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Control of Macrophage Cell Differentiation in Human Promyelocytic HL-60 Leukemia Cells by 1,25-Dihydroxyvitamin D_3 and Phorbol-12-myristate-13-acetate[1]

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ABSTRACT

Human promyelocytic leukemia cells (HL-60) were induced to differentiate into macrophage-like cells in a dose (3×10^{-10} to 10^{-7} M) and time (1 to 6 days)-dependent manner by 1,25-dihydroxyvitamin D_3 and the tumor promoter, phorbol-12-myristate-13-acetate. Differentiation was determined by an increase in the percentage of morphologically mature cells, in lysozyme and nonspecific esterase activities, and in reactivity with the murine OKM1 monoclonal antibody. Two HL-60 cell variants, designated as R-80 and B-II, were also examined. R-80 cells, which are resistant to induction of cell differentiation by phorbol-12-myristate-13-acetate, also exhibited resistance, although to a lesser degree, to induction of cell differentiation by 1,25-dihydroxyvitamin D_3. The resistance to the action of the two compounds is presumably not due to similar binding sites for the two inducers, since 1,25-dihydroxyvitamin D_3 was unable to compete for the phorbol diester binding sites as measured by [^{3}H]phorbol-12,13-dibutyrate binding. B-II cells were resistant to induction of cell differentiation by 1,25-dihydroxyvitamin D_3, phorbol-12-myristate-13-acetate, retinoic acid, and dimethyl sulfoxide.

Two-dimensional electrophoretic analysis of HL-60 cell protein patterns indicated that treatment of the HL-60 cells with 1,25-dihydroxyvitamin D_3, phorbol-12-myristate-13-acetate, retinoic acid, and dimethyl sulfoxide caused the cells to express various monocyte-macrophage and granulocyte marker proteins. None of the inducers caused a protein pattern identical to that of peripheral monocytes or granulocytes in the HL-60 cells, but the protein pattern of the HL-60 cells treated with 1,25-dihydroxyvitamin D_3 was the closest to that of peripheral blood monocytes.

These results indicate that 1,25-dihydroxyvitamin D_3 induces in the HL-60 cells a phenotype that resembles, but is not identical to, that of peripheral monocytes-macrophages.

INTRODUCTION

The human promyelocytic HL-60 leukemic cells are useful in studying the control of cell differentiation by chemical agents (6, 9, 22, 23, 27, 30, 32). PMA[3] and teleocidin, which are potent tumor promoters in a mouse skin model (17, 35), can induce in the HL-60 cells a macrophage-like phenotype at doses as low as 10^{-10} to 10^{-9} M (22, 27, 32). These 2 chemically unrelated agents seem to exert their biological response through a similar mechanism. Teleocidin competitively inhibits the specific binding of phorbol diesters to their cellular receptors (38) and, in the HL-60 cells, it induces differentiation characteristics similar to those induced by PMA (22). Further, an HL-60 cell variant that is resistant to PMA-induced cell differentiation exhibits a similar resistance to teleocidin but not to other inducers of cell differentiation such as DMSO or RA (6, 9, 20, 22).

Recently, it was reported that 1,25-$(OH)_2D_3$, the biologically active metabolite of vitamin D_3 (19), induced in the HL-60 cells a number of differentiation markers at low doses (30, 37). The biological activity of 1,25-$(OH)_2D_3$, as for both PMA and teleocidin (12, 21, 36, 38), requires binding to specific cellular receptors (10, 11, 16, 29). These studies raised the possibility that 1,25-$(OH)_2D_3$ may, in the HL-60 cells, induce differentiation processes via a mechanism similar to the action of PMA and teleocidin. To examine this possibility, we compared the ability of 1,25-$(OH)_2D_3$ and PMA to induce cell differentiation in HL-60 cells and in 2 HL-60 cell variants which are resistant to PMA-induced cell differentiation (22, 24, 28). In addition, we investigated the ability of 1,25-$(OH)_2D_3$ to inhibit specific phorbol diester receptor binding (38) as a means to establish whether 1,25-$(OH)_2D_3$, like teleocidin, shares similar binding sites with phorbol diesters. Cell differentiation in the HL-60 and variant cells was characterized by morphological, enzymatic, and antigenic markers as well as by a 2-dimensional electrophoretic analysis of newly synthesized cellular proteins.

Our results indicate that 1,25-$(OH)_2D_3$ induces in the HL-60 cells a macrophage phenotype similar to but not identical to that induced by PMA. Furthermore, 1,25-$(OH)_2D_3$, unlike teleocidin, does not decrease the binding of phorbol esters to their receptors. However, on the basis of a resistance of an HL-60 cell variant to both 1,25-$(OH)_2D_3$ and PMA, we can assume that these 2 agents may require a similar cellular event in the induction of a macrophage-like cell differentiation in the HL-60 cells.

