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Invited Review

Human osteoclast ontogeny and pathological bone resorption

N.A. Athanasou[1,2] and A. Sabokbar[1]

[1]Nuffield Department of Orthopaedic Surgery and [2]Department of Pathology, Nuffield Orthopaedic Centre, Oxford, UK

Summary. Monocytes and macrophages are capable of degrading both the mineral and organic components of bone and are known to secrete local factors which stimulate host osteoclastic bone resorption. Recent studies have shown that monocytes and macrophages, including those isolated from neoplastic and inflammatory lesions, can also be induced to differentiate into cells that show all the cytochemical and functional characteristics of mature osteoclasts, including lacunar bone resorption. Monocyte/macrophage-osteoclast differentiation occurs in the presence of osteoblasts/bone stromal cells (which express osteoclast differentiation factor) and macrophage-colony stimulating factor and is inhibited by osteoprotegerin. Various systemic hormones and local factors (eg cytokines, growth factors, prostaglandins) modulate osteoclast formation by controlling these cellular and humoral elements. Various pathological lesions of bone and joint (eg carcinomatous metastases, arthritis, aseptic loosening) are associated with osteolysis. These lesions generally contain a chronic inflammatory infiltrate in which macrophages form a significant fraction. One cellular mechanism whereby pathological bone resorption may be effected is through generation of increased numbers of bone-resorbing osteoclasts from macrophages. Production of humoral factors which stimulate mononuclear phagocyte-osteoclast differentiation and osteoclast activity is also likely to influence the extent of pathological bone resorption.

Key words: Osteoclast, Macrophage, Bone Resorption, Mononuclear phagocytes

Introduction

Bone resorption occurs physiologically throughout life, being necessary for the growth of bone as well as bone remodelling, which occurs continuously throughout adult life. Pathological bone resorption results when this process does not accord with physiological demands; it may be generalised, involving the entire skeleton (eg hyperparathyroidism), or localised with the formation of one or more discrete osteolytic lesions (eg skeletal metastasis). Both physiological and pathological bone resorption is carried out largely, if not exclusively, by highly specialised, multinucleated cells called osteoclasts. An increase in osteolysis is thus usually associated with an increase either in the number or the activity of bone-resorbing osteoclasts.

This review will focus on the process of osteoclast ontogeny, in particular those studies which have analysed human osteoclast formation. These investigations have considerably clarified the nature of osteoclast precursors and the osteoclast lineage; they have also provided information on the cellular and humoral mechanisms involved in the increase in osteoclast numbers (and consequently osteolytic activity) found at sites of pathological bone resorption. This review is in three parts. First, the characteristic structure and function of osteoclasts, as well as a number of other defining features of these cells will be described; then, experimental work on osteoclast ontogeny and cellular and humoral influences on this process will be presented; finally, the manner in which pathological bone resorption in man results from disturbances in osteoclast formation will be discussed.

Osteoclast structure, function and phenotypic characteristics

Osteoclast structure

Morphologically, the osteoclast is a large multinucleated cell which is found on the surface of trabecular and cortical bone. Osteoclasts are uncommonly seen in normal mature bone but are prominent in areas of active bone resorption where one or more osteoclasts lie in depressions on the bone surface called resorption or Howship's lacunae (Ham and Cormack, 1979). The osteoclast has an abundant pale-staining cytoplasm which is strongly (tartrate-resistant)

Offprint requests to: Dr. N.A. Athanasou, Nuffield Department of Orthopaedic Surgery, Nuffield Orthopaedic Centre, Oxford OX3 7LD, UK. Fax: (01865) 227354

acid phosphatase positive. Osteoclasts measure up to 100 μm in diameter and contain a variable number of round or oval nuclei (2-100), having finely dispersed chromatin and one or two prominent nucleoli per nucleus (Gothlin and Ericsson, 1976; Ham and Cormack, 1979).

The cytoplasm of osteoclasts contains numerous mitochondria, which lie mainly between the nuclear zone and the cell border furthest away from the bone surface; they contain a well-developed Golgi apparatus, numerous lysosomes and vacuoles and a relatively small amount of rough endoplasmic reticulum (Gothlin and Ericsson, 1976; Pierce et al., 1991). The cell surface of the osteoclast which lies in contact with the bone matrix contains a ruffled border, a complex network of branching and anatomosing microvillous membrane folds which extend into the bone being resorbed, and a clear zone which is devoid of organelles but rich in cytoskeletal actin filaments. Resorption and degradation of the underlying bone matrix occurs by the release of proteolytic enzymes and hydrogen ions across the ruffled border. The matrix deep to the ruffled border shows a loss of mineral with numerous detached crystals. Beneath the ruffled border, within the osteoclast cytoplasm, there are numerous membrane-bound vesicles and vacuoles. Many single membrane-bound dense bodies in which acid phosphatase can be demonstrated are present in the cytoplasm. Mononuclear cells which contain similar morphological features have been found near osteoclasts in bone (Rifkin et al., 1980). These cells have been designated pre-osteoclasts. Both macrophages and macrophage polykaryons do not exhibit a ruffled border or clear zone (Sutton and Weiss, 1966).

Osteoclast-related enzymes

Tartrate-resistant acid phosphatase (TRAP) is expressed in high levels in the osteoclast and is present in lysosomes, Golgi apparatus, and extracellular channels of the ruffled border (Minkin, 1982; Andersson et al., 1992). Although TRAP is expressed in a variety of tissues (eg lung, gut and kidneys), bone appears to express the highest levels of TRAP in normal tissues. TRAP is not specific to osteoclasts as its presence has been demonstrated in macrophage polykaryons and multinucleated giant cells (Hattersley and Chambers, 1989). The precise role of TRAP is uncertain. It is important for bone resorption and development but does not appear to be essential for bone resorption as shown by the fact that TRAP gene knockout mice do not develop osteopetrosis (Roodman et al., 1996).

