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Role of Vitamin D in Bone Resorption

Tatsuo Suda, Naoyuki Takahashi, and Etsuko Abe

Department of Biochemistry, School of Dentistry, Showa University, Tokyo 142, Japan

Abstract The idea that vitamin D must function at the bone site to promote bone mineralization has long existed since its discovery as an anti-rachitic agent. However, the definite evidence for this is still lacking. In contrast, much evidence has accumulated that $1\alpha,25(OH)_2D_3$ is involved in bone resorption. $1\alpha,25(OH)_2D_3$ tightly regulates differentiation of osteoclast progenitors into osteoclasts. Osteoclast progenitors have been thought to belong to the monocyte-macrophage lineage. $1\alpha,25(OH)_2D_3$ greatly stimulates differentiation and activation of mononuclear phagocytes. Recent reports have indicated that differentiation of mononuclear phagocytes into osteoclasts is strictly regulated by osteoblastic cells, the process of which is also stimulated by $1\alpha,25(OH)_2D_3$. In the differentiation of mononuclear phagocytes into osteoclasts, the target cells for $1\alpha,25(OH)_2D_3$ appear to be osteoblastic stromal cells. Osteoblastic cells produce several proteins such as BGP, MGP, osteopontin and the third component of complement (C3) in response to the vitamin. They appear to be somehow involved in osteoclast differentiation and functions. Thus, $1\alpha,25(OH)_2D_3$ seems to be involved in the differentiation of osteoclast progenitors into osteoclasts directly and also by an indirect mechanism involving osteoblastic cells. The precise role of osteoblastic cells in osteoclast development has to be elucidated in the future.

Key words: bone mineralization, anti-rachitic agent, osteoclast, osteoblast, mononuclear phagocytes

Vitamin D was discovered as an anti-rachitic agent to prevent the diseases of rickets and osteomalacia. In vitamin D deficiency, osteoblasts elaborate normal collagen molecules, but the collagen fibrils in bone fail to mineralize and are unable to carry out the structural role of the skeleton. Administration of vitamin D into rachitic animals and humans rapidly restores the mineralization of bone. Thus, the idea that vitamin D must function at the bone site to promote mineralization has long existed. However, the definite evidence for this is still lacking, though it has been reported that the calcium uptake [1], the number of receptors for insulin-like growth factor I (IGF-I) [2] and the synthesis of transforming growth factor β (TGFβ) [3] in osteoblastic cells are increased by $1\alpha,25$-dihydroxyvitamin D_3 [$1\alpha,25(OH)_2D_3$]. Instead, there is a strong concept that vitamin D is not required for bone mineralization if plasma calcium and phosphorus are maintained in a normal range. The latter concept suggests that the primary function of vitamin D is to elevate the plasma calcium and phosphorus to supersaturating levels by stimulating intestinal absorption of calcium and phosphorus, then to support mineralization of bone.

In contrast, much evidence has accumulated that $1\alpha,25(OH)_2D_3$ is involved in the mobilization of calcium from bone. Stimulation of osteoclastic bone resorption by $1\alpha,25(OH)_2D_3$ appears to occur by a mechanism involving osteoblasts. This corresponds with the preferential localization of $1\alpha,25(OH)_2D_3$ receptors in osteoblasts in bone. It is believed that osteoclasts lack $1\alpha,25(OH)_2D_3$ receptors. In this article, we want to review the recent progress in the understanding of the action of vitamin D in osteoclastic bone resorption.

STRUCTURES AND FUNCTIONS OF OSTEOCLASTS

Osteoclasts are multinucleated cells present only in bone. It is believed that osteoclast progenitors are of extra-skeletal origin, and they are recruited to bone via the blood stream. Osteoclast progenitors then proliferate and differentiate into mononuclear preosteoclasts, and fuse with each other to form multinucleated osteoclasts. Osteoclasts have a unique morphology

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Address reprint requests to Tatsuo Suda, Department of Biochemistry, School of Dentistry, Showa University, 1-5-8 Hatanodai, Shinagawa-Ku, Tokyo 142, Japan.

