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Mechanisms by Which Cells of the Osteoblast Lineage Control Osteoclast Formation and Activity

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Abstract The cells of bone are of two lineages, the osteoblasts arising from pluripotential mesenchymal cells and osteoclasts from hemopoietic precursors of the monocyte-macrophage series. Resorption of bone by the multinucleate osteoclast requires the generation of new osteoclasts and their activation. Many hormones and cytokines are able to promote bone resorption by influencing these processes, but they achieve this without acting directly on osteoclasts. Most evidence indicates that their actions are mediated by cells of the osteoblast lineage. Evidence for hormone- and cytokine-induced activation of osteoclasts requiring the mediation of osteoblasts comes from studies of resorption by isolated osteoclasts. However, consistent evidence for a specific "activating factor" is lacking, and the argument is presented that the isolated osteoclast resorption assays have not been shown convincingly to be assays of osteoclast activation. The view is presented that osteoblast-mediated osteoclast activation is the result of several events in the microenvironment without necessarily requiring the existence of a specific, essential osteoclast activator. On the other hand, a specific promoter of osteoclast differentiation does seem likely to be a product of cells of the stromal/osteoblast series. Evidence in favour of this comes from studies of osteoclast generation in co-cultures of osteoblast/stromal cells with hemopoietic cells. Conflicting views, maintaining that osteoclasts can develop from hemopoietic cells without stromal intervention, might be explained by varying criteria used in identification of osteoclasts. Osteoblastic and osteoclastic renewal, and the interactions of these lineages, are central to the process of bone remodeling. © 1994 Wiley-Liss, Inc.

Key words: osteoblasts, osteoclasts, hormones, cytokines, hemopoietic cells

Of the two major cell types of bone, the osteoblast and the osteoclast, bone formation results from osteoblast activity and bone resorption from osteoclast activity. These two processes are very tightly coupled, so that when a specific amount of bone is resorbed, the same amount is formed to replace it. This process, known as bone remodelling, is continuous throughout life and is necessary for the maintenance of a structurally sound skeleton. Essential features of remodelling are the exquisitely regulated formation of osteoblasts and osteoclasts, and the processes of communication between these cell families, which is the topic of this discussion.

These two main cell types are considered to arise from different lineages. The osteoblast arises from a multipotential primitive mesenchymal precursor, capable of differentiating along the chondrocyte, adipocyte, myocyte, or osteoblast lineages [Owen, 1985]. Osteoclasts, on the other hand, are derived from blood-borne precursors of the monocyte-macrophage series. Because of the difficulty of isolating and characterizing bone cells, the study of bone cell biology is a relatively new discipline. However insights of the last 10 years have revealed the great functional and developmental interdependence of these two lineages, with development of a concept of cells of the osteoblast lineage controlling the formation and activity of osteoclasts, through the actions of a number of cytokines and growth factors generated locally in bone. Production of many of these is under the control of circulating hormones [reviewed in Martin et al., 1993b; Mundy, 1993; Horowitz, 1993], which can influence the production of some cytokines and also synergize with some of them in their actions upon bone cells. In view of the tight coupling of bone resorption and formation, such a relationship is perhaps not surprising. This discussion

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focuses on what is known and what is hypothesized on the intercellular processes by which cells of the osteoblast lineage control osteoclast formation and activity.

Although osteoblasts have traditionally been considered to be responsible for bone formation, and are recognized in bone as plump cells lining trabeculae and actively engaged in the synthesis of bone components, it is clear that the term should describe a family of cells which includes stromal cells of the bone marrow cavity and which differ substantially in their properties at different stages of development and at different sites in bone. At the simplest level, mature osteoblasts comprise three main sub-populations: osteoblasts which synthesize matrix, lining cells which lie as flattened, nonsynthesizing cells along trabecular surfaces, and osteocytes which are situated deep in bone and communicate by a canalicular system with surface osteoblasts and lining cells. However the origin of osteoblasts from pluripotential stem cells present in the stromal fibroblastic system of the bone marrow strongly implies that the various stages of osteoblast differentiation are represented within the marrow and bone compartments. Cells at various stages of differentiation would be expected to exhibit to varying extents some of the differentiation features which have come to be recognised as "osteoblastic." From studies in vitro [Stein et al., 1989; Aubin et al., 1990] and in vivo [Strauss et al., 1990; Yoon et al., 1987; Zhou et al., 1994] a developmental sequence of osteoblast differentiation is proposed (Table I) in which the maturing osteoblast progressively acquires the ability to express certain genes. How these differentiation processes occur is not the subject of this overview, but suffice to say that there is accumulating evidence that retinoids, transforming growth factor beta (TGFβ), and the bone morphogenetic protein (BMP) family are likely to be very important players.

