33, and 42). NFκB, nuclear factor κ B; TRAF6, TNF receptor-associated factor 6; nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1; PYK2, Proline-rich Tyrosine Kinase 2; MitF, microphthalmia-associated transcription factor; ClC-7; Chloride Channel 7; PLEKHM1, pleckstrin homology domain containing, family M (with RUN domain) member 1; osteopetrosis associated transmembrane protein 1; CA2, carbonic anhydrase II; AE2, anion exchanger 2.

receptor activator of nuclear factor κB (RANK) ligand (RANKL) and macrophage-colony stimulating factor, undergo differentiation and fusion resulting in large multinucleated cells characterized by expression of a series of osteoclast markers, such as tartrate-resistant acid phosphatase (TRACP), matrix metalloproteinase (MMP)-9, cathepsin K, carbonic anhydrase II (CAII), the a3 subunit of the vacuolar $[H^+]$-ATPase, chloride channel 7 (ClC-7), osteopetrosis-associated transmembrane protein 1, and the calcitonin receptor (34–41). Osteoclastogenesis and the molecules involved in this process are summarized in Fig. 1, but are not discussed in any further detail because several excellent reviews have been published recently on this topic (1, 22, 33, 42).

Polarization and formation of the sealing zone, which is a specialized ring structure containing a high number of β-actin filaments, are the next steps in the life span of an osteoclast (43, 44). These processes require the $\alpha_v\beta_3$ integrin and the intracellular signal transducers c-src, Syk, and proline-rich tyrosine kinase 2, as well as the microphthalmia transcription factor (33, 45, 46), which appears to be an important regulator of osteoclastic gene transcription (47–49).

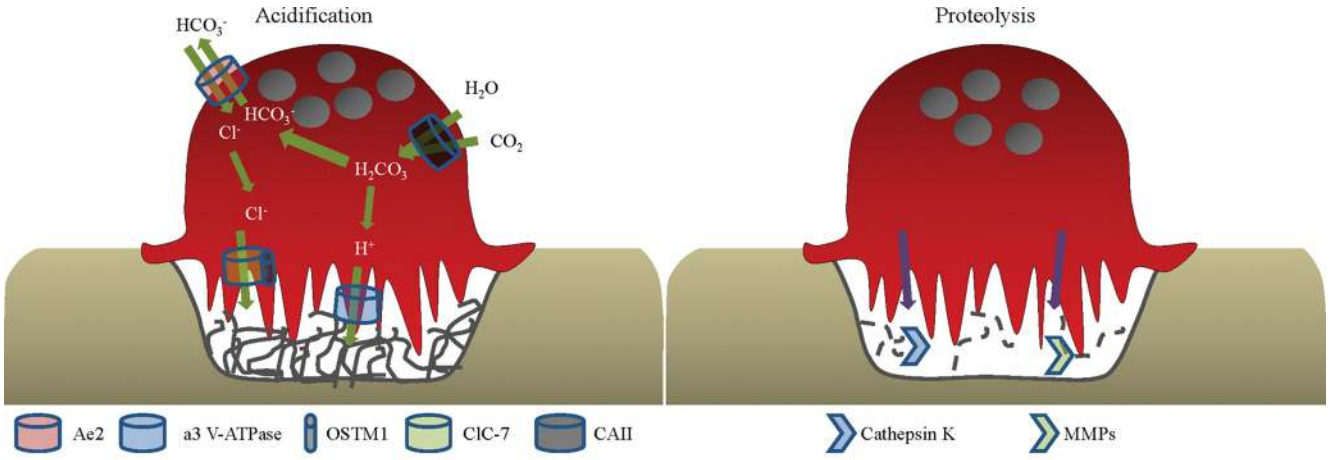
The final step of osteoclastogenesis is the activation of resorption, a process that is characterized by the formation of a ruffled border that is an intensely convoluted membrane present inside the sealing zone (43, 44). The formation of the ruffled border is not well-characterized; however, the signaling molecule Rab7 is required (50).

Bone resorption takes place at the ruffled border localized at the apical side of the osteoclasts, and it can be divided into two processes, namely acid secretion and proteolysis, although these processes likely occur at the same time (44, 51).

Bone resorption is initiated by active secretion of protons through a vacuolar type ATPase (V-ATPase) and passive transport of chloride through a chloride channel (52, 53). The secretion of hydrochloric acid leads to dissolution of the inorganic matrix of the bones (54). The osteoclastic V-ATPase is functionally specific and contains the a3 subunit, and accordingly, loss of a3 leads to osteopetrosis (37, 55–57). In both mice and man, the chloride channel ClC-7, has been shown to mediate chloride transport, thereby ensuring the electrochemical balance required for intense acidification (Fig. 2) (8, 39, 58). Recent data showed that ClC-7 functions as a proton-chloride antiporter (59, 60).

To generate the necessary levels of H^+ and Cl^-, the enzyme CAII catalyzes conversion of CO_2 and H_2O into H_2CO_3, which ionizes into H^+ and HCO_3^- (35), thereby providing the protons for the V-ATPase (27). Meanwhile, basolateral exchange of HCO_3^- ions for Cl^- by anion exchanger 2 (61–63) provides Cl^- ions required for the intense acidification occurring in the resorption lacuna. Interestingly, long bones differ from flat bones with respect to the molecular nature of the acidification machinery (62).

Proteolysis of the type I collagen matrix in bones is mainly mediated by the cysteine proteinase, cathepsin K. This enzyme is active at low pH in the resorption lacuna (Fig. 2) (64–68). The neutral MMPs also appear to play a minor role during organic matrix degradation; however, the exact role of MMPs is still being investigated (69) and is highly dependent on the bone type (38, 70, 71). The resorbed material is removed from the resorption pit by



Mutated gene/protein	Phenotypes	Osteoclast characteristics
ClCN7 / ClC-7	ADOII, IARO, ARO Severe osteopetrosis in mice	Decreased acid secretion Low resorption High numbers
TCIRG1 / a3 subunit	ARO Severe osteopetrosis in mice	Decreased acid secretion Low resorption High numbers
OSTM1/OSTM1	ARO	Low resorption High numbers
CA2/Carbonic anhydrase II	IARO Moderate osteopetrosis in mice	Altered internal pH regulation Low resorption Increased numbers
SLC4A2/Ae2	No human phenotype known Severe osteopetrosis in mice	Altered internal pH regulation Low resorption Normal numbers
CSTK / Cathepsin K	Pycnodysostosis Osteopetrosis-like disease in mice	Defective collagenolysis Low resorption Normal numbers
MMP-9/MMP-9	No human phenotype known Delayed long bone development	Normal resorption Normal numbers

For references see text

FIG. 2. *Top*, Schematic illustration of the differences between acid secretion and proteolysis during osteoclastic bone resorption, illustrating that the collagen matrix is removed by proteolysis after acidification. *Bottom*, Mutations/knockout in genes/proteins involved in bone resorption, phenotypes, and effect on osteoclasts. Ae2, Anion exchanger 2; a3 V-ATPase, a3 subunit of the osteoclast-specific V-ATPase. ClC-7, chloride channel 7; OSTM1, osteopetrosis associated transmembrane protein 1; CA2, carbonic anhydrase 2; MMP-9, matrix metalloproteinase 9.

uptake and transcytosis through the osteoclast (72, 73). After completing resorption, osteoclasts either undergo apoptosis or perform a further round of resorption (44) (Table 1).

In summary, the osteoclasts are highly specialized for both dissolution of the inorganic matrix and degradation of the organic matrix of the bones. These highly polarized cells are characterized by a unique set of membrane-bound molecules that ensure an efficient resorption of bone and other mineralized tissues. This complex machinery may be affected by a range of important parameters in physiology and pathology, and importantly in drug-induced situations that are important to identify and advance osteoclast research and biology.

IV. Osteoclast Subtypes in Physiological Situations

Osteoclast activities are essential for development, as well as remodeling of bone in response to aging and stress (6, 14, 15). Under normal physiological circumstances, the osteoclasts can be categorized into subgroups depending on the matrix on which they are positioned, the time of day, and the type of remodeling in which they participate. These different groups of osteoclasts have provided key information on skeletal maintenance.

A. Endochondral *vs.* intramembranous bone osteoclasts

Anatomically, two types of bones are present in the body, the long bones (*e.g.*, the femur and tibia) and the

flat bones (*e.g.*, the calvarium), with the main difference between these two types of bone being their development (74). Studies also indicate that osteoclasts on these two types of bones are functionally different with respect to both acid secretion and proteases involved in degradation (2).

Evidence that differences between resorption of flat and long bone exist was presented in 1999 (4). However, indications that even the acidification process is different have been published only recently (62). Data from mice deficient in the bicarbonate-anion exchanger Ae2 (Slc4a2) have shown that it is essential for bone resorption in long bones (61, 63), whereas it is not involved in bone resorption in calvariae (62), showing that distinct acid transport mechanisms are present in different subsets of osteoclasts. With respect to acid secretion into the resorption lacunae, it is presently not known whether any differences exist, although the absence of calvarial thickening in patients with defective ClC-7 strongly suggests that ClC-7 is not involved in resorption of the flat bones (75).

