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1α,25-DIHYDROXYVITAMIN D_3 INDUCES DIFFERENTIATION OF HUMAN PROMYELOCYTIC LEUKEMIA CELLS (HL-60) INTO MONOCYTE-MACROPHAGES, BUT NOT INTO GRANULOCYTES

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SUMMARY: The differentiating action of 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2D_3$] in hematopoietic cells was examined in 3 tumor cell lines. 1α,25-$(OH)_2D_3$ induced common differentiation-associated properties in macrophages and granulocytes similarly in mouse myeloblastic leukemia cells (Ml), human promyelocytic leukemia cells (HL-60) and human histiocytic monoblast-like lymphoma cells (U937). 1α,25$(OH)_2D_3$ markedly induced α-naphthyl acetate esterase activity, a typical marker of monocyte-macrophages, in Ml and HL-60 cells. In HL-60 and U937 cells, the vitamin also induced binding of the monoclonal antibody MAS 072, specific for monocyte-macrophages, but not of MAS 067, specific for granulocytes. These results clearly indicate that 1α,25$(OH)_2D_3$ induces differentiation of all cell lines examined preferentially along the monocyte-macrophage pathway.

In 1981, Abe et al. (1) discovered that 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$], an active metabolite of vitamin D_3, induces differentiation of mouse myeloblastic leukemia cells (Ml) into monocyte-macrophages. Subsequently, Miyaura et al. (2) and Suda et al. (3) reported that the vitamin also induced phagocytic activity, C3 rosette formation and the reduction of nitro-blue tetrazolium (NBT) by human promyelocytic leukemia cells (HL-60). The morphology of the HL-60 cells treated with 1α,25$(OH)_2D_3$ was very similar to the cells treated with retinoic acid (4), dimethyl sulfoxide (5), or actinomycin D (6) which are typical inducers of granulocyte differentiation. In addition, 1α,25$(OH)_2D_3$ made HL-60 cells adhere to the dish surface only weakly. From these results, we tentatively concluded that 1α,25$(OH)_2D_3$

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Abbreviations used: Ml, a mouse myeloid leukemia cell line; HL-60, a human myeloid leukemia cell line; U937, a human histiocytic monoblast-like lymphoma cell line; 1α,25$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; TPA, 12-O-tetradecanoylphorbol-13-acetate; NBT, nitro-blue tetrazolium.

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induces Ml cells to differentiate into monocyte-macrophages (1) and HL-60 cells into granulocytes (2). However, differentiation-associated properties such as phagocytosis, C3 rosette formation and NBT reduction are observed commonly in both monocyte-macrophage and granulocyte differentiation.

Very recently, McCarthy et al. (7) reported that 1α,25(OH)$_2$D$_3$ induces HL-60 cells to differentiate into monocyte-macrophages. We, therefore, re-examined the differentiating action of 1α,25(OH)$_2$D$_3$ in three different hematopoietic tumor cells (Ml cells, HL-60 cells and the human histiocytic monoblast-like lymphoma cells U937), using typical markers for either monocyte-macrophages or granulocytes. 1α,25(OH)$_2$D$_3$ induced differentiation of all cell lines examined into monocyte-macrophages.

MATERIALS AND METHODS

Chemicals: 1α,25(OH)$_2$D$_3$ was the generous gift of Dr. I. Matsunaga, Chugai Pharmaceutical Co. Ltd., Tokyo. Dexamethasone, retinoic acid and NBT were perchased from Sigma, St. Louis, and 12-O-tetradecanoylphorbol-13-acetate (TPA) from Consolidated Midland Corp., New York.

Cells and cell culture: The murine myeloid leukemia cell line (Ml; clone T22) were cultured in Eagle's minimal essential medium supplemented with twice the normal concentration of amino acids and vitamins and 10% heat-inactivated calf serum (Chiba Serum Institute, Chiba, Japan). The human promyelocytic leukemia cell line (HL-60) and the human histiocytic lymphoma cell line (U937) were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum (GIBCO, Grand Island, New York). All cells were inoculated at 1 x 10^5 cells/ml and inducers dissolved in ethanol were added to keep the final ethanol concentration at 0.1%. Control cultures were given the same volume of ethanol. The cells were cultured at 37°C for 3 days in a humidified atmosphere of 5% CO_2/95% air.