With that background to the osteoblast-stromal cell series, we propose to discuss how these cells influence the osteoclast lineage. Most of the discussion is based on data obtained from in vitro systems, with experiments carried out in bone organ cultures, and with stromal and osteoblastic cell cultures, co-cultures with hemopoietic cells, and with mixed bone marrow cultures.

Osteoclasts are multinucleated cells derived from blood-borne precursors of the monocyte-macrophage series which differentiate to form cells capable of bone resorption [reviewed in Baron et al., 1993]. The mature osteoclast is rich in tartrate-resistant acid phosphatase (TRAP) and possesses a ruffled membrane border on the surface facing the bone mineral. Pre-fused osteoclasts, containing a single nucleus, have long been recognised from both in vitro and in vivo studies as fully functional osteoclasts. Osteoclasts are richly endowed with lysosomal enzymes. Production of carbonic anhydrase II plays an important part in the process of acid production, as does a powerful Na/H exchange system which allows the production of acid locally at the site of resorption to facilitate the extracellular activity of certain lysosomal enzymes, e.g., cathepsins, which degrade demineralised collagen. Mammalian osteoclasts express on their surfaces many receptors for calcitonin, which is a potent inhibitor of osteoclast activity. On the other hand, the hormones and local factors which are capable of promoting bone resorption, e.g., parathyroid hormone (PTH), PTH-related protein, $1,25(OH)_2$ vitamin D_3, and cytokines, do so only indirectly by acting first on cells of the osteoblast lineage. This is illustrated conceptually in Figure 1. Promotion of resorption is the result of two mechanisms, one being the generation of new osteoclasts from precursors, and the other the activation of existing osteoclasts in bone. Both systemic factors and locally produced molecules make use of cells of the osteoblast lineage in promoting bone resorption. This seems to be the case with each of the two main pathways of resorption, that is, osteoclast activation and osteoclast formation.

TABLE I. Expression of Genes During the Process of Differentiation of Osteoblasts.

Early	Intermediate	Late
Pro-α(1)I collagen	Osteonectin	Osteocalcin
Growth hormone receptor	Alkaline phosphatase	
Bone sialoprotein	Osteopontin	
Biglycan	Matrix gla-protein	
Bone morphogenetic proteins		
Receptors for retinoic acid		

OSTEOCLAST ACTIVATION: IS THERE AN OSTEOBLAST-DERIVED OSTEOCLAST "ACTIVATOR"?

It has seemed logical to suggest that the activation of existing osteoclasts should be one im-

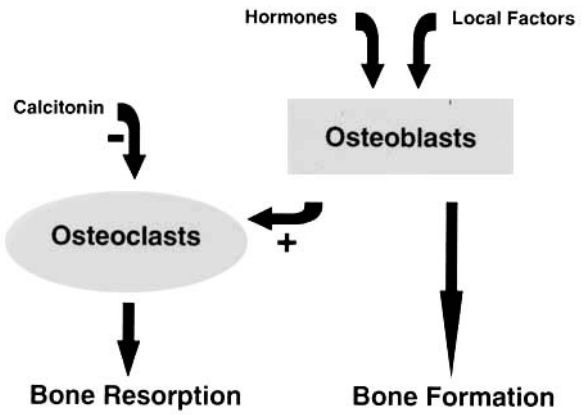

Fig. 1. In addition to being responsible for bone formation, cells of the osteoblast lineage mediate the actions of bone-resorbing hormones and cytokines.

portant mechanism by which bone resorption might be initiated. How important this is in vivo has never been established on a quantitative basis, although the injection of bone resorbing agents in vivo can lead to rapid (within 30 min) changes in the apparent state of activity of cells identifiable as osteoclasts. In fact the evidence in support of treatment-induced activation of osteoclasts relies heavily on in vitro studies.