Extensive research into the proteolytic processes involved in resorption of flat and long bones clearly demonstrates that the proteolytic processes involved in degradation of these two types of bone matrix are distinct (4, 71). Osteoclasts in flat bones preferentially appear to engage in MMP-mediated bone resorption, although cathepsin L seems to be involved, too. Osteoclasts in long bones primarily depend on cysteine proteinases, in particular cathepsin K (4, 71). TRACP also appears to be involved in bone resorption, and more so in calvarial bone (76–78). When osteoclasts generated from human peripheral blood are seeded on cortical bone, they primarily depend on cathepsin K, whereas when cathepsin K activity is blocked, there is some compensatory bone resorption mediated by MMPs (69). How these osteoclasts behave on bone substrates other than cortical bone is presently not known (Table 1).

Data suggesting that the bone matrix could play a role in the control of osteoclastic activities were presented in a study showing compositional differences between long bone and flat bone matrices, including differences in the presence of putative cysteine proteinase inhibitors (79). The functional significance of these data still remains to be fully elucidated, although they clearly illustrate the importance of understanding how a given context affects the osteoclasts.

B. Chondroclasts

It has long been discussed whether chondroclasts are a "real" cell type or whether they simply are osteoclasts that reside on cartilage instead of bone (77, 80–82). Chondroclasts are mainly important in endochondral bone development, and in addition there is some evidence that chondroclasts may also play a role in both rheumatoid arthritis (RA) and osteoarthritis (83–85). The term chondroclast derives from the localization of these cells on calcified cartilage as seen in the expanding growth plates during endochondral ossification (80, 86). For their formation, these cells are dependent on the presence of macrophage-colony stimulating factor and RANKL, as are bone-resorbing osteoclasts (87–90).

Most of the evidence for the functionality of chondroclasts is derived from studies of the longitudinal growth of long bones, *i.e.*, metatarsals and tibias isolated from mouse embryos (38, 80, 91–93). First, bone/cartilage resorption in these models is still dependent on acid secretion as evidenced by mice with mutations in the acid secretion process, *i.e.*, ClC-7-deficient and a3 V-ATPase-deficient mice, as well as mice unable to form sealing zones, *i.e.*, c-src-deficient mice, in which the massive bones mainly consist of calcified cartilage due to the defective resorption process (56, 94, 95). Similar findings have been noted in the corresponding human disease(s) (27, 96, 97). Interestingly, the ruffled border is less prominent in the chondroclasts than osteoclasts, potentially suggesting that lower levels of acid secretion are required for dissolution of this matrix (98, 99). The main difference between chondroclasts and osteoclasts is in the profiles of enzymes necessary for tissue degradation. Resorption by chondroclasts does not appear to depend as much on cathepsin K as does resorption by osteoclasts. Cathepsin K-deficient mice show no evidence of calcified cartilage in the marrow cavity of long bones, indicating that the removal of calcified cartilage during endochondral ossification occurs, although there are indications that the process is delayed (65, 100). Of importance is the observation of a massive compensation by MMPs in the absence of cathepsin K, which obscures the interpretation of data from cathepsin K-deficient systems (69, 70, 101–103). Finally, one study has indicated that TRACP is activated and secreted at the ruffled border in cathepsin K-deficient osteoclasts, and only in cells that play a role in the removal of calcified cartilage (104) (Table 1).

Although chondroclasts are involved in the degradation of a different matrix than osteoclasts, an interesting observation is that bone formation is tightly coupled to resorption of the mineralized matrix, as has clearly been demonstrated in studies of endochondral ossification (86). It is more likely that bone formation is coupled to chondroclast numbers because release of molecules from degradation of cartilage, which in composition is far from bone, would be expected to be different from molecules released during bone resorption; however, this has never been studied in detail.

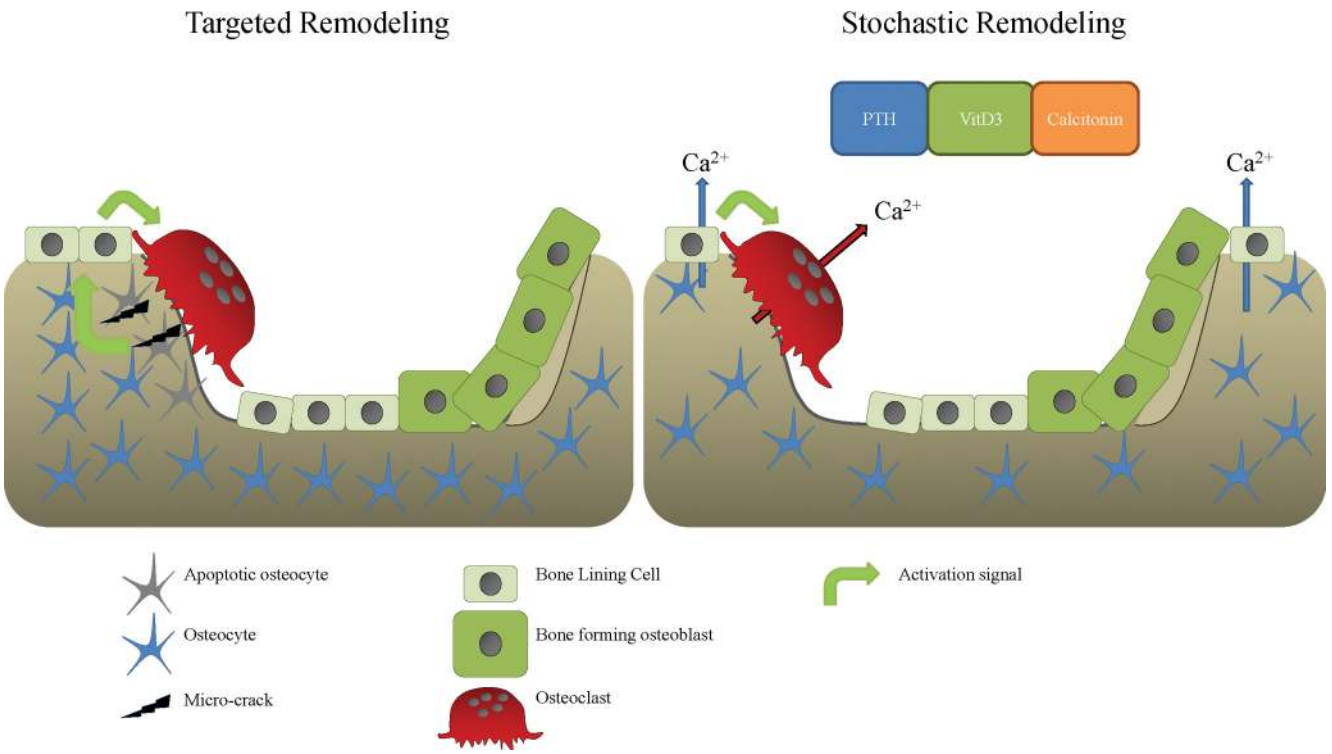


FIG. 3. Schematic illustration of stochastic *vs.* targeted remodeling. The figure illustrates the local nature of targeted remodeling, which is activated at specific sites after the formation of microcracks and leads to removal of the microcrack and restoration of the damaged bone. Stochastic remodeling, on the other hand, is of a systemic nature and is activated by low calcium levels in the circulation leading to PTH release. Other hormones, such as vitamin D3 (VitD3), and potentially calcitonin also play roles in stochastic remodeling. It appears that there are two levels of calcium homeostasis—one mediated by osteocytes independent of osteoclasts, and one including the osteoclasts—although the balance between these two ways of releasing calcium still remains to be fully understood.

C. Osteoclasts involved in targeted and stochastic remodeling

Two different modes of remodeling have been proposed: targeted and stochastic. Targeted bone remodeling takes place at specific sites, whereas stochastic remodeling occurs more randomly (19, 105, 106). The first type is primarily performed to replace microdamaged bone and thus to maintain the load-bearing capacity of the skeleton. The second type of remodeling appears random with respect to localization, although it may be involved in maintaining integrity of the bones, independent of damage. This process is hormonally regulated (105, 107).

The balance between these two modes of bone remodeling has not been fully elucidated yet, but studies in dogs indicate that approximately 30% of all remodeling is targeted and the remaining 70% is stochastic (105). With respect to the osteoclasts mediating these two types of remodeling, most studies have focused on how targeted remodeling is controlled.

It appears that the bone matrix contains signals regulating the activity of the osteoclasts (108). A recent study demonstrated that aged bones were more readily resorbed than young bones, thus supporting the hypothesis that the bone matrix composition influences remodeling rates (7). Furthermore, areas of microdamage, which are characterized by high numbers of apoptotic osteocytes, are preferentially and rapidly degraded by osteoclasts. This further supports the possibility that changes in the bone matrix and the balance between live and dead osteocytes determine which areas will be remodeled (108). Finally, a recent study demonstrated that targeted ablation of osteocytes led to a dramatic increase in osteoclast activity (109) (Table 1). These data indicate that death of osteocytes is a key point in induction of osteoclast activity (Fig. 3).

Taken together with the finding that bones of different age lead to different levels of osteoclastogenesis (7), it appears that osteoclast functionality is at least partially controlled by osteocyte-derived molecules, which are sequestered in the bone matrix.