MATERIALS AND METHODS

Cells and Culture Conditions. HL-60 cells were provided by Dr. R. C. Gallo (National Cancer Institute, Bethesda, Md.) (9, 23). An HL-60 cell variant resistant to induction of cell differentiation by phorbol diesters, designated as R-80, was isolated by culturing HL-60 cells for 80 subcultures (5- to 8-day intervals) in the presence of increasing concentrations of PMA (Chemicals for Cancer Research, Eden Prairie, Minn.) (20, 22, 24, 36). The R-80 cells were subcultured 4 to 6 times in the absence of PMA prior to their use in the various experiments. Another HL-60 cell variant resistant to induction of cell differentiation by DMSO, designated as B-II, was a gift from Dr. P. G. Major (Sidney Farber Cancer Institute, Harvard Medical School, Boston, Mass.) (28). The cells were cultured in bacterial and tissue culture Petri dishes (Falcon Plastics, Oxnard, Calif.)

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[3] The abbreviations used are: PMA, phorbol-12-myristate-13-acetate; DMSO, dimethyl sulfoxide; RA, retinoic acid; 1,25-$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; RPMI 1640, Roswell Park Memorial Institute Medium 1640; PBS, phosphate-buffered saline (NaCl, 8 g/liter; KCl, 0.2 g/liter; $MgCl_2 \cdot 6H_2O$, 0.1 g/liter; $Na_2HPO_4 \cdot 7H_2O$, 2.16 g/liter); PDBU, phorbol-12,13-dibutyrate.

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in RPMI 1640 supplemented with 20% fetal bovine serum, penicillin (100 units/ml), and streptomycin (100 μg/ml) (Grand Island Biological Co., Grand Island, N. Y.) at 37° in an atmosphere of 5% CO_2 in air in humidified incubators. The 1,25-$(OH)_2D_3$ and PMA were dissolved in a final concentration of 0.01% DMSO, and RA was dissolved in 0.1% DMSO in culture medium. DMSO at these concentrations did not affect cell growth or the expression of the various differentiation markers. Control cultures were treated with DMSO at a final concentration of 0.1% in culture medium.

Evaluation of Morphological Differentiation. For morphological evaluation of cell differentiation, control and treated cells were removed from the Petri dishes, pelleted by centrifugation, and resuspended in 1 ml fresh medium. Approximately 0.1 ml of the cell suspension was spread on a glass microscope slide, air dried, and stained with Wright-Giemsa. Differential cell counting was performed on 200 to 400 stained cells from at least 2 preparations for each experimental point.

Lysozyme and Nonspecific Esterase Activities. Lysozyme activity was determined by a decrease in turbidity of a cell suspension of *Micrococcus lysodeikticus*, measured at an absorbance of 450 nm at 25°, as described (34). The reaction mixture (3 ml) contained 2.5 ml of substrate (0.12 mg bacteria per ml of 67 mM potassium phosphate buffer, pH 6.24) and 0.5 ml of cell-free supernatant from control or treated cultures. The μg equivalent of lysozyme activity was estimated by comparing the cell-free supernatant activity with that of a standard activity curve using purified hen egg white lysozyme as a standard (Sigma Product L-6876). Results are expressed in μg equivalents/10^7 cells/10 ml of medium (25).

Nonspecific esterase activity was determined cytochemically using α-naphthyl acetate as the substrate (40). Cells spread on a microscope slide were fixed with cold formalin-acetone and incubated in a Coplin jar for 45 min at room temperature in a staining solution that contained 63.5 ml phosphate buffer (0.1 M, pH 7.6), 4.2 ml hexazotized *p*-rosalinin (obtained by mixing an equal volume of 4% sodium nitrite and 4% *p*-rosalinin), and 3.5 ml α-naphthyl acetate solution (28.6 mg/ml of ethylene glycol monomethyl ether). Prior to use, the pH of the staining solution was adjusted to pH 6.1, and the solution was filtered through filter paper to remove precipitated material.

Reactivity with OMK1 Monoclonal Antibody. The murine OKM1 monoclonal antibody, which exhibits reactivity with human blood monocytes and granulocytes (15), was obtained from Ortho Pharmaceutical Corp., Raritan, N. J. Reactivity with the OKM1 antibody was detected in the presence of a fluorescein-labeled goat anti-mouse antibody. The immunofluorescence was determined after 10^6 cells, suspended in 200 μl of cold PBS, were incubated with 5 μl of reconstituted OKM1 monoclonal antibody (diluted 1:40 with buffer) for 30 min at 4°. The cells were washed twice with ice-cold buffer and resuspended in 100 μl of fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin for 30 min at 4°. After 2 additional washes with buffer containing 30% glycerol, cell suspensions of 0.05 ml were dropped on glass slides, and the cells were covered with a coverslip and examined for reactivity with the antibody. Each experimental point was based on an analysis of 200 cells from 2 separate preparations.

