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Review

Recent advances in osteoclast biology and pathological bone resorption

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Summary. The osteoclast is a bone-degrading polykaryon. Recent studies have clarified the differentiation of this cell and the biochemical mechanisms it uses to resorb bone. The osteoclast derives from a monocyte/macrophage precursor. Osteoclast formation requires permissive concentrations of M-CSF and is driven by contact with mesenchymal cells in bone that bear the TNF-family ligand RANKL. Osteoclast precursors express RANK, and the interaction between RANKL and RANK (which is inhibited by OPG) is the major determinant of osteoclast formation. Hormones, such as PTH/PTHrP, glucocorticoids and $1,25(OH)_2D_3$, and humoral factors, including TNFα, interleukin-1, TGFß and prostaglandins, influence osteoclast formation by altering expression of these molecular factors. TNFα, IL-6 and IL-11 have also been shown to promote osteoclast formation by RANKL-independent processes. RANKL-dependent/independent osteoclast formation is likely to play an important role in conditions where there is pathological bone resorption such as inflammatory arthritis and malignant bone resorption. Osteoclast functional defects cause sclerotic bone disorders, many of which have recently been identified as specific genetic defects. Osteoclasts express specialized proteins including a vacuolar-type H^+-ATPase that drives HCl secretion for dissolution of bone mineral. One v-ATPase component, the 116 kD V_0 subunit, has several isoforms. Only one isoform, TCIRG1, is up-regulated in osteoclasts. Defects in TCIRG1 are common causes of osteopetrosis. HCl secretion is dependent on chloride channels; a chloride channel homologue, CLCN7, is another common defect in osteopetrosis. Humans who are deficient in carbonic anhydrase II or who have defects in phagocytosis also have variable defects in bone remodelling. Organic bone matrix is degraded by thiol proteinases, principally cathepsin K, and abnormalities in cathepsin K cause another sclerotic bone disorder, pycnodysostosis. Thus, bone turnover in normal subjects depends on relative expression of key cytokines, and defects in osteoclastic turnover usually reflect defects in specific ion transporters or enzymes that play essential roles in bone degradation.

Key words: CLIC5, Vitamin D, M-CSF, CAII

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Introduction

Bone resorption occurs continuously throughout life to maintain skeletal mass and calcium balance. This involves coupled bone formation and resorption, which are carried out by osteoblasts and osteoclasts respectively. The osteoclast is a multinucleated cell specialised to carry out lacunar resorption (Fig. 1). Osteoclasts are not commonly seen in normal adult bone but are often found at sites of osteolysis in diseases affecting bones and joints. Cellular and hormonal/humoral factors which influence the extent of pathological bone resorption act by regulating the activity and number (i.e. formation and survival) of osteoclasts.

Osteoclast origin and cell lineage

It is now well-established that osteoclasts are of haematopoietic rather than mesenchymal bone stromal cell origin. Evidence to support this conclusion was initially provided by experiments employing parabiosis or marrow transplants (Walker, 1973, 1975). Further studies showed that osteoclast precursors are derived from the pluripotential haematopoietic stem cell from which other leucocytes are derived and that the osteoclast lineage shares marrow precursors with monocytes and macrophages, including committed precursors (CFU-GM) for cells of this lineage (Kurihara et al., 1990; Alvarez et al., 1991). More recently,

transgenic and stem cell differentiation studies have provided unequivocal evidence as to the haematopoietic nature of osteoclasts, and conversely have shown that the chondrocytes and osteoblasts arise from mesenchymal stem cells (Tondravi et al., 1997; Pittenger et al., 1999). The human mononuclear osteoclast precursor circulates in the monocyte fraction of peripheral blood. It expresses the monocyte/macrophage integrins CD11b-c and the lipopolysaccharide receptor antigen CD14 (Athanasou and Quinn, 1990; Fujikawa et al., 1996), and is entirely negative for phenotypic markers of the mature osteoclast, such as tartrate-resistant acid phosphatase (TRAP), vitronectin receptor (VNR, the α_v ß3 integrin), calcitonin receptors and bone resorption (Ross et al., 1993, Roux et al., 1997). Although these precursors do not represent a distinct subpopulation in terms of expression of mononuclear phagocyte markers, only a minority of circulating mononuclear cells (approximately 2-5%) within the human monocyte fraction appear to be capable of osteoclast differentiation (Fujikawa et al., 1996). Rapid and dramatic changes in the phenotype of these mononuclear precursor cells occur in the process of osteoclast differentiation; this involves stepwise loss and acquisition of specific phenotypic markers for macrophages and osteoclast respectively (Takahashi et al., 1994; Faust et al., 1999).

