The Role of 1α,25-Dihydroxyvitamin D_3 in the Myeloid Cell Differentiation (42911)

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Until quite recently, the only known function of vitamin D was a regulator of the extracellular calcium homeostasis. However, specific cytosol or nuclear receptors for 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$) were recently found in almost all tissues, and this raised the question of whether 1α,25-$(OH)_2D_3$ has more subtle functions in a wide variety of cells and tissues besides bone and intestine (1).

In 1981, we clearly demonstrated that 1α,25-$(OH)_2D_3$ suppresses growth and induces differentiation of mouse myeloid leukemia cells (M1) (2). We extended this original observation to other types of cells, in particular focusing on hemopoietic cells. A wide variety of the effects of 1α,25-$(OH)_2D_3$ indicate that 1α,25-$(OH)_2D_3$ is not only a calcium-regulating hormone, but also a potent inducer of cell differentiation. We review here recently discovered functions of 1α,25-$(OH)_2D_3$ in inhibiting proliferation and inducing differentiation and fusion of hemopoietic cells.

Modulation of Growth and Differentiation of Hematopoietic Cells by 1α,25-$(OH)_2D_3$

Osteoclasts, the principal bone-resorbing cells, are considered to be derived from immature bone marrow cells of the monocyte-macrophage lineage (3, 4). Of the several systemic and local factors known to affect bone resorption, 1α,25-$(OH)_2D_3$ is one of the most potent inducers. This hormone markedly increases the number of osteoclasts and then stimulates bone resorption (5, 6). 1α,25-$(OH)_2D_3$ receptors have been found in colony-forming units in culture (precursors of macrophages and granulocytes), differentiated monocytes-macrophages, and activated T lymphocytes (7, 8). However, the mechanism by which 1α,25-$(OH)_2D_3$ alters osteoclast population was not known until quite recently.

1α,25-$(OH)_2D_3$ induces differentiation of not only mouse M1, but also human promyelocytic leukemia cells (HL-60) (9–11) and human histiocytic monoblast-like lymphoma cells (U937) (12, 13). Like other typical inducers such as 12-*O*-tetradecanoylphorbol-13-acetate (TPA), retinoic acid, and dexamethasone, 1α,25-$(OH)_2D_3$ markedly induced phagocytosis and nitro-blue tetrazolium-reducing activity in those myeloid leukemia cells. However, at that time, we could not conclude that 1α,25-$(OH)_2D_3$ induced the selective differentiation of those myeloid cells into monocytes-macrophages, since these differentiation-associated properties are observed commonly in both monocyte-macrophage and granulocyte differentiation.

HL-60 is a very useful cell line in determining the direction of differentiation, since HL-60 cells have been known to be induced to differentiate selectively into granulocytes by retinoic acid or dimethyl sulfoxide and into monocytes-macrophages by TPA or interferon-γ (IFN-γ). We therefore examined the differentiating action of 1α,25-$(OH)_2D_3$ using HL-60 cells and monoclonal antibodies specific for either differentiation. 1α,25-$(OH)_2D_3$ markedly induced binding of the monoclonal antibody MAS 072, which is specific for monocytes-macrophages, whereas retinoic acid did not affect the binding of MAS 072 (14). In contrast, when HL-60 cells were treated with either 1α,25-$(OH)_2D_3$, retinoic acid, or TPA, only retinoic acid increased the binding of MAS 067, which is specific for granulocytes. Neither 1α,25-$(OH)_2D_3$ nor TPA affected the binding of MAS 067 (14). These results clearly indicate that 1α,25-$(OH)_2D_3$ induces differentiation of HL-60 cells selectively into monocytes-macrophages. This conclusion was confirmed in several other cell lines of myeloid leukemia cells and also in normal bone marrow cells (11, 15–17). 1α,25-$(OH)_2D_3$ selectively induces macrophage differentiation of HL-60 cells even in the presence of 100-fold retinoic acid (18). Thus, it is concluded that 1α,25-$(OH)_2D_3$ is a potent natural inducer in the monocyte-macrophage differentiation (1).