Immunohistochemical studies have shown that osteoclasts contain high levels of the enzyme carbonic anhydrase II (Gay and Mueller, 1974; Delaisse and Vaes, 1992). This enzyme appears to play an important role in bone resorption as antisense constructs to this enzyme can block bone resorption both in organ cultures and isolated osteoclast cultures (Laitala and Vaananen, 1994). During the process of bone resorption, osteoclasts are capable of degrading type I collagen by release of cysteine proteases, termed cathepsins (Delaisse and Vaes, 1992; Inaoka et al., 1995). The collagenolytic activity of cathepsins has been shown to be elevated in bone organ cultures. Studies using group-specific inhibitors of cysteine protease have markedly inhibited PTH-induced bone resorption. Five different types of cathepsin have now been identified (K, O, L, B, D). Cathepsins B and L have been shown to be elevated in rat osteoclasts and high levels of cathepsin K are present in osteoclasts isolated from osteoarthritic hips and from giant cell tumour of bone (Goto et al., 1994; Shi et al., 1995). Recent studies have shown that matrix metalloproteinase protein-9 (MMP-9) is produced by osteoclasts in human bone tissue (Okada et al., 1995). High levels are present in osteoclasts, suggesting an important role for this enzyme in the degradation of collagen that occurs in bone resorption. MMP-9 cleaves collagen types I, III, IV and V. It has been shown that MMP-9 is expressed only during human osteoclast differentiation even before the expression of such markers such as TRAP, VNR and CTR (Wucherpfennig et al., 1994).

Osteoclast antigenic phenotype

Like macrophages and macrophage polykaryons, human osteoclasts strongly express leukocyte common antigen (CD45) and several other macrophage-associated antigens (eg CD13, CD15, CD68 and CD54) (Athanasou et al., 1987; Athanasou and Quinn, 1990). Several monoclonal antibodies, directed against CD45 and CD68 that react with human osteoclasts (eg PD7/26, KP1) are particularly useful as they identify osteoclasts in decalcified sections (Athanasou et al., 1991a,b). Osteoclasts, however, do not express other macrophage-associated markers such as CD11/18, CD14 and HLA-DR (Athanasou and Quinn, 1990). Thus, the human osteoclast expresses a restricted range of macrophage markers, a feature which is useful in distinguishing osteoclasts from macrophage polykaryons. Human and other animal osteoclasts have also been shown to strongly express the alpha chain of the vitronectin receptor (CD51) (Horton et al., 1985; Horton and Chambers, 1986); this is an integrin molecule that is involved in both cell-cell and cell-matrix interactions. This antigen is, however, weakly expressed by macrophage polykaryons and other cells of the mononuclear phagocyte system and therefore cannot be regarded as entirely osteoclast-specific (Athanasou et al., 1990). Other antigens strongly expressed by human osteoclasts are those recognised by the monoclonal antibodies 121F (Oursler et al., 1985) and Kn22 (Kukita and Roodman, 1989); these markers, however, have not been as extensively investigated for their specificity and utility as osteoclast markers.

Osteoclast receptors

Osteoclasts express receptors for calcitonin (CT) and

possibly other systemic hormones. Osteoclastic activity is directly and specifically inhibited by CT (Chambers and Magnus, 1982). Autoradiographic demonstration of receptors that bind CT is often used to identify mammalian osteoclasts (Nicholson et al., 1986). It has been shown that CT receptor expression is exhibited by committed (post-mitotic) osteoclast precursors late in osteoclast differentiation (Takahashi et al., 1995). PTH receptors are mainly present on osteoblasts but have also recently been demonstrated on mammalian osteoclasts and their precursors (Teti et al., 1991; Agawarla and Gay, 1992); these appear to mediate PTH-stimulated proliferation of osteoclast precursors. In coculture studies of osteoblasts with haematopoietic cells, it has been shown that osteoclast precursors may respond directly to PTH, but mature osteoclasts, even though they may express PTH receptors, do not appear to respond directly to this hormone. Whether oestrogen receptors are present on osteoclasts is controversial but in general these have not been identified by most observers (Roodman, 1996).

In contrast to osteoblasts, human osteoclasts express high levels of pp60c-src, a non receptor-related tyrosine kinase (Horne et al., 1992). It has been shown that high levels of this protein are localised in the ruffled border of resorbing osteoclasts (Tanaka et al., 1992). Since blocking pp60c-src activity inhibits bone resorption (Soriano et al., 1991), the expression of this kinase is now known to be related to the bone resorbing activity of the osteoclasts. Mutant mice having disrupted proto-oncogene c-src, are known to develop osteopetrosis; multinucleated osteoclasts are formed but they lack ruffled borders. Using specific inhibitors of pp60c-src, *in vitro* osteoclastic bone resorption and *in vivo* IL-1 induced hypercalcaemia was blocked, thus suggesting that pp60c-src plays an important role in regulating osteoclast activity.

Integrins are a family of cell surface glycoproteins that promote cell adhesion (Horton, 1985). Mammalian osteoclasts express three integrin receptors: αvß3, α2ß1 and αvß1. αvß3, the vitronectin receptor (VNR), is the major integrin expressed by osteoclasts and is a useful osteoclast marker (see above). Extensive characterisation of the VNR has revealed a role for this receptor in osteoclast adhesion as well as bone resorption. It is known that VNR recognises bone matrix proteins, including bone sialoproteins, in an RGD-dependent manner. Moreover, interference with integrin function, eg by adding anti-receptor antibodies or RGD-peptides, can result in blocking of bone resorption. The presence of receptors for M-CSF, IL-6 and IL-11 on mammalian osteoclasts has also been reported (Roodman, 1996).