Identification of molecular factors mediating osteoclast differentiation

In vitro systems of osteoclast formation from marrow and circulating mononuclear precursors established the necessity for the presence of macrophage-colony stimulating factor (M-CSF) and contact with an osteoblast/bone stromal cell population (Jimi et al., 1996). M-CSF, which is produced at varying levels by many mesenchymal cells including osteoblasts, is an absolute requirement for the proliferation and differentiation of osteoclast progenitors (Tanaka et al., 1993). M-CSF acts though c-fms, the M-CSF receptor, which has been demonstrated to be essential for normal osteoclast differentiation (Yoshida et al., 1990). Fms is a transmembrane tyrosine kinase-receptor; it belongs to a large superfamily that includes the insulin receptor. Fms is an integral membrane component in monocyte/macrophage family cells. Its second messengers include the intracellular tyrosine kinase src, which is also required for normal osteoclast differentiation (Soriano et al., 1991). As direct cell-cell interaction between mononuclear phagocyte progenitors and bone stromal cells appeared to be essential for osteoclastogenesis in vitro (Fujikawa et al., 1996; Jimi et al., 1996), it was postulated that a membrane-associated factor on bone stromal cells is essential for osteoclast differentiation. Direct attempts to isolate this osteoclast activating factor produced many candidate cytokines but were unsuccessful. However, a protein that did the reverse, blocking osteoclast development in vitro and in vivo, was discovered by two groups in 1997. This osteoclastogenesis inhibitory factor, osteoprotegerin (OPG) turned out to be a new dimeric ~50 kD tumour necrosis factor (TNF) decoy receptor (Simonet et al., 1997; Tsuda et al., 1997; Yasuda et al., 1998). Several such secreted TNF-binding proteins are known (the decoy receptors DcR1/TRID, DcR2/TRUNDD and DcR3, as well as soluble alternative transcripts of Fas and DR3/TNFRSF25). OPG-deficient mice exhibit osteoporosis; transgenic mice overexpressing OPG develop osteopetrosis (Simonet et al., 1997; Mizuno et al., 1998; Lacey et al., 1998). Expression cloning to find the proteins that bind OPG identified a previously characterized TNF-family protein, TRANCE (TNF-related activation-inducing cytokine), which had been shown to activate the TNF-family receptor RANK (receptor activator of nuclear factor-κB) a T-lymphocyte product which is a survival factor for dendritic cells (Anderson et al., 1997; Wong et al., 1997). This TNF-family ligand is now generally named after its receptor, RANK-ligand or RANKL.

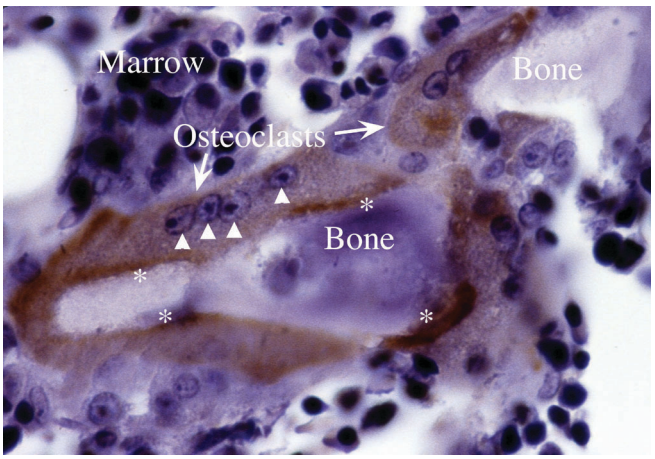

Fig. 1. Osteoclasts in medullary trabecular bone. This section, showing abundant osteoclasts in a calcium-starved laying hen, is stained with hematoxylin to show cells and nuclei (blue), and is labelled with antibody to the vacuolar H^+-ATPase (brown) which is expressed prominently at the osteoclast-bone interface (asterisks) (Blair et al., 1989). Osteoclasts are multinucleated (arrowheads). This is a frozen section; osteoclasts are degradative cells with abundant proteinases; in bone fixed by conventional means osteoclasts are often autolysed. The field shown is 125 μm wide and was photographed using 40x objective lens.

RANKL exists in cell membrane-bound and soluble forms but is mainly a membrane-bound protein in vivo (Hofbauer et al., 2000). As with any TNF, however, its receptor-binding domain alone is active. TNFs generally function as trimers or larger complexes, which cause the association in the target membrane of multiple activated receptor subunits. In the presence of M-CSF, RANKL induces osteoclast formation from spleen cells and monocyte/macrophage cell populations in the absence of bone stromal cells (Kong et al., 1999). This generally requires serum-containing medium and can be finicky, varying between cell preparations and serum lots; this

has led many investigators to believe that additional uncharacterized growth factors or cell-cell interactions may be involved in this process. Nevertheless, RANKL knockout mice exhibit osteopetrosis and defects in tooth eruption that are associated with an absence/deficiency of osteoclasts (Kong et al., 1996). These findings indicate that RANKL is a key stromal cell factor required for osteoclast formation during normal bone turnover in mice.