Stochastic remodeling, while occurring at random sites, is centrally regulated by hormones such as PTH, vitamin D3, and potentially calcitonin, and its main role is the regulation of calcium homeostasis (110–112). It has even been questioned to what extent this process depends on the presence of osteoclasts because patients with nonfunctional osteoclasts have normal calcium homeostasis (110–112). Yet, when they are calcium-deprived, osteopetrotic patients fail to correct their calcium levels, indicating that osteoclasts, which are either absent or nonfunctional in

these patients, do play a role in calcium homeostasis, and thus stochastic remodeling (Fig. 3) (113).

In summary, targeted remodeling is beginning to be understood in detail, and it is a tightly regulated and coupled process involving osteocytes, osteoclasts, and cells of the osteoblast lineage. On the other hand, stochastic remodeling and the role it plays in calcium homeostasis are still not very well understood, although there are indications that there is a level of regulation by the activity of osteoclasts.

D. Trabecular and cortical osteoclasts

Bone remodeling does not occur with the same frequency in cortical and trabecular bone. Every year, 25% of the trabecular bone matrix, but only 4% of the cortical matrix, is remodeled (114). Interestingly, most *in vitro* osteoclast experiments are based on cortical bone (or dentine) substrates, which are either slowly remodeled or not remodeled at all (7, 102, 114–116). Studies have shown that bones endogenously contain signals regulating osteoclastogenesis and resorption and that these signals appear to be related to the age of the bone (7, 117) (Table 1). Thus, an interesting question is whether osteoclasts themselves are indeed different when derived from different matrices or whether the difference is matrix related. Furthermore, systemic regulation is likely to be involved in controlling which bones are resorbed to some extent.

These data also correlate with evidence indicating that remodeling of different bone compartments can be either primarily targeted, such as in the cortex, or primarily stochastic, as seen in some parts of trabecular bone (106). A further understanding of this could provide directions for the development of novel drugs producing optimal benefit at the sites where it is most needed, *i.e.*, leading to a better fracture reduction than that presently obtained.

E. Diurnal variation in osteoclasts or osteoclast activity?

Bone resorption markers measured in serum may be interpreted as indicating the net result of all osteoclast subtypes and activity levels at one particular time. A wide range of factors, known and unknown, may influence the interpretation (15). Diurnal variation is a well-established and important parameter of bone turnover. Postprandially, bone resorption decreases by approximately 50% compared with that of fasting individuals, but during the night, bone resorption increases to an equally large degree (118–120). Several investigations have demonstrated that the circadian variation in bone resorption is induced in part by food intake (121–123), which, at least partially, involves the peptide hormone glucagon-like peptide (GLP) 2 (124). Interestingly, the osteoclast number does not appear to depend on the time of day, further emphasizing differences between osteoclast number and activity (32) (Table 1). An interesting aspect of this is that targeting nocturnal resorptive activity appears to lead to inhibition of bone resorption, whereas not attenuating bone formation (125–127), thereby highlighting an interesting prospect of reducing bone resorption in a specific, nocturnal manner.

In summary, studies of osteoclasts under different physiological conditions, such as those listed above, have highlighted the heterogeneity of these cells. Furthermore, these studies highlighted the importance of the balance between bone resorption and bone formation, a tightly regulated phenomenon that rarely is disturbed under physiological conditions. Finally, how the heterogeneity of the osteoclasts affects bone formation is presently not well understood, but a further understanding of this process could help optimal treatment of diseases involving alterations in bone remodeling.

V. Osteoclast Subtypes in Pathological Situations

Changes in osteoclast activity and number have been detected in several diseases, ranging from illnesses involving excessive bone resorption, such as osteoporosis and Paget's disease; to those involving secondary activation of osteoclasts, such as osteolytic metastases and RA; to diseases involving defective osteoclast differentiation and/or function, such as osteopetrosis. These different types of diseases have shed important light on osteoclastic function with respect to obtaining the right type of treatment. They have also shed light on a very central aspect in bone biology, the coupling principle. The coupling principle describes the phenomenon that bone formation follows bone resorption, which leads to a complete restoration of the bone removed during bone resorption (17).

A. Osteoporotic osteoclasts

1. Changes in osteoclastogenic potential in osteoporosis

An important aspect of osteoporosis is whether the number of osteoclast precursors in the circulation increases, and, if so, whether the osteoclastogenic potential of these cells is increased. Eghbali-Fatourechi *et al.* (128) showed that the overall number of cells expressing RANKL is increased in postmenopausal women compared with premenopausal or estrogen-treated women, clearly indicating that the bone marrow microenvironment, including stromal, T, and B cells, changes in a proosteoclastic direction when estrogen is reduced. These data were supported by a recent study from the same authors showing that bone marrow cells isolated from estrogen-treated or control postmenopausal women displayed

reduced osteoclastogenic potential (129) (Table 1). Estrogen was shown to have a dual mode of action—the first leading to overall lower RANKL expression by bone marrow cells, and the second reducing the osteoclastogenic response to RANKL (130). Interestingly, aging of mice was also shown to increase the osteoclastogenic potential of bone marrow cells, both by up-regulation of RANKL production and by increasing precursor sensitivity to RANKL (131).

In vitro studies of the changes in cellular activity of osteoclasts from osteoporosis patients are limited, but these have indicated both an accelerated osteoclastogenesis and resorption (132, 133). Furthermore, a key cell in the up-regulation of osteoclastogenesis appears to be the T cell, which responds to lowered estrogen levels by increasing RANKL production (134).

The main issue with all the studies of osteoporotic osteoclasts and their precursors is the use of mixed cell populations, which clouds the interpretation of the results, and therefore these aspects of osteoclastic function still require further investigation. Furthermore, with the recent publication of the possibility of assessing the "anabolic potential" of osteoclasts (11, 13), it would be of interest to investigate the anabolic capacity of osteoporotic osteoclasts and thus shed light on the imbalance between bone resorption and bone formation in osteoporosis.

2. A direct role for sex steroids on osteoclasts

The role of estrogen on cells belonging to the osteoclastic lineage has been studied extensively, with several findings indicating that estrogen suppresses osteoclastogenesis but not the resorptive activity of mature osteoclasts (135–137). Androgens, such as dihydrotestosterone, exhibit similar effects to estrogen on osteoclasts *in vitro*, although this has not been studied in great detail (138). Finally, a recent study using mice deficient for the estrogen receptor (ER)-α, specifically in mature osteoclasts, showed bone loss in female, but not male, mice (139). This demonstrated that estrogen likely plays a direct role in bone resorption by even mature osteoclasts (139). Although the authors used the cathepsin K promoter to ensure specific knock-down of the ER-α in osteoclasts, cathepsin K is also expressed in preosteoclasts, albeit to a lower extent (139). More studies are needed to investigate the role of ER-α in mature osteoclasts specifically. Interestingly, osteoclasts in different bone sites preferentially express different ERs, with cortical osteoclasts mainly expressing ER-α and trabecular osteoclasts mainly expressing ER-β (140), whereas from a functional point of view ER-α appears to be more relevant for trabecular, not cortical, bone (141). Furthermore, the expression pattern also differs between mature and differentiating osteoclasts; ER-α is mainly expressed in immature cells, and ER-β is present at all stages of osteoclastogenesis (137). Again, there appear to be different osteoclastic subtypes, which also appear to be relevant in the context of bone loss rates in different bone compartments during osteoporosis (114). As indicated above, an important point is the difference between genders (139). In mice, the gender-based difference between cortical apposition and endocortical resorption that becomes more apparent with increasing age might be explained by differences in ER expression (142). With respect to changes in the osteoclasts after menopause, a couple of studies have clearly demonstrated that bone resorption, as well as bone formation, increases in women after menopause (143–145), and these changes become more explicit in high- and low-turnover patients (145). Although bone formation increases as a function of the increased resorption, it does not match bone resorption, thereby illustrating the importance of understanding the interplay between osteoclasts and osteoblasts in detail.

B. Changes in osteoclast activities with increasing bone matrix age

Numerous studies have investigated the control of osteoclast activity as a function of changes in biochemical properties of the bone matrix. Aging leads to accumulation of different biochemical modifications of the bone matrix, such as advanced glycation end-products (AGEs), homocysteine, increased calcium concentration, as well as some modifications of the collagen matrix (146).

Recent studies have indicated that these modifications of the bone matrix itself actually modulate the activity of the osteoclasts to a certain extent (7, 117). Homocysteine, which accumulates in bone and in circulation with age, was shown to activate osteoclastogenesis and bone resorption (147) (Table 1). AGEs are modifications of proteins that accumulate in various tissues with age, and they have been implicated in the pathology of osteoporosis (146, 148). Some evidence indicating a direct regulation of osteoclast activity by AGEs has been published, but these studies are contradictory. One study shows activation of resorption by AGE-modified proteins (149), whereas the other study shows the opposite (117); however, quite different techniques were used.

