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THE ROLE OF VITAMIN D IN BONE AND INTESTINAL CELL DIFFERENTIATION

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INTRODUCTION

Vitamin D_3 is well known to be metabolized first in the liver to 25-hydroxyvitamin D_3 [25(OH)D_3] and subsequently in the kidney to 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$] or 24,25-dihydroxyvitamin D_3 [24,25$(OH)_2D_3$] before it functions in the two major target organs of vitamin D: intestine and bone. Of these two dihydroxy metabolites, 1α,25$(OH)_2D_3$ is the active form of vitamin D_3. 1α,25$(OH)_2D_3$ binds to a receptor in the target cells and causes transcription of the specific genes that code for several

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proteins including a calcium-binding protein called *calbindin-D*. Calbindin-D is somehow involved in the intracellular transport of calcium. Thus, $1\alpha,25(OH)_2D_3$ is a steroid hormone that is produced by the kidney and regulates calcium metabolism (5).

Pike et al (30) first isolated the $1\alpha,25(OH)_2D_3$ receptor from chick intestine. The molecular weight of the receptor protein was 60 kd. The $1\alpha,25(OH)_2D_3$-binding domain was located at the *C*-terminal region and the DNA-binding domain at the *N*-terminal region. Like other steroid hormone receptors, the DNA-binding domain had two zinc-binding fingers. The DNA-binding domains of the respective steroid hormone receptors were highly homologous (10). Very recently, Mader et al (20) pointed out the importance of the *N*-terminal zinc-finger portion in the DNA-binding domain. All of the receptors had four cysteines in each zinc-finger portion bound to the zinc atom. They also reported that steroid hormone receptors could be classified into two groups; the receptors for progesterone, mineralocorticoids, androgen, and glucocorticoids belonged to the first group, and those for estrogen, the vitamin A metabolite retinoic acid, thyroid hormone T_3, and $1\alpha,25(OH)_2D_3$ belonged to the second group (20). The researchers reported the particular importance of the three specific amino acids for the activation of the target genes of the respective hormones. Significantly those three amino acids are exactly the same in the retinoic acid, thyroid hormone, and $1\alpha,25(OH)_2D_3$ receptors. These similarities suggest that vitamin D acts in a way similar to vitamin A and thyroid hormone.

Abe et al (1) made a breakthrough in studies on the $1\alpha,25(OH)_2D_3$ action by clearly demonstrating that $1\alpha,25(OH)_2D_3$ induces differentiation of mouse myeloid leukemia cells (M1) into monocyte-macrophages. We (46) extended this original observation to other types of cells, focussing in particular on hematopoietic cells, cancer cells, and skin epidermal cells. We also found that $1\alpha,25(OH)_2D_3$ prevented tumor promotion induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) and mezerein in a mouse skin chemical carcinogenesis system (4). The results gathered over the past several years suggest that $1\alpha,25\text{-}(OH)_2D_3$ is not only a regulator of mineral homeostasis but also a biological response modifier in immune responses and carcinogenesis systems. Like retinoic acid, $1\alpha,25(OH)_2D_3$ influences the proliferation and differentiation of several normal and tumor cells, and like the glucocorticoids and estrogens, $1\alpha,25(OH)_2D_3$ has immunoregulatory activities.

In fact, several investigators have found abnormalities of the immunohematopoietic system in children with rickets (44). Rickets caused by vitamin D deficiency is often associated with an increased frequency of infections and impaired phagocytosis by neutrophils (53). Administration of vitamin D corrects these irregularities. Nevertheless, vitamin D deficiency or $1\alpha,25(OH)_2D_3$ receptor defects do not usually cause clear deficiencies of the

hematopoietic-immune system in vivo. This finding suggests that $1\alpha,25(OH)_2D_3$ is not absolutely necessary for maintaining the hematopoietic-immune system normally but may cooperate with other growth factors and systemic hormones in the regulation of hematopoiesis and immune responses.

In contrast, the absolute importance of $1\alpha,25(OH)_2D_3$ for the maintenance of mineral homeostasis is established. In vitamin D deficiency or $1\alpha,25(OH)_2D_3$ receptor defects, disorders of mineral and bone metabolism dominate the clinical picture. In this review, we show that the ability of $1\alpha,25(OH)_2D_3$ to induce cell differentiation is closely linked to its classic functions in regulating calcium metabolism in the two major target organs of vitamin D: intestine and bone.