RANKL mRNA is most abundant in skeletal tissues and lymphoid tissues that are active in mediating an immune response (e.g. lymph node, thymus, spleen, Peyer's patches) (Kong et al., 1996). RANKL is expressed by hypertrophic chondrocytes and RANKL expression is most abundant in the growth plate and the metaphyseal periosteum in adult mouse bone (Akiyama et al., 1999; Kartsogiannis et al., 1999). RANKL is also expressed by endothelial cells and some fibroblasts and it is found at lower levels in other tissues such as heart, skeletal muscle, and lung (Kartsogiannis et al., 1999). Animals injected with RANKL only form osteoclasts in bone and not in other tissues. This observation indicates that there are other specific factors present in the bone microenvironment which are required for the differentiation of osteoclasts from precursor cells.

In contrast to RANKL, OPG is expressed in high concentrations in a variety of tissues including endothelial cells, fibroblasts, smooth muscle cells, monocyte, dendritic cells, B lymphocytes and cancer cells (Kartsogiannis et al., 1999). OPG is mainly produced by cells of the osteoblast lineage in bone. It inhibits the differentiation, survival and fusion of osteoclast precursors, induces osteoclast apoptosis and inhibits mature osteoclast activity. Decoy receptors such as OPG typically have relatively broad binding specificity, and OPG is known to bind at least one other TNF-family protein, the TNF-related apoptosis inducing ligand (TRAIL) (Emery et al., 1998).

RANKL-RANK signalling

RANKL, via its receptor RANK, activates nuclear factor-κB (NF-κB). Activation of NF-κB is not a unique property of RANK. It is shared by a number of other receptors including those of TNF. Further, RANK activates other intermediate signals including TNF-receptor-associated factors (TRAFs), 2, 5 and 6 (Kim et al., 1999; Wong et al., 1999). TRAFs are signalling molecules that act as second messengers to a variety of IL-1/Toll receptors as well as the TNF family (Chung et al., 2002). TRAFs 2,5, and 6 are not specific to RANK. TRAF 6 is a key IL-1 intermediate signal. From these pathways, a basis for reinforcement of RANKL signalling or alternative osteoclast differentiation entirely by other TNFs and IL-1 can be inferred, and indeed this idea is supported by recent studies (vide infra). Additional regulatory pathways activated by RANK include the c-Jun N-terminal kinase (Kim et al., 1999), which is downstream of a variety of cytokines, and src (Wong et al., 1999), which mediates signals of tyrosine kinase receptors.

The NF-κB signal also interacts with DNA-transcription-cofactor Smad proteins (Lopez-Rovira et al., 2000). These interactions may be important in the co-operative effect of 1,25 dihydroxyvitamin D-3 [1,25$(OH)_2D_3$] and transforming growth factor ß (TGFß) in osteoclast differentiation (Yanagisawa et al., 1999). Smad genes were identified as homologues of the Drosophila mad (mothers against decapentaplegic) and *Caenorhabditis elegans* sma genes (Liu et al., 1996). Smads regulate a variety of transcription factors and only some of them (R-Smads) interact with receptors, of which two principal subtypes mediate effects of TGFß–superfamilies in the broad classes of TGFs/activins (AR-Smads 2 and 3) and BMPs/GDFs (BR-Smads 1, 5, and 8) (Miyazono et al., 2001). Smad activity is regulated by kinases, including the serine/threonine kinase activity of the TGFß–superfamily type I and II receptors themselves. Smad3, an AR-smad, is a cofactor for the vitamin D receptor (Yanagisawa et al., 1999), TGFß (Yanagisawa et al., 1999), and NF-κB (Lopez-Rovira et al., 2000).

The core NF-κB signalling pathway has been extensively studied. In the cytoplasm, NF-κB is present as an inactive complex with inhibitor kB (IkB). IL-1, TNFα and RANK activate kinases that phosphorylate IkB, leading to ubiquitin-related degradation of the inhibitory complex which allows nuclear localization of NF-κB (Mercurio and Manning, 1999). Acting via TRAF-6, IL-1 activates pathways replacing the late stages of RANK signalling (Lomag et al., 1999). The TRAF pathways are also activated by TNFα (Kobayashi et al., 2000). This convergence on NF-κB emphasizes its role in osteoclast differentiation. There are two forms of NF-κB that have redundant functions. When both are eliminated, a defect in osteoclast differentiation and osteopetrosis occurs (Iotsova et al., 1997).