Interestingly, AGEs are accumulated in diabetes, and they have been speculated to be involved in the increased fracture rates observed in patients with this disease (150, 151). Another intriguing finding is the induction of apoptosis in osteoblasts by AGEs (152), which potentially could play a role in the imbalance between osteoclast and osteoblast function during osteoporosis and aging. These findings are all preliminary in nature, and they await confirmation from independent research groups. However, once again they illustrate the heterogeneity of osteoclasts,

in this case as a function of matrix age, and the importance of understanding this phenomenon.

C. Osteoclast-rich osteopetrosis

The most frequently occurring forms of osteopetrosis are those caused by mutations in either the a3 subunit of the V-ATPase, ClC-7, or osteopetrosis-associated transmembrane protein 1. Osteoclasts from patients with mutations in these genes or proteins and from knockout mice have been studied quite extensively (8, 39, 56, 58, 95, 153–156).

Microscopic analyses of cells from patients with defective acid secretion by osteoclasts indicated defective ruffled border formation, but also accumulation of material in vesicles, indicating hampered transcytosis (157). Apart from confirming the defective acid secretion and thereby bone resorption, when either ClC-7 or the a3 subunit is mutated (8, 39, 56, 58, 95, 153, 154), these studies also shed light on important aspects of bone remodeling.

In vitro studies indicate that osteoclasts with impaired acid secretion have higher survival rates than cells with normal secretion, due to the reduced release of proapoptotic signals during resorption (159). This observation correlates well with the high numbers of osteoclasts observed *in vivo* in this group of patients, as well as with findings in mice with attenuated acidification of the resorption lacunae (95, 97) (Table 1). Furthermore, significantly increased resorbed areas are seen during impaired acid secretion, but the resorption pits are shallow, indicating a disturbed activity of the osteoclasts (97). More importantly, these studies highlighted that bone formation in these patients is ongoing—a process that appears to be correlated to the increased number of osteoclasts rather than bone resorption (96, 154, 160, 161). These findings contrast with the classical perception that bone formation always follows bone resorption in a tightly coordinated manner and illustrate the importance of the actual presence of osteoclasts to maintain bone formation.

D. Osteoclast-poor osteopetrosis

Several murine forms of osteoclast-poor osteopetrosis have been described in the literature (1, 42). In general, whereas the mutations express a pronounced osteopetrotic phenotype and few or no osteoclasts are present, the phenotypes are less severe than the phenotypes of the different osteoclast-rich osteopetrotic mutations (1). These data strongly suggest that the osteoclasts are indeed involved in the production of anabolic signals for bone formation (3, 15).

Studies of mice deficient in c-src and c-fos, a key molecule involved in ruffled border formation and a key signal transducer for osteoclastogenesis, clearly demonstrated that osteopetrosis was due to nonfunctional osteoclasts or the absence of osteoclasts, respectively (162, 163). Interestingly, these two groups of mice have opposing phenotypes with respect to bone formation. The osteoclast-rich c-src knockouts have increased bone formation (164), and the osteoclast-poor c-fos knockouts have decreased bone formation (165). The anabolic effects of PTH are present in the c-src$^{-/-}$ mice but are blunted in the c-fos$^{-/-}$ mice (166), indicating that osteoclasts are central for bone formation (Table 1).

Two recent studies identified mutations in the genes for RANK and RANKL as the causes of osteopetrosis in a novel group of patients (28, 29). No indications of osteoclasts were found in these patients (28, 29), which is consistent with previous observations in mice deficient in both RANKL and RANK (87, 90). Patients with mutations in either RANKL or RANK have a pronounced osteopetrotic phenotype and classical histological hallmarks of osteopetrosis including unresorbed primary spongiosa. However, although limited data have been published, the osteopetrotic phenotype appears to be less severe than the one observed in the osteoclast-rich forms (1). Thus, mutations within the RANK/RANKL/osteoprotegerin (OPG) system can lead to osteoclast-poor osteopetrosis with low bone formation in mice and men.

Interestingly, alterations in osteoblast function, such as changes in the production of RANKL and OPG, may have the same effect. Stabilizing osteoblastic β-catenin in transgenic mice, thus mimicking constitutive activation of the canonical Wnt signaling pathway, was followed by an up-regulation of OPG in relation to RANKL (31, 167). As expected, the mice developed osteoclast-poor osteopetrosis with failure of tooth eruption, a classical phenomenon in murine osteopetrosis. Mutations within *LRP5* related to the Wnt signaling pathway have underscored the fundamental importance of this pathway for regulation of bone mass. The osteoporosis pseudoglioma syndrome was found to be caused by loss of function mutations in the gene for *LRP5* (168). In contrast, mutations affecting the first propeller of the coreceptor, presumed to be followed by chronic activation of the Wnt pathway, were found in various forms of monogenic human osteosclerotic phenotypes (169). Among these, autosomal dominant osteopetrosis type 1 has been well characterized clinically, biochemically, histomorphometrically, and biomechanically (75). Autosomal dominant osteopetrosis type 1 is an osteoclast-poor osteopetrotic phenotype with increased biomechanical competence and no low-energy fractures. Osteoclast profiles are markedly decreased (97), bone formation seems to be normal, and OPG levels in the circulation increased (170). However, when investigating os-

teoclasts *ex vivo* from these patients, they express normal bone resorptive capacity (30).

In summary, osteoclast-poor osteopetrosis can arise in murine mutations/transgenics or humans when the OPG/RANKL/RANK system is affected directly or indirectly. These findings underscore this cytokine system as a key regulator of osteoclastogenesis. Moreover, the phenotypes seem to be less affected than the osteoclast-rich forms, the reason for which is so far unresolved, although there are indications that reductions in bone formation are involved (1, 28, 29).

E. Pycnodysostotic osteoclasts

An interesting subtype of osteoclasts with defective bone resorption is observed in patients with pycnodysostosis. Pycnodysostosis is caused by loss of function or loss of expression mutations in the cysteine proteinase cathepsin K, which in humans causes dwarfism and poor bone quality due to defective remodeling of the bones (36, 171–173). Few studies examining the phenotype of pycnodysostotic osteoclasts have been published. Microscopic analyses of the osteoclasts have shown significantly increased amounts of demineralized collagen matrix in the resorption pit, but also inside the osteoclasts, indicating disturbed resorption and trafficking of resorbed components (174, 175). A study of biochemical markers of bone turnover showed that C-terminal crosslinked telopeptide of type I collagen (CTX-I) release was reduced, whereas production of the MMP-generated type I collagen fragment carboxyterminal telopeptide of type I collagen (ICTP) was increased (67) (Table 1). Several studies of cathepsin K-deficient mice have been published, and whereas they confirm that cathepsin K is essential for degradation of the organic matrix in bone (65, 66, 176), there are also several differences between the human and mouse phenotypes (103). Furthermore, in cathepsin K-deficient mice, bone formation parameters are highly increased (100). These findings have not been replicated in pycnodysostosis patients in whom the bone matrix is disordered (177), and a clinical case study indicated that anabolic response to PTH was absent (174). Two recently published clinical studies have shown that whereas bone resorption markers are strongly reduced, bone formation is also suppressed in women treated with the cathepsin K inhibitor odanacatib (178). In a monkey study monitoring bone formation by histomorphometry reductions in bone formation, rates were shown in the trabecular bone compartment, whereas bone formation was increased in the cortical compartment (179–181).

In conclusion, cathepsin K mediates cleavage of type I collagen in the resorption lacunae, but its secondary effects on bone formation are bone type-dependent and still need to be investigated further.

F. Other diseases characterized by increased osteoclast activity

Apart from hypogonadal osteoporosis, several diseases are characterized by accelerated osteoclastogenesis and function. Although the etiology of these diseases is different, there are interesting overlaps and discrepancies that provide highly useful information about osteoclastic function and secondary effects on bone formation under different circumstances (182).

1. Pagetic osteoclasts

Paget's disease is a late-onset disease that is quite common in the elderly Caucasian population, where it affects approximately 3% of individuals (182). The disease is characterized by focal increases in osteoclast numbers, nuclearity, and size, which leads to localized bone destruction, although surrounding osteoblasts also are activated (183) (Table 1). The identified causes of the disease include mutations in four different genes, *TNFRSF11A*, *TNFRSF11B*, *VCP*, and *SQSTM1* (182, 184–186). These genes encode RANK, OPG, p97, and p62, all of which are involved in the regulation of osteoclastogenesis. The mutations all result in different subtypes of Paget's (182, 184–186). These mutations render the osteoclast precursors more sensitive to RANKL stimulation, resulting in a higher number of osteoclasts, and potentially also explaining the presence of giant osteoclasts (185, 187, 188). Interestingly, a recent study in mice indicated that the most common mutation in p62 does not make the osteoclasts Pagetic alone, although it sensitizes them to other yet-to-be-described causes of Paget's (189, 190).

