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Role of Cytokines in Bone Resorption

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Abstract Osteoclastic bone resorption is modulated in humans by powerful osteotropic factors which are generated in the immediate vicinity of bone resorbing surfaces. These factors are released from marrow mononuclear cells and from some bone cells, and some are actually incorporated into the noncollagenous bone matrix from where they are released when bone is resorbed. They are likely important not only in the control of normal bone remodeling, but also in a number of disease states associated with disordered remodeling. In this review, current concepts of the effects of these factors on cells in the osteoclast lineage will be discussed. © 1993 Wiley-Liss, Inc.

Key words: osteoclasts, bone resorption, cytokines, src

In the last 20 years, it has become apparent that the process of bone resorption is regulated not only by systemic hormones such as parathyroid hormone and 1,25 dihydroxyvitamin D, but in addition by a large number of powerful osteotropic factors which are generated locally in the bone cell microenvironment. These factors probably have more important effects on osteoclast function than do the systemic hormones, whose major role is the control of extracellular fluid calcium homeostasis. Bone resorption is the process by which small packets of bone throughout the skeleton are removed by osteoclastic resorption to be later replaced by osteoblastic bone formation. Since the resorption of bone occurs in discrete packets which are geographically and chronologically separate one from another, it has always appeared likely that the primary control of this process is mediated by local factors generated in the microenvironment of each bone remodeling packet.

The first observation that cytokines may stimulate osteoclastic bone resorption was made over 20 years ago, when it was demonstrated that peripheral blood leukocytes activated by antigens to which they had previously been exposed or by phytohemagglutinin released a factor into their cell culture media which stimulated osteoclasts to resorb bone in organ cultures of fetal rat long bones [1,2]. This lymphokine, as it was thought to be at the time, was termed osteoclast activating factor, or OAF. Since that time, it has become recognized that OAF is comprised of a number of discrete peptides, some of which stimulate osteoclastic bone resorption and some of which inhibit osteoclastic bone resorption. It has also become apparent that the factors which comprise this activity may be important in the bone loss which occurs in a number of pathologic states, and particularly chronic inflammatory diseases which cause bone loss, certain neoplasms associated with bone resorption, in osteopetrosis where failure of production of cytokines is likely important, and, most importantly, in the bone loss associated with the postmenopausal state and aging, namely osteoporosis.

The cytokines which are involved in osteoclastic bone resorption are produced by marrow mononuclear cells and by bone cells, and some may be even incorporated into the nonmineralized bone matrix and released during the process of osteoclastic bone resorption [3,4]. The mechanisms which control release of these factors in the bone cell microenvironment are not clear. However, it is apparent that these factors act at different sites on osteoclast formation and activation, and the regulation of their production in the bone marrow microenvironment is different for each cytokine. There is considerable overlap among the cytokines which stimulate osteoclastic bone resorption and cytokines

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which are involved in normal blood cell formation. This is hardly surprising, since the osteoclast is derived from a similar marrow-derived hematopoietic precursor as the formed elements of the blood. Many experiments performed over the last 10 years have indicated that colony stimulating factors, in particular monocyte-macrophage colony stimulating factor (M-CSF) and granulocyte-macrophage colony stimulating factor (GM-CSF), have important effects on osteoclast formation. Other cytokines which influence leukocyte formation, such as interleukin-1 and tumor necrosis factor, are also important in osteoclast generation.

Some of the more important cytokines which have been shown to regulate osteoclastic bone resorption will now be discussed.

INTERLEUKIN-1

Interleukin-1 is a powerful stimulator of osteoclastic bone resorption. This was first recognized over 10 years ago, when conditioned media enriched in interleukin-1 were found to stimulate bone resorption in organ cultures of neonatal mouse calvaria [5]. Interleukin-1 has similar activity in other types of bone resorbing assay systems, including organ cultures of fetal rat long bones, cultures of human and murine marrow mononuclear cells, and isolated avian and rodent osteoclasts. Interleukin-1 has multiple effects at all stages in the osteoclast lineage. It stimulates proliferation of osteoclast precursors [6]. In addition, it also enhances differentiation of committed precursors to form mature multinucleated cells. There is also controversial evidence to suggest that it activates mature osteoclasts to form ruffled borders and resorb bone, an effect which is apparently mediated indirectly through other types of cells in the osteoclast environment [7]. On the other hand, there is also evidence to suggest that interleukin-1 may have an inhibitory effect on the mature cell [8].