Labeling of Cells and 2-Dimensional Electrophoresis. Cells for the 2-dimensional analysis were incubated for 2 days with or without the inducers in the growth medium and subsequently for 18 hr in RPMI 1640 lacking methionine (Grand Island Biological Co.; Selectamine Kit), supplemented with 10% fetal bovine serum, antibiotics, and [^{35}S]methionine (60 μCi/ml, 1000 Ci/mmol; Amersham Corp., Arlington Heights, Ill.). Human monocytes were prepared from peripheral blood as described previously (18). Cells were solubilized in a solution containing 9 M urea, 4% Nonidet P-40, 2% mercaptoethanol, and 2% ampholytes (pH 9 to 11; LKB, Bromma, Sweden) at a final pH of 9.5. Two-dimensional electrophoresis was performed using the 7- × 7-inch ISO-DALT system (3, 4). The gels were then dried and autoradiographed on Kodak XAR-2 film for 1 week.

Binding of [^{3}H]PDBU to Intact Cells. The binding of [^{3}H]PDBU (specific activity, 6.4 Ci/mmol; Life System Co., Newton, Mass.) to intact cells (36) was assayed in 1.5 ml high-density polypropylene micro-test tubes (Bio-Rad Laboratories; No. 223-9501). Each tube contained, in a total volume of 260 μl RPMI 1640 (lacking serum), 1 to 4 × 10^6 HL-60 cells, 31 nM [^{3}H]PDBU in the presence or absence of excess nonradioactive PDBU (42 μM) (Chemicals for Cancer Research), as well as other ligands as specified. The ligands [PMA, teleocidin, RA, and 1,25-$(OH)_2D_3$] were analyzed for their ability to inhibit [^{3}H]PDBU binding to HL-60 cells. Therefore, they were incubated with the HL-60 cells (at 4°) 15 min prior to the addition of [^{3}H]PDBU. The cells used in the various reactions had been washed 3 times with cold PBS, pH 7.4, and resuspended in serum-free RPMI 1640. All reactions took place on ice in a 4° cold room.

The reaction mixtures were thoroughly mixed and allowed to incubate for 45 min. The final DMSO concentration was less than 1%. The reaction was terminated by the addition of 0.5 ml ice-cold PBS. The tubes were immediately centrifuged at 12,000 × *g* in an Eppendorf microcentrifuge (Brinkman Instrument Co., Westbury, N. Y.) for 5 min at 25°. After centrifugation, a 100-μl aliquot was removed to determine the amount of free [^{3}H]PDBU. The remaining supernatant was carefully aspirated off, and the cells were washed 3 times with aliquots of 0.5 ml ice-cold PBS. The end of the micro-test tube containing the pelleted cells was cut off and placed overnight in a scintillation vial containing a solubilizing solution of 1 ml of 1% lauryl sulfate and 10 mM dithiothreitol. The counting efficiency for [^{3}H]PDBU under these conditions was about 50%. Specific binding was calculated as the difference between the total binding and nonspecific binding. The latter was determined in the presence of excess nonradioactive PDBU. The nonspecific binding of [^{3}H]PDBU was 12 ± 5% (S.D.) of the total binding.

RESULTS

Induction of a Macrophage Cell Differentiation in HL-60 Cells by Treatment with 1,25-$(OH)_2D_3$ or PMA. During a period

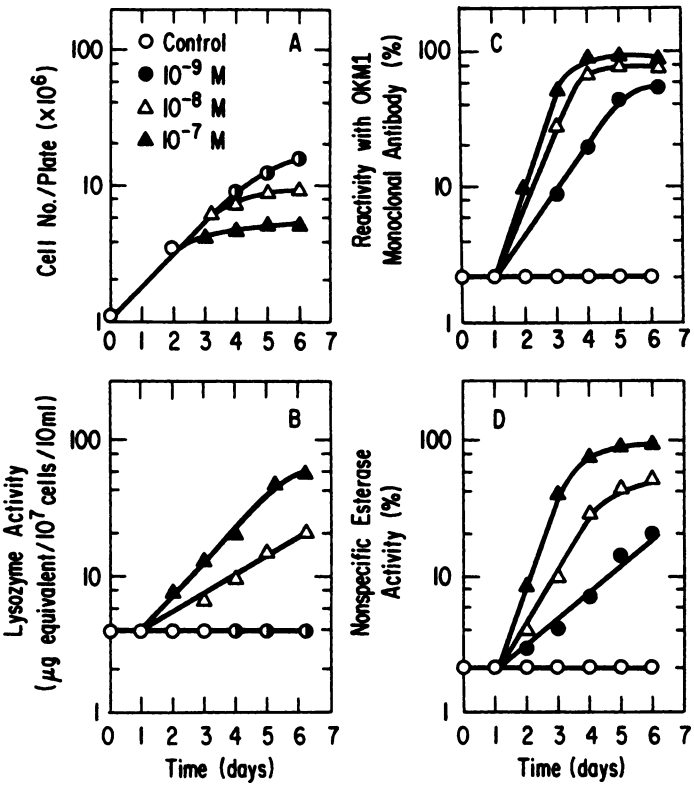

Chart 1. Cell growth and induction of markers of cell differentiation in HL-60 cells at various times after treatment with different concentrations of 1,25-$(OH)_2D_3$.

