Abstract

Osteoclast activation is a critical cellular process for pathological bone resorption, such as erosions in rheumatoid arthritis (RA) or generalized bone loss. Among many factors triggering excessive osteoclast activity, cytokines such as IL-1 or tumour necrosis factor (TNF)-α play a central role. New members of the TNF receptor ligand family (namely receptor activator of nuclear factor-κB [RANK] and RANK ligand [RANKL]) have been discovered whose cross-interaction is mandatory for the differentiation of osteoclasts from haemopoietic precursors, in both physiological and pathological situations. Osteoprotegerin, a decoy receptor which blocks this interaction, decreases osteoclast activity and could have a fascinating therapeutic potential in conditions associated with upregulated bone resorption.

Keywords: bone cytokines, differentiation, osteoclast, osteoprotegerin, RANK, RANKL

Introduction

Bone remodelling is a continuous physiological process that occurs in adult skeleton in which bone resorption is followed by new bone formation, maintaining mechanical strength and structure. Bone cells that are responsible for this coupled process include bone-resorbing cells (osteoclasts, which are derived from haematopoietic cells of the monocyte/macrophage lineage) and bone-forming cells (osteoblasts, which are of mesenchymal origin). The bone resorption process is involved in many clinical situations that are relevant to the work of rheumatologists, such as focal bone destruction or erosion in RA and other inflammatory arthritides, and the diffuse bone loss that is encountered in osteoporosis.

Osteoclast differentiation: basic mechanisms and new insights

Osteoclast progenitor cells are recruited from haematopoietic compartments, and then proliferate and differentiate toward mature osteoclasts. During this multistep differentiation process postmitotic osteoclast precursors progressively express osteoclast-associated markers, such as...
as calcitonin receptor and tartrate-resistant acid phosphatase, as they lose some of their macrophage characteristics. Then, mononuclear preosteoclasts fuse together to form multinucleated giant cells. Terminal osteoclast differentiation eventually leads to active bone-resorbing cells [1].

Role of osteoblast/stromal cells in osteoclast differentiation

Biological models of in vitro osteoclast differentiation have been developed that have facilitated detailed study of many of the factors involved in the regulation of this process. The most commonly studied models are cultures of mouse bone marrow or cocultures of haematopoietic cells with bone-derived stromal cells, which give rise to large numbers of bone-resorbing osteoclasts [2]. Studies based on these models have found that mesenchymally derived stromal cells play a critical role in supporting and stimulating osteoclast differentiation, a process that probably necessitates cell–cell contact between osteoclast precursors and stromal cells [3,4]. In some human models, however, a cellular interaction between osteoclast precursors and stromal cells is not always required [5–7].

Local and hormonal factors that are involved in osteoclast differentiation

Bone resorption is closely controlled in vivo by cellular and hormonal factors, which affect not only osteoclast activity, but also osteoclast formation. Parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] increase bone resorption, primarily via an indirect mechanism that is mediated by osteoblasts [2]. Oestrogens have a negative impact on osteoclast differentiation, and oestrogen deficiency leads to increased osteoclast differentiation and activation [8]. The cytokines IL-1, IL-6 and TNF-α are known to increase bone resorption by stimulating both osteoclast activity and differentiation. This effect involves, at least in part, prostaglandin production [9,10]. The major role of macrophage colony-stimulating factor (M-CSF) has been pointed out in M-CSF-deficient (op/op) mice, which develop an osteopetrosis that is characterized by the absence of osteoclasts [11]. Studies using murine cocultures [12] have shown that M-CSF acts both on proliferation and on differentiation of precursor cells. Local injections of M-CSF in rat metaphyseal bone also increase in situ osteoclast differentiation and bone resorption [13]. Other cytokines stimulate bone resorption at least partially by increasing osteoclast differentiation, such as leukaemia inhibiting factor and IL-11 [14,15]. Conversely, some cytokines such as IL-4 or IFN-γ have been shown to inhibit osteoclast differentiation in vitro [16]. The role of transforming growth factor-β is more complex; it decreases osteoclast precursor proliferation and bone resorption activity [17,18], but it also increases the expression of two osteoclastic markers – vitronectin receptor and calcitonin receptor [19,20]. Most of the cytokines that regulate osteoclast differentiation are produced in the bone microenvironment, mainly by osteoblast/stromal cells, further emphasizing the key role of these cells in osteoclast differentiation.

A new interactive system in osteoclast bone resorption

The recent discovery of new members of the TNF receptor ligand family has pointed out the crucial role of RANK and RANKL in osteoclast differentiation and activation [21*, 22*] (Fig. 1).