Osteoclast functional characteristics

Time-lapse cinematographic studies of osteoclasts in tissue culture have shown them to be highly motile cells that participate actively in bone resorption (Goldhaber, 1960). The cell border of the osteoclast is vigorously active and restless (Chambers and Magnus, 1982). Calcitonin and other inhibitors of bone resorption cause a cessation of osteoclast motility and induce a characteristic shrinking and retraction of cytoplasmic processes. By definition, osteoclasts are bone resorbing cells and the most specific defining criterion for identifying a cell as an osteoclast is demonstration of the unique ability of this cell to form resorption lacunae in bone (Boyde et al., 1984; Chambers et al., 1984). This is best demonstrated by scanning electron microscopy whereby osteoclasts cultured on a mineralised substrate such as bone or dentine can be seen to have formed lacunar resorption pits. Fully mature osteoclasts begin resorption of a mineralised substrate almost directly after they have been isolated from bone. Other cell types such as monocytes, macrophages and tumour cells have been shown to be capable of degradation of the inorganic and organic bone matrix components, but they have not been shown to be capable of lacunar bone resorption (Chambers and Horton, 1984).

A number of distinct osteoclast characteristics are listed in Table 1. They are particularly useful in distinguishing osteoclasts from morphologically similar macrophage polykaryons and related giant cells.

Osteoclast ontogeny and cellular and humoral factors influencing osteoclast formation

Studies on osteoclast ontogeny

Much recent research has been directed towards defining the ontogeny of the osteoclast and the factors associated with the origin, recruitment and formation of these multinucleated cells. Osteoclasts form by fusion of their mononuclear precursors. This process is subject to

Table 1. Defining criteria of osteoclasts and their presumed precursors.

Morphological
Light microscopy
Multinuclearity
Location at sites of bone resorption
Transmission electron microscopy
Ruffled borders (osteoclast-specific)
Clear zones
Enzyme histochemistry
Expression of tartrate-resistant acid phosphatase
Expression of tartrate-resistant trinucleotide phosphatase
Expression of type-II carbonic anhydrase isoenzyme
Expression of matrix metalloproteinase-9
Immunohistochemistry/immunology
Expression of restricted range of leukocyte or macrophage-associated antigens (CD13, CD15, CD45, CD54, CD68, and CD51 [vitronectin receptor])
No expression of CD11/18, CD14, HLA-DR, Fc and C_{3b} receptor
Functional behaviour
Ability to form resorption lacunae on a bone substrate (osteoclast specific)
Response to calcitonin
Ability to bind calcitonin (possess calcitonin receptors)
High levels of pp60c-src

hormonal control and occurs rapidly. Originally, osteoclasts and osteoblasts were thought to arise from a single precursor cell (Tonna and Cronkite, 1961), but it is now generally accepted that osteoclasts are formed by fusion of circulating mononuclear precursor cells which are themselves of haematopoietic origin. Parabiosis experiments between irradiated and normal animals showed that the cells that fuse to form osteoclasts in a healing fracture in the irradiated rat must have been derived from the non-irradiated rat through the circulation (Gothlin and Ericsson, 1973). Experiments with quail-chick chimaeric bone grafts (Jotereau and LeDouarin, 1978) and mouse models of the disease osteopetrosis (Walker, 1973) also showed that osteoclasts form by fusion of marrow-derived cells which are present in the circulation.

Although the haemopoietic origin of the osteoclast is now well-recognised, the precise identity and nature of the mononuclear precursor cells which fuse to form these cells has for many years been controversial. Several models of osteoclast formation have been proposed. In the first, osteoclasts were thought to derive from a specialised line of osteoclast progenitors in haematopoietic tissues; these progenitors were thought to originate either from a unique primordial (osteoclast-specific) stem cell or an early osteoclast-specific derivative of the pluripotential haematopoietic stem cell (Chambers, 1985). However, a unique stem cell for osteoclasts has not been identified. This model was originally based on several non-specific criteria for defining osteoclast precursors. The finding of leukocyte common antigen, which is expressed by all nucleated progeny of the pluripotential haematopoietic stem cell, on osteoclasts also argued strongly against human osteoclasts (and their circulating precursors) being derived from a stem cell that is distinct from that which gives rise to other peripheral blood leukocytes (Athanasou et al., 1987).

In the second model, osteoclasts are thought to share a common marrow progenitor with mononuclear phagocytes such as the colony-forming unit for granulocytes and macrophages (CFU-GM) but subsequently to form their own osteoclast-specific cell line in the circulation and in bone (Burger et al., 1982; Thesingh, 1986). This hypothesis was promoted by the results of long-term avian and mammalian haematopoietic (bone-marrow and spleen) culture systems (Takahashi et al., 1988a; Alvarez et al., 1990). In general, all these systems require the addition of 1,25 dihydroxy-vitamin D_3 [1,25 $(OH)_2D_3$] for the formation of TRAP-positive multinucleated cells, some of which have been shown to be capable of bone resorption. Takahashi et al. (1988b), using long-term murine marrow and spleen culture systems, showed that osteoblastic or other specific bone-derived stromal cells are an absolute requirement for the differentiation of osteoclast precursors of haematopoietic origin into functional osteoclasts. These culture systems have also shown that a number of factors (eg cytokines and growth factors) influence osteoclast formation from precursors in haemopoietic tissues (Suda et al., 1995). In particular, it has been shown that macrophage-colony stimulating factor (M-CSF) is required for both the proliferation and differentiation of osteoclast precursors (see below) (Tanaka et al., 1993). However, these studies on osteoclast formation from marrow precursors provided relatively little information on the nature of circulating osteoclast precursors or the manner in which these cells develop into osteoclasts.

In the third model it is proposed that osteoclasts are formed directly by fusion of mononuclear progenitors present in the blood monocyte fraction. A number of observations related to unusual giant cell lesions of extra-skeletal tissues, eg breast carcinoma with stromal giant cells, giant tumour of tendon sheath, showed that this might be possible (Athanasou et al., 1989, 1991a,b). In these studies, osteoclast-like giant cells which appeared to exhibit the ultrastructural features and antigenic phenotype of macrophage polykaryons were found to be capable of lacunar bone resorption in a manner identical to that of osteoclasts. It was subsequently shown by Udagawa et al. (1990) that murine monocyte and alveolar macrophage populations contain osteoclast precursors which form osteoclasts when these cells are cocultured with the pre-adipocytic bone stromal cell line, ST2 in the presence of $1,25(OH)_2D_3$ and glucocorticoids. Monocyte-depleted cell populations of peripheral blood failed to form colonies on ST2 cell layers, whereas the monocyte-enriched fraction formed several colonies of mononuclear and multinucleated cells positive for TRAP. These findings were later confirmed using murine monocytes and osteoblast-like cells, where it was also found that, when as few as 100 peripheral blood mononuclear cells were cocultured with osteoblasts, then osteoclast differentiation still occurred (Quinn et al., 1994). This indicated that the proportion of cells in the monocyte fraction capable of osteoclastic differentiation was surprisingly high and argued strongly against such cells being a population of specialised predetermined circulating osteoclast precursors.