In Paget's patients, biochemical markers of both bone resorption and bone formation are increased, showing an overall increase in bone turnover at the affected sites. However, bone resorption clearly exceeds bone formation (182). Whether the osteoclasts in Paget's behave differently from those in healthy individuals during bone resorption is presently unknown. In particular, it is not known whether osteoclasts in Paget's require acidification to resorb bone, or whether cathepsin K is the main protease, although answers to these questions might be of value in the development of new therapies for Paget's. Furthermore, an explanation for the localized nature of Pagetic lesions has still not been found. Even under the extreme circumstances seen in Pagetic lesions, bone formation is coupled to osteoclastic parameters, although whether this is due to increased osteoclast numbers or activities is not known. Moreover, in this case a treatment type eliminating the activity of both types of cells is most likely to be preferred because the increase in bone formation occurring is part of the pathology, and likely will provide no benefit for the bones if maintained. Thus, bisphosphonate, which strongly attenuated overall bone

turnover, appears to be highly relevant in the context of Paget's disease (191).

2. Osteolytic osteoclasts

Several forms of cancer can metastasize to bone and form osteolytic metastases (192–196). Once the cancer has reached the bone, tumor and bone interact in a vicious cycle in which tumor-secreted factors, such as PTHrP, stimulate bone cells, which in turn release growth factors and cytokines that promote further tumor cell growth (192, 197). The activation of osteoclastogenesis induced by tumor cells has been shown to involve a switch in the RANKL/OPG ratio favoring osteoclastogenesis and activation, leading to release of the tumorigenic factor TGF-β, and thereby inducing the vicious cycle (198). As a function of the increased numbers of osteoclasts and accelerated bone resorption, a marked up-regulation of osteoblast activities is also observed (199, 200).

The activity of osteoclasts in metastases has been monitored closely using biochemical markers of bone turnover (199, 200), and these studies have indicated that bone resorption by tumor-induced osteoclasts to some extent depends on MMP activity, rather than cathepsin K, because the type I collagen fragment ICTP is released in high amounts (199, 201) (Table 1). Animal models of breast cancer bone metastases are to some extent sensitive to inhibitors of both cathepsin K and MMPs (101, 202–204); however, clinical data for MMP inhibitors have been disappointing (205, 206). An interesting question is whether these agents, to be effective, have to inhibit MMPs before the tumors actually metastasize. For cathepsin K inhibitors, the data indicate a beneficial effect on the release of the bone resorption marker N-terminal crosslinked peptide of type I collagen, and an increase in ICTP levels (204). However, further information is needed to draw reliable conclusions on the usefulness of cathepsin K inhibitors for metastatic bone disease.

In contrast, treatments ablating both osteoclasts and the increased osteoblast activity, such as denosumab and bisphosphonates, reduce the destructive capacity of the metastasis and, importantly, the afflicted pain. However, they do not appear to affect the cancer cells (192, 207–210), although there are some indications that the bisphosphonates affect the life span of the cancer cells as well as reducing osteolysis (211).

In summary, from a treatment point of view, there are several similarities between Paget's and osteolytic metastases. The optimal approach appears to involve a strategy of reducing overall bone turnover toward the normal range, such as with the use of denosumab or bisphosphonates An intriguing possibility would be to target only the areas undergoing destruction, but whether this is feasible is presently not known.

3. Arthritic osteoclasts

Later stages of RA are characterized by massive bone destruction caused by osteoclasts (212, 213). However, there are several indications that these osteoclasts are not classical bone-resorbing osteoclasts but include cells that degrade calcified cartilage (83, 214) (Table 1). Several studies have indicated that TNF-α at least partially drives osteoclastogenesis in RA (215), as exemplified by mice overexpressing human TNF-α with massive joint destruction including bone erosion (216). Furthermore, TNF-α-neutralizing antibodies, such as infliximab, or soluble TNF-α receptor antagonists, such as etanercept, provide amelioration of RA in humans (217, 218). Apart from TNF-α, RANKL is, not surprisingly, a crucial factor in osteoclastogenesis during RA (87), and mice deficient in RANKL are protected against bone, but not cartilage, erosion (219). Treatment with OPG of mice with collagen-induced arthritis also leads to amelioration of bone destruction, while having a markedly lower effect on cartilage degradation (220). In addition, a study in which TNF-α overexpressing mice were crossed with mice deficient in c-fos (*i.e.*, deficient in osteoclasts), showed no bone destruction but clear evidence of cartilage destruction (221). Furthermore, human clinical trials using denosumab have demonstrated that the RANKL/RANK axis is a key player in RA and that inhibition of RANKL signaling may provide a useful treatment option (222). Finally, more recent evidence has indicated that IL-1α and IL-1β both play a partial role in bone resorption and cartilage degradation (223). Anakinra, which is a soluble IL-1 receptor antagonist, is also used for treatment of RA, although it appears to be less effective than the TNF-α inhibitors (217). In addition, tocilizumab (anti-interleukin-6 receptor inhibitor) has shown promise in preventing RA progression through an effect including a reduction in osteoclast numbers (158, 324).

Because osteoclasts play a significant role in RA, bisphosphonates appear to be an attractive treatment option. Early evidence has indicated that zolendronate may be useful (224), although this has not been fully established yet (225). Furthermore, interpretation of the effects of bisphosphonates in RA is often clouded by glucocorticoid treatment of the same patients because glucocorticoids are associated with rapid systemic bone loss, independent of RA (226, 227). However, in both collagen-induced arthritis in rats and in the TNF-α transgenic mouse model, zolendronate was effective in reducing both bone and cartilage destruction (228, 229).

The bone resorption process in RA is still not completely understood despite several studies into the molecular mechanisms. The role of cathepsin K has been extensively studied, and the data are somewhat conflicting

(230–232). Overexpression of cathepsin K has been shown to accelerate joint destruction in mice (231), and overexpression of cathepsin K has been observed in humans with RA (233, 234). However, studies in the TNF-α overexpression model crossed with cathepsin K-deficient mice showed that cathepsin K plays only a marginal role in bone resorption in RA (232), a finding supported by a case study showing severe arthritis in a pycnodysostotic patient (235), although there are still controversies with respect to the role of cathepsin K in RA (236).

Other cathepsins have not been explored in detail, and their expression patterns do not indicate a particular effect on osteoclast function in RA (237).

Under some circumstances, MMPs also play a role in bone resorption (2, 81). Studies showing that the MMP-derived collagen type I fragment, ICTP, is increased in RA could indicate that osteoclasts used MMPs to digest matrix under these circumstances (238, 239). Infliximab treatment has been shown to reduce ICTP levels, as well as osteoclast numbers (240), further indicating that osteoclasts utilize MMP-mediated bone degradation in RA. However, a direct link between the production of ICTP and osteoclasts has not been demonstrated yet.

Whether acid secretion by osteoclasts is needed for bone destruction in RA is also not clear. Because bone destruction is likely to occur as a result of MMP activity, the need for acidification may be reduced when compared with "classical" bone resorption (69), although this is still not fully understood. Another possibility is that MMP-mediated collagen type I degradation is mediated by another cell type, although this still remains to be clarified. A case study of arthritis in a case of autosomal dominant osteopetrosis type II (241) showed a lack of bone degradation, whereas cartilage degradation was abundant, thereby mimicking the situation seen in osteoclast-deficient systems (221) and indicating that bone resorption in RA depends fully on acid secretion.

In summary, development of severe RA involves osteoclasts, and a reduction of bone resorption by these cells is desired. This may be obtained through inhibition of inflammation and thereby bone and cartilage destruction, as seen with anti-TNF-α therapy. Alternatively, therapies such as denosumab that target the osteoclasts directly may also be useful, although these fail to eliminate inflammation and only partially prevent cartilage degradation (220). The optimal therapy could be a combination of antiinflammatory and antiosteoclastic measures, although this is presently not known.

In summary, studies of osteoclasts under pathological circumstances have highlighted some important phenomena. First, osteoclasts themselves, not just their resorptive activity, mediate bone formation and therefore perform an important secondary role in bone remodeling, which is of importance when developing novel treatments for osteoporosis (15). Second, excessive and local activation of osteoclasts occurs in several diseases, and interestingly the osteoclasts appear to switch subtype with respect to their resorption machinery. These findings highlight the importance of characterizing the function of osteoclasts under pathological circumstances to optimize treatment strategies.

VI. Drug-Induced Osteoclast Subtypes

A. Existing drugs

Several antiresorptive drugs for the treatment of osteoporosis, as well as glucocorticoids and PTH treatment, are known to alter osteoclasts in various ways. These drugs all provide critical information on osteoclast function, and furthermore, they have also played a great role in illustrating the interplay between osteoclasts and osteoblasts, as will be described in the following section.

1. Bisphosphonates

Bisphosphonates have long been associated with induction of apoptosis in osteoclasts, and the mechanism of action underlying the apoptotic effect depends on whether or not the bisphosphonates contain nitrogen (242). Both classes of bisphosphonates bind to the bone matrix and are taken up by the osteoclast during bone resorption. The simple bisphosphonates are metabolized into toxic ATP analogs, thereby inducing osteoclast apoptosis *in vitro* (242). The nitrogen-containing bisphosphonates exert their function by inhibiting the mevalonate pathway, which leads to the generation of an ATP analog known to induce apoptosis in osteoclasts *in vitro* (242). The antiresorptive potency of the nitrogen-containing bisphosphonates *in vivo* is controlled by mineral binding affinity and by their ability to inhibit the mevalonate pathway (242).