The relation between suppression of cell growth and induction of cell differentiation is most interesting. In order to investigate this relation, we examined the effect of 1α,25-$(OH)_2D_3$ in a mouse myelomonocytic leukemia cell line (WEHI-3), which is known to produce high levels of interleukin 3. 1α,25-$(OH)_2D_3$ strikingly increased the proportion of cells accumulating in the G_0/G_1 phase and decreased that in the S phase (19). The phenotype of the surface antigens became positive in macrophage-associated surface markers (Mac-1 and Ia antigens) after treatment with 1α,25-$(OH)_2D_3$. Furthermore, 1α,25-$(OH)_2D_3$ markedly inhibited interleukin 3 production by WEHI-3 cells (19). It is therefore concluded that 1α,25-$(OH)_2D_3$ inhibits proliferation of

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WEHI-3 cells by blocking transition of the cells from the G_0/G_1 to the S phase, resulting in induction of the G_0/G_1-arrested cells to differentiate into macrophages (Fig. 1). The relation between the suppression of cell growth by $1\alpha,25\text{-}(OH)_2D_3$ and interleukin 3 production has to be examined in the future.

Survival of Leukemic Mice by Vitamin D Treatment

The marked *in vitro* effect of $1\alpha,25\text{-}(OH)_2D_3$ on cell growth and differentiation in hematopoietic cells led us to examine the possibility of whether *in vivo* administration of vitamin D compounds to tumor-bearing mice would decrease their leukemogenicity. There have been several reports about growth inhibition of myeloid leukemia cells by $1\alpha,25\text{-}(OH)_2D_3$ in short-term cultures (2, 10, 11, 17). However, when leukemic cells are cultured with some differentiation inducers for a longer time, they tend to become resistant to them. Thus, we examined the effects of $1\alpha,25\text{-}(OH)_2D_3$ on proliferation of mouse M1 cells in a long-term culture to develop an effective therapeutic strategy using vitamin D compounds.

Growth in the M1 cells cultured for 20 days with 10^{-8} *M* $1\alpha,25\text{-}(OH)_2D_3$ was suppressed almost completely (20). However, $1\alpha,25\text{-}(OH)_2D_3$-resistant cells appeared on about Day 25 after continuous treatment with $1\alpha,25\text{-}(OH)_2D_3$ and they started to grow again thereafter. In contrast, when M1 cells were treated simultaneously with 10^{-8} *M* $1\alpha,25\text{-}(OH)_2D_3$ and non-cytotoxic doses of antileukemic drugs such as arabinofuranosylcytosine and daunomycin, resistant cells did not appear for at least 35 days and a striking growth inhibition was observed (20).

On the basis of these *in vitro* findings, we further examined whether simultaneous treatment with vitamin D compounds plus antileukemic drugs could prolong the survival time of syngeneic SL mice inoculated with M1 cells. We used 1α-hydroxyvitamin D_3 (1α-$(OH)D_3$) instead of $1\alpha,25\text{-}(OH)_2D_3$, since 1α-$(OH)D_3$ was more effective than $1\alpha,25\text{-}(OH)_2D_3$ in prolonging the survival of leukemic mice (21). SL mice inoculated with 3×10^5 M1 cells were treated with 1α-$(OH)D_3$ and daunomycin, separately or in combination, three times a week. The mean survival time of untreated leukemic mice was about 19 days, whereas that of mice treated with either 1α-$(OH)D_3$ or daunomycin alone was about 27 days. In contrast, the mean survival time of mice treated with 1α-$(OH)D_3$ in combination with daunomycin was about 35 days (20). These results suggest that the combination therapy with vitamin D compounds and low doses of antileukemic drugs may provide a new effective therapeutic strategy in the future.

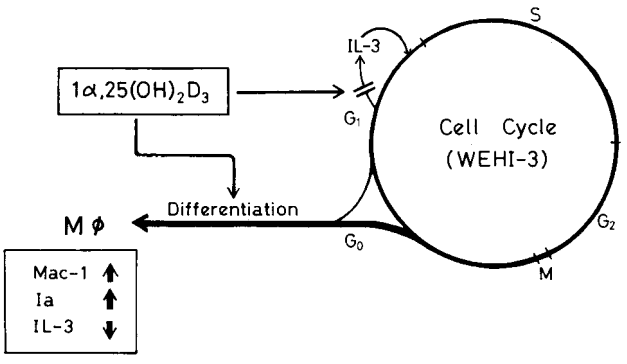

Figure 1. A proposed model for $1\alpha,25\text{-}(OH)_2D_3$ action in inhibiting growth and inducing macrophage differentiation in WEHI-3 cells.