THE ROLE OF VITAMIN D IN INTESTINAL CELL DIFFERENTIATION

Intestinal mucosal cells have a relatively short life span. They originate in progenitor cells present in the crypt region of the villi and differentiate and migrate toward the villus tips, where they are extruded into the lumen within 4 to 5 days (Figure 1) (16). The villus length of vitamin D–deficient rats and chicks is only 70–80% of that in vitamin D–supplemented animals, and the administration of vitamin D into vitamin D–deficient animals elongates the

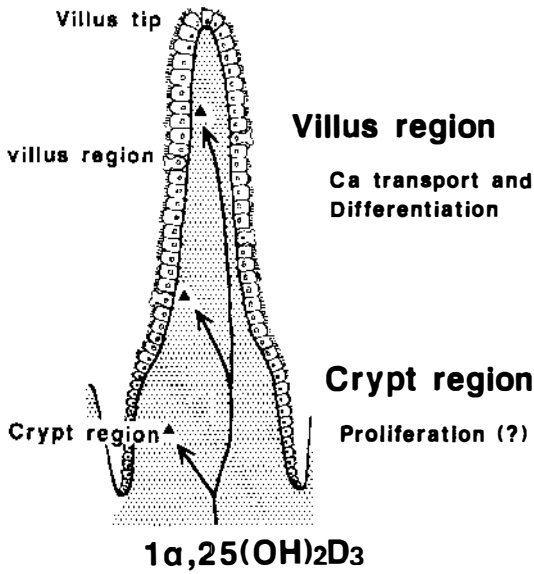

Figure 1 A schematic representation of the intestinal villus and the possible sites of action of $1\alpha,25(OH)_2D_3$.

villus length to a normal range. This finding suggested that vitamin D is somehow involved in the regulation of growth and differentiation of intestinal mucosal cells (3, 42), but only quite recently was the biochemical mechanism of vitamin D in regulating the growth and differentiation of intestinal mucosal cells known (38).

Polyamines are ubiquitous polycationic compounds of low molecular weights. The compounds are involved in cell proliferation and differentiation in many tissues (28). Figure 2 illustrates the pathways of polyamine metabolism. Three major polyamines (putrescine, spermidine, and spermine) are synthesized sequentially from ornithine (29). First, ornithine is decarboxylated to putrescine by ornithine decarboxylase (ODC), a rate-limiting enzyme of polyamine biosynthesis. This reaction is specifically inhibited by α-difluoromethylornithine (DFMO). S-Adenosylmethionine is similarly decarboxylated by S-adenosylmethionine decarboxylase (SAMDC) to form decarboxylated S-adenosylmethionine. The propylamino group of decarboxylated S-adenosylmethionine is transferred to putrescine to form spermidine. Spermine is similarly formed from spermidine by adding another propylamino group of the decarboxylated S-adenosylmethionine. The transfer of the propylamino group to putrescine and spermidine is catalyzed respectively by spermidine and spermine synthases. Methylglyoxal bis(guanylhydrazone) (MGBG) is a potent inhibitor of SAMDC, which suppresses spermidine and spermine synthesis.

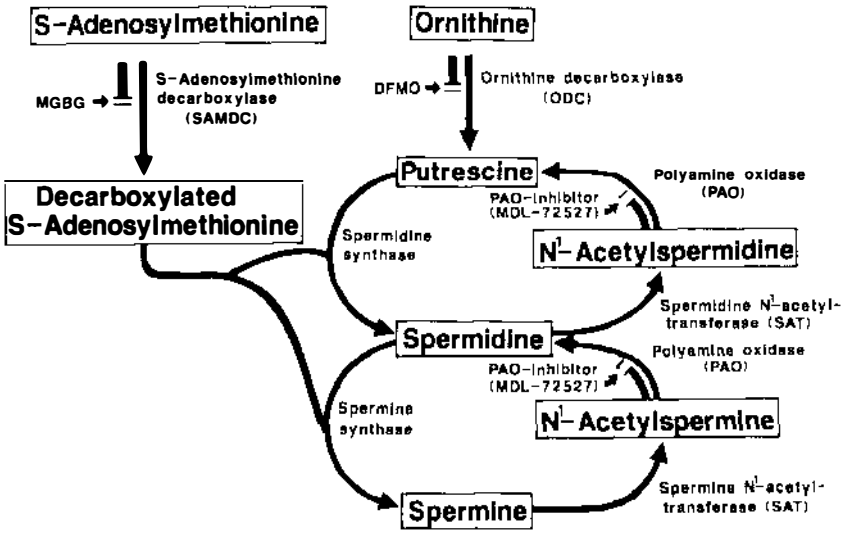

Figure 2 The pathways of polyamine metabolism and the sites where its inhibitors (DFMO, MGBG, and MDL-72527) act. DFMO = α-difluoromethylornithine, MGBG = methylglyoxal bis(guanylhydrazone), MDL-72527 = N^1, N^4-bis(2,3-butadienyl)-1,4-butanediamine.