Regulation of RANKL-induced osteoclast formation by calciotropic hormones and cytokines

Experiments *in vitro*, using a variety of culture systems, have shown that osteoblast and bone stromal cell RANKL expression is up-regulated, and OPG expression downregulated by calciotropic hormones and bone cytokines that stimulate bone degradation, including PTH, 1,25$(OH)_2D_3$, glucocorticoids, IL-1, IL-11, TNFα and prostaglandin-E_2 (Hofbauer et al., 1999; Kitazawa et al., 1999; Nakashima et al., 2000; Chung et al., 2001; Horwood et al., 2001; Koseki et al., 2002; Li et al., 2002). Some growth factors that affect monocyte-lineage cells as well as mesenchymal cells, such as TGFß, down-regulate RANKL expression in osteoblasts (Quinnet al., 2001), but increase osteoclast differentiation in cultures of haematopoietic cells incubated with RANKL and M-CSF (Koseki et al., 2002); this may in part be due to TGFß stimulation of osteoblast OPG expression and pre-osteoclast RANK

expression (Thirunavukkarasu et al., 2001). Stimuli that promote osteoclast formation typically decrease OPG expression in osteoblast lineage cells (Murakami et al., 1998). Estradiol is an important case where many complex observations have been reported and a consistent mechanism is not clear; for a summary see (Cao et al., 2003). Consensus in vitro observations on RANKL/OPG and some *in vivo* correlative data on osteoclast formation and activity are summarized in Table 1.

The cytokines other than RANKL that affect osteoclast differentiation are probably of importance in pathological conditions where large amounts of these factors are produced. IL-1, IL–6 and TNFα can be made by macrophages as well as mesenchymal cells with a variety of stimuli. They are produced by mesenchymal cells in postmenopausal osteoporosis (Marie et al., 1993), and by synovial fibroblasts and macrophages in rheumatoid arthritis (Ridderstad et al., 1991). IL-1 and IL-6 are coordinated with RANKL or TNFα expression in stromal cells (Atkins et al., 2000), and are stimulated by PTH (Onyia et al., 1997). IL-1, IL-6 and TNFα may be autocrine or paracrine stimuli (Tani-Ishii et al., 1999), and their signals are also regulated by secondary factors. IL-6 induces osteoclast differentiation via gp130 (Suda et al., 1995). The IL-1 decoy receptor, IL-1RII, can limit expression of pro-apoptotic TNF family proteins by monocyte family cells that are involved in arthritic destruction of joints (Bessis et al., 2000). Other important cytokines in inflammation and repair include TGFß and prostaglandin E_2. TGFß is typically identified with macrophages and is prominent in inflammatory tissues (Cutolo et al., 1993), and prostaglandin E2 appears to be produced by osteoblasts and to play a major role in the repair of bone (Zhang et al., 2002). There has been considerable interest recently in T-cell cytokines such as IL-7 and IL-17 (Lubberts et al., 2003; Toraldo et al., 2003), although the role of T-cells in bone loss is still not well characterized.

Aside from the RANKL pathway, the M-CSF/fms receptor pathway also has alternative stimuli. At least two tyrosine kinase receptors related to Fms are expressed in bone. Met and Kit are tyrosine kinase receptors related to Fms, which are expressed in osteoclasts and precursors (Gattei et al., 1996; Grano et al., 1996). The ligands for these receptors, scf and hgf, are expressed by osteoblasts, (Grano et al., 1996; Blair et al., 1999). Their physiological functions are uncertain. However, the intracellular signals stimulated by Met, Kit, and Fms have prominent common effects mediated by src and cbl, suggesting that Met and Kit may modify or augment the osteoclast-differentiation function of Fms under some circumstances.

RANKL–induced osteoclast formation and the role of cytokines in pathological bone resorption

Although it is probable that changes in RANKL expression or RANK signalling occur in clinical bone diseases characterised by pathological bone resorption, it should be noted that as yet such links are theoretical and not proven. Despite the transgenic mouse data on osteopetrosis and RANKL, there have not been clinical reports linking RANKL defects to osteopetrosis.

Table 1. Regulation of expression of RANKL relative to OPG by hormones and cytokines. Osteoblasts and mesenchymal cells are major RANKL-expressing cells in normal bone turnover. In rheumatoid arthritis, the lymphoid inflammatory infiltrate may also be a major producer of osteoclast-inducing RANKL. For brevity, references list only first author and year; see references for complete authors.