of 6 days of growth in culture, about 2% of the untreated HL-60 cells exhibited nonspecific esterase activity characteristic of monocytes-macrophages (Fig. 1; Chart 1). A similar percentage of the cells reacted with OKM1 monoclonal antibody which detects a surface antigen present in monocytes and granulocytes (15, 33). The HL-60 cells also retain a small amount of lysozyme activity (4 μg equivalents/10^7 cells/10 ml of growth medium) (Chart 1).

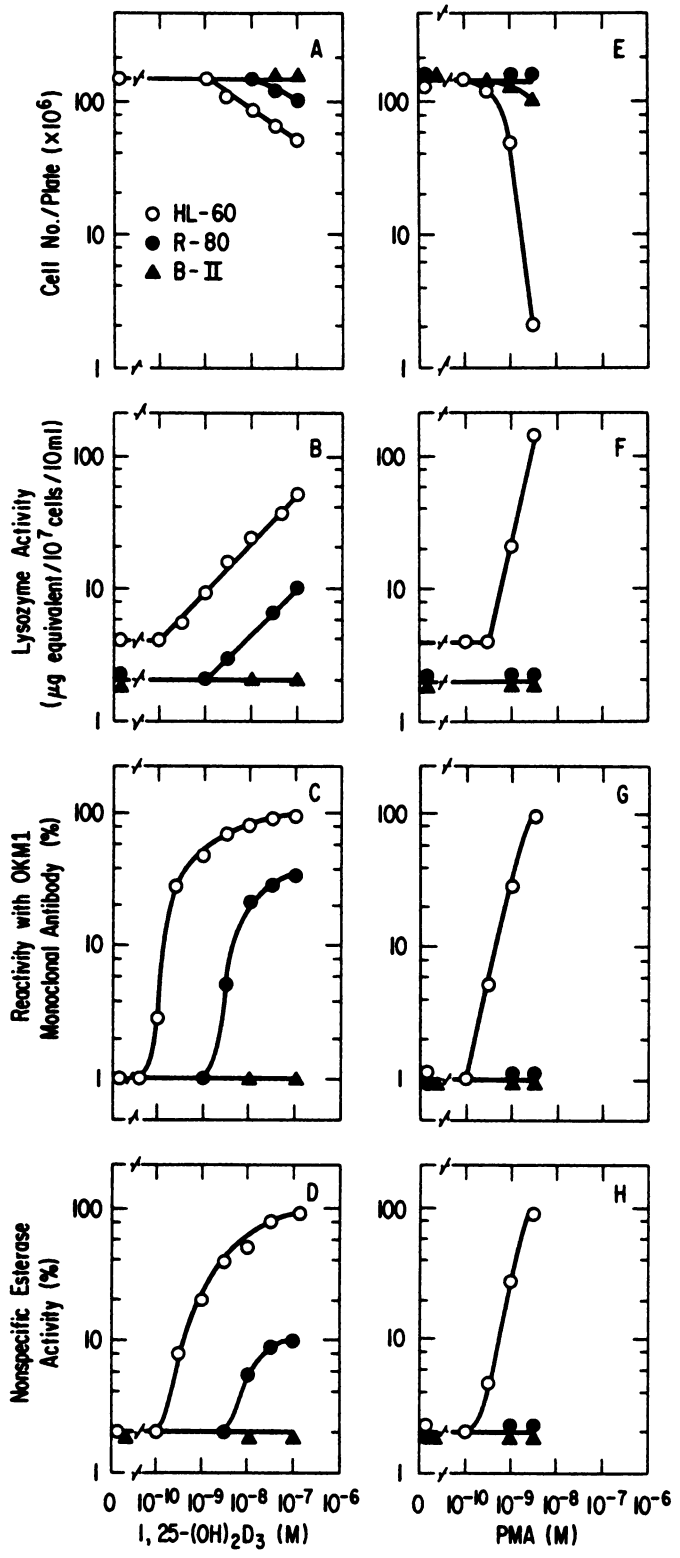

Chart 2. Induction of markers of cell differentiation in HL-60, R-80, and B-II cells after treatment for 6 days with different concentrations of 1,25-$(OH)_2D_3$ or PMA.