In addition to the sequential metabolism of polyamines, several research groups have proved recently that there is a metabolic pathway of the higher polyamines (spermidine and spermine) back into putrescine (9a, 10a, 21a, 29). The enzymatic basis for such a reversal of pathway has now been established. It involves two sequential reactions by spermidine/spermine N^1-acetyltransferase (SAT) and polyamine oxidase (PAO) (Figure 2). SAT is a rate-limiting enzyme for the reversal pathway from spermidine or spermine back into putrescine. PAO catalyzes the conversion of acetylated spermidine or spermine into lower polyamines. PAO is specifically inhibited by N^1,N^4-bis(2,3-butadienyl)-1,4-butanediamine (MDL-72527). No specific inhibitor of SAT is currently known.

Vitamin D reportedly elongates the intestinal villus and accelerates the enterocyte migration in both vitamin D–deficient chicks and rats (3, 42). For the past few years, we have been studying the mechanism by which $1\alpha,25(OH)_2D_3$ elongates the villus. The rapidly proliferating and maturing intestinal villus mucosa provides a good model for studying the role of vitamin D on polyamine metabolism. In a previous report (49), we demonstrated that the receptor specifically bound to $1\alpha,25(OH)_2D_3$ was similarly distributed along the duodenal villus from the crypt region to the villus tip (Figure 1). The duodenal activity of ODC and the tissue content of putrescine increased greatly after a single intravenous injection of $1\alpha,25(OH)_2D_3$ into vitamin D–deficient chicks (40). Administration of $1\alpha,25(OH)_2D_3$ also greatly enhanced the duodenal activity of SAT (39). This result suggests that vitamin D regulates both the classic sequential pathway and the reversal pathway of polyamine metabolism.

We examined the time course of change in the duodenal activities of ODC and SAT and the duodenal content of putrescine after a single injection of $1\alpha,25(OH)_2D_3$ into vitamin D–deficient chicks. A significant increase in the duodenal SAT activity occurred as early as 30 min after the $1\alpha,25(OH)_2D_3$ injection. The SAT activity reached a maximum at 2 h, then gradually declined. In contrast, the duodenal ODC activity did not increase until 2 h after the $1\alpha,25(OH)_2D_3$ injection. The ODC activity attained a maximum at 6 h, then gradually declined. Obviously the induction by $1\alpha,25(OH)_2D_3$ of the SAT activity preceded that of the ODC activity in the chick duodenum. The duodenal content of putrescine changed in parallel with the ODC activity but not with the SAT activity. Of the various metabolites of vitamin D_3 examined, only $1\alpha,25(OH)_2D_3$ increased the duodenal ODC and SAT activities, at a dose level of 0.625 μg. The dose level of $1\alpha,25(OH)_2D_3$ required to induce 50% of the maximal increase in the duodenal SAT activity was only one tenth that required to induce the ODC activity. The SAT activity was induced by $1\alpha,25(OH)_2D_3$ in many vitamin D target organs such as the duodenum, kidney, pancreas, and the bursa of fabricius but not in the liver. The degree of the stimulation of the SAT activity was the greatest in the

duodenum. In contrast, the induction of the ODC activity by $1\alpha,25(OH)_2D_3$ occurred only in the intestine.

Steeves & Lawson (43) reported that besides ODC activity, $1\alpha,25(OH)_2D_3$ enhanced SAMDC activity in chick duodenum. The SAMDC activity was increased by $1\alpha,25(OH)_2D_3$ in a biphasic manner with maximal responses at 1 and 6 h. SAMDC is a rate-limiting enzyme of spermidine and spermine synthesis. A possible explanation for the induction of SAMDC by $1\alpha,25(OH)_2D_3$ is that the increased putrescine content stimulated SAMDC activity, since putrescine is a potent inducer of the SAMDC activity.

In the previous report (38), we examined the effect of prior administration of DFMO on the increase in the duodenal content of polyamines induced by $1\alpha,25(OH)_2D_3$. Although prior administration of DFMO completely blocked the $1\alpha,25(OH)_2D_3$-induced ODC activity, it did not suppress the increase of putrescine concentration in the duodenum. In fact, the putrescine concentration increased linearly for up to 6 h after a single injection of $1\alpha,25(OH)_2D_3$, irrespective of the presence or absence of DFMO. In contrast, the duodenal spermidine content decreased after a single injection of $1\alpha,25(OH)_2D_3$, similarly in the presence or absence of DFMO. The duodenal spermine level did not vary after the $1\alpha,25(OH)_2D_3$ injection.