Induction of Fusion of Macrophages by $1\alpha,25\text{-}(OH)_2D_3$

We next examined the possibility of whether $1\alpha,25\text{-}(OH)_2D_3$ induces fusion of macrophages. Because similar $1\alpha,25\text{-}(OH)_2D_3$ receptors have been found both in the differentiated macrophages and activated T lymphocytes (7, 8), we investigated the direct action and the lymphocyte-mediated indirect action of $1\alpha,25\text{-}(OH)_2D_3$ on macrophage fusion. We used alveolar macrophages as precursor cells for multinucleated giant cell formation, since alveolar macrophages often fuse *in vivo*.

As a source of lymphocytes, spleen cells were obtained from ddy strain male mice and cultured with either phytohemagglutinin (PHA), concanavalin A (Con A), or $1\alpha,25\text{-}(OH)_2D_3$. After being cultured for 3 days, the spleen cells were removed by centrifugation and conditioned media were obtained. Alveolar macrophages obtained from mice of the same strain were cultured for 3 days either with conditioned media or directly with PHA, Con A, or $1\alpha,25\text{-}(OH)_2D_3$. Fusion rate of macrophages was expressed as percentages of the total number of nuclei within giant cells to the total number of nuclei counted.

Figure 2 shows the time course of change in fusion of alveolar macrophages directly induced by $1\alpha,25\text{-}(OH)_2D_3$ (right) or by a spleen cell-mediated indirect

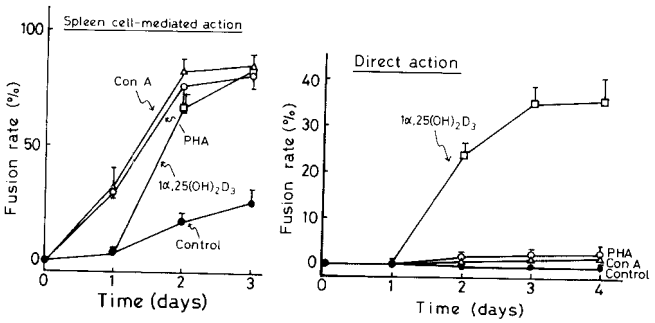

Figure 2. Time course of change in the fusion of alveolar macrophages induced either by exposure to conditioned media obtained from spleen cell cultures treated for 3 days with 15 μg/ml of PHA or Con A or 12 n*M* $1\alpha,25\text{-}(OH)_2D_3$ (left), or by direct exposure to 15 μg of PHA or Con A or 12 n*M* $1\alpha,25\text{-}(OH)_2D_3$ (right). The fusion rate of macrophages was expressed as percentages of the total number of nuclei within giant cells containing at least three nuclei in a cell to the total number of nuclei counted. From Reference (22); reproduced by permission of the National Academy of Sciences.

mechanism (left). By the indirect mechanism, as much as 80% of the macrophages fused to form multinucleated giant cells on Day 3, similarly with each of the three conditioned media from spleen cell cultures (Fig. 2, left) (22). The conditioned media contain both mitogens or $1\alpha,25\text{-}(OH)_2D_3$ and also a macrophage fusion factor (MFF) produced by spleen cells. Thus, the question remains whether the stimulating effect of conditioned media on macrophage fusion is due to a direct action of PHA, Con A, or $1\alpha,25\text{-}(OH)_2D_3$, or to an indirect action involving MFF released from the stimulated spleen cell cultures. However, $1\alpha,25\text{-}(OH)_2D_3$ added to the macrophages directly induced fusion of about 35% of the cells on Day 3. In contrast, neither PHA nor Con A induced fusion (Fig. 2, right) (22).

Therefore, it is concluded that $1\alpha,25\text{-}(OH)_2D_3$ is capable of inducing fusion of mouse alveolar macrophages by a direct mechanism and also by a spleen cell-mediated indirect mechanism (Fig. 3). PHA and Con A also induce production of a lymphokine (MFF) which exhibits macrophage fusion activity (22, 23).

After we reported the direct action of $1\alpha,25\text{-}(OH)_2D_3$ in inducing macrophage fusion, several laboratories have confirmed the direct action of $1\alpha,25\text{-}(OH)_2D_3$ on fusion of hemopoietic cells, using HL-60 cells (16), feline, baboon, and human bone marrow mononuclear cells (24, 25) and human blood monocytes (26). Thus, it is concluded that $1\alpha,25\text{-}(OH)_2D_3$ has a common function in directly inducing fusion of hemopoietic cells of the monocyte-macrophage lineage.