The idea that bone resorbing agents must act indirectly stems from the observation that isolated osteoblasts of various origins were noted to respond to the bone resorbing hormones and to possess receptors for them, in the face of the lack of any evidence for receptors or direct responses to the hormones in osteoclasts. Thus the idea developed that the bone-resorbing hormones must act first on osteoblasts, most likely lining cells, which would then be responsible for the activation of existing osteoclasts, and probably also the generation of new osteoclasts from precursors [Martin et al., 1979; Rodan and Martin, 1981]. The same conclusion was reached by arguing that, since osteoclasts were most likely derived from a non-bone cell (of hemopoietic origin), it would be logical that their activities in bone should be directed by authentic bone cells, and it was proposed that osteoblasts should be responsible for this [Chambers, 1980].

At the time these views were formulated, methods did not exist to test the hypothesis, but in the next several years this was addressed in experiments using cells isolated from newborn rat bone and plated onto thin slices of cortical bone [Chambers and Fuller, 1985; Chambers, 1985; Thomson et al., 1986, 1987; Evely et al., 1991]. Resorption by the isolated osteoclasts was assessed by measuring the areas or numbers of resorption pits produced by the cells in response to treatment. When cells are isolated in this way from newborn rodent bone, the marrow is washed out and osteoclasts are removed from the endosteal surfaces. When plated onto thin wafers of bone they are inevitably mixed with a large excess of other cells, many of them osteoblasts and stromal cells. In order to prepare relatively highly purified osteoclast cultures, the period for cell adhesion is limited to a short time, 10 or 15 min, during which osteoclasts become firmly attached while many of the other cells can be removed by vigorous washing. When cultures such as this are treated overnight with bone-resorbing agents (e.g., PTH, IL-1, TNFα, etc.), no stimulation of resorption is detected [Chambers, 1985; Thomson et al., 1986, 1987; Evely et al., 1991]. These can be described as "functionally pure" cultures of osteoclasts. Alternatively, deliberate contamination of these cultures with osteoblasts can be achieved by allowing long settlement periods before washing, or by adding osteoblasts (or surrogate osteoblasts in the form of certain osteogenic sarcoma cells) to the cultures. In these conditions, the bone-resorbing agents are now able to stimulate resorption. Thus the presence of osteoblasts allows the resorbing agents to produce their effects, and so the concept has developed that the osteoblasts in these conditions produce a "resorption stimulator," which through some undefined mechanism is able to activate the osteoclasts on the surface.

The search for such a specific osteoclast stimulating factor has so far been unsuccessful. In the experience of some groups, activation of resorption by "functionally pure" cultures of osteoclasts (see above) could be produced by the transfer of conditioned medium from osteoblasts which had been stimulated by resorbing agents [McSheehy and Chambers, 1986; Collin et al., 1992]. This observation has not been sufficiently consistent to allow characterisation or purification of such an activity. Besides, it could be explained by the presence in the conditioned medium of growth-promoting activity capable of enhancing the numbers of non-osteoclasts in the cultures during the experimental period. This is particularly a possibility in the studies of Collin et al. [1992], in which the resorption-stimulating activity was released constitutively into cell supernatants, without the need for

stimulation of the cells by agents such as PTH. The counter argument is that the cultured osteoblasts produce a specific substance which acts directly upon the osteoclasts, allowing the "functionally pure" cultures to respond. A very labile osteoblast product might be responsible for such an activity, and this possibility is raised particularly by the observations of Gallwitz et al. [1993] that a 5-lipoxygenase product of arachidonic acid might achieve this.

Apart from the failure so far to identify a specific osteoclast stimulator likely to be common to all the known stimulators of bone resorption, a difficulty with these experiments is that it has not been rigorously established that the difference between resorption by "functionally pure" osteoclast cultures and "osteoblast-contaminated" cultures can be explained solely by osteoclast activation by a specific osteoblast product. It remains possible that the presence of a large number of osteoblasts provides a microenvironment in which the osteoclasts function more efficiently. Second, and perhaps more importantly, from the beginning of the culture period the osteoblasts and other accessory cells increase in number, so that what is a "functionally pure" culture at the beginning of an experiment is not so after a period of time, which would be likely to vary between laboratories and experimenters, using even slightly different cell preparation methods. This might be best illustrated by the data obtained from prolonged cultures of isolated rat osteoclasts, growing on bone for several days [Fenton et al., 1993]. The osteoblast numbers increased 3-fold from 24 h to 48 h, and cultures which were not responsive to PTH in the first 24 h became responsive thereafter. Furthermore the actual numbers of osteoclasts continued to increase beyond 24 h, consistent with the generation of new osteoclasts under the culture conditions. It is therefore possible, even in cultures of less than 24 h duration, that if sufficient numbers of osteoblasts and other accessory cells are provided, this could result in the generation of functional osteoclasts from a pool of precursors at various stages, present within the original isolate. At present there is nothing to exclude this possibility and therefore we question whether the isolated osteoclast assay, measuring resorption pits on bone slices, is genuinely a measure of osteoclast *activation,* or whether it might reflect alterations in osteoclast generation. If this were correct, the use of this experimental system would be of limited value, since it would essentially be measuring something similar to that being assessed in osteoclast formation systems such as mixed bone marrow cultures, but perhaps with even greater variability and difficulty of standardization.