and function to resorb calcified bone by making resorption pits (Howship's lacunae). The most characteristic feature of osteoclasts is the presence of ruffled borders and clear zones (Fig. 1). The ruffled border is a complex structure of deeply infolded finger-like plasma membranes adjacent to the bone surface. The ruffled border is surrounded by a clear zone, which serves for the attachment of osteoclasts to the bone surface to maintain a microenvironment favorable for bone resorption. Osteoclasts have abundant vitronectin receptors, which appear to be involved in their attachment to bone [4]. Tartrate-resistant acid phosphatase (TRAP) has been widely used as a specific histochemical marker of osteoclasts in bone tissues, but its precise role in osteoclasts is not known. Calcitonin inhibits bone resorption by directly acting on preosteoclasts and osteoclasts to reduce their number and activity. The expression of calcitonin receptors is one of the most reliable markers of mammalian osteoclasts [5].

The resorbing area under the ruffled border is acidic, which favors dissolution of mineral (Fig. 1). Recently, it has been shown that the proton pump of the vacuolar H^+-ATPase type exists in the ruffled border membrane of osteoclasts [6]. In bone-resorbing osteoclasts, hydrogen ions are provided by carbonic anhydrase II, which catalyzes the hydration of CO_2 to H_2CO_3. Secretion of hydrogen ions by osteoclasts generates an equal amount of cytoplasmic base equivalents, principally as HCO_3^-. Osteoclasts have a chloride/bicarbonate exchanger, which normalizes the intracellular pH when osteoclasts resorb bone actively [7].

DISCOVERY OF BONE-RESORBING ACTIVITY OF VITAMIN D

In 1952, Carlsson [8] first reported that vitamin D functions in the process of calcium mobilization from calcified bone. He reached this conclusion using rats which developed severe hypocalcemia by maintaining on a vitamin D-deficient, low calcium diet for several weeks. Three days after a single administration of 2.5 μg (100 IU) of vitamin D_3 to these animals, plasma calcium rose from 5.0 to 8.5 mg/dl. Since there was very little calcium in the diet, it was thought that the calcium must have derived from bone,

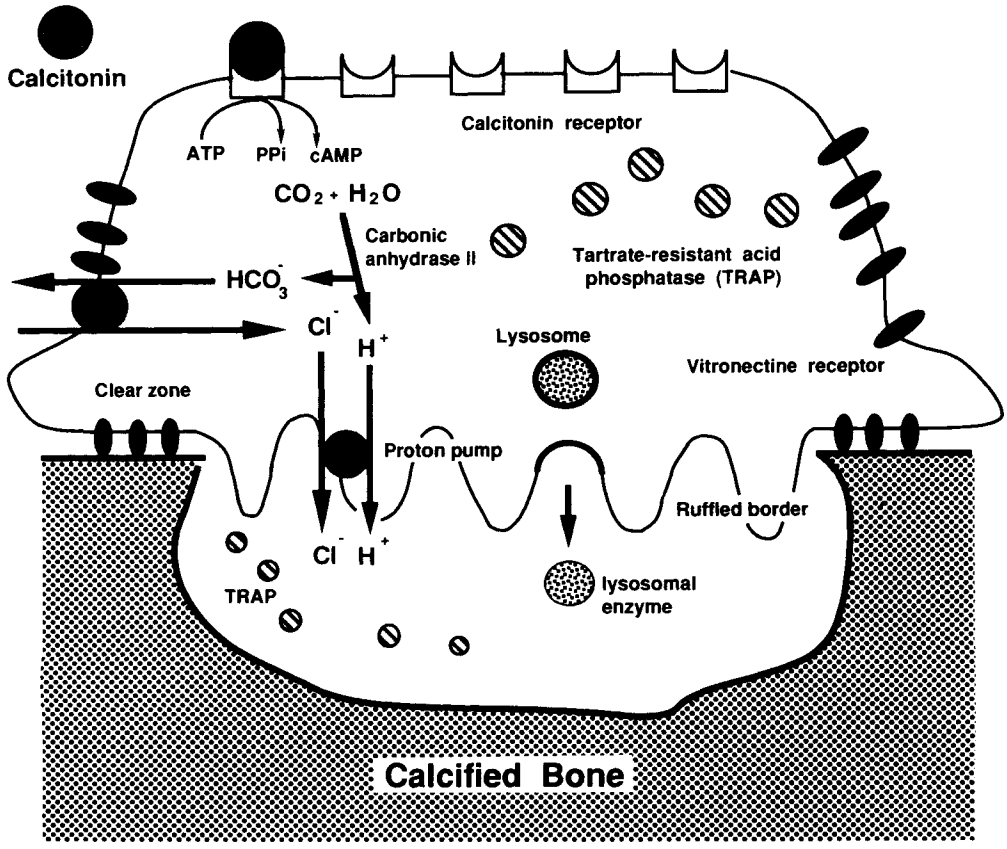

Fig. 1. A schematic representation of typical structures and functions of osteoclasts.