Polyamines are Involved in the $1\alpha,25\text{-}(OH)_2D_3$-Induced Fusion of Macrophages

While examining further the mechanism of the $1\alpha,25\text{-}(OH)_2D_3$-induced fusion of macrophages, we found that polyamines are involved in this mechanism. Mouse alveolar macrophages incubated with $1\alpha,25\text{-}(OH)_2D_3$ began to fuse at 36 hr and the fusion rate increased linearly for up to 60 hr (27). Addition of as much as 0.05–5 mM α-difluoromethylornithine, a specific inhibitor of ornithine decarboxylase, did not inhibit fusion appreciably, but addition of 0.05–5 μM methylglyoxal bis (guanylhydrazone) (MGBG), an inhibitor of S-adenosylmethionine decarboxylase, strikingly inhibited fusion (27). When macrophages were treated with both 12 nM $1\alpha,25\text{-}(OH)_2D_3$ and 5 μM MGBG for the first 12 hr and incubated further for 60 hr in fresh medium containing $1\alpha,25\text{-}(OH)_2D_3$ but not MGBG, fusion was significantly inhibited, suggesting that the $1\alpha,25\text{-}(OH)_2D_3$-induced synthesis of polyamines precedes fusion. The inhibition by MGBG of the $1\alpha,25\text{-}(OH)_2D_3$-induced fusion was restored completely by adding 1 μM spermidine or spermine or 100 μM putrescine (27). None of the polyamines alone induced fusion. MGBG suppressed the $1\alpha,25\text{-}(OH)_2D_3$-induced incorporation of [^{3}H]leucine into the trichloroacetic acid-insoluble fraction in macrophages, but its inhibitory effect was restored completely by adding 1 μM spermidine. When macrophages were incubated with [^{14}C]ornithine, the polyamine that accumulated most was [^{14}C]spermidine (27). $1\alpha,25\text{-}(OH)_2D_3$ further enhanced the accumulation of [^{14}C]spermidine. The accumulation of [^{14}C]putrescine and spermine was not appreciably altered by the $1\alpha,25\text{-}(OH)_2D_3$ treatment. Adding MGBG almost completely suppressed the accumulation of [^{14}C]spermidine and spermine, but it enhanced the accumulation of [^{14}C]putrescine considerably (27). These results indicate that spermidine is an important intracellular mediator of the $1\alpha,25\text{-}(OH)_2D_3$ action in inducing protein synthesis, which in turn induces fusion of alveolar macrophages.

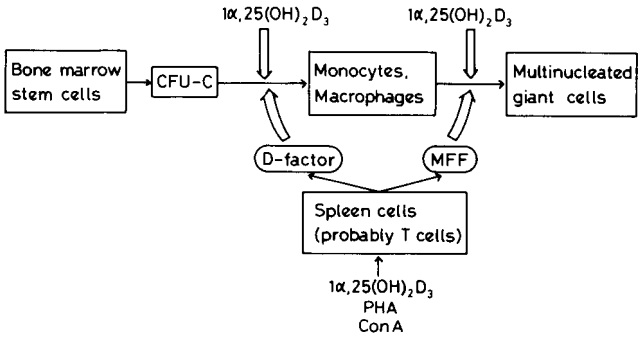

Figure 3. Involvement of $1\alpha,25\text{-}(OH)_2D_3$, PHA, and Con A in the differentiation of hematopoietic cells into monocytes-macrophages and multinucleated giant cells.

Spermidine-Dependent Proteins Responsible for Fusion of Macrophages

We next examined the nature of spermidine-dependent proteins responsible for macrophage fusion by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis of [^{35}S]methionine-labeled proteins. $1\alpha,25\text{-}(OH)_2D_3$ increased synthesis of 14 proteins at 24 hr before it initiated fusion at 36 hr after the vitamin addition (Table I). When spermidine synthesis was inhibited by adding MGBG, the enhanced synthesis of 9 in the 14 proteins induced by $1\alpha,25\text{-}(OH)_2D_3$ was greatly diminished with a concomitantly occurring inhibition of fusion. Addition of spermidine with MGBG and $1\alpha,25\text{-}(OH)_2D_3$ recovered the synthesis of these nine proteins and the MGBG-inhibited fusion as well. The synthesis of three proteins in nine was induced in a similar manner by interferon-γ (IFN-γ), retinoic acid (RA), or lipopolysaccharide (LPS) (Table I). These compounds have been known to induce activation but not fusion of mouse alveolar macrophages (28). The apparent M_r of the remaining six proteins were 142,000, 98,000, 78,000, 60,000, 50,000, and 42,000, respectively.