Assay of the properties of differentiated cells: Phagocytic activity was measured by determining the percentage of the cells that phagocytosed yeasts which were heat-killed and opsonized with fresh mouse or human AB serum. Cells with C3 receptors were determined according to the method of Lotem and Sachs (8) by measuring rosette formation with sheep erythrocytes coated with rabbit anti-sheep erythrocyte antibody and mouse complement. NBT reduction was assayed as reported previously (9). The percentage of the cells containing intracellular blue-black formazan deposits was determined. α-Naphthyl acetate esterase activity was examined in cytocentrifuge preparations using a Sigma's kit.

Determination of cell surface antigens: Binding of monoclonal antibodies to the cell surface antigens was determined by indirect immunofluorescence. The MAS series monoclonal antibodies (065 IgG$_1$, 066 IgM, 067 IgM and 072 IgG$_{2b}$) were perchased from Sera-Lab, Cambridge, UK. and the secondary antibodies (goat anti-mouse IgG and IgM) conjugated with fluoresceine isothiocyanate from Tago Immunodiagnostic Inc., Burlingame, Calif. and Cappel Laboratories Inc., West Chester, Phila., respectively.

RESULTS AND DISCUSSION

It has been reported that 0.12 - 120 nM of 1α,25(OH)$_2$D$_3$ induces differentiation of both Ml and HL-60 cells in a dose-dependent manner (1, 2). 1α,25(OH)$_2$D$_3$ at 120 nM induced phagocytic activity, C3 rosette formation and reduction of NBT similarly in Ml, HL-60 and U937 cells (Table 1). Dexamethasone at 1000 nM induced these differentiation-associated properties of Ml cells to a level similar to that by 120 nM of 1α,25(OH)$_2$D$_3$. Retinoic acid at 1000 nM and TPA at 1 nM also induced differentiation of HL-60 and U937 cells (Table 1). Dexamethasone is a typical inducer of monocyte-macrophage differentiation in Ml cells (10). TPA is a typical inducer of monocyte-macrophage differentiation in HL-60 (11) and U937 cells (12). Retinoic acid induces differentiation of HL-60 cells into granulocytes (4) and U937 cells into monocyte-macrophages (13). U937 cells are induced to differentiate only into monocyte-macrophages (12 - 14). The reduction of

Table 1. Induction of differentiation-associated properties of Ml, HL-60 and U937 cells by typical inducers.

Cell lines	Treatment		Phagocytosis	C3 receptor	NBT reduction
		nM	%	%	%
Ml	Control		1.1 ± 0.2	2.8 ± 0.5	0.3 ± 0.2
	1α,25(OH)$_2$D$_3$	120	55.9 ± 1.9	28.8 ± 2.3	6.8 ± 4.0
	Dexamethasone	1000	54.0 ± 2.2	23.5 ± 3.1	14.0 ± 1.6
HL-60	Control		2.3 ± 0.4	9.5 ± 1.6	4.1 ± 0.8
	1α,25(OH)$_2$D$_3$	120	70.6 ± 3.3	67.3 ± 4.8	87.2 ± 2.1
	Retinoic acid	1000	50.6 ± 2.1	42.5 ± 2.1	77.6 ± 2.2
	TPA	1	57.3 ± 3.4	53.6 ± 3.4	9.5 ± 1.4
U937	Control		1.3 ± 0.1	11.1 ± 1.5	6.7 ± 1.1
	1α,25(OH)$_2$D$_3$	120	48.2 ± 1.6	45.7 ± 5.1	34.5 ± 5.0
	Retinoic acid	1000	37.5 ± 1.7	36.5 ± 2.9	33.8 ± 7.8
	TPA	1	54.6 ± 2.1	41.7 ± 4.2	18.6 ± 1.0

All cells were inoculated at 1 x 10^5 cells/ml and each culture was incubated with one of the compounds. The concentration of each compound used was optimal to obtain maximal response. After incubation for 3 days, each differentiation-associated property was tested. The values show the percentage of the total cells having each of the differentiation-associated properties. Data are means ± S.E.M. of 3 - 5 determinations.