Although *in vitro* data clearly show that bisphosphonates induce apoptosis, analyses of osteoclast numbers in iliac crest biopsies failed to show a reduction in the number of osteoclasts when patients were treated with bisphosphonates (243–245). On the other hand, bisphosphonates reduce systemic levels of TRACP 5b and cathepsin K, both markers of osteoclast number (32, 246–248), potentially indicating that osteoclasts undergo systemic apoptosis, which correlates well with the expected effects of bisphosphonates (242) (Table 1). Other studies have shown that when bisphosphonate therapy continues for more than 1 yr, the number of circulating osteoclast precursors is reduced, and these reductions are speculated to be related to reduced serum RANKL levels (249, 250).

A recent study of biopsies from alendronate-treated patients showed the presence of giant hypernucleated, detached, and frequently apoptotic osteoclasts, and the number of these abnormal osteoclasts correlated with the cumulative dose of bisphosphonate (251). Although interesting, the biological implications of this finding are not clear yet.

One potential explanation for the discrepancies in scoring osteoclasts in the iliac crest biopsies is the very low number of osteoclasts observed in general. Recent reports have also debated the clinical relevance of studying iliac crest biopsies because they are from non-weight-bearing bones and these are different from weight-bearing bones (252–254), and in general more data are needed to draw a final conclusion on the osteoclastic response to bisphosphonates.

On the other hand, the effect on reduction of bone resorption measured both by biochemical markers and by bone histomorphometry (activation frequency) confirms a potent reduction in bone resorption, and the level of reduction is often down to the lower range of premenopausal levels, although this depends heavily on the efficacy of the individual bisphosphonate (242, 243, 245, 255–258).

With respect to secondary effects on bone formation, measurement of biochemical markers of bone turnover shows a marked reduction in bone formation markers, and the effects are maintained throughout the treatment period, although this again is dependent on the individual bisphosphonate (242, 243, 245, 255–258). Biopsy studies have confirmed that bone formation is reduced when compared with placebo, and although the reduction in bone formation rates is dependent on the individual bisphosphonate, the data indicate that bone formation is not completely suppressed but is reduced to the lower postmenopausal levels (243, 245, 258). The FLEX study (Fracture Intervention Trial Long-term Extension), although showing continued reductions in vertebral fractures, increase in bone mineral density (BMD), and reduction of bone turnover markers with alendronate, did not show a significant reduction in bone formation rates when comparing patients stopping alendronate to patients continuing treatment; however, the numbers of biopsies were low (259).

All in all, there is no doubt about the fracture-preventing effects of bisphosphonates; however, knowledge of the effect of bisphosphonates on osteoclasts *in vivo* is quite limited. Apoptosis of the osteoclasts most likely explains the reduction in bone resorption. Furthermore, although the extent of the secondary reduction in bone formation is still discussed, it appears to be clinically relevant, and it most likely is the explanation for the attenuation of the BMD increase seen after the first year of treatment.

a. Osteonecrosis of the jaw (ONJ) and bisphosphonates. Bisphosphonate therapy, especially in the case of malignancy-induced bone loss, has been connected to the occurrence of ONJ, mainly due to the ability of bisphosphonates to strongly suppress bone turnover (260–262). Although the probability of ONJ is very low for the dosing regimens used for treatment of osteoporosis, there has still been a lot of debate about whether ONJ is the result of the massive suppression of bone turnover in the jaw (262). Interestingly, alveolar bone of the jaw is very similar to bone matrix in the long bone, *i.e.*, it contains the classical cell types as well as the lamellar structure (263). Furthermore, bone remodeling occurs normally in alveolar bone, although the rate of remodeling has been estimated to be up to 10-fold higher than the corresponding rate in long bones (263–266). In ONJ, the number of osteoclasts has been investigated, and it appears that the osteoclasts are absent from the lesions (267, 268), although opposing evidence also exists (269) and thus more studies are needed.

It has been speculated that massive suppression of osteoclast function, and thus bone turnover, in this high-turnover compartment is what causes ONJ to occur; however, there are several other factors involved, such as tooth extraction or infections, and the overall causality is still not clear (262). One point of particular interest is whether this phenomenon is specific for bisphosphonates or whether it will happen with other very potent and long-lived antiresorptives; however, this is presently not known.

2. Selective estrogen receptor modulators/hormone replacement therapy

Because cessation of estrogen production is a major cause of osteoporosis (3, 14) and both estrogen and SERMs are used for treatment of osteoporosis, several studies have been conducted to clarify their effect on osteoclasts.

Estrogen has been shown to exert direct antiosteoclastic effects at several stages of osteoclastic differentiation and function, namely osteoclastogenesis, resorption, and apoptosis. Direct inhibition of the formation of multinucleated osteoclasts is thought to be caused by suppression of RANKL-induced c-*Jun* and basal c-*Jun* N-terminal kinase activity in osteoclast precursor cells (135, 136). In nonpurified osteoclast-precursor systems, estrogen was found to inhibit osteoclastic differentiation in a human system (270), possibly via down-regulation of the $\alpha_v\beta_3$ integrin (271). Two studies of estrogen have been conducted using CD14+ osteoclast precursors. As mentioned earlier, one study showed significant inhibition of osteoclastogenesis (137), whereas the other showed no direct effect on osteoclast precursors (272). To date, there is no explanation for this discrepancy.

Studies of the effects of SERMs on osteoclasts have shown that tamoxifen inhibits osteoclastogenesis directly, whereas raloxifene and ospemifene only inhibited osteoclasts through up-regulation of the expression of OPG by osteoblasts (273). Although early studies showed an effect of raloxifene on osteoclastogenesis, these were conducted using mixed cell populations and therefore most likely reflect the increase in OPG (274).

Mature osteoclasts have also been shown to respond directly to estrogen (275, 276). These studies showed that both the activity and the production of the lysosomal enzymes are down-regulated by estrogen (277, 278), possibly explaining the reduction in resorption by the down-regulation of cathepsin K and TRACP (36, 66, 77, 279) (Table 1).

In summary, *in vitro* data clearly demonstrate that estrogen and SERMs reduce osteoclast numbers via inhibition of osteoclastogenesis, and potential effects on bone resorption and apoptosis might add to the *in vivo* effect.

Although some studies of osteoclasts in patients treated with either HRT or SERMs have been conducted, the effects of both estrogen and SERMs on bone remodeling indices based on histomorphometry are quite modest (280–284). Overall, these studies show a reduction in activation, frequency, and depth of resorption, as well as—where detectable—a small decrease in osteoclast numbers. Reduced bone formation rates were also observed, confirming the coupled nature of inhibition mediated by estrogen and SERMs (280–284). These data are corroborated by biochemical markers of bone turnover, which clearly demonstrated a coupled reduction in bone resorption and bone formation (32, 285–287), and furthermore explain the plateau effect observed in BMD measurements after 1 yr (285).

In summary, many of the numerous studies of the *in vitro* mode of action of HRT and SERMs show a reduction in osteoclastogenesis. In alignment, *in vivo* studies of these therapies on osteoclasts confirm that osteoclastogenesis is lower than in the untreated population, and importantly, these also confirm the secondary decrease in bone formation.

3. Calcitonin

Calcitonin is a natural peptide hormone produced by parafollicular cells (C cells) of the thyroid gland. Calcitonin possesses potent antiresorptive effects (288), and binding of calcitonin to the calcitonin receptor on osteoclasts induces a rapid change in the cytoskeletal structure of the osteoclasts *in vitro*, which in turn leads to a reduction in bone resorption without inducing apoptosis of the cells (102, 289, 290). Calcitonin in either an injectable or a nasal form has been approved for treatment of osteoporosis; however, because it only prevents about 35% of vertebral fractures, most likely due to low exposure, the clinical usefulness is limited (122). Recent studies have indicated that a recently developed oral formulation of salmon calcitonin will lead to improved efficacy because it has been optimized with respect to pharmacokinetic and pharmacodynamic properties. This has led to a 10-fold higher exposure and thereby a greater reduction in bone resorption parameters. Thus, this agent will most likely provide improved efficacy in preventing fractures (291), and although it remains to be proven in long-term clinical trials, the phase II data are promising (127).

The mode of action of oral calcitonin is a transient suppression of the nocturnal rise in bone resorption obtained by giving the treatment at the right time of day—in the evening (292), which results in a reduction in bone resorption, but no effect or very modest secondary effects on bone formation (127) (Table 1). These findings are further supported by other clinical studies showing that calcitonin may inhibit bone resorption without affecting bone formation, a finding observed independent of administration route (293–296).

There are histological indications that calcitonin attenuates ruffled border formation by osteoclasts (296–298), and this appears to be the mode of action underlying the antiresorptive effects of calcitonin *in vivo*, thereby elaborating on the previously described transient reduction in bone resorption (292).

Studies of mice lacking the calcitonin receptor indicated that bone formation was increased, and thus that calcitonin is a suppressor of bone formation (299, 300). These studies were conducted mainly in young mice. A recent study in mice deficient in the calcitonin receptor specifically in osteoclasts failed to reproduce this finding (301, 302). However, considering the very modest, or nonexistent, suppression of bone formation in patients treated with calcitonin, the mice data appear of low relevance in the clinical setting (127, 293–296).