If there is no specific osteoclast activator functioning as a common mediator of the effects of the many endocrine and paracrine promoters of bone resorption, how might osteoblasts contribute to osteoclast activation? One possibility is that they contribute only by promoting osteoclast formation, but that the osteoclast is generated as an active cell. This is perhaps unlikely because so many diverse functions of the osteoclast are necessary. These include the synthesis and release of lysosomal enzymes, acid production, synthesis of calcitonin receptor, and cell motility, any of which require regulation. These functions are so many and diverse that it seems more likely that many local products contribute to osteoclast activity, rather than that a single "activator" can orchestrate the osteoclast's resorptive capacity. One example of a complex property of the osteoclast is its motility, since as it resorbs it moves across the bone surface. We have proposed that the plasminogen activator (PA)-inhibitor system might contribute to this property as one of a number of possible functions of this serine protease system in bone, where its production and that of its inhibitors is very tightly regulated by a number of hormones and cytokines [Allan et al., 1990, 1991; Fukumoto et al., 1992; Martin et al., 1993a,b]. For example, inactive single chain urokinase (sc uPA) produced by osteoblasts [Fukumoto et al., 1992] could bind to uPA receptors on osteoclasts, and become activated, generating plasmin at those sites. The resulting pericellular proteolysis would contribute to cell motility, which would cease when the appropriate part of the cell reaches PAI-1, which is stored in the matrix. Plasminogen-dependent movement of the osteoclast has been demonstrated in vitro [Grills et al., 1990]. Access of the motile osteoclast to the mineralized bone surface might in turn be dependent on osteoblast behaviour, but not necessarily upon a specific osteoblast product. The latter describes the concept of the trabecular lining cells serving to separate osteoclasts from the bone surface, but when they contract upon responding to a resorbing agent, they provide access to the mineral surface by the osteoclasts, which then begin to resorb [Jones and Boyde, 1976; Rodan and Martin, 1981].

If osteoblast activation of osteoclasts results from the sum of many events in the microenvironment rather than from the action of a single "activator," how many of these events are essential? It is of interest to note the evidence of Sahni et al. [1993] that brief treatment of osteoblasts with bisphosphonates results in reduced ability of the osteoblasts to activate resorption in the isolated osteoclast assay with resorption pit measurements. This might be consistent with there being relatively few processes involved in the osteoblast-osteoclast interactions under these conditions. An alternative explanation, at least as interesting, would be that the bisphosphonates induce production of a resorption inhibitor by the osteoblasts. However the question remains unanswered yet, and requires more precise analysis of the behaviour of mature osteoclasts.

OSTEOCLAST FORMATION: IS THERE A SPECIFIC OSTEOCLAST-PROMOTING FACTOR PRODUCED BY THE OSTEOBLAST LINEAGE?

There is little doubt that the formation of new osteoclasts from precursors is an important part of the response to bone-resorbing hormones.

Several in vitro systems have provided strong evidence that accessory cells are necessary for the generation of osteoclasts from hemopoietic precursors. Fetal mouse bone rudiments containing no osteoclast progenitors, when co-cultured with murine bone marrow cells, provoked differentiation into bone-resorbing osteoclasts [Burger et al., 1982]. Similar conclusions were reached from studies in which the same type of fetal bone rudiments were co-cultured with hemopoietic stem cells either of mouse bone marrow origin [Scheven et al., 1986] or from IL-3-dependent hemopoietic stem cell lines [Hagenaars et al., 1989].