It has since been established that the human osteoclast precursor circulates in the monocyte fraction (Fujikawa et al., 1996a,b). Formation of multinucleated cells exhibiting the osteoclast phenotypic characteristics of TRAP, VNR, CTR and the ability to form resorption lacunae is seen when cells of the monocyte fraction are cultured in contact with specific bone-derived stromal cells in the presence of $1,25(OH)_2D_3$ and M-CSF. Osteoclast formation from human monocytes requires human M-CSF to be added as rodent M-CSF, which would be secreted by supporting bone stromal cells, does not bind to the human M-CSF receptor. The importance of M-CSF in human osteoclastogenesis has been further confirmed by Sarma and Flanagan (1996) who used long term human bone marrow cultures to show that M-CSF induced a substantial increase in osteoclast formation and bone resorption above that induced by $1,25(OH)_2D_3$ alone.

Fujikawa et al. (1996a,b) also showed that circulating mononuclear osteoclast precursors express the phenotypic characteristics of monocytes and not osteoclasts ie they are CD11/18 and CD14 positive and do not exhibit TRAP, VNR and CTR expression or possess the ability to carry out lacunar bone resorption. This has recently been confirmed by the work of Massey et al. (1998) who showed that the circulating peripheral blood mononuclear cells from which osteoclasts form are CD14 positive rather than CD14 negative. Thus, by all current criteria, osteoclast precursors do not appear to represent a distinct population of circulating cells and should be regarded as mononuclear phagocytes that express monocyte/macrophage markers. However, it should be appreciated that only a minority of mononuclear cells (approximately 2-5%) within the human monocyte fraction appear to be capable of osteoclast differentiation and that *in vitro* osteoclast phenotypic characteristics are only acquired after monocytes or macrophages have been cultured (in the presence of $1,25(OH)_2D_3$ and M-CSF) in contact with bone-derived stromal cells. This suggests that the mononuclear phagocyte osteoclast precursors in these cell populations undergo osteoclast differentiation in bone itself, where all these cellular and humoral elements would be present in the bone microenvironment (Fig. 1).

A number of recent studies have suggested that the above cellular and humoral requirements for osteoclast formation described above are not strictly required under specific conditions. Matayoshi et al. (1996) showed that the presence of bone marrow stromal cells is not essential for osteoclastogenesis. They mobilised CD34+ cells into the circulation by treating healthy donors with G-CSF. Using magnetic-activated cell sorting, they obtained a purified CD34+ population which did not contain any stromal cells (as assessed by lack of the stromal-cell marker; Stro-1) and showed that, following the addition of a mixture of IL-1, IL-3 and GM-CSF to this CD34+/Stro-1 negative population, osteoclastic bone resorbing cells were generated. Their findings thus suggested that osteoclasts can originate from uncommitted haematopoietic stem cells which are mobilised into the peripheral circulation by G-CSF, and that osteoclast formation may occur in the absence of specific pro-osteoclastogenic stromal cells. More recently Higuchi et al. (1998) showed that treatment of human peripheral blood mononuclear cells with antifusion regulatory protein-1 (CD98) results in the generation of multinucleated cells which are TRAP and CT receptor positive and capable of bone resorption. These studies are important in identifying factors which are likely to play a major role in osteoclast formation. They also indicate that there may be more than one cellular mechanism associated with osteoclast formation.

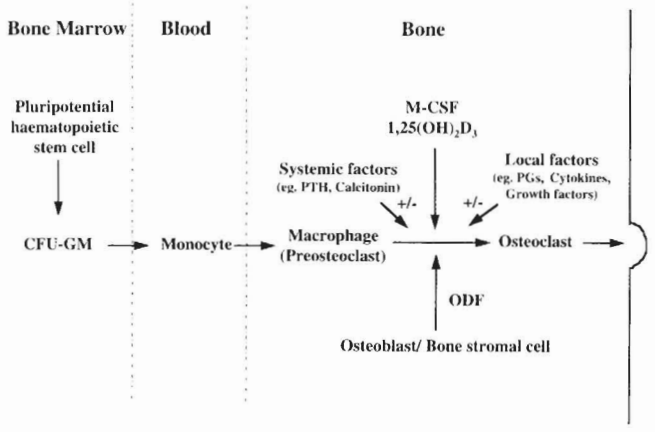

Fig. 1. The cell lineage of human osteoclasts.

Bone stromal cells and osteoclast formation

It is notable that *in vitro* experiments using cocultures of bone-derived stromal cells and osteoclast precursors of haematopoietic or of monocyte or macrophage origin, lacunar bone resorbing cells form only after several days of incubation. This process appears to involve a number of steps associated with the proliferation and differentiation of mononuclear osteoclast precursors into mature functional osteoclasts; this is associated with the loss and acquisition of specific phenotypic markers for macrophages and osteoclasts respectively (Takahashi et al., 1994). It has been shown that mononuclear osteoclast precursors primarily proliferate during the first four days of culture before maturing into osteoclastic cells (Tanaka et al., 1993). This process is associated with rapid and dramatic changes in the phenotype of these cells in culture. It has been shown that mononuclear preosteoclasts which express the macrophage marker, non-specific esterase, are present at sites of bone resorption before mononuclear cells that contain both TRAP and non-specific esterase (Baron et al., 1986). This again suggests that mononuclear osteoclast precursor cells that fuse to form multinucleated osteoclasts are cells of the monocyte-macrophage lineage. Such mononuclear phagocytes are not unique to bone as shown by the fact that macrophages isolated from extraskeletal tissues can also be induced to form osteoclasts (Udagawa et al., 1990; Quinn et al., 1996).