Treatment of HL-60 cells for more than 24 hr with 1,25-$(OH)_2D_3$ at doses higher than 3×10^{-10} M resulted in a time- and dose-dependent increase in nonspecific esterase and lysozyme activities, in an increase in reactivity of the cells with OKM1 monoclonal antibody, and in a decreased cell growth (Charts 1 and 2; Fig. 1). At the end of 6 days of treatment with doses higher than 3×10^{-8} M, more than 80% of cells stained for nonspecific esterase activity, and more than 90% of the cells exhibited reactivity with OKM1 monoclonal antibody. Lysozyme activity was elevated to about 50 μg equivalents/10^7 cells/10 ml (Chart 2) after treatment with 10^{-7} M 1,25-$(OH)_2D_3$. This treatment also produced about 25% cell attachment to the surface of the Petri dishes, another macrophage characteristic. The different monocyte-macrophage characteristics induced by 1,25-$(OH)_2D_3$ in HL-60 cells were similar to those induced by PMA (22, 23, 27, 32, 33), although the kinetics of differentiation marker acquisition were not necessarily identical (Chart 2).

To characterize further the nature of the differentiation processes induced by 1,25-$(OH)_2D_3$ and PMA, we included in our studies 2 additional inducers of cell differentiation, namely, DMSO and RA (6, 9, 22), as well as 2 additional HL-60 cell variants. The B-II cell variant was included because of its resistance to DMSO-induced cell differentiation (28), while the R-80 cell variant was included for its resistance to PMA-induced cell differentiation (22). The results indicated that B-II cells were resistant not only to DMSO-induced cell differentiation but also to the 3 other inducers of cell differentiation tested (Chart 2). The R-80 cells were resistant to PMA-induced cell differentiation and somewhat resistant to differentiation induced by 1,25-$(OH)_2D_3$ (Table 1; Chart 2). The R-80 cells, however, were as susceptible as the parental HL-60 cells to cell differentiation induced by DMSO and RA. These 2 agents induced in the R-80 cells a granulocytic phenotype characterized by an increase in the percentage of cells with banded or segmented nuclei (Table 1).

These results indicate that both 1,25-$(OH)_2D_3$ and PMA induce in the HL-60 cells a phenotype that resembles that of monocytes-macrophages. The R-80 cells, which are resistant to PMA-induced cell differentiation, were also resistant to induction of cell differentiation by 1,25-$(OH)_2D_3$. No such resistance could be established with DMSO or RA which induce in the HL-60 and R-80 cells a phenotype that resembles that of granulocytes.

Inhibition of Specific [^{3}H]PDBU Binding to Intact HL-60 Cells by Inducers of Cell Differentiation. The resistance of R-80 cells to cell differentiation induced by both PMA and 1,25-$(OH)_2D_3$ implies that these 2 agents may share a common event or pathway in the induction of cell differentiation in the HL-60 cells. An initial event in the interaction of these inducers with susceptible cells is their binding to specific cellular receptors. It was therefore of interest to determine whether these inducers share common binding sites in the HL-60 cells. To study this possibility, we tested the ability of 1,25-$(OH)_2D_3$ to inhibit the specific binding of phorbol diesters as measured by the binding of [^{3}H]PDBU to intact HL-60 cells (36). For comparison, we included teleocidin, a nonphorbol compound, which has been shown to compete for the PDBU binding sites (21, 38), and RA which unlike PMA, teleocidin, and 1,25-$(OH)_2D_3$ induces a granulocytic phenotype

in both R-80 and HL-60 cells (6, 22). In agreement with previous reports, PMA at doses above 4×10^{-9} M inhibited [^{3}H]PDBU-specific binding to HL-60 cells by more than 90% (Chart 3). A similar inhibition of binding was also observed with 9×10^{-9} M teleocidin. The 1,25-$(OH)_2D_3$, on the other hand, did not inhibit [^{3}H]PDBU binding even at doses as high as 5×10^{-6} M, nor was there an inhibition of [^{3}H]PDBU binding with 3×10^{-7} M RA. In a similar approach, we have shown that PMA at 7×10^{-10} to 7×10^{-6} M did not inhibit the specific binding of [^{3}H]1,25-$(OH)_2D_3$ to its cytosolic receptor (37) (data not shown).

These results indicate that 1,25-$(OH)_2D_3$, unlike teleocidin, does not seem to interact with the specific phorbol diester receptor sites. It thus appears that the common events in the induction of cell differentiation by PMA and 1,25-$(OH)_2D_3$ involve events following the binding step.