The development of mouse bone marrow culture systems which allowed reproducible assay of osteoclast-forming capability has added greatly to understanding of the process of osteoclast development [Takahashi et al., 1988a,b; Shinar et al., 1990; Suda et al., 1992]. The conclusion from these experiments is that the recruitment of osteoclasts from precursors is an indirect effect mediated by cells of the osteoblast lineage and perhaps by other cells of the bone marrow stroma.

In studying osteoclast formation in cultures of mouse bone marrow, Takahashi et al. [1988c] noted that osteoclast formation took place in close apposition to clusters of osteoblast-like cells, suggesting that the latter were contributing to osteoclast formation. This led to experiments in which spleen cells were used as a source of osteoclasts in co-cultures with osteoblasts, which they were able to do provided that the two cell types were grown on the same surface [Takahashi et al., 1988a]. In these experiments the cells needed to be living, since fixed osteoblasts were ineffective in mediating osteoclast formation. The ability to support osteoclast formation in co-cultures with hemopoietic cells was not confined to osteoblasts, but certain bone marrow-derived stromal cells were also effective [Udagawa et al., 1989; Yamashita et al., 1990]. The requirement for co-culture on the same surface, with no osteoclasts formed if the two cell types were separated by filters, strongly implied that cell-cell contact is necessary for the promotion of osteoclast formation by osteoblasts or stromal cells [see also Akatsu et al., 1991]. Such contact might be necessary to allow the action of a membrane-associated molecule capable of promoting osteoclast formation. This is the explanation we favour, although it is also possible that the matrix, by trapping cytokines and serving as a reservoir for them, might mediate these events.

An essential feature of such experiments has been the use of stringent criteria for the identification and quantitation of osteoclasts in cultures. The criteria necessary are TRAP activity, calcitonin receptors (by receptor autoradiography), and the ability to form resorption pits on slices of bone or dentine [Takahashi et al., 1988b,c; Hattersley and Chambers, 1989; Shinar et al., 1990]. Anything less than this is open to criticism because giant polykarya can form under these culture conditions, and TRAP staining alone does not distinguish such cells from osteoclasts.

HORMONES AND CYTOKINES, INCLUDING CSFS, IN THE REGULATION OF OSTEOCLAST FORMATION

The systemic hormones, PTH (and PTHrP) and 1,25$(OH)_2$ vitamin D_3, have been thoroughly studied as stimulators of osteoclast formation in bone marrow cultures and in co-cultures of osteoblast/stromal cells with hemopoietic cells [Suda et al., 1992]. In addition to systemic factors, however, the bone marrow microenvironment clearly plays an essential role as a source of cytokines which are powerful

stimulators of osteoclast formation, and this must surely be significant for the bone remodelling process. Cytokines are multifunctional peptides that regulate cell growth and differentiation. Unlike hormones they are produced locally from diverse sources, acting as paracrine or autocrine regulators. They are released rapidly, possess relatively unstable mRNAs, and their actions are typified by intricate interactive networks involving more than one cytokine, that serve to amplify responses. Cytokines relevant to bone cell function are IL-1, IL-6, IL-11, TNFα, TNFβ, LIF, M-CSF, GM-CSF, and IL-3. Of these, IL-1, IL-6, and TNFα are products of the monocyte-macrophage series, but they are also produced by stromal cells and osteoblasts. The latter are also the cells of origin for M-CSF, GM-CSF, LIF, and IL-11. Cytokine production by bone cells may be enhanced by other cytokines. For example, IL-6 production is enhanced by IL-1 and TNFα, and LIF production enhanced by TNFα. Conversely, production of these cytokines may be inhibited by glucocorticoids or by estrogen.

These cytokines play important roles in the formation of osteoclasts. For example, a mutation in the coding region of the M-CSF gene in the mouse impairs ability to form multinucleated osteoclasts, resulting in one variant of murine osteopetrosis, the *op/op* mouse [Felix et al., 1990; Yoshida et al., 1990]. It is not yet clear exactly how M-CSF acts in osteoclast development, but it appears to play a role in both proliferation and differentiation of osteoclast progenitors [Takahashi et al., 1991; Tanaka et al., 1993]. On the other hand, M-CSF has been found to inhibit the bone resorbing activity of isolated osteoclasts [Hattersley et al., 1988], and osteoclasts have been found to be rich in M-CSF receptors [Hofstetter et al., 1992]. As is the case with M-CSF, both GM-CSF and IL-3 reduce bone resorption in organ culture [Lorenzo et al., 1987]. All three cytokines inhibit the generation of osteoclasts in mouse bone marrow cultures. However, if marrow hemopoietic cells are pretreated with CSFs before co-culture with osteoblast/stromal cells and 1,25(OH) D, each of the CSFs enhances osteoclast formation, with M-CSF the most effective [Takahashi et al., 1991]. The conclusion from these various observations is that M-CSF, GM-CSF, and IL-3 secreted by cells in the bone marrow (stromal-osteoblasts) contribute to the development of osteoclast-like cells by enhancing proliferation of precursors. In the case of M-CSF, this is also necessary for the differentiation of osteoclasts later in the development pathway.