RANKL, also called osteoclast differentiation factor (ODF), TNF-related induced cytokine (TRANCE), or osteoprotegerin ligand (OPGL)

Because osteoblast–stromal cell interactions with osteoclast precursors are required for subsequent osteoclast differentiation, an ODF expressed by these cells and recognized by osteoclast precursors was suspected. Such a factor was identified as RANKL [23**,24**]. RANKL is a membrane-bound TNF-related factor that is expressed by osteoblast/stromal cells. That the presence of RANKL is vital in osteoclast differentiation is now well established, and its soluble recombinant form has been tested in a number of in vitro and in vivo studies. In in vitro murine or human osteoclast differentiation models, soluble RANKL enables osteoclast precursors to differentiate in the presence of M-CSF, even in the absence of osteoblast/stromal cells [25**,26]. Bone resorption activity is increased, as well as the osteoclast survival [23**,26]. Mice that are defective for RANKL develop a form of osteopetrosis. They are characterized by the absence of osteoclasts, although osteoclast progenitors are present and are able to differentiate into bone-resorbing osteoclasts in the presence of normal osteoblast/stromal cells [27**].

In addition, the soluble form of RANKL has been shown to be produced by human fibroblasts transfected with an expression vector for RANKL and by in vitro activated murine T cells [23**,28]. However, it is not clear whether this soluble form plays a role in vivo in normal bone homeostasis or in pathological processes that are characterized by increased bone resorption.

RANK

Osteoclast precursors express RANK, a membrane-bound TNF receptor that recognizes RANKL through a direct cell–cell interaction with osteoblast/stromal cells [29**]. Recent studies [29**,30] demonstrated that this receptor is essential for the transduction of signals that lead to osteoclast differentiation. An overexpression of soluble RANK results in osteopetrosis, with a decreased number of osteoclasts [30]. Conversely, mice that are deficient for RANK develop a severe osteopetrosis that is characterized by the absence of osteoclasts. In addition, osteoclast precursors in these mice are unable to differentiate to osteoclasts in vitro, in the presence of RANKL and M-CSF [31].
New members of the TNF receptor ligand family: role of RANKL (ODF, TRANCE, OPGL) and its receptor RANK in osteoclast differentiation. RANKL, a membrane-bound TNF-related factor, is expressed by osteoblast/stromal cells and is upregulated by osteotropic factors such as 1,25(OH)2D3, PTH, IL-6 or IL-11. Osteoclast (OC) precursors express RANK, a membrane-bound TNF receptor, that recognizes RANKL through cell–cell interaction with osteoblast/stromal cells. This interaction enables osteoclast precursors to differentiate in the presence of M-CSF. Osteoprotegerin (OPG) is a member of the TNF receptor family that lacks a transmembrane domain and represents a secreted TNF receptor. OPG recognizes RANKL, and this decoy receptor blocks the interaction between RANK and RANKL, leading to inhibition of osteoclast differentiation and activation.

Osteoprotegerin
Osteoprotegerin is a member of the TNF receptor family that lacks a transmembrane domain and represents a secreted receptor. Osteoprotegerin recognizes RANKL, and this decoy receptor blocks the interaction between RANK and RANKL, leading to an inhibition of osteoclast differentiation and activation [32••,33••]. Overexpression of osteoprotegerin in transgenic mice results in a form of osteopetrosis that is characterized by a defect in osteoclast differentiation [32••]. By contrast, osteoprotegerin-deficient mice develop severe osteoporosis because of increased osteoclast differentiation and function [34••]. In vitro studies have demonstrated the strong inhibitory action of osteoprotegerin on osteoclast differentiation, as well as on the bone-resorbing activity of osteoclasts [32••,33••].

Role of RANK/RANKL and osteoprotegerin in osteoclast differentiation
RANKL/osteoprotegerin balance, signal transduction and osteoclast differentiation
Recent data suggest that M-CSF and RANKL are two major factors involved in osteoclast differentiation. M-CSF is required for both proliferation and differentiation, and RANKL (which is not a growth factor) is required for differentiation into mature osteoclasts and for osteoclast activity [26]. In bone tissue, osteoprotegerin and RANKL are expressed by osteoblast/stromal cells, and the ratio of these products may modulate the ability of these cells to stimulate osteoclast differentiation/activity, as well as the rate of bone resorption [21].

In addition, it has been shown [26] that the interaction between RANKL and RANK results in a transduction signal in preosteoclasts and in mature osteoclasts that may activate nuclear factor-κ (NF-κB). The role of NF-κB in the osteoclast differentiation has been previously demonstrated in mice with a double knockout for the p50 and p52 NF-κB subunits, in which a defect of osteoclast differentiation leads to an osteopetrosis [35,36]. Other intracellular events are activated by transduction signals, such as c-jun terminal kinase, and TNF receptor-associated factors, which regulate activation of NF-κB and/or c-jun terminal kinase [21].