To further examine the intestinal polyamine metabolism induced by $1\alpha,25(OH)_2D_3$, we compared the relative contribution of ODC and SAT in the duodenal synthesis of putrescine induced by $1\alpha,25(OH)_2D_3$, by comparing the conversion of [^{3}H]ornithine into [^{3}H]putrescine with that of [^{14}C]spermidine into [^{14}C]putrescine using the in situ duodenal loop method (38). The amounts of putrescine generated from ornithine and spermidine were calculated from the conversion rate and the endogenous duodenal concentration of each substrate. The duodenal content of putrescine measured by high-pressure liquid chromatography was well correlated with the amount of putrescine synthesized from spermidine but not from ornithine. To confirm the relative importance of the interconversion in the $1\alpha,25(OH)_2D_3$-induced putrescine synthesis, we examined the effect of MDL-72527, an inhibitor of PAO, on the $1\alpha,25$-$(OH)_2D_3$-induced duodenal synthesis of putrescine (41). Prior administration of MDL-72527 completely suppressed the $1\alpha,25(OH)_2D_3$-induced duodenal synthesis of putrescine. The duodenal spermidine level decreased similarly in both groups of birds treated with $1\alpha,25(OH)_2D_3$ alone or with $1\alpha,25(OH)_2D_3$ plus MDL-72527. N^1-acetylspermidine accumulated only in the birds treated with $1\alpha,25(OH)_2D_3$ plus MDL-72527.

Figure 3 summarizes the pathway of duodenal synthesis of putrescine and the role of $1\alpha,25(OH)_2D_3$ in this process. $1\alpha,25(OH)_2D_3$ produces a marked increase in duodenal formation of putrescine by two different pathways, one from ornithine and the other from spermidine. The increase in duodenal

putrescine content coincided with the decrease in duodenal spermidine level. This result indicates that SAT has a larger role than ODC in the $1\alpha,25(OH)_2D_3$-induced synthesis of putrescine in duodenal tissues. The question then became why spermidine was not exhausted from the epithelial cells. To solve this problem, we examined the distribution of ODC and SAT along the villus mucosa. The duodenal epithelial cells were sequentially isolated by the nonenzymatic method of Weiser (52). In vitamin D–deficient chicks, the ODC activity was very low in the villus region and increased toward the crypt region (38). Six hours after a $1\alpha,25(OH)_2D_3$ injection, the ODC activity increased markedly in the entire villus region. In contrast, SAT activity was distributed in an opposite pattern. In vitamin D–deficient chicks, SAT activity was also very low, but it increased greatly, especially in the villus region, after a $1\alpha,25(OH)_2D_3$ injection.

Figure 4 shows the polyamine metabolism in intestinal epithelial cells. These cells are characterized by rapid cell turnover, and there is a typical gradient of differentiation from the crypt region to the villus tip. In the crypt cells, putrescine, spermidine, and spermine are synthesized sequentially from ornithine. During the differentiation and migration of the crypt cells toward the villus tip, polyamine metabolism changes considerably. In the villus cells, putrescine is thought to be generated mainly from spermidine. This interconversion of polyamine metabolism is supported by the reciprocal distribution of the ODC and SAT activities along the duodenal villus mucosa: the ODC activity is high in the crypt region and the SAT activity is high in the villus region. In the villus mucosa, the tissue content of spermidine is approximately 50 times higher than that of ornithine. Thus, spermidine is considered to be a more suitable substrate than ornithine for the putrescine synthesis in the villus region.

The question is the role of putrescine in modulating cell growth and differentiation in intestine. To address this issue, we administered DFMO

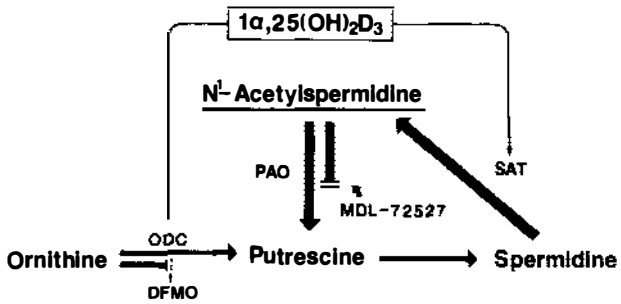

Figure 3 The pathways of putrescine synthesis induced by $1\alpha,25(OH)_2D_3$ in the chick intestine. Abbreviations: see Figure 2.