However none of these hemopoietic growth factors fulfils criteria which would be expected of one which is specific for osteoclast formation. The claim has been made that an "osteoclast colony-stimulating factor" has been identified and isolated [Lee et al., 1991]. However the biological assay used in that isolation work was the mixed marrow culture system (containing both stromal and hemopoietic elements). Therefore the material isolated had no actions which distinguished it from several cytokines and hormones capable of promoting osteoclast formation with the mediation of stromal cells/osteoblasts. No convincing evidence was produced in that or in subsequent work from the same group that the isolated factor could promote authentic osteoclast formation from purely hemopoietic cells. Lee et al. [1992] showed that the activity which they had isolated promoted formation of TRAP-positive cells from bone marrow cells cultured in Bacto agar, as did IL-3 and stem cell factor.

The results are similar to those of Kurihara et al. [1989], using spleen cells from 5-FU-treated mice. On the other hand, when strict criteria for osteoclast identification were used, none of the CSFs were able to induce osteoclast differentiation in semi-solid cultures of mouse bone marrow cells [Takahashi et al., 1991]. Furthermore, Chambers et al. [1993] have established a number of osteoclastogenic cell lines from the *H*-2K^{b}tsA58 transgenic mouse, but the osteoclastogenesis with these cell lines still required the presence of stromal cells and 1,25(OH)D.

The stromal cell-hemopoietic cell co-culture data provides the strongest evidence for the existence of such a factor. This indicates the existence of a contact-dependent process, which may be a stromal cell membrane molecule requiring contact between the stromal cell and a hemopoietic precursor which has been primed by exposure to the hemopoietic growth factors, M-CSF, GM-CSF, and IL-3. This hypothetical substance has been termed "stromal osteoclast forming activity" [Chambers et al., 1993], or "osteoclast differentiation factor" [Suda et al., 1992]. Although involvement of matrix factors is not excluded, we favour the idea that such a molecule exists [Suda et al., 1992], even though some workers have argued that osteoblastic stromal cells are not required for osteoclast differen-

tiation [Kurihara et al., 1989, 1990]. Much of the lack of agreement is in the interpretation of the data. In experiments in which very strict criteria of osteoclast identification have been applied, the need for stromal/osteoblast participation is convincing. This is not so when a single criterion is used, as in the case of TRAP staining in the experiments of Lee et al. [1991, 1992], and insufficient attention paid to the resorption capabilities of the generated cells.

HOW HORMONAL AND CYTOKINE RESORPTION STIMULATORS WORK

The known stimulators of bone resorption fall into three main groups: PTH, PGE, and PTHrP, which act through cyclic AMP, $1,25(OH)_2$ vitamin D_3 acting as a steroid hormone, and the cytokines IL-1, TNFα, IL-6, and IL-11. In all the latter cases the signal transduction mechanisms are not well understood, but they are likely to be different from those employed by the cyclic AMP group and $1,25(OH)_2D_3$. In all these cases, osteoblastic stromal cells are needed for osteoclast formation to proceed from hemopoietic precursors, and it is possible that a common pathway for each of these agents is the membrane stromal factor capable of programming the final stages of osteoclast differentiation. Such a possibility is promulgated in Figure 2.