A feature of most systems of osteoclast formation from marrow or circulating mononuclear precursors is the necessity for the presence of osteoblasts or a specific bone stromal cell population. The manner in which osteoblasts/stromal cells transduce signals that induce osteoclastogenesis has recently been discovered. A cytokine, termed osteoclastogenesis inhibitory factor (OCIF) molecular weight of 60kD as a monomer was identified in the conditioned medium of human embryonic lung fibroblasts (Tsuda et al., 1997). This cytokine inhibited osteoclastogenesis stimulated by $1,25(OH)_2D_3$, PTH and interleukin 11 in a dose-dependent manner but did not inhibit bone resorption by mature osteoclasts. This molecule was found to be

identical to osteoprotegerin (OPG), a novel member of the TNF receptor superfamily (Simonet et al., 1997). OPG was known to block ovariectomy-associated bone loss in rats. Using an osteoblast/marrow stromal cell-spleen cell coculture system of osteoclastogenesis, a genetically engineered soluble form of the ligand for OPG/OCIF was then found to induce osteoclast formation from spleen cells in the absence of osteoblast/stromal cells (Lacey et al., 1998; Yasuda et al. 1998). The addition of OPG/OCIF abolished osteoclastic cell formation induced by this protein. Bone resorbing factors upregulated expression of the ligand for OPG/OCIF on osteoblast/stromal cells. This membrane-bound protein was termed osteoclast differentiation factor (ODF) and appears to be the much sought after ligand which mediates the essential signal which osteoblasts/bone stromal cells deliver to osteoclast progenitors. ODF was found to be identical to TRANCE/RANKL which enhances T-cell growth and dendritic cell function.

Molecular mechanisms involved in osteoclast formation

A number of other studies on genetic mutations in animals with osteopetrosis, a disease characterised inadequate osteoclastic bone resorption, have also provided insight into the molecular mechanisms of bone resorption. First, it was found that osteopetrotic *op/op* mutant mice do not express functional M-CSF receptors and hence lack the ability to form osteoclasts (Felix et al., 1990; Yoshida et al., 1990). Such osteopetrosis appears to be due to a defect in osteoblasts/marrow stromal cells as osteoblasts isolated from *op/op* mice cannot support osteoclastic differentiation *in vitro* unless M-CSF and $1,25(OH)_2D_3$ are added. The impairment in osteoblasts was suggested on the basis that $1,25(OH)_2D_3$-stimulated osteblasts. Osteopetrotic toothless rats failed to activate osteoclasts to resorb bone *in vitro* cocultures; defects in the osteoblast signalling pathway have been implictaed in such impairment (Odgren et al., 1997). Studies on mice lacking the *c-fos* proto-oncogene/transcription factor have shown that these mice develop osteopetrosis due to a lack of osteoclast development. Interestingly, lack of *c-fos* expression leads to an increase in the number of macrophages in the tissues of these mice, suggesting that *c-fos* is an important factor in the branching of the osteoclast and macrophage cell lineages (Grigoriadis et al., 1994). It also indicates that this proto-oncogene may promote differentiation of bipotential macrophage/osteoclast precursors into bone-resorbing polykaryons. More recently, it has been shown that antibodies to *c-fos* cause a significant inhibition of the proliferation of osteoclast progenitors but have no effect on macrophage differentiation. *c-fos* expression is upregulated in Pagetic osteoclasts and prolonged expression of *c-fos* can enhance osteoclast differentiation (Hoyland and Sharpe, 1994). The gene encoding *c-src* is also important in osteoclast formation and function. Osteoclast progenitor cells from *c-src* deficient mice do not form mature osteoclasts in coculture with osteoblasts. Osteoclasts express high levels of *c-src*; it is located on the ruffled border membrane and in cytoplasmic organelles (Horne et al., 1992)

Humoral factors influencing osteoclast formation

A number of systemic and locally acting factors are known to influence osteoclast formation and function. A number of these factors and their influence on osteoclast formation are discussed below. It should be noted, however, that the effect of various humoral factors on osteoclast formation and activity is often difficult to separate as is the relative importance of each of these factors on pathological bone resorption. A summary of the effects of a number of these factors on osteoclast formation is shown in Table 2.

M-CSF is one of a family of haematopoietic growth or colony factors (CSFs) that stimulate the clonal growth of haematopoietic progenitors *in vitro* and *in vivo*. It is produced by stromal cells, macrophages, endothelial cells and T-lymphocytes. As noted above, M-CSF is essential for the proliferation and differentiation of osteoclast progenitors. M-CSF acts through *c-fms*, the M-CSF receptor, which has been demonstrated on osteoclasts, and its signal transduction is mediated by a tyrosine kinase (Weir et al., 1993; Suda et al., 1997). M-CSF may also be a mediator of PTH-stimulation of osteoclastic bone resorption by osteoblasts. Other CSFs are also known to promote osteoclast formation but the mechanism of their action is less clear than for M-CSF. GM-CSF in human marrow cultures has been shown to promote osteoclast formation but in murine marrow cultures did not increase osteoclast formation (Roodman,

Table 2. Systemic and local factors that influence osteoclast formation.

FACTOR	FORMATION
Systemic Factors	
PTH	+
$1,25(OH)_2D_3$	+
Calcitonin	-
Androgens/oestrogens	-
Corticosteroids	+
Local factors	
Prostaglandins	±
IL-1	+
IL-6	+
IL-4	-
IL-11	+
IFN-γ	-
TGFα	+
TGFß	-
TNFα	+
TNF ß	+
M-CSF	+
GM-CSF	+

+: stimulation; -: inhibition; ±: stimulation/inhibition (effect is dependent on stromal cell type).