the only tissue to provide this calcium. In 1972, Raisz et al. [9] clearly demonstrated using organ cultures that the metabolite of vitamin D_3 responsible for bone resorption was indeed $1\alpha,25(OH)_2D_3$. The mechanism of action of $1\alpha,25(OH)_2D_3$ in bone resorption, however, was not known until quite recently.

$1\alpha,25(OH)_2D_3$ STIMULATES OSTEOCLAST-LIKE CELL FORMATION BY A MECHANISM INVOLVING OSTEOBLASTS

Several lines of evidence have indicated that osteoclasts are derived from cells of the monocyte-macrophage lineage. In 1981, we reported that $1\alpha,25(OH)_2D_3$ induces differentiation of mouse myeloid leukemia cells (Ml) into mature macrophages [10]. Subsequently, we demonstrated that $1\alpha,25(OH)_2D_3$ induces not only differentiation but also activation and fusion of some macrophages [11,12]. From these results, we hypothesized that $1\alpha,25(OH)_2D_3$ induces bone resorption by stimulating differentiation and fusion of osteoclast progenitors into osteoclasts.

In 1988, we developed an efficient mouse marrow culture system [13]. When marrow cells were cultured with $1\alpha,25(OH)_2D_3$, a number of TRAP-positive osteoclast-like cells were formed. They apparently satisfied major criteria of osteoclasts described in a previous section [13,14]. Interestingly, TRAP-positive multinucleated cells formed in mouse marrow cultures were negative for non-specific esterase (NSE), a marker of monocyte-macrophages, but some mononuclear cells were positive for both TRAP and NSE [13]. This is consistent with the possibility that osteoclasts are derived from cells of the mononuclear phagocyte family.

In the course of investigating osteoclast formation in mouse marrow cultures, we found that TRAP-positive osteoclast-like cells were formed mainly near the colonies of alkaline phosphatase (ALP)-positive cells (possibly osteoblastic cells), suggesting that osteoblastic cells are somehow involved in osteoclast formation. To examine this possibility, we developed a co-culture system, in which primary osteoblastic cell populations and spleen cells were co-cultured in the presence of $1\alpha,25(OH)_2D_3$ [15]. Many TRAP-positive mononuclear cells and multinucleated cells were formed only in the co-cultures in response to $1\alpha,25(OH)_2D_3$. No TRAP-positive cells appeared in separate cultures of either osteoblastic cells or spleen cells even in the presence of $1\alpha,25(OH)_2D_3$. These results confirm again that osteoblastic cells are required for the differentiation of osteoclast progenitors into osteoclasts.

The primary osteoblastic cell populations could be replaced by marrow-derived clonal stromal cells (MC3T3-G2/PA6 and ST2) [16] and bone-derived clonal osteoblastic cells (KS-4) [17] in co-cultures with spleen cells. Clonal stromal cells of non-bony origin (ST13, BALB-3T3 and NIH-3T3) could not support osteoclast differentiation in co-cultures with spleen cells. At present, it is difficult to identify the common phenotype in the above three positive clonal cells which could support osteoclast differentiation. It may be important to point out that most of the clonal stromal cells which can support osteoclast differentiation are endowed with the ability to support hemopoiesis in co-cultures with bone marrow cells [16]. This also may indicate that there is a common mechanism between hemopoiesis and osteoclast differentiation.