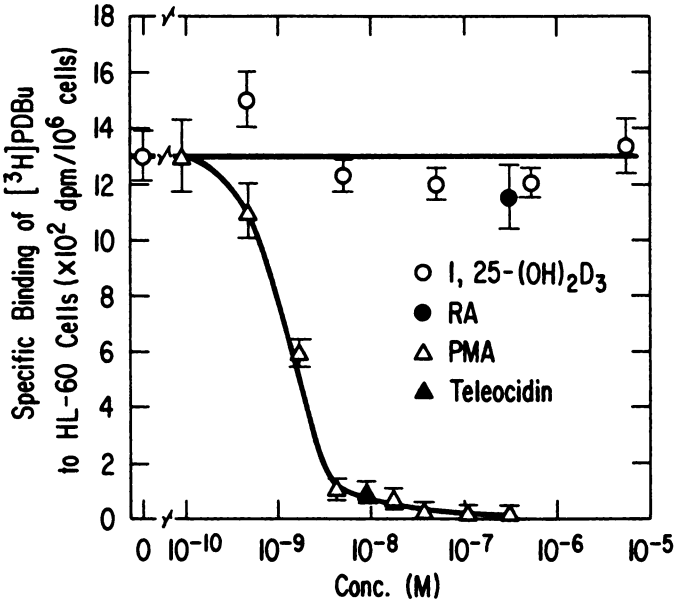

Chart 3. Inhibition of the specific binding of [^{3}H]PDBU to intact HL-60 cells by 1,25-$(OH)_2D_3$, RA, PMA, or teleocidin. Each *point* represents the mean for 3 separate experiments done in triplicate; *bars*, S.E. Control value was $13.2 \pm 0.8 \times 10^2$ dpm/10^6 cells.

Synthesis of Monocyte-Macrophage Marker Proteins in HL-60 Cells after Treatment with Different Inducers of Cell Differentiation. In order to describe further the macrophage phenotype induced by 1,25-$(OH)_2D_3$ and PMA, we used 2-dimensional gel electrophoresis to analyze the newly synthesized proteins in HL-60 cells after treatment with a number of inducers of cell differentiation. For comparison, we included monocytes and granulocytes from human peripheral blood. Treatment with 1,25-$(OH)_2D_3$, PMA, RA, and DMSO caused, as previously reported for some of the inducers (5, 8, 26), either the disappearance or appearance of a fraction of the cell proteins. Among the proteins that did not exhibit marked changes were actin and β_2-microglobulin (Fig. 2; Table 2). Treatment of the HL-60 cells with 1,25-$(OH)_2D_3$ resulted in the synthesis of 2 proteins (labeled D in Fig. 2) that are presumably specific to this inducer and 5 monocyte-macrophage marker proteins (Table 2) (13, 18) (Fig. 2, *rectangles*). Treatment with PMA, DMSO, and RA did not cause the synthesis of the 2 proteins specific to 1,25-$(OH)_2D_3$. These treatments did, however, cause the cells to express a number of the monocyte-macrophage marker proteins. Another apparent monocyte-macrophage marker protein, labeled as M:1 (Fig. 2), was synthesized at high levels in PMA- and DMSO-treated HL-60 cells and at lower levels in RA- and 1,25-$(OH)_2D_3$-treated cells. HL-60 cells treated with 1,25-$(OH)_2D_3$ also synthesized 2 major cytoplasmic proteins, Mit:5, which is a mitochondrial protein of monocytes and granulocytes, and C:1 (Calgon 1), a protein expressed at high levels by granulocytes and at significant levels by monocytes (Fig. 2; Table 2). Neither of these 2 proteins are expressed at significant levels in untreated HL-60 cells (Fig. 2; Table 2). One of the 2 (C:1) was also detected after treatment with RA and DMSO.

PMA has specific effects in the HL-60 cells (such as the induction of protein P:1) that are not produced by the other inducers. However, these changes also occur in other cell types

Table 1

Induction of cell differentiation in HL-60 and R-80 cells after 6 days of treatment with 1,25-$(OH)_2D_3$, PMA, DMSO, or RA

Inducer	Concentration	Cell no. (× 10^6/Petri-dish)		Lysozyme activity (μg equivalents/10^7 cells/10 ml)		Cells exhibiting nonspecific esterase activity (%)		Cells reacting with OKM1 monoclonal antibody (%)		Morphologically mature cells (%)		Cells with banded or segmented nuclei (%)	
		HL-60	R-80	HL-60	R-80	HL-60	R-80	HL-60	R-80	HL-60	R-80	HL-60	R-80
Control		14	15	4	2	2	2	1	1	5	2	<1	<1
1,25-$(OH)_2D_3$	10^{-7} M	6	10	50	6	85	10	91	36	88	8	5	<1
PMA	3×10^{-9} M	0.2	14	135	2	88	2	86	2	89	4	3	<1
DMSO	1.5%	2	4	23	8	5	6	46	39	87	90	53	56
RA	10^{-6} M	4	7	4	3	5	3	53	47	84	72	36	33

Table 2

Occurrence of marker proteins in HL-60 cells after treatment with 1,25-$(OH)_2D_3$, PMA, RA, or DMSO and in untreated peripheral blood monocytes and granulocytes

	C:1	Mit:5	P:1	D	M:1	R[a]:1	R:2	R:3	R:4	R:5
HL-60 control	±[b]	−	−	−	±	−	−	−	±	−
HL-60 + RA	+	−	−	−	+	−	−	±	+	+
HL-60 + DMSO	+	±	−	−	+	±	−	±	+	+
HL-60 + PMA	−	−	+	−	+	±	−	+	±	+
HL-60 + 1,25-$(OH)_2D_3$	+	+	−	+	+	+	+	+	+	+
Monocyte-macrophage[c]	+	+	−	−	+	+	+	+	+	+
Granulocyte[c]	+	+	−	−	−	−	−	±	−	−

[a] R, rectangles in Fig. 2.
[b] −, absence of marker; +, presence of a specific marker protein; ±, not very abundant.
[c] Based on previous marker proteins characterized previously (18).

including CEM cells (33) treated with PMA and thus are probably not related to the specific phenotype induced by PMA in the HL-60 cells.