NBT in HL-60 and U937 cells induced by TPA appeared smaller than that induced by $1\alpha,25(OH)_2D_3$ or retinoic acid (Table 1). This may be because the assay system of NBT reduction contains 100 ng/ml TPA as a stimulant for the production of hydrogen oxide and superoxide needed to reduce NBT. Incubation of the cells with TPA prior to the assay may diminish sensitivity of the cells to the phorbol ester.

The differentiation-associated properties shown in Table 1, however, are commonly observed in both monocyte-macrophage and granulocyte differentiation. We therefore examined the differentiating action of $1\alpha,25(OH)_2D_3$ in hematopoietic tumor cells using specific markers either for granulocytes or macrophages. α-Naphthyl acetate esterase activity has been believed to be specific for monocyte-macrophages (15). About 35% of the Ml cells and 70% of the HL-60 cells became positive in the esterase activity after treatment with 120 nM of $1\alpha,25(OH)_2D_3$ for 3 days (Fig. 1A and B). Dexamethasone at 1000 nM and TPA at 1 nM also made about 35% of the Ml cells and 20% of the HL-60 cells, respectively, positive in the esterase activity (Fig. 1A and B), whereas retinoic acid at 1000 nM did not induce the esterase activity of HL-60 cells at all (Fig. 1B). U937 cells were 100% positive in the esterase activity even in the absence of any inducers (data not shown).

Figure 2 shows the antigenic expression on the cell surface of HL-60 induced by $1\alpha,25(OH)_2D_3$, retinoic acid and TPA. Monoclonal antibodies MAS 072 specific for human monocyte-macrophages and MAS 065, 066 and 067 specific for human granulocytes were used to examine selective differentiation of HL-60 cells. Binding of MAS 072 to the control HL-60 cells was very small (< 1%), but was greatly increased after incubation with 12 or 120 nM of $1\alpha,25(OH)_2D_3$. Retinoic acid did not affect the binding of MAS 072 to HL-60 cells (Fig. 2A). Although TPA made HL-60 cells adhere strongly to the dish surface and induced morphologically the cells to differentiate into typical macrophages, they did not react with MAS 072 (Fig. 2A). The inability of the HL-60 cells to express the antigen specific for monocyte-macrophages by TPA may indicate that the phorbol ester does not mimic the phys-