If that is indeed the case, we would expect these agents to converge in their actions at some points before invoking the stromal osteoclast-forming activity. Indeed there is increased evidence for sharing of pathways among these agents, highlighted recently by studies with IL-11. This is a recently discovered cytokine, a product of bone marrow stromal cells [Paul et al., 1990] which is a powerful promoter of osteoclast formation and bone resorption in co-cultures of osteoblasts and marrow cells [Tamura et al., 1993; Girasole et al., 1994]. What is most interesting and relevant to the present discussion is that both PTH and $1,25(OH)_2D_3$-stimulated osteoclastogenesis were inhibited completely by neutralizing antiserum against IL-11 [Girasole et al., 1994], and each of these hormones also increased IL-11 production in the cultures. Moreover IL-1 and TNFα effects were partially (50–70%) inhibited by the same antiserum. In addition, the IL-11 induction of osteoclast formation in these cultures is prevented by blockade of prostaglandin synthesis [Girasole et

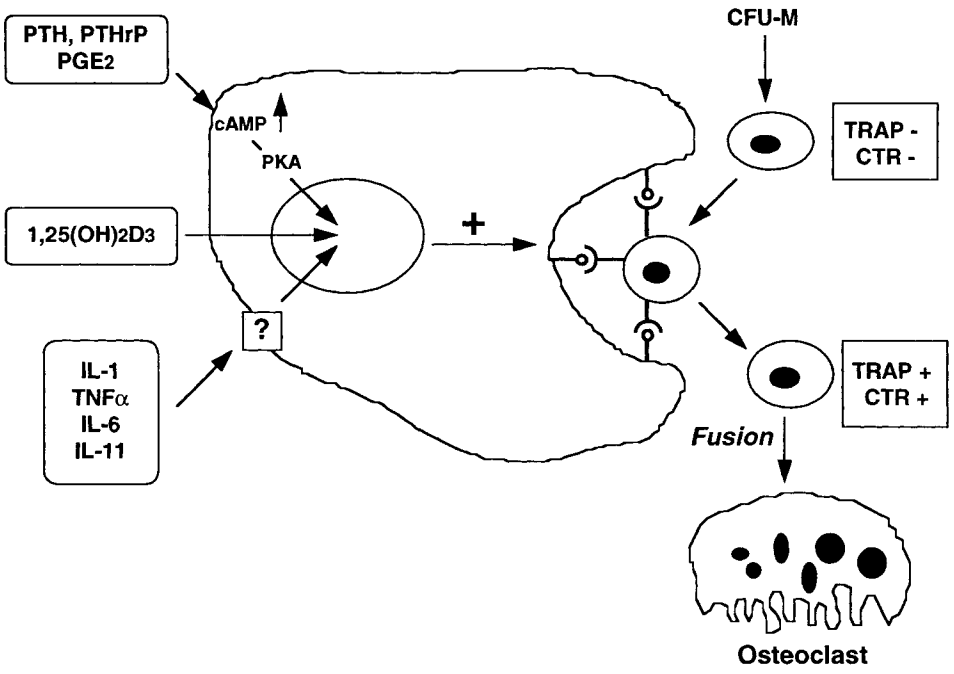

Fig. 2. Central role of the stromal osteoblast in enhancing osteoclast formation from hemopoietic precursors. This model proposes promotion of osteoclast formation by a factor associated with the stromal cell membrane. It is the common mediator of the effects of the three main classes of stimulators of osteoclast formation, one group acting through cyclic AMP and protein kinase A, one through a steroid hormone response pathway, and the cytokine stimulators with varied signalling pathways after interaction with specific cell-surface receptors.

al., 1994], as has been found to be so for $1,25(OH)_2D_3$ induction in similar cultures [Shinar and Rodan, 1990]. Thus IL-11 may occupy a central position in the control of osteoclast formation, a process which is influenced by many cytokines and hormones, and which is surely subject to much redundancy in the roles of these factors.

In the case of IL-6, also a product of stromal cells, the interactions are more complex. Recent studies have involved estrogen in regulating IL-6 production, and for the first time provide a possible explanation for estrogen's effect on bone resorption. Estrogen withdrawal has long been known to result in enhanced bone loss; this is the basis for post menopausal osteoporosis. Ovariectomy in the mouse results in increased osteoclast formation in marrow cultures from these animals [Kalu et al., 1990]. Of great interest is the fact that anti-IL-6 antibody prevents that increase, the data indicating a role for IL-6 in the promotion of osteoclast formation in the estrogen-deplete state [Jilka et al., 1992; Horowitz, 1993]. Interestingly, there is indirect evidence that in women following removal of the ovary there is an increased production of IL-1, TNFα, and GM-CSF by cultures of peripheral blood monocytes, and this can be reversed by estrogen replacement. Observations such as this, together with the finding in IL-6-deficient transgenic mice that these animals could maintain their bone mass after ovariectomy [Poli et al., 1994] all focus upon the importance of cytokines in cellular interactions in bone. The formation, actions, and interactions among those cytokines are complex and under the influence of circulating hormones. Unravelling these networks is a great challenge and a number of new approaches arise from these recent discoveries. Although characterization of the stromal osteoclast forming activity which has been discussed in this