Very recently, McInnes and Rennick (29) reported that recombinant mouse interleukin 4 (rIL-4) induced formation of multinucleated cells in bone marrow mononuclear cell cultures. IL-4 was originally found as

Table I. A List of Proteins of which Synthesis is Enhanced by 1α,25-$(OH)_2D_3$, IL-4, IFN-γ, RA, and LPS in Mouse Alveolar Macrophages

	1α,25-$(OH)_2D_3$[a]	IL-4[a]	IFN-γ[b]	RA[b]	LPS[b]
Activation	+	+	+	+	+
Fusion	+	+	−	−	−
Band					
1 (M_r = 172,000)	++*	+*	+		
2 (M_r = 142,000)	++*				
3 (M_r = 107,000)	+++				
4 (M_r = 98,000)	+*	+*			
5 (M_r = 78,000)	+*	+*			
6 (M_r = 72,000)	++*		++	+	+
7 (M_r = 64,000)	+			+	
8 (M_r = 60,000)	++*				
9 (M_r = 53,000)	++*	++*		+	+
10 (M_r = 50,000)	++*	++*			
11 (M_r = 49,000)	++		+	++	+
12 (M_r = 42,000)	+++*				
13 (M_r = 36,000)	++		+	++	++
14 (M_r = 34,000)	++		++		++
15 (M_r = 75,000)			+		++
16 (M_r = 57,000)			+++	+	++
17 (M_r = 48,000)				+	

[a] Plus marks with asterisk indicate spermidine-dependent synthesis of proteins and those without asterisk indicate spermidine-independent synthesis of protein.
[b] Spermidine dependency of protein synthesis was not examined.

a factor involved in the growth and differentiation of immature B cells in the supernatants of mitogen-stimulated mononuclear cell cultures (30). This lymphokine has been called B cell stimulatory factor 1. Recent reports have demonstrated that B cell stimulatory factor acts on not only B cells but also other hematopoietic cells, including macrophages (31–33). These results indicate that B cell stimulatory factor 1 (IL-4) has an important role in the regulation of growth and differentiation of various hematopoetic cells. We confirmed that mouse rIL-4 induces fusion of mouse alveolar macrophages. However, the maximal fusion rate of alveolar macrophages obtained by as high as 3000 units/ml of rIL-4 was only 40–45%, whereas that induced by the conditioned media of mitogen-stimulated spleen cell cultures attained a level as high as 70–80%. Although some other unknown factor(s) may also be involved in inducing fusion of alveolar macrophages, IL-4 is considered to be a powerful candidate for MFF.

We found that rIL-4 also induced fusion of alveolar macrophages by a spermidine-dependent mechanism and it increased synthesis of five proteins (172,000, 98,000, 78,000, 53,000, and 50,000) in nine proteins induced by 1α,25-$(OH)_2D_3$ through a spermidine-dependent mechanism. These results suggest that the three spermidine-dependent proteins (98,000, 78,000, and 50,000) commonly induced by 1α,25-$(OH)_2D_3$ and IL-4 are involved in the fusion of mouse alveolar macrophages (Table I) (Tanaka *et al.*, submitted for publication). This suggests that there is a common mechanism in the fusion of alveolar macrophages induced by the respective compounds. A possible explanation is that 1α,25-$(OH)_2D_3$ induces IL-4 synthesis, which in turn promotes fusion of macrophages. However, it should be noted that the maximal fusion rate obtained by rIL-4 was lower than that induced by 1α,25-$(OH)_2D_3$. Whether 1α,25-$(OH)_2D_3$ induces expression of mRNA of IL-4 in macrophages and whether 1α,25-$(OH)_2D_3$ and IL-4 increase the synthesis of above common proteins by respective receptor-mediated mechanisms are of considerable interest and are currently under investigation.