THE MECHANISM OF ACTION OF $1\alpha,25(OH)_2D_3$ IN OSTEOCLAST DIFFERENTIATION

For understanding the role of $1\alpha,25(OH)_2D_3$ in osteoclast differentiation, it is important to know that how its signal is transduced for osteoclast development. We reported that when spleen cells and either primary or clonal osteoblastic cells were co-cultured with each other but separated by a membrane filter, no osteoclast-like cells were formed even in the presence of $1\alpha,25(OH)_2D_3$ [15–17]. We also reported that osteoclast formation induced by PGs [18], PTH [13,19], PTHrP [19] and IL-1 [20] is similarly mediated by osteoblastic cells. Cyclic AMP-mediated signal transduction has been suggested to be commonly involved in the osteoclast development induced by those bone-resorbing agents. In contrast, $1\alpha,25(OH)_2D_3$ appears to induce osteoclast formation by a mechanism independent of cAMP. PTH stimulates both cAMP production and osteoclast-like cell formation in co-cultures with KS-4 cells [17], whereas PTH induces neither cAMP production nor osteoclast-like cell formation in co-cultures with ST-2 cells [16]. These results indicate that bone-resorbing agents act directly on osteoblastic stromal cells. Therefore, it is suggested that $1\alpha,25(OH)_2D_3$ and cAMP have to activate the same or an overlapping gene in osteoblastic cells in order to support osteoclast development (Fig. 2). Such a factor(s) produced by osteoblastic cells must be expressed on the plasma membrane of osteoblastic cells or embedded into bone

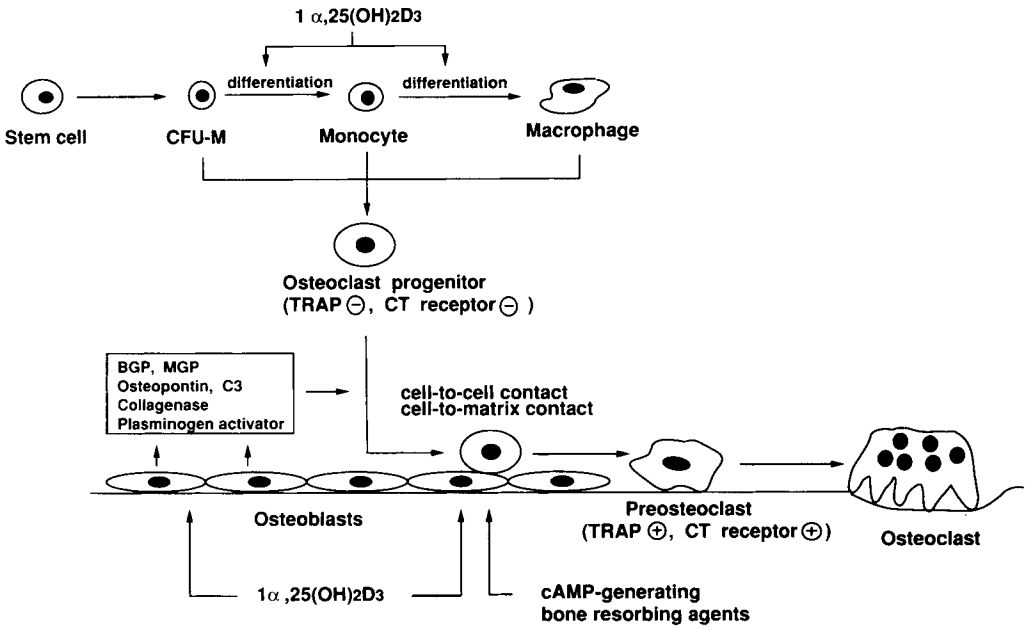

Fig. 2. A hypothetical view for the action of $1\alpha,25(OH)_2D_3$ in osteoclast differentiation.

matrix, and play a critical role in its recognition system through a cell-to-cell or cell-to-matrix contact mechanism. Isolation and identification of such a factor(s) will be one of the most important research projects in osteoclast biology.