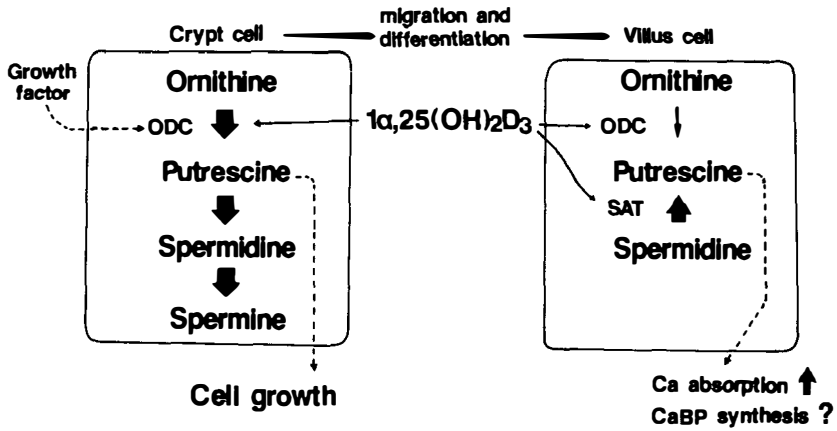

Figure 4 The differences in polyamine metabolism between the proliferative crypt cells and the differentiated villus cells in the intestinal villus mucosa. Abbreviations: see Figure 2; also: Ca = calcium, CaBP = vitamin D-dependent calcium binding proteins.

and MDL-72527 to one-day-old chicks to produce putrescine-depleted birds. MDL-72527 was given orally twice a day for four days. DFMO was given by dissolving it in drinking water. The birds were killed on day four, and the activity of intestinal calcium absorption and the villus length were measured. The average villus length in the control birds was 923 ± 28 μm. Administration of DFMO and MDL-72527 reduced the duodenal content of putrescine to 10% of the control level. It also reduced the duodenal villus length. The average length of the villi of putrescine-depleted birds was 604 ± 20 μm. The reduced villus length recovered to 853 ± 24 μm, about 90% of the control, as early as 12 h after putrescine was administered (unpublished results). The activity of duodenal calcium absorption was also reduced to 60% of the control level in chicks treated with DFMO and MDL-72527. Administering putrescine to birds given the two inhibitors restored the duodenal calcium absorption almost completely. These results clearly indicate that putrescine is involved in the development of intestinal villus mucosa and calcium absorption, and vitamin D acts to induce enzymes concerned with putrescine metabolism.

THE ROLE OF VITAMIN D IN BONE CELL DIFFERENTIATION

The effect of vitamin D on bone is extremely complicated. Vitamin D deficiency increases nonmineralized bone matrix (osteoid tissues), resulting in rickets in children and osteomalacia in adults. Administration of $1\alpha,25(OH)_2D_3$ to vitamin D–deficient animals stimulates osteoclastic bone

resorption, then cures rickets and osteomalacia. As described previously, $1\alpha,25(OH)_2D_3$ promotes the absorption of calcium and phosphorus across the intestinal epithelial cells. This metabolite also suppresses parathyroid hormone secretion, probably directly and also indirectly via an increase in serum calcium concentration. Whether $1\alpha,25(OH)_2D_3$ promotes osteoblastic bone mineralization directly or it acts on mineralization indirectly by providing sufficient calcium and phosphorus to newly formed bone matrix remains unclear. However, recently developed in vitro techniques and a number of cell lines established from bone tissues have allowed researchers to show that $1\alpha,25(OH)_2D_3$ regulates the function and differentiation of both osteoblasts and osteoclasts.

Osteoblasts

The evidence that the $1\alpha,25(OH)_2D_3$ 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 (23, 45). Indeed, $1\alpha,25(OH)_2D_3$ acts on the cells of the osteoblast phenotype to produce several noncollagenous proteins (Table 1). Bone tissues contain two vitamin K–dependent calcium-binding proteins, bone Gla protein (BGP, osteocalcin) (32) and matrix Gla protein (MGP) (33). BGP consists of 49 amino acid residues including 3 Gla residues, and MGP comprises 79 amino acid residues with 5 Gla residues. The similarity of the amino acid sequences between BGP and MGP indicates that BGP and MGP arose from a common gene ancestor. BGP is soluble in water and preferentially bound to the mineral phase (32), but MGP is insoluble in water and anchored to the matrix phase (33). Northern blot analysis of RNAs from a number of tissues in rats has revealed that BGP mRNA is found only in bone, a result that is consistent with the specific expression of BGP in bone, but MGP mRNA is found in almost all tissues examined (9). Price & Baukol (31) first demonstrated that the osteoblastlike rat osteosarcoma cells, ROS 17/2.8, can produce BGP in response to $1\alpha,25(OH)_2D_3$. The BGP concentration in serum and bone was markedly reduced in animals fed a vitamin D–deficient diet and increased after the administration of $1\alpha,25(OH)_2D_3$. This result suggests the importance of $1\alpha,25(OH)_2D_3$ in BGP synthesis in vivo (17). The promoter region of the rat BGP gene (56) and human BGP gene (12) contains a domain responsive to $1\alpha,25(OH)_2D_3$. The synthesis of MGP is also reported to be stimulated by $1\alpha,25(OH)_2D_3$ in another osteosarcoma cell line, UMR 106 (8).