1996). GM-CSF and other CSFs may act in a sequential fashion in the stepwise mechanism of osteoclast formation, expanding the population of primed mononuclear phagocyte osteoclast precursors. These cells would then be pushed towards osteoclast differentiation by other factors such PTH or $1,25(OH)_2D_3$. This is in contrast to the effect of adding $1,25(OH)_2D_3$ or GM-CSF together when no change in osteoclast formation is generally seen.

Interleukin-1 (IL-1) is a cytokine which promotes both osteoclastic bone resorbing activity and osteoclast formation in murine and human marrow cultures (Pfeilschifter et al., 1989; Roodman, 1996). Its bone resorbing effect is mediated by osteoblasts (Thomson et al., 1986). IL-1 stimulates the growth and differentiation of CFU-GM and can increase the number of committed mononuclear osteoclast precursors (Uy et al., 1995a). IL-1 is produced by various stromal cells, monocytes, macrophages and tumour cells, and is likely to play an important role in the osteolysis associated with inflammatory and neoplastic lesions in bone (Vaes, 1988). IL-1 has been identified in tumours associated with hypercalcaemia; elevated levels of this cytokine have been reported in isolated marrow cells derived from patients with myeloma (Roodman, 1996).

Tumour necrosis factor α (TNFα) and TNFß have both been shown to stimulate both the formation and activity of osteoclasts (Thomson et al., 1987; Roodman, 1996). It has also been shown that TNFα potentiates the stimulatory effects of IL-1 on osteoclast formation. Although *in vitro* effects of TNFß have been reported in myeloma organ cultures, increased levels of this cytokine have not been found in patients with myeloma (Alsina et al., 1996). TNFα, on the other hand, has been implicated in the pathogenesis of hypercalcaemia of malignancy. TNFα potentiates the effects of IL-1 on osteoclast formation and both cytokines are commonly found in elevated concentrations in pathological conditions where osteolysis predominates eg aseptic loosening, rheumatoid arthritis (Chiba et al., 1994; Skerry and Gowen, 1995). Both IL-1 and TNF are also thought to be associated with the bone loss that occurs in osteoporosis (Kitazawa et al., 1984).

Interleukin-6 (IL-6) is a multifunctional, locally acting cytokine that exerts its influence through a cell surface receptor made up of a membrane-bound IL-6 receptor and a signal-transducing 130kd glycoprotein (Suda et al., 1995). IL-6 is not thought to stimulate the formation of osteoclasts from precursors alone but rather in combination with its soluble receptor. IL-6 is produced by many cells in the bone microenvironment, including marrow stromal cells, monocytes, macrophages, osteoblasts and osteoclasts. The soluble IL-6 receptor is formed by limited proteolysis of the cell membrane-bound IL-6 receptor and is present normally in the circulation. *In vitro* studies have shown that osteoclasts are formed when the soluble IL-6 receptor is added with IL-6 to cocultures of murine bone marrow precursors and osteoblasts. It is thought that a cellular interaction between osteoblastic cells and osteoclastic progenitors is necessary for IL-6 to stimulate the formation of osteoclasts in bone. IL-6 is thought to play an important role in osteoporosis and Paget's disease of bone (Manolagas, 1995; Roodman, 1995) as well as in the osteolysis associated with Gorham Stout disease and rheumatoid arthritis (Devlin et al., 1996; Kotake et al., 1996).

Interleukin-11 (IL-11) and *leukaemia inhibitory factor* (LIF) also utilise the 130-kilodalton glycoprotein as a signal transducer (Suda et al., 1995). IL-11, which is produced by bone marrow stromal cells, induces formation of osteoclasts in cocultures of murine bone marrow and osteoblasts (Girasole et al., 1994). It appears to play a major role as a controlling factor in the formation of osteoclasts, possibly acting as a common mediator of the effects of $1,25(OH)_2D_3$, PTH, IL-1 and TNF. The effect of IL-11 on osteoclast formation may be mediated via prostaglandins.

Other growth factors and cytokines have also been shown to influence the formation of osteoclasts. These include *transforming growth factor* ß (TGF ß), *interleukin-4* (IL-4) and *interferon* γ (IFN γ). TGF ß, produced by osteoblasts and osteoclasts, is known to inhibit both the proliferation and fusion of human osteoclast precursors (Roodman, 1996). It may act as a coupling factor regulating osteoclast formation by osteoblasts and possibly osteoclasts themselves through activation of latent TGF ß or secretion of TGF ß itself. IL-4, produced by activated T-cells, is also known to inhibit the formation of osteoclasts in bone marrow cultures and cocultures of mononuclear phagocytes and osteoblasts (Shioi et al., 1991). Interferons, particularly IFNγ, are also known to inhibit both osteoclast formation and activity (Takahashi et al., 1986). Other local factors influencing osteoclast formation and activity include *oxygen free radicals* which stimulate osteoclast formation and bone resorption (Garrett et al., 1990). PTH and IL-1 stimulation of osteoclastic bone resorption may induce production of oxygen free radicals which in turn may act to mediate the effect of these substances on osteoclast formation and activity.

Prostaglandins (PGs), such as PGE_2, influence bone cell function by stimulating cAMP production (Kaji et al., 1996; Akatsu et al., 1989). PGs have been reported to be involved in the stimulatory effects of hormones and cytokines such as $1,25(OH)_2D_3$, IL-1 and IL-11 (Akatsu et al., 1989, 1991; Suda et al., 1995). It has also been shown that PGs can be substituted for $1,25(OH)_2D_3$ as a differentiation factor in cultures of bone marrow stromal cells (Collins and Chambers 1992). The effect of PGs, however, on the formation of osteoclasts in culture is known to differ profoundly depending on the type of stromal cells supporting osteoclast formation. It has been shown that cocultures of monocytes and rat osteoblast-like UMR 106 cells inhibit osteoclast formation whereas coculture with the bone marrow stromal cell, ST2, stimulates osteoclast formation (Quinn et al., 1997). PGE_2 also appears to stimulate the formation of osteo-

clasts in human bone cell-human monocyte cocultures (Athanasou and Neale: Unpublished observation). These findings indicate that the effect of PGs on the formation of osteoclasts from monocyte precursors is highly dependent upon the type of stromal cell supporting osteoclast differentiation.