Besides importance of direct interaction between osteoblastic cells and osteoclast progenitors, osteoblastic cells produce soluble factors involving osteoclast development. Macrophage colony-stimulating factor (M-CSF) is a soluble factor produced by osteoblastic cells which is essential for inducing osteoclast development. It is known that osteopetrotic *op/op* mice are devoid of osteoclasts congenitally. Wiktor-Jedrzejczak et al. [21] first reported the possibility that osteoclast deficiency in *op/op* mice is due to the failure of hemopoietic stromal cells to release M-CSF. Evidence for this was unequivocally proved by Yoshida et al. [22], who clearly demonstrated an extra thymidine insertion at base pair No. 262 in the coding region of M-CSF gene in *op/op* mice. This insertion generated a stop codon, TGA, 21 base pairs downstream. From these results, they concluded that the M-CSF gene of *op/op* mice cannot code the functionally active M-CSF protein. Administration of recombinant human M-CSF restored the impaired bone resorption of *op/op* mice in vivo [23]. We also showed that osteoclast deficiency in *op/op* mice is due to a defect in osteoblastic cells, but not in spleen cells [24]. The M-CSF produced by osteoblastic cells plays a critical role in osteoclast development. At present, there is no evidence that $1\alpha,25(OH)_2D_3$ regulates the production of M-CSF.

The finding that M-CSF is essential for osteoclast development indicates again that osteoclasts are derived from the monocyte-macrophage lineage. More recently, we demonstrated that alveolar macrophages preferentially differentiate into osteoclast-like multinucleated cells in response to $1\alpha,25(OH)_2D_3$, when they were co-cultured with ST2 cells [25]. No osteoclast-like cells were formed, when alveolar macrophages were cultured without ST2 cells even in the presence of $1\alpha,25(OH)_2D_3$. These results together with our previous findings that $1\alpha,25(OH)_2D_3$ stimulates differentiation and activation of mononuclear phagocytes indicate the importance of $1\alpha,25(OH)_2D_3$ in inducing osteoclast development in two different aspects: one is the stimulation of monocytic differentiation of hemopoietic immature cells, and the other is the commitment of the differentiation of mononuclear phagocytes into osteoclasts by a mechanism involving osteoblastic cells (Fig. 2). The $1\alpha,25(OH)_2D_3$ receptors present in mononuclear phagocytes may disappear during their differentiation into osteoclasts.

PROTEINS PRODUCED BY OSTEOBLASTIC CELLS IN RESPONSE TO $1\alpha,25(OH)_2D_3$

The evidence that $1\alpha,25(OH)_2D_3$ receptors are present in osteoblasts but not in osteoclasts

indicates that the major target cells for $1\alpha,25(OH)_2D_3$ in bone are osteoblasts. Indeed, $1\alpha,25(OH)_2D_3$ acts on cells of the osteoblast phenotype to produce several non-collagenous proteins.

The bone tissue contains two vitamin K-dependent calcium-binding proteins: bone Gla protein (BGP, osteocalcin) and matrix Gla protein (MGP). BGP is soluble in water and preferentially bound to the mineral phase, whereas MGP is insoluble in water and anchored to the matrix phase. Price and Baukol [26] first demonstrated that the osteoblast-like rat osteosarcoma cells, ROS 17/2.8, can produce BGP in response to $1\alpha,25(OH)_2D_3$. The promoter region of the rat BGP gene [27] and human BGP gene [28] contains a domain responsive to $1\alpha,25(OH)_2D_3$. It is suggested that BGP stimulates osteoclast development by chemotaxis of osteoclast progenitors or other unknown functions [29]. The synthesis of MGP has also been reported to be stimulated by $1\alpha,25(OH)_2D_3$ in another osteosarcoma cell line, UMR 106 [30]. Osteopontin (bone sialoprotein I) is a 44 kDa glycoprotein that contains the residues Arg(R)-Gly(G)-Asp(D), a sequence identical to the cell binding domain of fibronectin and several other cell-adhesion molecules [31]. An immunologic examination has revealed that osteopontin is present in bone matrix, osteoblasts and osteocytes [32]. The levels of osteopontin mRNA were markedly increased by $1\alpha,25(OH)_2D_3$ in osteoblastic cells such as ROS 17/2.8 [33] and MC3T3-El [34]. The promoter region of the human osteopontin gene has been reported to contain a domain responsive to $1\alpha,25(OH)_2D_3$ [35]. Since the vitronectin receptors expressed on the plasma membrane of osteoclasts recognize the R-G-D sequence, osteopontin may serve as an adhesion molecule between osteoclasts and bone.