Osteopontin (bone sialoprotein I) is a 44-kd glycoprotein that contains the residues Gly-Arg-Gly-Asp-Ser, a sequence identical to the cell-binding domain of fibronectin and several other cell-adhesion molecules (27). An immunologic examination revealed that osteopontin is present in bone matrix, osteoid, osteoblasts, and osteocytes (21). The levels of osteopontin mRNA

Table 1 Bone matrix proteins, the synthesis of which is regulated by $1\alpha,25(OH)_2D_3$

Proteins	Characteristics	Regulation by $1\alpha,25(OH)_2D_3$	Reference
Bone Gla protein (BGP, osteocalcin)	Water-soluble protein composed of 49 amino acid residues containing 3 Gla residues with calcium-binding capacity	↑	12, 16, 3
Matrix Gla protein (MGP)	Water-insoluble protein composed of 78 amino acid residues containing 5 Gla residues	↑	9, 33
Osteopontin (bone sialoprotein I)	44-kd glycoprotein containing the specific cell binding sequence -Gly-Arg-Gly-Asp-Ser-	↑	21, 25, 2
Type I collagen	Major structural protein composed of three individual polypeptides: two α1[I] and one α2	↑ ↓	2, 7, 15 14, 17, 3

were markedly increased by $1\alpha,25(OH)_2D_3$ in osteoblastic cells such as ROS 17/2.8 (55) and clonal mouse osteoblastic MC3T3-E1 cells (25). Although the physiologic role of BGP, MGP, and osteopontin in bone metabolism remains to be elucidated, the dependence of $1\alpha,25(OH)_2D_3$ on the synthesis of those matrix proteins suggests the importance of this hormone in the regulation of bone matrix formation.

The effect of $1\alpha,25(OH)_2D_3$ on bone collagen synthesis is controversial. In vitro studies using primary cell cultures from human trabecular bone (2), human osteosarcoma cells MG-63 (7), and clonal mouse osteoblastic cells MC3T3-E1 (15) have indicated that $1\alpha,25(OH)_2D_3$ increases incorporation of proline into collagenase-digestible proteins. In contrast, studies using organ cultures of rodent calvariae (34), isolated human osteoblastlike cells (54), and ROS 17/2.8 (14) have all indicated that $1\alpha,25(OH)_2D_3$ has catabolic effects on collagen synthesis in vitro. The promoter of the rat α1(I) collagen gene was recently isolated and sequenced. The α1(I) promoter contained a *cis*-active element regulated by $1\alpha,25(OH)_2D_3$ receptors in rat osteoblastic cells (18). These conflicting results may be explained by the dual functions of $1\alpha,25(OH)_2D_3$: stimulation of the differentiation of mesenchymal progenitor cells into osteoblasts and inhibition of mature osteoblast functions. Clearly, further studies are needed to evaluate the effects of $1\alpha,25(OH)_2D_3$ on the differentiation of mesenchymal cells into osteoblasts and the functions of mature osteoblasts, separately.

Osteoclasts

Osteoclasts are multinucleated cells with bone-resorbing activity. The origin of osteoclasts differs from that of osteoblasts. Various studies have established that the osteoclasts are formed by the differentiation and fusion of mononuclear osteoclast progenitors derived from hematopoietic stem cells (24). When $1\alpha,25(OH)_2D_3$ is administered to vitamin D–deficient animals, osteoclastic bone resorption is enhanced and the number of osteoclasts increases. Two different mechanisms induce osteoclastic bone resorption: the activation of quiescent osteoclasts and the recruitment of new osteoclasts.

With respect to osteoclast activation, McSheehy & Chambers (22) reported that in response to various systemic and local bone-resorbing factors including $1\alpha,25(OH)_2D_3$, osteoblastlike cells produce and release a factor that stimulates quiescent osteoclasts to induce bone resorption. $1\alpha,25(OH)_2D_3$ has no direct effect on the isolated osteoclasts, indicating that hormone activates osteoclasts through a mechanism involving osteoblastlike cells. The precise nature of this factor is not currently known.