RANK/RANKL, immune cells and osteoclast differentiation
RANK and RANKL have been shown to be expressed in dendritic cells and T lymphocytes, respectively, in which they appear to be important regulators of the interactions between these cells [37,38]. These data suggest that, apart from osteoclast differentiation and activation, RANK and RANKL are involved in the immune system as suggested by the mice knockout models. RANKL-deficient mice lack lymph nodes and exhibit defects in differentiation of T and B lymphocytes [27••], and RANK-deficient mice exhibit a marked deficiency in B cells in the spleen and lack lymph nodes [31].

Regulation of RANKL and osteoprotegerin expression
Recent studies have demonstrated that osteotropic factors and hormones such as PTH, 1,25(OH)2D3, IL-11, IL-1β, TNF-α or prostaglandin E2 upregulate RANKL expression in osteoblast/stromal cells (Fig. 1). In addition, osteoprotegerin expression is downregulated by prostaglandin E2, and is upregulated by oestrogens [21•]. RANKL expression has not yet been extensively studied.

An integrated view and clinical implications
An emerging concept is that cytokines and hormonal factors that are involved in bone resorption may act by a common final pathway involving RANKL and RANK [21•]. In accordance with this concept, a recent in vivo study [39••] has shown that a recombinant chimaeric Fc fusion form of osteoprotegerin inhibited hypercalcemia and bone resorption induced by IL-1β, TNF-α, PTH and 1,25(OH)2D3 in mice. This convergence theory is probably not exclusive because recent studies [40,41] have suggested that the effects of TNF-α or IL-6 may involve different effectors.

Therapeutic perspectives
The concept presented above will probably lead to new therapeutic approaches in several diseases that are characterized by excessive bone resorption. Thus, osteoprote-
gerin (a specific inhibitor of RANKL) or an analogue may be used to block the excess of bone resorption in pathological conditions such as hyper-resorption of malignancy, in which this pathway seems to be primarily involved [42••], or in osteoporosis, in which oestrogen deficiency could lead to decreased production of osteoprotegerin and subsequent increased bone resorption.

Bone erosions in rheumatoid arthritis

Rheumatoid arthritis is another interesting clinical model for the study of the role of RANK/RANKL in bone erosions, and as a therapeutic target for osteoprotegerin. Rheumatoid arthritis is characterized by progressive bone and cartilage destruction as a result of chronic synovitis. Numerous studies have pointed out the role of cytokines such as TNF-α or IL-1 in the joint destruction [43]. Recent studies suggest that RANKL mRNA is highly expressed in synovial tissues from patients with RA, but not in normal synovial tissues. This expression is detected in synovial fibroblasts, as well as in activated T cells derived from RA synovial tissues, suggesting that these cells may contribute to osteoclast formation at the specific sites of bone destruction in RA [44•,45•]. In addition, in rat adjuvant-induced arthritis, RANKL is expressed on the surface of activated T cells isolated from affected rats, and may be secreted in T cell cultures. Activated T cells could therefore directly induce osteoclastogenesis through membrane-bound and soluble RANKL [28]. These data suggest that RANKL may have a major pathophysiological importance in the bone and joint destruction observed in inflammatory arthritides such as RA. Activated T cells, which play a central role in the pathogenesis of RA, may (in addition to stromal cells) contribute to the osteoclast-mediated bone resorption via RANKL expression [46••].

Conclusion

Osteoclasts are multinucleated cells that are formed by fusion of osteoclast precursors from haematopoietic origin. These cells are responsible for bone resorption and osteoclast differentiation and represent an evident point of control of bone resorption. Bone resorption is closely regulated in vivo by many cellular and hormonal factors, which affect not only osteoclast activity, but also osteoclast formation. The recent discovery of new members of the TNF receptor ligand family (ODF, TNF-related induced cytokine, osteoprotegerin ligand) have emphasized the crucial role of RANKL, which is expressed by osteoblast/stromal cells, and its receptor RANK, which is expressed by osteoclast cells, in osteoclast differentiation and activation. This system is completed by osteoprotegerin, which is a secreted TNF receptor. Osteoprotegerin recognizes RANKL, and this decoy receptor blocks the interaction between RANK and RANKL. A number of osteotropic factors and hormones may modulate bone resorption via this common final pathway, which may represent a potential therapeu-

tic target in pathologic processes that are characterized by excessive bone resorption.

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