Production and/or secretion of collagenase activity by osteoblastic cells are stimulated by $1\alpha,25(OH)_2D_3$ [36]. Also, osteoblastic cells produce tissue plasminogen activator, the activity of which is stimulated by $1\alpha,25(OH)_2D_3$ [37]. Plasminogen activator converts plasminogen to plasmin, which activates latent collagenase. These enzymes induced by **$1\alpha,25(OH)_2D_3$** are responsible for the removal of osteoid seams, which is essential for the initiation of bone resorption.

Very recently, we purified a 190 kDa protein from conditioned media of ST2 cell cultures treated with $1\alpha,25(OH)_2D_3$ and identified it as the third component of mouse complement (C3) [38]. Northern and western blot analysis revealed that the production of C3 by ST2 and primary osteoblastic cells was strictly dependent on $1\alpha,25(OH)_2D_3$, but the production by hepatocytes was not. Adding $1\alpha,25(OH)_2D_3$ together with anti-mouse C3 antibody to bone marrow cultures greatly inhibited the formation of TRAP-positive osteoclast-like cells, though the addition of C3 alone induced no TRAP-positive cell formation [38]. These results indicate the possibilities that C3 is one of the factors produced by osteoblasts in response to $1\alpha,25(OH)_2D_3$, and that C3 is somehow involved in osteoclast differentiation with other $1\alpha,25(OH)_2D_3$-dependent factors.

It is important to determine whether the vitamin D-dependent production of C3 by osteoblastic cells is biologically relevant to bone metabolism. The circulating level of C3 in mice was about 1.5 mg/ml, which was at least 500- to 1,000-fold higher than that of the C3 concentration in the conditioned media of ST2 and primary osteoblastic cells treated with $1\alpha,25(OH)_2D_3$ for 3 days [39] (Fig. 3). However, when $1\alpha,25(OH)_2D_3$ was administered into vitamin D-deficient mice, the calvarial level of C3 greatly increased, whereas the serum level was not changed. This may indicate the validity of the old concept that there is a bone fluid compartment which is separated by the circulating blood stream [40].

CONCLUSIONS

$1\alpha,25(OH)_2D_3$ tightly regulates differentiation of osteoclast progenitors into osteoclasts.

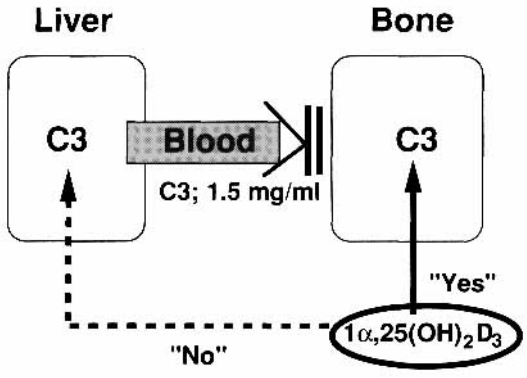

Fig. 3. A schematic representation of C3 production by osteoblasts and hepatocytes. The production of C3 by osteoblasts is dependent on $1\alpha,25(OH)_2D_3$, but that by hepatocytes is not. The bone C3 concentration is not influenced by the extremely high circulating levels of C3. This corresponds with the old idea that there is a bone fluid compartment separated by the circulating blood stream.

Differentiation and activation of mononuclear phagocytes (possibly osteoclast progenitors) are greatly stimulated by $1\alpha,25(OH)_2D_3$. Differentiation of mononuclear phagocytes into osteoclasts is also stimulated by $1\alpha,25(OH)_2D_3$ by a mechanism involving osteoblastic cells. In the latter differentiation, the target cells for $1\alpha,25(OH)_2D_3$ appear to be osteoblastic stromal cells, which produce BGP, MGP, osteopontin, C3, and probably an unidentified osteoclast differentiation-inducing factor(s) in response to the vitamin. These proteins appear to be somehow involved in osteoclast differentiation. Further studies are needed to elucidate the precise role of these proteins in osteoclast differentiation. Isolation of an osteoclast differentiation-inducing factor(s) induced by $1\alpha,25(OH)_2D_3$ is one of the most important research projects in osteoclast biology. Elucidation of the underlying mechanism of signal transduction induced by $1\alpha,25(OH)_2D_3$ will throw a light on the unsolved problems in the skeletal action of vitamin D.

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