The recent development of bone marrow culture systems has greatly contributed to our understanding of osteoclast differentiation. The formation of tartrate-resistant acid phosphatase (TRACP, a marker enzyme of osteoclasts)–positive multinucleated cells has been demonstrated in several marrow cultures of mice (50), felines (11), baboons (36), and humans (19). Bone-resorbing hormones such as $1\alpha,25(OH)_2D_3$ and parathyroid hormone stimulated osteoclastlike multinucleated cell formation, whereas calcitonin inhibited it in several marrow cultures. In mouse marrow cultures, TRACP-positive mononuclear and multinucleated cells showed specific binding of [^{125}I]-calcitonin. Calcitonin also stimulated cAMP production in parallel with the appearance of TRACP-positive multinucleated cells (47). Although the TRACP-positive multinucleated cells formed in mouse marrow cultures stained negative for nonspecific esterase (a marker enzyme of macrophages), some mononuclear cells stained positive for both TRACP and nonspecific esterase (37). This result indicates that osteoclasts arise from progenitor cells derived from the monocyte-macrophage family (Figure 5). When mouse marrow cells were cultured on dentine slices in the presence of $1\alpha,25$-$(OH)_2D_3$, multinucleated cells with ruffled borders and clear zones formed numerous resorption lacunae (37, 50). Thus, the multinucleated cells formed in mouse marrow cultures in response to $1\alpha,25(OH)_2D_3$ satisfy the major criteria for osteoclasts.

In mouse marrow cultures, TRACP-positive multinucleated cells were formed only near the clusters of alkaline phosphatase–positive stromal cells. This finding confirms the pioneering work of Rodan & Martin (35), who first suggested that osteoblastic cells are somehow involved in osteoclast formation. We also demonstrated that osteoclastlike multinucleated cells are formed

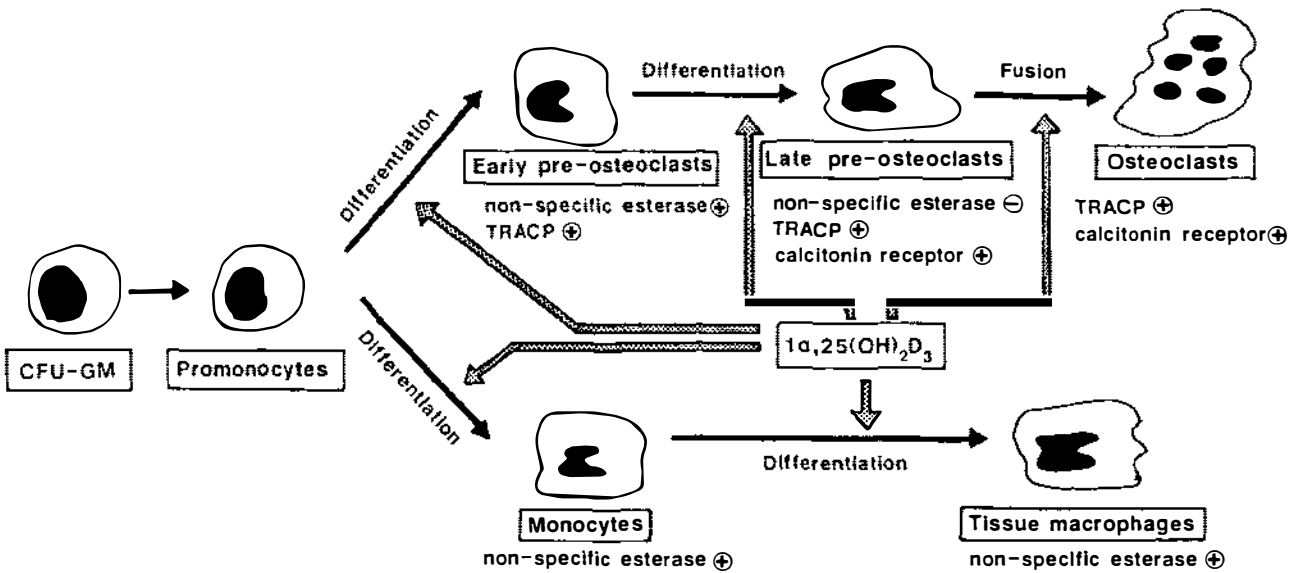

Figure 5 A hypothetical model for osteoclast differentiation and its regulation by $1\alpha,25(OH)_2D_3$. The granulocyte-macrophage colony-forming unit (CFU-GM) contains osteoclast progenitors. Promonocytes differentiate into early preosteoclasts, which express markers of both osteoclasts (TRACP) and monocyte-macrophages (nonspecific esterase). They differentiate into late preosteoclasts, which possess calcitonin receptors and lose monocyte markers. Late preosteoclasts then fuse to form multinucleated osteoclasts.

in cocultures of mouse spleen cells and osteoblastlike cells derived from mouse calvariae in the presence of $1\alpha,25(OH)_2D_3$ (48). Direct contact of spleen cells with osteoblastlike cells was essential for the formation of osteoclastlike multinucleated cells. No TRACP-positive cells appeared when spleen cells and osteoblastlike cells were cocultured separated from each other by a membrane filter (48).