1,25 dihydroxy vitamin D_3 ($1,25(OH)_2D_3$) is also likely to act through osteoblasts to stimulate osteoclast formation. $1,25(OH)_2D_3$ receptors have been localised on osteoblasts and are not present on osteoclasts (Narbaitz et al., 1983). Monocytes and macrophages are known to possess receptors for $1,25(OH)_2D_3$and $1,25(OH)_2D_3$ is also a secretory product of these cells (Gordon, 1986). $1,25(OH)_2D_3$ induces the differentiation of murine and human myeloid and monoblastic leukaemia cells along the monocyte pathway (Bar-Shavit et al., 1983). It also enhances substrate adhesion, enzyme production and expression of mononuclear phagocyte-associated antigens. $1,25(OH)_2D_3$ also promotes the fusion of monocytes and macrophages to form macrophage polykaryons (Abe et al., 1983). $1,25(OH)_2D_3$ appears to act on bone stromal cells and osteoclast precursors to promote osteoclast formation. In particular, it appears to be necessary for the maturation of osteoclast precursor cells to form polykaryons that are capable of lacunar bone resorption.

PTH is also known to act through osteoblasts and bone-marrow stromal cells to stimulate the activity and formation of osteoclasts (McSheehy an Chambers, 1986). Both PTH and $1,25(OH)_2D_3$ appear to act predominantly on post-mitotic osteoclast precursors in bone (Uy et al., 1995b). Their effect may be mediated by PGs and modulated by cytokines such as IL-1, TGF α, IL-6. All of these agents appear to act through a mechanism mediated by cyclic adenosine 3',5'-monophosphate (cAMP) and protein kinase A (Roodman, 1995). PTH-related peptide (PTH-rP) is thought to act similarly (Martin and Udagawa, 1998).

Calcitonin is known to inhibit the function of osteoclasts (Chambers and Magnus, 1982; Chambers et al., 1985a,b). It has also been shown to inhibit osteoclast formation from mononuclear precursors in the monocyte fraction (Quinn et al., 1994). Its physiological role in inhibiting bone resorption, however, remains uncertain.

Oestrogens and *androgens* act to inhibit osteoclast formation *in vitro* and *in vivo* (Roodman, 1996). Osteoblasts are known to contain oestrogen receptors but evidence as to whether osteoclasts contain oestrogen receptors is contradictory. Oestrogens inhibit IL-6 production by bone marrow stromal cells and oestrogen deficiency leads to an increase in CFU-GM and osteoclast numbers (Jilka et al., 1992). Similar effects have been reported with regard to androgens. Bone loss associated with oestrogen deficiency may also be mediated via IL-1 and TNFα (Kitazawa et al., 1994). Administration of an IL-1 receptor antagonist to ovariectomised animals decreases bone loss and bone resorption. Ovariectomy increases stromal cell secretion of IL-1 and the formation of osteoclast-like cells in culture. TNFα appears to act in concert with IL-1 in this system to enhance osteoclast formation and bone resorption. Oestrogens and androgens also promote osteoclast apoptosis both *in vitro* and *in vivo*.

Osteoclast formation and pathological bone resorption

Osteoclasts are commonly found at sites of osteolysis associated with systemic bone diseases such as hyperparathyroidism, and Paget's disease, and localised osteolytic lesions, such as bone tumours or inflammatory lesions (eg osteomyelitis and aseptic loosening). In these conditions, there is commonly an increase in the absolute number of osteoclasts; this increase would, as a consequence, lead to an increase in resorptive activity. Thus, one means whereby pathological bone resorption is likely to be effected is through disturbances in the mechanisms that govern the formation of these multinucleated cells.

In systemic bone resorbing conditions, such as hyperparathyroidism, it has been shown that the number of osteoclasts increases rapidly (within hours) after the administration of PTH (Bingham et al., 1969). Given the current status of what is known about osteoclast formation, this finding suggests that the rapid increase in osteoclast numbers that occur in this condition results either from a rapid mobilisation of mononuclear phagocyte-osteoclast precursors from peripheral blood or, more likely, PTH stimulation of the formation of mature osteoclasts from a primed cell population of resident mononuclear phagocyte osteoclast precursors present in bone. PTH and other systemic osteotropic hormones may also influence osteoclastic resorption by regulating the production of cytokines which influence osteoclast formation. The synthesis of IL-6 and IL-11, both products of stromal cells and osteoblasts, is promoted by PTH, $1,25(OH)_2D_3$ and PTHrP (Suda et al., 1995; Roodman, 1996). Using cultures of bone marrow cells, it has been shown that IL-6 plays a central role in the pathogenesis of osteoporosis associated with loss of gonadal function (Manolagas, 1995). The production of IL-6 by cultured bone marrow cells and osteoblastic cell lines is inhibited by oestrogen, and oestrogens inhibit the transcriptional activity of the human IL-6 gene promoter.

IL-6 has also been reported to be involved in the pathogenesis of bone resorption associated with Paget's disease (Roodman, 1995; Roodman et al., 1992). Osteoclast-like multinucleated cells which form in long term cultures of human bone marrow cells from patients who have Paget's disease produce high levels of IL-6 within the culture medium (Kukita et al., 1990). This conditioned medium has been found to promote the formation of osteoclasts in cultures of normal human bone marrow cells. The bone marrow and peripheral blood plasma from patients who have Paget's disease also contain elevated levels of IL-6. Osteoclasts from patients who have Paget's disease also express mRNA

for IL-6 and the nuclear transcription factor for IL-6 (Roodman, 1995). This is in contrast to normal osteoclasts which have been shown to express only mRNA for IL-6. Thus, both the increased production of IL-6 and the response to this cytokine may lead to an autocrine amplification of the stimulation of the formation and activity of osteoclasts by IL-6. Stromal cells from the microenvironment of the bone marrow appear to be the main source of IL-6 and they are involved in the increased formation of osteoclasts that is seen in Paget's disease. IL-6 produced by bone marrow stromal cells is also thought to play a role in the osteolysis associated with multiple myeloma (Roodman, 1995). In addition, IL-6 has been found to promote the resorption of bone that is associated with rheumatoid arthritis and vanishing bone disease (Devlin et al., 1996; Kotake et al., 1996).