Further studies are needed to understand this potential dissociation of bone resorption and bone formation. It may be that this dissociation occurs because calcitonin disappears quickly from the circulation and thus is a completely reversible treatment (122). An interesting question is whether calcitonin treatment may result in better bone quality than potent antiresorptives due to the lack of effect on bone formation and the lower suppression of bone resorption, which is expected to lead to a slow, yet prolonged increase in BMD (6, 303).

4. Parathyroid hormone

Although PTH does not appear to affect osteoclasts directly because these cells do not appear to express the PTH receptor, PTH nonetheless affects osteoclast function on many different levels (10). *In vitro* studies of the

effects of PTH on osteoclasts all show that PTH induces osteoclastogenesis and that induction of a transient RANKL expression is essential for this effect (10). However, PTH has mainly been studied in relation to its powerful anabolic effects on osteoblasts (10). Intermittent dosing of PTH in human subjects results in a marked increase in bone formation markers, and secondarily in activation of bone resorption through increased RANKL expression (9, 304, 305). Bone histomorphometric and biochemical marker studies confirm the increase in bone turnover (306–308) (Table 1).

The anabolic mode of action of PTH has been debated extensively. Studies show that PTH directly activates bone formation by osteoblasts when given intermittently (309, 310). In mouse models that are either deficient in osteoclasts or deficient in bone resorption, data suggest that the anabolic effect of PTH is dependent on the presence of mature osteoclasts, but not on their activity (165, 166, 311). Furthermore, initial clinical trials combining alendronate and PTH showed that alendronate blunted the anabolic effect of PTH (312, 313), and there were indications that even pretreatment with alendronate led to a blunting of the PTH response (314). On the other hand, animal studies indicate that PTH can be combined with a bisphosphonate (315, 316), but, as noted by Johnston *et al.* (315), there are marked differences in the doses of PTH used in rodents and in humans.

Collectively, PTH exerts marked regulation of bone turnover (15), including the activation of osteoclasts. The potential anabolic role of osteoclasts and, especially, how to achieve the right osteoclast subtype are debated intensely. Future studies will most likely explain this complex interplay between bone cells and thus guide the right combination of PTH and antiresorptive.

5. Strontium ranelate

Strontium ranelate is approved for treatment of osteoporosis, albeit only in Europe, through its ability to reduce fracture risk in patients (317–321). The mode of action has been studied extensively, and yet it is not fully clear exactly how it works *in vivo*. Bone biopsies have been investigated, and these indicated small increases in bone formation and mineralization rates but no changes in bone resorption or osteoclast parameters, thus indicating that strontium ranelate stimulates novel bone formation (322). These data were supported by analysis of biochemical markers of bone turnover demonstrating increased bone formation (308, 323), while also showing a modest decrease in bone resorption markers (323, 325).

In vitro studies support the hypothesis that strontium ranelate has a dual effect, namely inhibition of bone resorption while stimulating bone formation (326–329). Furthermore, strontium has also been shown to increase OPG expression by osteoblasts (330).

In summary, strontium ranelate is a very interesting molecule with respect to effects on osteoclasts, and several lines of *in vitro* evidence indicate that it reduces osteoclast function (326). However, the relevance of the effect on osteoclasts is still debated, and thus the overall effects of this "uncoupling" molecule are still not fully understood.

6. Glucocorticoids

Glucocorticoids are used to overcome inflammatory conditions, such as inflammatory bowel diseases and RA (331). Glucocorticoid use is associated with severe bone loss due to strongly attenuated bone formation (332). This attenuation of bone formation leads to a rapid acceleration in the number of fractures in glucocorticoid-treated patients (332), especially in trabecular bone compartments such as vertebrae (333). Glucocorticoid treatment is the most common cause of secondary osteoporosis (333), and thus patients on glucocorticoids are often treated with antiresorptives (334).

In vivo, glucocorticoids inhibit osteoblastogenesis, the generation of bone-forming osteoblasts, and promote apoptosis of osteoblasts and osteocytes, which is consistent with the well-known inhibition of bone formation (335).

In contrast, the cellular effects of glucocorticoids on osteoclasts are a subject of controversy. *In vivo*, the effects appear to fall into two categories, one being a short-lived acceleration of osteoclastogenesis and bone resorption, whereas the other is a reduction in osteoclast numbers, which is not well-characterized with respect to exposure time to glucocorticoids (335–340) (Table 1). Interestingly, a study by Kim *et al.* (340) showed that the detrimental effect of glucocorticoids on bone formation was absent when the glucocorticoid receptor was ablated specifically in osteoclasts in mice.

In vitro studies of glucocorticoids are often conducted in the presence of contaminating cells, and because glucocorticoid treatment also promotes RANKL and reduces OPG expression in osteoblasts, it is unclear exactly to what extent they influence the osteoclasts (341, 342). Two recent studies showed that glucocorticoid treatment hyperactivated osteoclasts and thus suggests that glucocorticoids indeed have a direct effect on bone resorption (343, 344). Yet some studies show the opposite (340). Overall, the results appear to be very context-dependent, illustrating the complex nature of the biological effects of glucocorticoids.

Measurements of biochemical markers of bone turnover in human subjects on glucocorticoid therapy provided diverse results, which appeared to be dependent on the dose of glucocorticoid used (331, 345, 346). However, biochemical marker data indicate that bone resorption

increases short term, whereas bone formation is attenuated long term (345) in response to glucocorticoid therapy, which corresponds well to mouse studies (335–339). The short-term increase in bone resorption and long-term suppression of bone formation are also observed with histomorphometry in mice (347, 348).

In summary, glucocorticoids exert detrimental effects on bone, and whereas the effects on osteoclasts are not completely clear yet, further investigation of the effect on the coupling between osteoclasts and osteoblasts could explain the overall beneficial effect of antiresorptives on a syndrome mediated primarily by suppressed bone formation (227, 349). These findings further highlight the importance of understanding the interplay between bone cells to provide the optimal treatment.

B. Future treatments

A series of interesting targets for osteoporosis treatment are currently under investigation. The targets of these treatments to some extent employ novel modes of action on osteoclasts. These novel modes of action are of importance when investigating whether they may have secondary effects on bone formation, and subsequently on bone quality.

1. Denosumab

Denosumab is a fully humanized monoclonal antibody to RANKL; it has gone through a phase III fracture efficacy trial in which it was shown to reduce fracture rates by 68% in vertebrae and 40% in hip (351); and it was recently accepted for treatment of severe osteoporosis in both the United States and Europe.

In line with *in vitro* studies of inhibition of RANKL (210, 352), denosumab prevents osteoclastogenesis, blocks bone resorption, and increases osteoclast apoptosis. It induces a massive reduction of osteoclasts *in vivo* and, thereby, almost complete suppression of bone resorption in both humans and mice (352–354) (Table 1). Denosumab treatment also leads to a marked suppression of bone formation markers in humans (353, 354), as well as a marked suppression in bone formation rates measured by histomorphometry in animal models (352, 355). Thus, denosumab treatment is consistent with the classical perception of coupling.

A key point with respect to denosumab is whether the suppression is too severe and could lead to detrimental effects on bone quality long term (6). However, as is the case with bisphosphonate treatment, this is not clear at present.

2. Cathepsin K inhibitors

Cathepsin K is a critical protease for degradation of the type I collagen matrix in the resorption pits during bone resorption by osteoclasts (36, 65, 66). Studies conducted in pycnodysostosis patients before the final identification of cathepsin K showed massive accumulation of nondigested bone collagen fibers in the resorption pit below the osteoclasts (175). These findings were matched by those from investigations in cathepsin K-deficient mice (66), demonstrating a critical role for cathepsin K in degradation of the organic matrix. Further studies in cathepsin K-deficient systems have indicated that cells of the osteoblast lineage, namely bone-lining cells (68); cells of hematopoietic origin (69); and a general up-regulation of the osteoclastic stimuli, osteoclast numbers, and proteases, especially RANKL and MMPs (103), are involved in compensating for the lack of cathepsin K. Interestingly, a hallmark of the absence of cathepsin K function is the presence of the MMP-derived collagen fragment ICTP, which is seen in pycnodysostosis patients, cathepsin K-deficient mice, and cell cultures (67, 69, 103, 356), strongly indicating a compensation by MMPs in the absence of cathepsin K (Table 1).