More recently, we showed that two marrow-derived stromal cell lines, MC3T3-G2/PA6 and ST2, can be substituted for the primary osteoblastlike cells in inducing osteoclastlike multinucleated cells in the cocultures with spleen cells (51). The adherent stromal cell layer formed in long-term marrow cultures (Dexter culture) reportedly provides a suitable microenvironment for the growth and differentiation of hematopoietic progenitors (6). MC3T3-G2/PA6 and ST2 per se do not express the phenotypic properties of osteoblasts, but they do support the growth and differentiation of hematopoietic progenitors when cocultured with marrow cells (13, 26). MC3T3-G2/PA6 cells support the growth of hematopoietic stem cells through a mechanism of the cell-to-cell contact between them (13). Similarly, the direct contact of spleen cells with the stromal cells was required for the differentiation of osteoclast precursors into TRACP-positive mononuclear and multinucleated cells (48). Therefore, either direct contact of osteoclast precursors (possibly the monocyte-macrophage family) with marrow-derived stromal cells or a closed microenvironment formed by the two types of the cells is probably necessary for osteoclast development (Figure 6). $1\alpha,25(OH)_2D_3$ appears to have an

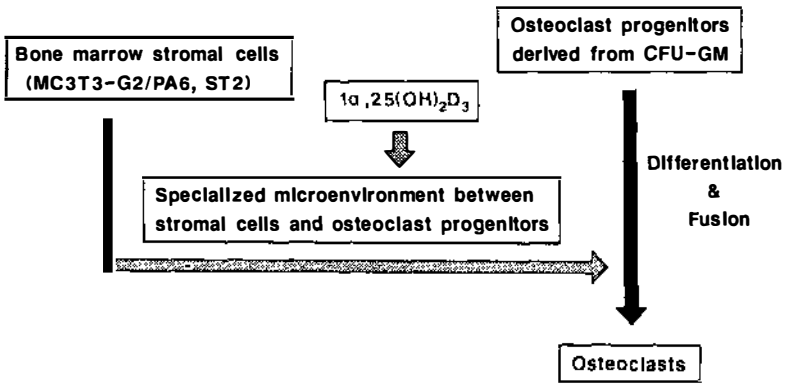

Figure 6 A hypothetical model for the role of marrow-derived stromal cells in osteoclast formation. Osteoclast progenitors derived from the granulocyte-macrophage colony-forming unit (CFU-GM) differentiate into multinucleated osteoclasts under the control of marrow-derived stromal cells. The specialized microenvironment between the marrow-derived stromal cells and the osteoclast progenitors is necessary for osteoclast formation. $1\alpha,25(OH)_2D_3$ probably provides a suitable microenvironment to support osteoclast differentiation.

important role in maintaining such a microenvironment, which supports osteoclast differentiation. This hypothesis is also supported by the fact that no osteoclastlike cells were formed in a separate culture of either spleen cells or peripheral blood mononuclear cells that contained no marrow-derived stromal cells (50).

The conclusion is, therefore, that $1\alpha,25(OH)_2D_3$ induces osteoclastic bone resorption through both activation and recruitment of osteoclasts. The former process is mediated by a factor produced by osteoblasts and the latter through a mechanism involving marrow-derived stromal cells. Further studies should help clarify how these stromal cells form the microenvironment suitable for osteoclast formation and identify the target cells of $1\alpha,25(OH)_2D_3$ (osteoclast precursors, stromal cells, or both) in osteoclast differentiation.

CONCLUSIONS

The ability of $1\alpha,25(OH)_2D_3$ to induce differentiation of various immature cells into functioning cells is closely linked to its classic functions in regulating mineral metabolism in the two major target organs of vitamin D. $1\alpha,25(OH)_2D_3$ elongates the villi of the duodenal tissues in putrescine-deficient chicks by a mechanism involving putrescine synthesis. This process results in a marked increase in intestinal calcium absorption. $1\alpha,25(OH)_2D_3$ also induces differentiation and fusion of osteoclast progenitors to form multinucleated osteoclasts by a mechanism involving cells of the osteoblast lineage. We expect that a better understanding of the differentiation-inducing activity of $1\alpha,25(OH)_2D_3$ will provide important clues to the physiology and pathophysiology of disorders in mineral metabolism.

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