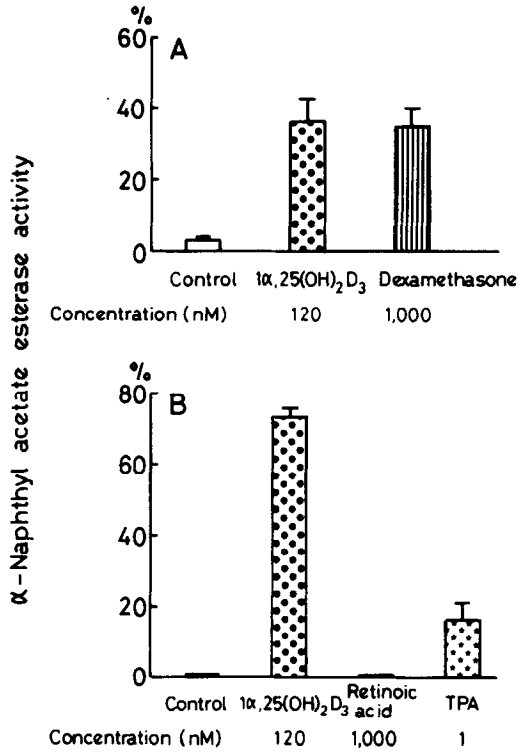

Fig. 1 Expression of α-naphthyl acetate esterase activities in Ml (A) and HL-60 cells (B) induced by $1\alpha,25(OH)_2D_3$ ( ), dexamethasone ( ), retinoic acid ( ) or TPA ( ). Cells were inoculated at 1×10^5 cells/ml and cultured with either vehicle ( ) or one of the compounds. Concentrations of each compound used were optimal to obtain maximal response. After incubation for 3 days, at least 500 cells were assayed for α-naphthyl acetate esterase activities. Data are means ± S.E.M. of 3 determinations.

iologic signals for the acquisition of the surface membrane antigen, as suggested by Graziano et al. (16). Monoclonal antibodies MAS 065, 066 did not bind to either the control or the treated HL-60 cells (data not shown), whereas MAS 067 reacted with 8% of the control HL-60 cells. When HL-60 cells were treated with 1000 nM of retinoic acid, about 45% of the cells showed binding of MAS 067. Neither $1\alpha,25(OH)_2D_3$ nor TPA increased the proportion of the cells that reacted with MAS 067 (Fig. 2B). When U937 cells were cultured with 12 or 120 nM of $1\alpha,25(OH)_2D_3$, the proportion of the cells that reacted with MAS 072 increased from the control level (< 1%) to 15 - 20%.

These results clearly indicate that $1\alpha,25(OH)_2D_3$ induces differentiation of three different hematopoietic tumor cells (Ml, HL-60 and U937 cells) into monocyte-macrophages, but not into granulocytes. This conclusion is in ac-

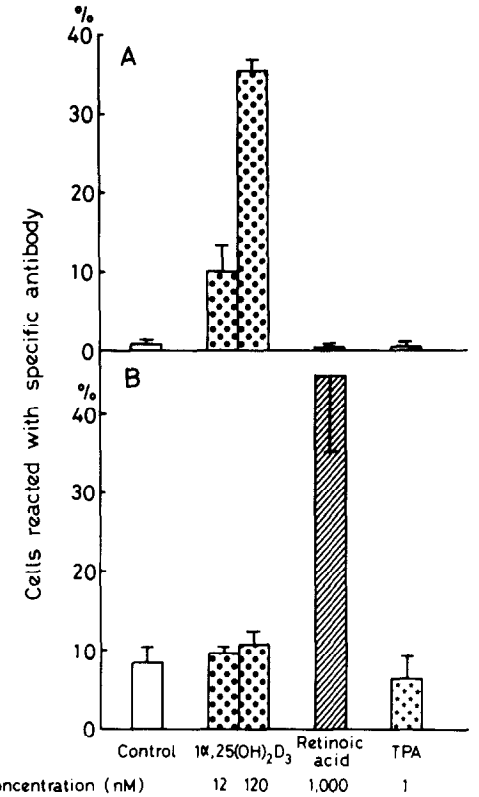

Fig. 2 Comparison of antigenic expression on the cell surface of HL-60 induced by several compounds. Cells were cultured for 3 days with either vehicle (□), 12 or 120 nM of 1α,25(OH)$_2$D$_3$ (▣), 1000 nM of retinoic acid (▨), or 1 nM of TPA (▣). Antigenic expression was examined by indirect immunofluorescence with monocyte-macrophage-specific monoclonal antibody MAS 072 (A) and granulocyte-specific monoclonal antibody MAS 067 (B). Data are means ± S.E.M. of 3 - 4 determinations.

cord with the results reported by McCarthy et al. (7). They also reported that 1α,25(OH)$_2$D$_3$-induced in vitro differentiation of normal human bone marrow cells into monocyte-macrophages (7). Thus, it appears that 1α,25(OH)$_2$D$_3$ induces differentiation of both normal and tumor hematopoietic cells preferentially along the monocyte-macrophage pathway.

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