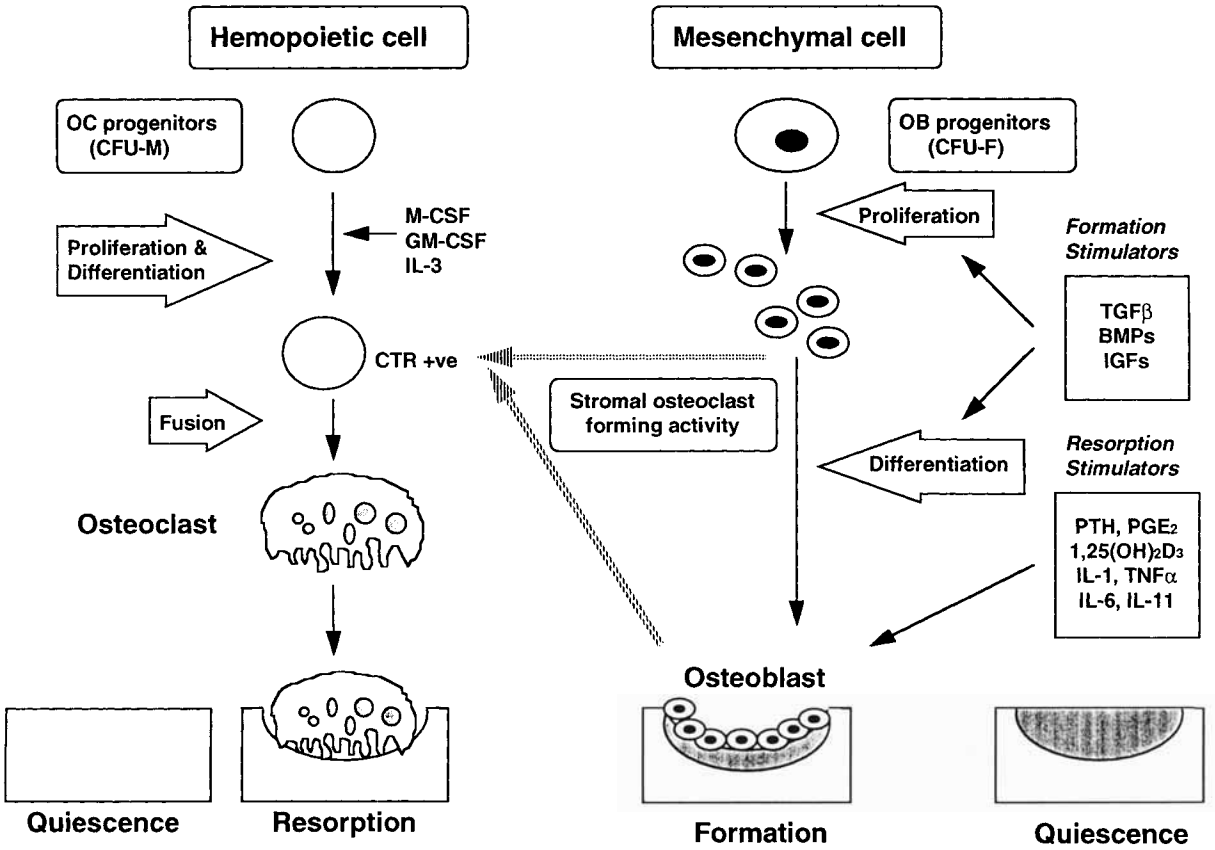

Fig. 3. Interactions between the osteoblast and osteoclast lineages during differentiation, highlighting the importance of locally generated control elements in the renewal of the osteoblast and osteoclast populations, and hence to the process of bone remodelling. Hemopoietic growth factors, the products of members of the osteoblast lineage, stimulate proliferation of hemopoietic precursors of osteoclasts. Cells at various stages of osteoblast differentiation are capable of promoting osteoclast differentiation, mediating the actions of the bone resorption stimulators. The growth factor stimulators of bone formation are also locally produced.

overview would be of major interest, it may not provide all the answers.

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