CYTOKINE OR HORMONE	RATIO OF RANKL TO OPG	COMMENT	KEY REFERENCE
$1,25(OH)_2D_3$	Increase	In vivo results correlate poorly	Koseki, 2002; Horwood, 2001; Kitazawa, 1999; Murakami, 1998; Suda 2003
Estradiol	Variable	Sex and receptor dependent; also affects RANK signalling	Cao 2003, Saika 2001, Lindberg 2001, Srivasta 2001
Glucocorticoids	Increase		Kitazawa 1999; Chung, 2001;
PTH	Increase		Koseki et al., 2002; Horwood, 2001; Murakami, 1998;
IL-1	Increase		Nakaxhima, 2000; Hofbauer, 1999; Murakami, 1998
IL-6	None	Affects osteoclast formation via macrophage receptors	Nakaxhima, 2000; Hofbauer, 1999
L-7	Increase	Increases RANKL in T-cells	Toraldo, 2003
IL-11	Increase		Horwood, 2001; Nakaxhima 2000
IL-17	Increase	T-cell derived cytokine active ininduced murine arthritis	Lubberts, 2003
PGE2	Increase		Koseki et al., 2002, Li, 2002: Murakami, 1998
TGFß	Decrease	In macrophages, stimulates osteoclast formation	Koseki et al., 2002, Quinn 2001; Thirunavukkarasu, 2001
TNFα	Increase	Affects macrophage response as well as osteoblasts	Nakaxhima, 2000; Hofbauer 1999

RANKL polymorphisms have not been identified in Paget's disease (Wuyts et al., 2001), but mutations in the RANK and OPG genes have been noted in familial expansile osteolysis and hyperostosis corticalis deformans juvenilis ("juvenile Paget's disease") (Hughes et al., 2000; Whyte et al., 2002). One possibility is that in humans, the RANK system is so critical that only mild defects, which may not be characterized for some time, are survivable.

Changes in OPG, which is a circulating decoy receptor that regulates the activity of TNF family proteins, have been implicated in bone disease. Glucocorticoid therapy reduces circulating OPG (Sasaki et al., 2001) and may contribute to secondary osteoporosis in this way. OPG may be important in the physiological response to bone loss, since the level of OPG is increased in postmenopausal women (Yano et al., 1999). Recombinant OPG has been proposed as a therapeutic agent for osteoporosis and related disorders, but its utility is still under study. There is also interest in OPG polymorphisms as possible causes of sporadic osteoporosis and other disorders (Langdahl et al., 2002).

Bone loss in arthritis appears to be related to TNFα expression, and TNFα has been implicated in alternative mechanisms of osteoclast differentiation (Kudo et al., 2002). This topic is of some importance as it has been shown that TNFα–blocking therapy produces sustained improvement in rheumatoid arthritis (Taylor, 2003). There is also interest in the effect of OPG on bone loss in arthritis. OPG deficiency appears to be important in the progress of TNFα–mediated arthritis (Haynes et al., 2003). OPG directly ameliorates osteoclastic activity in animal models of arthritis, although the mechanism involved is unclear (Redlich et al., 2002; Romas et al., 2002). A direct clinical relationship linking TNFα therapy to normalization of serum OPG and RANK, however, has not been shown (Ziolkowska et al., 2002). Further, TNFα directly up-regulates OPG in human mesenchymal stem cells (Brandstrom et al., 2001), suggesting that TNFα effects on osteoclast formation are not due solely to RANKL activity. Elevated OPG has also been demonstrated in human rheumatoid arthritis (Feuerherm et al., 2001). These findings argue that TNFα–mediated augmentation of bone degradation may be important in vivo.

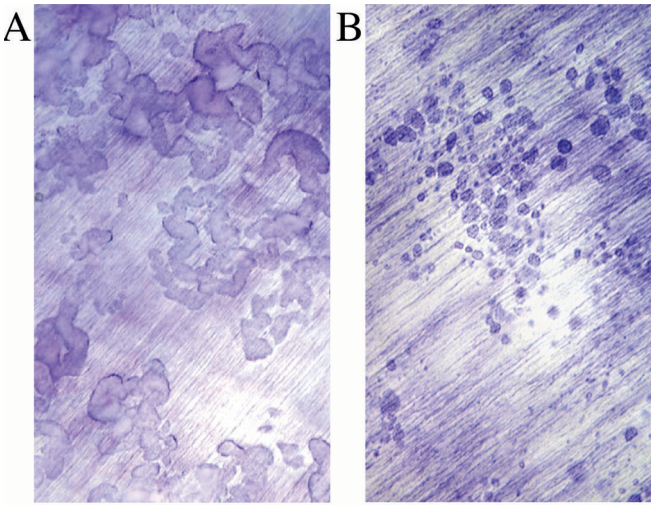

Fig. 2. Lacunar resorption by osteoclasts made with RANKL and M-CSF **(A)** and TNFα and M-CSF **(B)**. The resorption pits produced by cells cultured with TNFα are notably smaller than those produced by cells which differentiate in the presence of RANKL. Each photograph is ~200 μm wide and was photographed using a 10x objective lens.