Localised pathological bone resorption may result from a variety of causes, but it is most often associated with neoplastic and inflammatory lesions of bone. Tumour cells and inflammatory cells release numerous cytokines, prostaglandins and other local factors that enhance the bone-resorbing activity of mature osteoclasts; this effect is mediated indirectly by osteoclasts. Macrophages are a major component of the host cellular response to neoplastic and inflammatory lesions in bone (Bugelski et al., 1987). Stimulated by cytokines found in inflammatory lesions or produced by tumour cells, chemoattractant proteins are released that induce the recruitment of monocytes into these lesions. Amongst these inflammatory macrophages or tumour-associated macrophages (TAMs), are likely to be cells of macrophage phenotype that are capable of differentiation into functionally mature osteoclasts. It has been shown that TAMs derived from primary carcinomas of the breast in mice (Quinn and Athanasou, 1992), as well as murine inflammatory foreignbody macrophages derived from granulomas induced by the wear particles of implanted biomaterials, are capable of osteoclastic differentiation (Athanasou et al., 1992; Pandey et al., 1996). In the mouse, these macrophages require the same cellular and humoral conditions for osteoclast differentiation as those of osteoclast precursors derived from haematopoietic tissue; ie the presence of osteoblasts or other specific stromal cells and $1,25(OH)_2D_3$.

More recently, it has been shown that human macrophages, isolated from inflammatory and neoplastic lesions associated with osteolysis, are also capable of osteoclast differentiation under conditions similar to those noted for monocyte-osteoclast differentiation. Human TAMs (Quinn et al., 1998a), inflammatory macrophages isolated from the synovial membrane of joints affected by rheumatoid arthritis (Fujikawa et al., 1996b), and macrophages derived from the membrane surrounding an aseptically loose prosthesis (Sabokbar et al., 1997), have all been shown to be capable of differentiation into mature osteoclasts capable of extensive lacunar bone resorption. These precursors appear to be present in fact in all human tissue macrophage populations as shown by the fact that peritoneal macrophages, isolated from uraemic patients undergoing dialysis, are similarly capable of osteoclast differentiation (Quinn et al., 1998b). The cellular and humoral conditions required for human macrophage-osteoclast differentiation are effectively the same as for monocyte-osteoclast differentiation: ie there is generally a requirement for contact with a specific osteoblastic or other marrow stromal cell population as the presence of $1,25(OH)_2D_3$ and human M-CSF. It was found that macrophages derived from the arthroplasty pseudo-membrane surrounding loose prostheses capable of osteoclast differentiation occurred in the absence of M-CSF (Sabokbar et al., 1997). The addition of an antibody to M-CSF to arthroplasty-derived macrophage-osteoblast cocultures, however, was found to inhibit the extent of macrophage-osteoclast differentiation, indicating that M-CSF is endogenously produced by cells present in these cocultures.

This mechanism of macrophage-osteoclast differentiation is likely to be of particular significance in those lesions in which there is rapid destruction of bone associated with a heavy infiltrate of macrophages, such as in the form of aseptic loosening termed aggressive granulomatosis where rapid extensive osteolysis is seen in association with a pronounced foreign body macrophage response to the formation of numerous wear particles from implanted biomaterials (Santavirta et al., 1990). In this heavy inflammatory granulomatous response there is likely to be an increased number of immature mononuclear phagocytes with proliferative potential which are capable of undergoing osteoclastic differentiation. As a consequence, osteolysis occurs rapidly in this condition. Other osteolytic lesions characterised by a heavy infiltrate of macrophages (such as osteomyelitis, Gaucher's disease, sarcoidosis, or Histiocytosis X) may similarly generate osteoclast-like bone resorbing cells from within the macrophage infiltrate seen in these conditions. As $1,25\ (OH)_2D_3$ is a product of macrophages themselves (Nathan, 1987), it is likely to be important in granulomatous inflammatory conditions associated with osteolysis in bone (such as sarcoidosis, gout, tuberculosis, as well as aseptic loosening and other non-granulomatous inflammatory conditions) where numerous macrophages are seen in the inflammatory infiltrate.

In metastatic cancers, the number of TAMs is generally higher in earlier small metastases, which enlarge rapidly, decreasing later when the lesions enlarge more slowly (Bugelski et al., 1987). In bone metastases, osteoclasts and osteoclastic bone resorption are also known to be most prominent in the early phase when a tumour metastasis is becoming established in bone and osteolysis is proceeding rapidly; later, relatively few osteoclasts are evident and osteolysis proceeds more slowly (Galasko, 1976). As the number of TAMs and thus the capacity for osteoclast differentiation would appear to be increased in early metastases, this to some

extent explains the two more or less distinct phases of osteolytic bone destruction which occur in metastatic bone disease. Tumour cells are also known to produce factors that stimulate macrophage-osteoclast differentiation such as M-CSF (Kacinski, 1995).

Conclusion

In summary, recent studies have confirmed that the osteoclast is a member of the mononuclear phagocyte system: it is derived from the pluripotential haematopoietic stem cell and has a precursor that circulates in the monocyte fraction. This precursor expresses a monocyte-macrophage phenotype. In the cellular and humoral microenvironment of bone, these mononuclear phagocyte osteoclast precursors differentiate into mature lacunar bone-resorbing osteoclasts. Osteoblasts play a central role in this process, releasing an osteoclast differentiation factor which is necessary for osteoclast formation from precursor cell populations. Systemic and locally produced factors that act on osteoblasts, osteoclast precursors or mature osteoclasts themselves are known to influence this process and have been shown to play a role in several diseases of bone in which osteolysis is prominent.

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