Calcium is Essential in the Fusion of Alveolar Macrophages Induced by 1α,25-$(OH)_2D_3$

It has been reported that some divalent cations, in particular calcium ion, are involved in the fusion process of some cells such as erythrocytes, skeletal muscle cells, and myoblasts (34–36). Very recently, we also found that the calcium ion is essential for inducing fusion of macrophages. When alveolar macrophages were cultured with 1α,25-$(OH)_2D_3$ in a medium with graded concentrations (0.13–1.85 mM) of calcium, the fusion rate went down in parallel with the decrease of medium calcium (37). Neither calcium ionophore A23187 nor TPA induced fusion of freshly isolated macrophages, but the two compounds greatly promoted fusion of the macrophages pretreated with 1α,25-$(OH)_2D_3$ for 18 hr. The vitamin effect for the first 18 hr occurred in a similar manner irrespective of the medium calcium concentration (37). In contrast, millimolar orders of calcium were essential in the subsequent period of incubation (18–72 hr) for inducing fusion (37). The activation of macrophages measured by the induction of cytotoxicity and the enhancement of glucose consumption by 1α,25-$(OH)_2D_3$ occurred in a similar manner irrespective of the medium calcium concentration. These results clearly indicate that the fusion process of alveolar macrophages induced by 1α,25-$(OH)_2D_3$ can be divided into two phases: the calcium-independent priming phase (0–18 hr) and the calcium-dependent progression phase (18–72 hr). 1α,25-$(OH)_2D_3$ is necessary only in the priming phase and A23187 or TPA can be substituted for 1α,25-$(OH)_2D_3$ in the progression phase (Fig. 4). In the progression phase, changes of cytoskeletal proteins induced by calcium-dependent proteins such as calmodulin, calpain and gelsolin, phospholipase A_2-dependent arachidonic acid metabolism, or membrane phospholipase C-dependent phospholipid metabolism may be involved. The relationship between the proteins commonly induced by 1α,25-$(OH)_2D_3$ and IL-4 by a spermidine-dependent mechanism in the priming phase and the role of calcium in the progression phase is of

considerable interest and it is under investigation in our laboratory.

The Role of 1α,25-$(OH)_2D_3$ on Osteoclast Formation

The marked stimulating effects of 1α,25-$(OH)_2D_3$ on differentiation of myeloid cells into monocytes-macrophages and fusion of the differentiated macrophages prompted us to examine the role of 1α,25-$(OH)_2D_3$ on osteoclast formation. We developed a mouse marrow culture system to examine the formation of osteoclast-like multinucleated cells (MNC). 1α,25-$(OH)_2D_3$ greatly stimulated the formation of tartrate-resistant acid phosphatase (TRACP, a marker enzyme of osteoclasts)-positive MNC dose and time dependently (38). The number of clusters consisting of TRACP-positive mononuclear cells was also increased by 1α,25-$(OH)_2D_3$. Time course studies showed that these TRACP-positive mononuclear cell clusters appeared prior to the formation of MNC, suggesting that the TRACP-positive mononuclear cells are precursors of MNC. In the control culture, no TRACP-positive MNC was formed. Most of the TRACP-positive mononuclear cell clusters and MNC were found near the colonies of alkaline phosphatase-positive mononuclear cells (possibly osteoblasts) in the cultures (38).

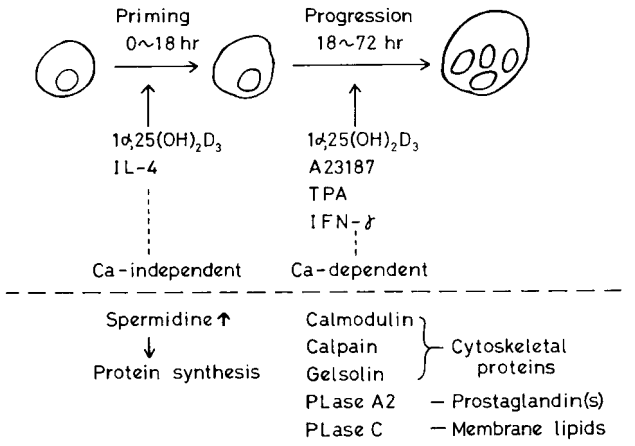

Figure 4. A possible mechanism of the two-step fusion process of alveolar macrophages.