To summarize the electrophoresis results, treatment of HL-60 cells with 1,25-$(OH)_2D_3$, PMA, DMSO, or RA caused the cells to express various monocyte-macrophage and granulocyte marker proteins. Cells treated with 1,25-$(OH)_2D_3$ differed in their protein pattern from cells treated with PMA or the other 2 inducers. None of the inducers caused a protein pattern identical to that of either monocytes-macrophages or granulocytes; treatment with 1,25-$(OH)_2D_3$ induced in the HL-60 cells the protein pattern closest to that of peripheral blood monocyte-macrophages.

DISCUSSION

Treatment of HL-60 cells with certain chemicals, including physiological agents, can cause the cells to acquire a phenotype that resembles that of either granulocytes or monocytes-macrophages (6, 9, 22, 27, 32). A member of this class of agents is the hormone 1,25-$(OH)_2D_3$ which regulates calcium metabolism (19, 39). Incubation of HL-60 cells with this hormone results in its binding to cellular receptors (37) as well as induction of a series of markers of differentiation (30, 37). Initial studies by Miyaura *et al.* (30) have shown that a proportion of HL-60 cells treated with 1,25-$(OH)_2D_3$ exhibits intermediate levels of morphological maturation, an acquired ability to phagocytize *Candida albicans*, and an increased level of C3 receptors. These authors therefore suggested that 1,25-$(OH)_2D_3$ induces in the HL-60 cells a granulocytic cell differentiation. However, some of the markers identified are shared by granulocytes and monocytes-macrophages.

To establish more definitely the pathway of cell differentiation induced in HL-60 cells by 1,25-$(OH)_2D_3$, we looked for a series of additional markers of differentiation. These included increases in activity of lysozyme (22, 25, 32) and nonspecific esterase (22, 32, 40), reactivity with OKM1 monoclonal antibody (15, 33), and the appearance of differentiation marker proteins in a 2-dimensional gel electrophoresis profile (13, 18). For comparison in these assays, we included treatment with PMA, which causes the HL-60 cells to acquire a phenotype that resembles that of macrophages (22, 27, 32), and DMSO and RA, which induce a granulocyte-like phenotype (6, 9, 22).

The results indicated that treatment of HL-60 cells with 1,25-$(OH)_2D_3$ caused an increase in lysozyme and nonspecific esterase activities, increased reactivity with OKM1 monoclonal antibody, and an increase in the fraction of morphologically mature cells; the inductions of these markers were both time and dose dependent. The increases in nonspecific esterase activity and in the synthesis of a number of monocyte-macrophage protein markers and the absence of a large fraction of cells with banded or segmented nuclei (characteristic of granulocytes) indicate that, in contrast to previous suggestions (30, 37), 1,25-$(OH)_2D_3$ treatment of HL-60 results in a cell type that more closely resembles that of a monocyte-macrophage than that of a granulocyte. Treatment of mouse myeloid leukemia cells with 1,25-$(OH)_2D_3$ has also been shown to yield cells with a macrophage-like phenotype (1). The monocyte-macrophage phenotype induced by 1,25-$(OH)_2D_3$ in the HL-60 cells was found to be similar but not identical with that induced by PMA.

An interesting result of the studies reported in this paper was the observation that the R-80 cells were resistant to the differentiation induced by both PMA and 1,25-$(OH)_2D_3$. These and the previous results suggest that these 2 inducers may affect a similar process which leads to the monocyte-macrophage-like phenotype. This process seems to involve a signal that follows the binding of these inducers to their specific receptors, since 1,25-$(OH)_2D_3$ was not able to compete for the phorbol diester binding sites. Various events including "down modulation" of specific binding (29, 36) and alterations in phospholipid (7, 20, 39) and calcium (19, 35, 39) metabolism, induced by both inducers, may perhaps be the common signal(s) that leads to the similar although not identical phenotype.