Although pro-inflammatory cytokines influence osteoclast formation by modulating RANKL and OPG expression on osteoblasts and other cells, as discussed above, these cytokines also induce osteoclast formation by a RANKL-independent mechanism (Kudo et al., 2002, 2003; Sabokbar et al., 2003). Mouse marrow cultures and cultures of human monocytes incubated with TNFα induce osteoclast differentiation from mononuclear precursors; the addition of IL-1 markedly stimulates the resorption of the osteoclasts which are formed in this way (Kobayashi et al., 2000; Kudo et al., 2002). In man, this RANKL-independent mechanism results in the formation of numerous small osteoclasts that produce relatively small resorption pits (Kudo et al., 2002); this process of osteoclast formation is thus quantitatively and qualitatively distinct from RANKL-dependent osteoclast formation where large resorption pits are produced (Fig. 2). IL-6 and IL-11 have also been shown to promote human osteoclast formation in a similar manner (Kudo et al., 2003).

It has been reported that TNFα stimulates osteoclast differentiation from macrophages only in rodent cell cultures in which there are permissive concentrations of RANKL (Lam et al., 2000) and, on this basis, it has been suggested that TNFα alone does not induce osteoclast formation. There is disagreement as to whether basal levels of RANKL are required for osteoclast formation in all species. RANK-deficient mice fail to make a significant number of osteoclasts, arguing for an essential role for RANK signalling in normal bone modelling (Dougall et al., 1999). However, these animals also cannot form lymph nodes, and it is unclear how other cytokine signalling processes are affected. Further, RANK and RANKL deficient mice, though osteopetrotic, are viable, so minor bone degradation may occur in mice without RANK signalling. OPG abolishes bone resorption in TNF-mediated arthritis (Redlich et al., 2002), but OPG is not absolutely specific for RANKL, so this could reflect effects on other TNF-family proteins.

The current findings suggest that there are two pathways of osteoclast formation, one RANKL-induced, the other either completely or mainly RANKL-independent and cytokine-induced. These pathways may operate under different tissue conditions and are not mutually exclusive (Fig. 3). Whether the alternative RANKL-independent mechanism operates with cells 'primed' by minimal RANKL signals is probably not functionally important. Rather, what is important, is the