Salmon calcitonin (CT) markedly inhibited the formation of TRACP-positive MNC but not of TRACP-positive mononuclear cell clusters induced by 1α,25-$(OH)_2D_3$ (38). By means of the autoradiography of ^{125}I-labeled CT and the measurement of CT-dependent cAMP production, it was shown that the TRACP-positive MNC formed *in vitro* possessed CT receptors (39). ^{125}I-Labeled CT was specifically bound to the TRACP-positive MNC and mononuclear cells (39). The CT-dependent cAMP production was induced in parallel with the increase in the number of TRACP-positive MNC induced by 1α,25-$(OH)_2D_3$ (39). Neither freshly isolated marrow cells nor the cells cultured without 1α,25-$(OH)_2D_3$ accumulated CT-dependent cAMP. CT also induced cytoplasmic contraction of marrow-derived MNC. These results suggest that the MNC induced by 1α,25-$(OH)_2D_3$ from mouse marrow cells possess CT receptors and respond to exogenous CT, as authentic osteoclasts do (39).

When marrow cells were cultured with 1α,25-$(OH)_2D_3$ on sperm whale dentin slices, many resorption lacunae were formed on the dentin surfaces (38). An electron microscopic study revealed that MNC located on the lacunae exhibited characteristic ruffled borders and clear zones. The surface dentin layer facing the ruffled borders was decalcified, whereas that facing the clear zones was not. Numerous fragments of dentin crystals were recognized both in the extracellular space

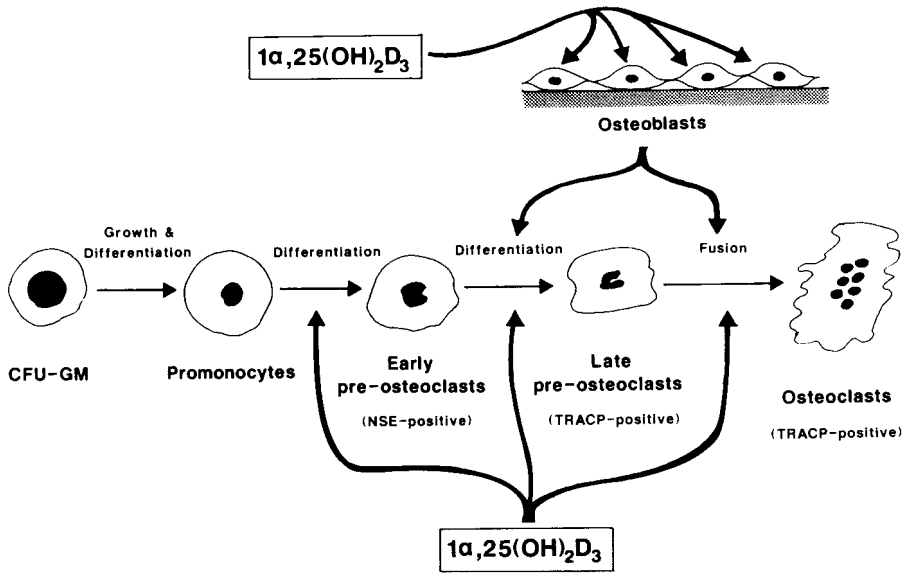

Figure 5. A possible mechanism of 1α,25-$(OH)_2D_3$ action in osteoclast formation.

of the ruffled borders and in the dark lysosomes located in the deep cytoplasm of MNC (Sasaki *et al.*, submitted for publication). These results clearly indicate that mouse marrow-derived MNC formed *in vitro* in the presence of $1\alpha,25\text{-}(OH)_2D_3$ satisfy all of the criteria of osteoclasts and that osteoblasts may play an important role in osteoclast formation (Fig. 5).

Conclusion

$1\alpha,25\text{-}(OH)_2D_3$ modulates not only extracellular calcium homeostasis, but also growth, differentiation, and fusion of hemopoietic cells. Two aspects of the effects of $1\alpha,25\text{-}(OH)_2D_3$ in modulating cell growth and differentiation appear important: first, $1\alpha,25\text{-}(OH)_2D_3$ regulates osteoclast formation; in other words, skeletal homeostasis by modulating differentiation and fusion of osteoclast progenitors. Second, $1\alpha,25\text{-}(OH)_2D_3$ appears to act as a biologic response modifier in cell growth and differentiation. We expect that a better understanding of the differentiating action of $1\alpha,25\text{-}(OH)_2D_3$ will provide important clues to the physiology and pathophysiology of several clinical states.

This review article has been written based on the presentation at the symposium entitled Newly Discovered Actions of 1,25-Dihydroxyvitamin D_3 chaired by Dr. H. F. DeLuca, President of SEBM, at the FASEB Meeting held in Las Vegas, May 1–6, 1988. I am grateful to Dr. H. F. DeLuca for giving me the opportunity to present our recent work at the symposium.

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