The effects of interleukin-1 on bone have also been tested extensively in vivo. Both interleukin-1α and interleukin-1β stimulate bone resorption systemically in normal mice, although the effects are much more prominent immediately adjacent to the site of injection [9,10]. The effects on bone are so significant that hypercalcemia results. This has been shown to be mediated totally by the effects of interleukin-1 on osteoclasts, since it does not occur in osteopetrotic mice which have ineffective osteoclastic bone resorption [12]. Part of the effects of interleukin-1 on bone resorption in vivo are prostaglandin-mediated, since they are blocked by inhibitors of prostaglandin synthesis such as indomethacin [10]. This is much more so for the local effects than for the systemic effects. Interleukin-1 causes sustained hypercalcemia, following a transient fall in the blood ionized calcium [11]. This short-lived decrease in blood ionized calcium is also prostaglandin-mediated, since it is inhibited by indomethacin [11].

The effects of interleukin-1 on bone formation are still difficult to discern. It appears likely that interleukin-1 inhibits bone formation in vivo unless it is administered intermittently, when bone formation is enhanced, probably because of the coupling phenomenon which links bone formation with previous bone resorption [10]. Under these circumstances, a wave of bone resorption is followed secondarily by prolonged bone formation, provided the bone forming cells are not exposed at the same time to interleukin-1 [10].

Interleukin-1 has been implicated in a number of disease states. It is present in increased amounts in the conditioned media of peripheral blood monocytes harvested from patients with postmenopausal osteoporosis, and its production under these circumstances is inhibited when patients are treated with estrogen [13,14]. It has thus been linked to the bone loss associated with the postmenopausal state. It is produced by a number of solid tumors which are associated with hypercalcemia [15,16], often in conjunction with other hypercalcemic mediators which are frequently expressed by hypercalcemic tumors such as the parathyroid hormone-related protein [15].

TUMOR NECROSIS FACTOR AND LYMPHOTOXIN

Tumor necrosis factor and lymphotoxin are two related cytokines which have powerful and identical effects on bone [17]. Many of their effects seem to overlap with those of interleukin-1. Tumor necrosis factor and lymphotoxin both stimulate osteoclastic bone resorption in organ cultures of fetal rat long bones [17] and in neonatal mouse calvariae [18,19] and stimulate the formation of mature osteoclasts from precursors [6]. Moreover, both factors activate mature osteoclasts, an effect which is probably mediated indirectly through intermediate cells [20].

Both tumor necrosis factor and lymphotoxin cause increased osteoclastic bone resorption and hypercalcemia in vivo [21,22]. This has been shown either by repeated injections, by prolonged infusions, or by the use of Chinese hamster ovarian cells stably transfected with human tumor necrosis factor and then inoculated into nude mice. The tumor-bearing nude mice become hypercalcemic [21].

Tumor necrosis factor and lymphotoxin have been implicated in the bone resorption associated with some diseases. Lymphotoxin is overproduced by established cell lines derived from rat and human models of myeloma [22]. Most of the bone resorbing activity produced by these cells in vitro can be inhibited by neutralizing antibodies to lymphotoxin. Tumor necrosis factor has also been implicated in the bone resorption and hypercalcemia associated with solid tumors [23–25]. In this circumstance, tumor necrosis factor is produced not by the solid cancer cells, but rather by host cells which are stimulated by the presence of the tumor. In one such tumor, the hypercalcemia was reversed by treatment of tumor-bearing nude mice with neutralizing antibodies to tumor necrosis factor [25]. The mechanisms for host cell stimulation are probably multiple. Granulocyte-macrophage colony stimulating factor (GM-CSF) and other factors have been shown to be involved [23].

Tumor necrosis factor may also be responsible for other paraneoplastic syndromes associated with malignancy. These include anemia, leukocytosis, cachexia, and hypertriglyceridemia [21,25,26].

INTERLEUKIN-6

Interleukin-6 has different effects on bone resorption from those of tumor necrosis factor and interleukin-1. Interleukin-6 stimulates the formation of osteoclasts from precursors [27,28]. Interleukin-6 also probably has effects on mature osteoclasts. Interleukin-6 is expressed by mature osteoclasts, and both neutralizing antibodies to interleukin-6 as well as antisense oligonucleotides inhibit the capacity of multinucleated osteoclasts to resorb bone [29].

Interleukin-6 has also been shown to cause hypercalcemia in vivo associated with increased osteoclastic bone resorption [30]. This has been demonstrated by inoculating Chinese hamster ovarian cells with interleukin-6 and showing that tumor-bearing nude mice develop hypercalcemia. Interleukin-6 has been implicated in the bone loss associated with Paget's disease [31], with increased generation of osteoclasts in Paget's disease and with the hypercalcemia associated with myeloma [32]. Interleukin-6 is certainly overproduced in some models of myeloma, and neutralizing antibodies to interleukin-6 have been shown to reduce the serum calcium in some of these patients.