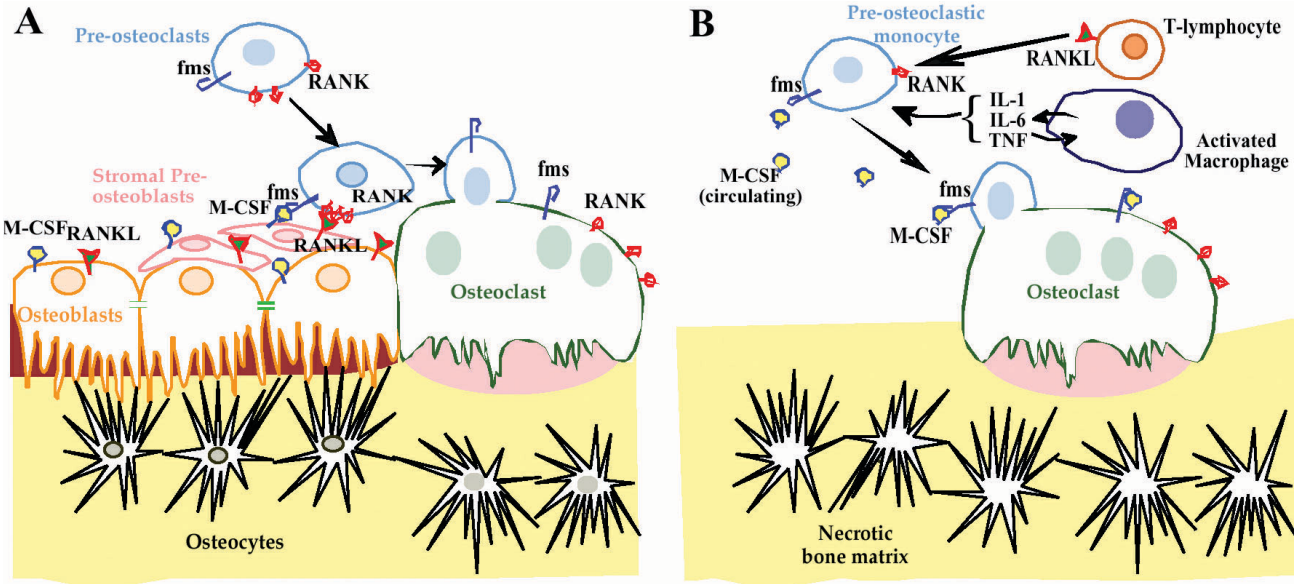

Fig. 3. Alternative pathways for osteoclast terminal differentiation. **A**. In normal bone turnover the macrophage-family signal M-CSF and the tumor necrosis factor-family signal RANKL are central to osteoclast formation, and the major sources are cell-surface presented growth and differentiation factors from mesenchymal cells including osteoblasts. However, circulating signals including 1,25 dihydroxyvitamin D3 and other factors not currently defined may be required, and this process is regulated by secondary hormones and cytokines (Table 1). **B.** In inflammatory and reparative situations, as in this osteoblast-denuded/ necrotic bone surface, normal sources of RANKL and M-CSF may be limited and secondary factors that stimulate the same pathways are of increased importance. These may include soluble growth factors produced by macrophages such as the tumor necrosis factor-family signal TNFα, transforming growth factor-ß, and interleukins 1 and 6; for clarity only some highly expressed cytokines are shown (see Table I1. RANKL is produced by T-lymphocytes that are present in inflammatory bone disease, so this signal may be present, albeit at reduced levels. However, in vitro osteoclast production can be accomplished without RANKL from CD 14 human blood monocytes.

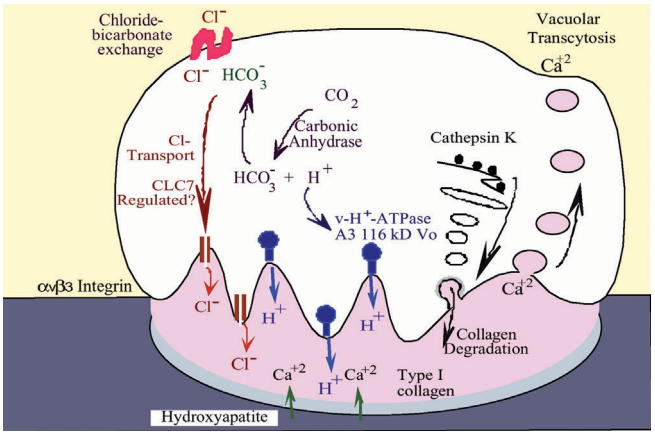

Fig. 4. Major biochemical and transport pathways in the osteoclast. The driving force is a vacuolar-type H^+-ATPase; this is electrically coupled to chloride transport. Chloride-bicarbonate exchange maintains intracellular pH neutrality. Other important features include a tight bone attachment, which is not well characterized but is known to depend on the vitronectin receptor, acid proteinase expression, and vacuolar transcytosis for removal of degraded bone products.

role of additional cytokines including TNFα in the local bone degradation in diseases such as rheumatoid arthritis. At sites of physiological (e.g. normal bone turnover) or pathological (e.g. osteoporosis) bone resorption, where few or no inflammatory cells are present and levels of inflammatory and reparative cytokines (Table 1) are low, RANKL-induced osteoclast formation would predominate. However, in inflammatory diseases of bone and joint, where numerous inflammatory cells and abundant cytokines are present, RANKL-independent osteoclast formation may predominate. It is interesting to note that glucocorticoids have opposing effects on these two mechanisms, promoting RANKL-induced but inhibiting TNF-induced osteoclast formation (Kudo et al., 2002). This is in keeping with the fact that systemic glucocorticoids induce osteoporosis yet control osteolysis that occurs secondary to inflammatory diseases such as rheumatoid arthritis (Neeck et al., 2002).

Osteoclast physiology and developmental defects

The basic mechanisms that mediate the removal of bone mineral by the osteoclast were defined by the 1990s (Fig. 4). However, the detailed structure of specific transport proteins is still an area of active investigation, and, as the mechanisms have been refined, this understanding has been of increasing practical value. An essential feature that differentiates the osteoclast from other macrophage polykaryons (giant cells) is massive expression of a cell-surface, vacuolar-like H^+-ATPase (Blair et al., 1989), which is required for the

energy-consuming step of the acid secretion that dissolves bone mineral. The protons derive indirectly from CO_2 in a process accelerated by carbonic anhydrase type II, absence of which leads to a type of osteopetrosis with variable severity and correlated renal and mental problems (Hunter et al., 1991; Alper, 2002). The v-H^+-ATPase is electrogenic so it must have an electrical balance, which is a Cl^- channel (Blair and Schlesinger, 1990; Blair et al., 1991), producing HCl. Cellular acid-base balance is maintained by a Cl^-/HCO^3-exchanger on the basolateral cell membrane (Teti et al., 1989). Osteoclasts secrete an acidic collagenase, cathepsin K, and a phosphatase active in acidic environments (tartrate-resistant acid phosphatase, or TRAP), which are required for efficient bone degradation under laboratory conditions (Tezuka et al., 1994; Hollberg et al., 2002). To date, TRAP is useful mainly as an osteoclast marker and deficiencies created in laboratory animals have no correlates in human disease (Hollberg et al., 2002). On the other hand, deficiency of cathepsin K is responsible for a sclerotic bone disorder, pycnodysostosis, also called Toulouse-Lautrec syndrome (Gelb et al., 1996). The osteoclast forms a tightly sealed bone attachment compartment that allows the extracellular acidification. This sealing process depends on the vitronectin receptor, the αvß3 integrin (Ross et al., 1993). Degraded calcium and collagen fragments are transported through the osteoclast by vacuolar transcytosis (Nesbitt and Horton, 1997; Salo et al., 1997).