An interesting study by Fuller *et al.* (357) showed that inhibition of cathepsin K in cultured osteoclasts led to augmented secretion of IGF-I. Furthermore, increased numbers of osteoclasts, containing granules of matrix proteins, have been observed in monkey studies of cathepsin K inhibitors (175, 181), thus indicating the potential of this protease for anabolic stimulation of the osteoblasts. Cathepsin K-deficient mice have been studied extensively, and recent experiments indicate that bone formation in trabecular bone is increased after cathepsin K administration and thus that bone resorption and bone formation are not coupled (100, 176). However, clinical studies of cathepsin K inhibitors, such as odanacatib, have shown that whereas a robust reduction in CTX and N-terminal crosslinked peptide of type I collagen occurred and no changes in TRACP 5b were observed, a significant decrease in the bone formation marker pro-peptide of collagen type I and nonsignificant reductions in bone formation rates by histomorphometry were seen (178). Furthermore, a study of osteoclast morphology as a function of cathepsin K inhibition in humans indicated increased size of the osteoclasts and the presence of large TRACP-positive vacuoles, yet no increase in osteoclast numbers (358). Studies in monkeys clearly demonstrated that bone formation in the trabecular compartments was dose-dependent and significantly reduced by cathepsin K inhibitors, whereas an induction of bone formation was observed at cortical sites (179, 180). Further studies are needed to clarify whether the osteoclasts in cathepsin K-deficient situations indeed signal to the osteoblasts. An indication came from a pycnodysostosis case study that showed no bone formation response to PTH (174), and

thus indicated that secretion of the coupling signals may be attenuated at least in human systems. A possible explanation for the lack of secondary anabolic effects induced by inhibition of cathepsin K is the presence of demineralized collagen fibers in the resorption pit, which are removed by bone-lining cells (68). Although it is not well understood how the presence of fibers and their subsequent removal affect osteoblasts, a study indicated that RGD sequences, which are numerous in collagen, antagonize osteoblast function (359).

These findings again illustrate the importance of carefully investigating the osteoclast subtype as a function of cathepsin K inhibition to more accurately predict potential secondary effects on bone formation.

3. Glucagon-like peptide-2

GLP-2 is a 33-amino acid peptide. GLP-2 is created by specific posttranslational proteolytic cleavage of proglucagon in a process that also liberates the related GLP-1 (124). GLP-2 is produced by the intestinal endocrine L cell and by various neurons in the central nervous system (124). Intestinal GLP-2 is cosecreted along with GLP-1 upon nutrient ingestion.

GLP-2 has in clinical settings been demonstrated to inhibit bone resorption (124–126) (Table 1). Reductions in bone resorption by exogenous GLP-2 require an intact gastrointestinal tract (125, 361, 362). The decreased meal-induced inhibition of bone resorption in jejunostomy patients, who lack a GLP-2 response, supports the view that GLP-2 plays a role in postprandial reduction in bone resorption (361, 362).

GLP-2 has in addition been suggested to inhibit bone resorption without affecting bone formation (125), highlighting this mode of inhibition of resorption for further investigation with respect to osteoclast subtypes.

4. Acid secretion inhibitors

Acid secretion by osteoclasts has been an interesting therapeutic target since the discovery that this process is controlled by the a3 subunit of the V-ATPase and ClC-7, both of which are quite specific to osteoclasts (37, 39, 56). Furthermore, *in vitro* studies of osteoclasts treated with inhibitors of these ion transporters have shown that the osteoclasts are unable to resorb bone and that they therefore survive longer (8, 159, 363), thereby mimicking the elevated numbers of osteoclasts observed in patients with mutations in the genes for a3 and ClC-7 (37, 97) (Table 1). In aged ovariectomized rats, early low-potency chloride channel inhibitors were able to prevent bone resorption by approximately 50%, as monitored by both BMD and the biochemical markers of bone resorption CTX-I or deoxypyridinoline, while augmenting the number of osteoclasts and showing no inhibition of bone formation markers (8, 364). Similar findings were published for an inhibitor of the V-ATPase (365). In a study of prosthetic implants coated with bafilomycin, osteoclast numbers were elevated, and indications of increased bone formation were observed (366). These studies were the first to provide proof of concept that inhibition of acidification is a really promising target for osteoporosis treatment. Most interestingly, bone formation levels, as measured by osteocalcin and by evaluation of the dynamic histomorphometry parameters mineral apposition rate and the mineralizing surface *vs.* bone surface, were not affected. These data therefore suggest that inhibition of acidification of the osteoclastic resorption lacunae results in an uncoupling of bone formation and bone resorption, thereby possibly improving the potential efficacy of the treatment. This is in contrast to other antiresorptive treatments where a secondary decrease in bone formation is observed (3, 367). These data also indicate that the subtype of osteoclasts obtained—nonresorbing yet alive—when targeting acid secretion is active with respect to bone formation, and thus might possibly be combined with PTH treatment in the future.

Finally, other compounds that appear to modulate the activity of osteoclasts are in development for osteoporosis. These include calcilytics, PTHrP, and sclerostin, but their effects on osteoclasts, which most likely are indirect, are not clear yet (368–370), and thus these will not be described further.

VII. The Bone Anabolic Effects of the Osteoclasts

Since the early discovery that osteoclast activities were involved in regulation of bone formation during targeted remodeling (17, 18, 371, 372), a series of studies have investigated the nature of this process.

The early studies focused mainly on the release of molecules from the bone matrix during bone resorption and identified molecules such as IGF-I and TGF-β (357, 373, 374). However, with the recent discovery that mature osteoclasts, not osteoclast precursors and not necessarily bone resorption, are needed for stimulation of bone formation (8, 9), a series of studies have investigated this phenomenon.

Zhao *et al.* (12) demonstrated that osteoclast-mediated expression of EphrinB2 and osteoblast-mediated expression of EphB4 were involved in a bidirectional communication between these cell types. EphrinB2 on osteoclasts stimulated bone formation by the osteoblasts via binding to EphB4, while EphB4 expression on osteoblasts in turn inhibited osteoclastogenesis via binding to EphrinB2 (12).

However, ephrin signaling requires close contact between the osteoclasts and their target cells. This has led to the speculation that ephrin signaling could be involved in the interplay between osteoclasts and bone-lining cells, which are found in close contact and appear to regulate the activity of each other (68, 375).

Stimulatory signals from osteoclasts directly to mature bone-forming osteoblasts are, on the other hand, likely to be paracrine because these cell types are not found in close contact (264). Both TGF-β and IGF-I are produced by the osteoclasts and are known to stimulate bone formation under various circumstances (376–380). In relation to these findings, it is interesting that the anabolic effect of PTH in mice was shown to be mediated through IGF-I (350), an effect that is absent in the absence of osteoclasts (166). This confirms that IGF-I is a coupling factor.

A recent study demonstrated the mature human osteoclasts, independent of their resorptive activity, secrete factors that activate nodule formation by the osteoblasts (11). This study was followed by a study showing that osteoclasts produce the anabolic factors bone morphogenetic protein 6, Wnt10b, and sphingosine-1-phosphate, again independent of bone resorption (13). Furthermore, inhibition of bone morphogenetic protein 6, Wnt10b, and sphingosine-1-phosphate led to inhibition of the osteoclast-mediated stimulation of bone formation *in vitro*. Finally, osteoclasts have also been shown to produce cardiotrophin-1 (CT-1), which activates bone formation by osteoblasts, although the role of CT-1 was clearly shown to be dependent on age because loss of CT-1 in newborn mice caused osteopenia, whereas in larger mice it caused mild osteopetrosis due to defective bone resorption (360).

In summary, the presence of mature osteoclasts is associated with the secretion of stimulation of bone anabolic signals, and whereas several candidate factors have been identified, a clear demonstration that removal of one of the molecules specifically in the osteoclasts *in vivo* leads to loss of bone formation is still missing.

VIII. Conclusions and Future Perspectives

Osteoclasts have traditionally been viewed as bone resorption "machines"; however, studies of osteoclasts have highlighted that these cells are highly context-specific, and the context of the individual osteoclasts is important for the continued regulation of bone remodeling.

As described in detail in this review, the osteoclasts possess at least two highly important functions: 1) bone resorption, a process that is highly dependent on a series of external stimuli, such as matrix type, remodeling status, hormones involved in calcium homeostasis, genotype, inflammation, and importantly also on intervention strategies; and 2) stimulation of bone formation by the osteoblasts, a process that as illustrated by studies conducted in osteopetrotic patients is, to a large extent, independent of bone resorption. It is presently not completely clear when the osteoclasts are anabolically active, yet it appears to be related to the presence of large multinuclear osteoclasts because bone anabolic responses are seen under these circumstances (3, 15).

Understanding osteoclast functioning may be useful for developing drugs that not only inhibit bone resorption but also enable bone resorption levels that ensure targeted remodeling and, importantly, support continued anabolic signaling from osteoclasts to osteoblasts in the bone remodeling compartment. This has the triple effect of: 1) maintaining a sufficient resorption level and thereby avoiding excessive aging of the bones; 2) sustaining a local stimulation of bone formation at the resorption site only; and 3) not initiating induction of bone formation in otherwise quiescent sites. Theoretically, this type of inhibition of bone resorption would allow a continuous, ongoing increase in BMD, which is in contrast to the effects of the presently approved antiresorptives where a plateau effect on BMD is observed within the first 12–18 months. This means that even with less powerful suppression of bone resorption, such as that seen with the oral formulation of salmon calcitonin, the long-term effects would surpass those of the bisphosphonates.

A deeper understanding of both the differences in the resorption process depending on circumstances and the knowledge relating to when the osteoclasts are anabolically active will aid in the identification of novel treatment opportunities for bone diseases.

Finally, the use of biochemical markers of bone turnover is becoming increasingly relevant for the continued understanding of osteoclasts. Markers provide systemic information on the outcome of a given treatment and can help answer questions such as whether glucocorticoids exert detrimental effects on bone formation, and whether antiresorptives antagonize bone formation secondary to bone resorption because of suppression of osteoclast numbers or activity.

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