Recently, interleukin-6 has been linked to the bone loss associated with oophorectomy [33]. In estrogen-deficient rats, bone loss due to osteoclastogenesis can be prevented by estrogen replacement therapy or by neutralizing antibodies to interleukin-6. Presumably, estrogen suppresses normal interleukin-6 expression by osteoblastic cells. These findings raise interesting implications for the relative roles of interleukin-6 and interleukin-1 (see earlier) in postmenopausal bone loss.

INTERLEUKIN-4

Interleukin-4 is a multifunctional cytokine which inhibits osteoclastic bone resorption in organ cultures of neonatal mouse calvariae [34]. It also has the capacity to inhibit the formation of osteoclasts from marrow mononuclear precursors. Transgenic mice overexpressing interleukin-4 develop an osteopenic syndrome which resembles that of osteoporosis [35]. Recent studies have suggested that interleukin-4 may actually enhance the formation of mineralized bone nodules in cultures of cells with the osteoblast phenotype [36].

GAMMA INTERFERON

Gamma Interferon is a cytokine which is a powerful inhibitor of bone resorption [37]. It has effects both on osteoclast precursors as well as on committed progenitors [38]. It does not appear to have any effects on mature cells. Recently, it has been used in vivo to lower the serum calcium in hypercalcemic tumor-bearing mice. Its toxicity limits its usefulness in vivo. It seems to be a more effective inhibitor on bone resorption mediated by cytokines such as interleukin-1 and tumor necrosis factor than by parathyroid hormone [39].

COLONY STIMULATING FACTORS

Since the osteoclast is derived from a hematopoietic precursor it shares with the formed elements of the blood, it is not surprising that a number of colony stimulating factors regulate

osteoclast formation. The best studied is monocyte-macrophage colony stimulating factor (M-CSF). M-CSF is responsible for the formation of osteoclasts from precursors in the neonatal period in mice. This has been shown unequivocally in the op/op murine variant of osteopetrosis, in which there is a naturally occurring defect in the coding region for the M-CSF gene [40]. In this murine model of osteopetrosis, there is a transient failure of osteoclast formation. The disease can be corrected by M-CSF [41]. These results show that M-CSF is required for normal osteoclast formation during this period in the mouse.

Recently, a colony stimulating factor has been identified for osteoclasts which seems to be relatively specific for the osteoclast lineage [25,42]. This factor has yet to be purified to homogeneity, and its relationship to other colony stimulating factors is still to be determined.

TRANSFORMING GROWTH FACTOR β

Transforming growth factor β is a powerful growth regulatory factor which is present in abundant amounts in the bone matrix [43] but is also produced by cells in the monocyte microenvironment including other types of bone cells. Transforming growth factor β has effects on bone resorption which are very complex [19,44]. It inhibits the formation of osteoclasts from precursors [45]. However, one of its effects on bone in vivo is to enhance bone resorption in the marrow cavity. It appears that some osteoclasts are stimulated by TGFβ and some are inhibited. It may depend on the other cells in the osteoclast microenvironment. If there are cells present in the microenvironment which respond to TGFβ by producing a local factor which stimulates osteoclasts, then bone resorption may be the overall response.

There are other growth regulatory factors present in the bone matrix which may regulate osteoclast activity. These include the heparin-binding fibroblast growth factors, bone morphogenetic proteins, insulin-like growth factors I and II, and platelet-derived growth factor [46]. It remains to be determined what the precise effects of these factors on osteoclasts are.

ROLE OF TYROSINE KINASES IN OSTEOCLASTIC BONE RESORPTION

Recently, it has been shown that a number of tyrosine kinases may be important in normal osteoclastic bone resorption. Observations with M-CSF referred to above demonstrating that M-CSF is required for normal osteoclast formation suggest that the M-CSF receptor, the receptor tyrosine kinase c-fms, may also be necessary for the production of normal osteoclasts. Moreover, recent studies in mice which are deficient in expression of the src proto-oncogene by gene knockout show that this nonreceptor tyrosine kinase is also essential for normal osteoclast action and ruffled border formation [12,47,48]. These data suggest that both receptor and nonreceptor tyrosine kinases may be required for normal osteoclast formation and action. The drug herbimycin A, which is an effective inhibitor of the src tyrosine kinase (among other tyrosine kinases), effectively lowers the serum calcium and decreases osteoclastic bone resorption in vivo [49].

In summary, there is now an abundance of evidence which indicates that local factors generated in the microenvironment of bone resorbing cells are powerful regulators of osteoclast function. Evidence now exists that one of these cytokines, namely M-CSF, is required for normal osteoclast formation in the neonatal period, and that others, particularly tumor necrosis factor and interleukin-6, are likely involved in the bone loss associated with some malignancies, and in the case of the latter, estrogen withdrawal. As knowledge in this field expands, it is likely that our understanding of the role that these cytokines play in other common diseases of bone loss will also be clarified. This is an important area of study, since understanding these mechanisms should lead to better therapies for these important bone diseases.

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