Recently, important discoveries have been made in osteoclastic membrane transporter isotypes. The v-type H^+-ATPase is composed of membrane (V_0) and cytoplasmic (V_1) subassemblies; the V_0 comprises a 17 kDa hydrogen channel and a 116 kD protein with several transmembrane passes. The V1 assembly is a nano-motor with rotation coupled to ATP hydrolysis and acid transport. The membrane anchoring V_0 subunit, particularly its 116 kD component, appear to be crucial for membrane insertion and the elevated H^+-ATPase expression in the osteoclast. Four isoforms of the 116 kD V_0 component are known, with the third variant, TCIRG1 (A3) the (only) one whose expression is amplified in the osteoclast (Li et al., 1999). Thus, defects in this protein would be expected to be associated with osteoclastic acid pumping defects, and this has proved not only to be correct but to be a surprisingly common cause of osteopetrosis (Frattini et al., 2000; Michigami et al., 2002), as well as a possible cause of variability in bone density (Carn et al., 2002). Another acid secreting cell related to skeletal metabolism, the renal intercalated cell, also has a unique 116 kD V0 subunit, the fourth described, which is required for expression and insertion of the v-ATPase in this cell (Smith et al., 2000, 2001).

A 62 KDa Cl^- channel isolated from avian osteoclast ruffled border by affinity to the stilbene anion channel inhibitor DIDS was reconstituted chloride transport in planar lipid bilayers, and was characterized as a rectifying channel with 16.7 pS conductivity (Alper, 2002). This channel, called Clor.62 (Schlesinger et al., 1997), has SH2 and SH3 domains and co-precipitates src. Src antisense and Clor.62 antisense reduced the activity of acid secretion and degradation of bone, so this channel is thought to carry the bulk of the chloride current balancing the H^+-ATPase. There is, however, no correlation to human disease. The human homologue is intracellular chloride channel 5 (CLIC5), one of a group of chloride channels typically expressed intracellularly and believed to function in capacities such as acid secretion, but which as yet are poorly characterized. Transgenic animals and other molecular methods may be useful in characterizing the role of this transporter in future, but have not been reported to date. On the other hand, transgenic mice deficient in a chloride channel analogue, CLC-7 (Brandt and Jentsch, 1995; Kornak et al., 1999), have osteopetrosis (Cleiren et al., 2001; Kornak et al., 2001). Defects in CLC7 are associated with osteopetrosis in several families. Most defects in CLC7 appear to be silent unless homozygous. While CLC7 expression is essential, lack of channel activity in Xenopus (Brandt and Jentsch, 1995), except possibly at low pH that also activates endogenous Xenopus chloride channels (Diewald et al., 2002), suggest the possibility that this is a regulatory membrane protein rather than the actual chloride conductance, which the avian osteoclast work suggests is a member of the CLIC family, possibly CLIC5.

There are multiple additional genetic bases for osteoclastic dysfunction. Transgenic mice deficient in src have osteopetrosis, and signals including cbl downstream of src are important (Tanaka et al., 1996), although clear clinical correlates are not available, possibly because defects in these central tyrosine-kinase pathways are embryonic-lethals. Free-radical production defects, such as in macrophage and neutrophil activity disorders and chronic granulomatous diseases, often also cause mild osteopetrosis (Yang et al., 1999; Madyastha et al., 2000). This suggests that free-radical production is also important to bone degradation, although the mechanism is unclear. Not all osteoclast defects correlate with any known pathway, though, suggestion that further cell fusion, protein transport or precursor defects, with or without immune disease, probably exist.

One further pathway that deserves mention is that HCl secretion is critical to the function of bone-adsorbed antiresorptive agents. Bisphosphonates are antimetabolites that bind hydroxyapatite, and are almost totally bound to bone mineral under physiological conditions (Carano et al., 1990). Osteoclasts, by dissolving the hydroxyapatite with acid, cause release of the bone-bound antimetabolites, an autoinhibitory process that, because of its relation to the unique osteoclast physiology, has very few side effects.

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