Analysis of the protein profiles in the 2-dimensional gel electrophoresis patterns indicates that the phenotype induced by PMA and to some degree by 1,25-$(OH)_2D_3$ was not identical with those of normal peripheral blood monocytes-macrophages. A study of reactivity with monoclonal antibodies against the surface antigens in differentiating myeloid leukemia cells as well as a study dealing with the expression of some functional markers in these cells led to a similar conclusion (14, 31). Thus, the phenotypes acquired after treatment with 1,25-$(OH)_2D_3$, PMA, DMSO, and RA represent either a limited pattern of the normal mature cells or a mixture between the monocyte-macrophage and granulocyte patterns with the different inducers controlling the degree to which a certain pattern is expressed. Of all the tested inducers, 1,25-$(OH)_2D_3$ induced in the HL-60 cells the pattern closest to that of monocytes-macrophages.

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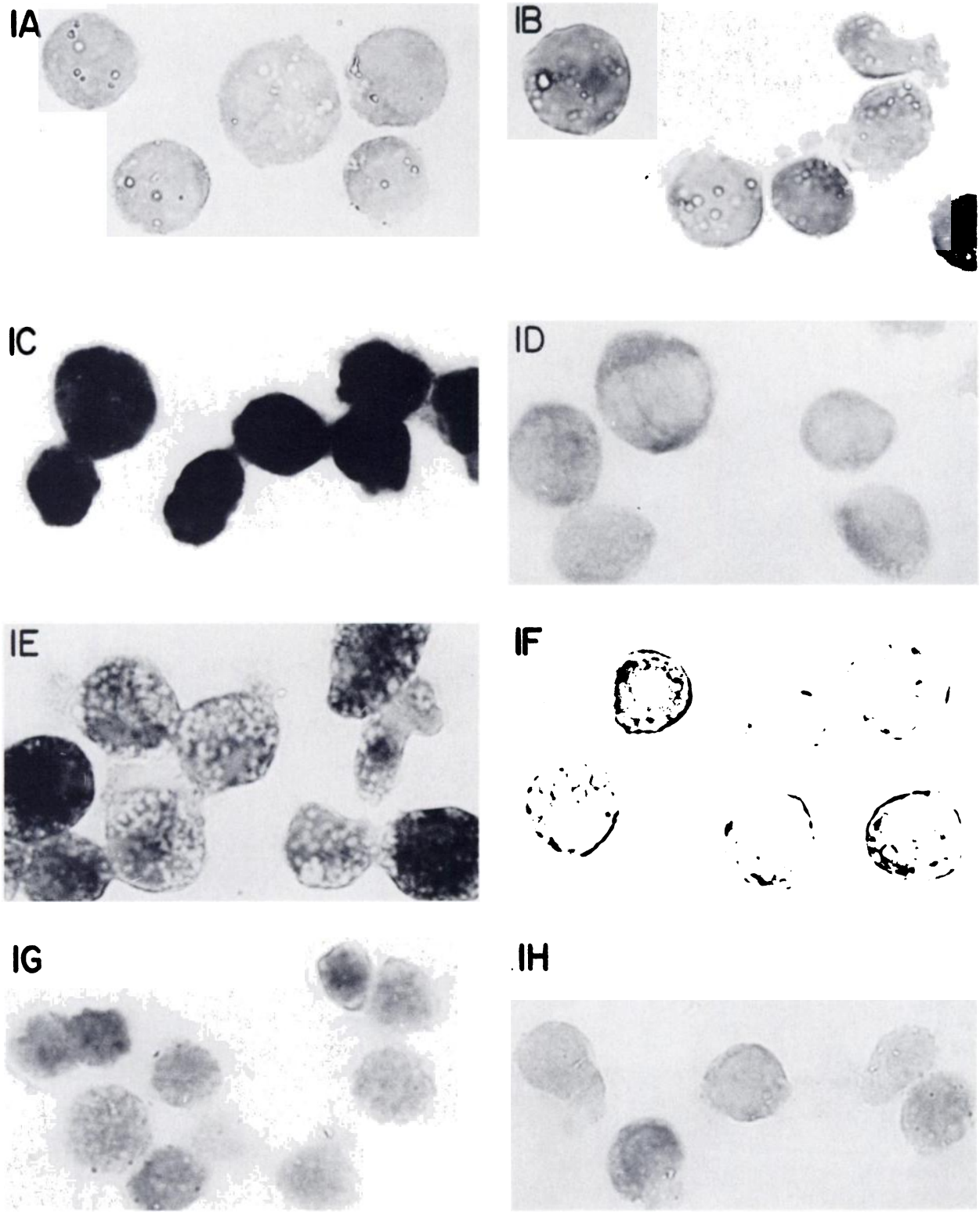

Fig. 1. Staining for nonspecific esterase activity in control cells and cells at the end of 6 days of treatment. *A*, *C*, *E*, and *G*, HL-60 cells; *B*, *D*, *F*, and *H*, R-80 cells. *A* and *B*, control; *C* and *D*, 10^{-7} M 1,25-$(OH)_2D_3$; *E* and *F*, 3×10^{-9} M PMA; *G* and *H*, 1.5% DMSO. × 700.

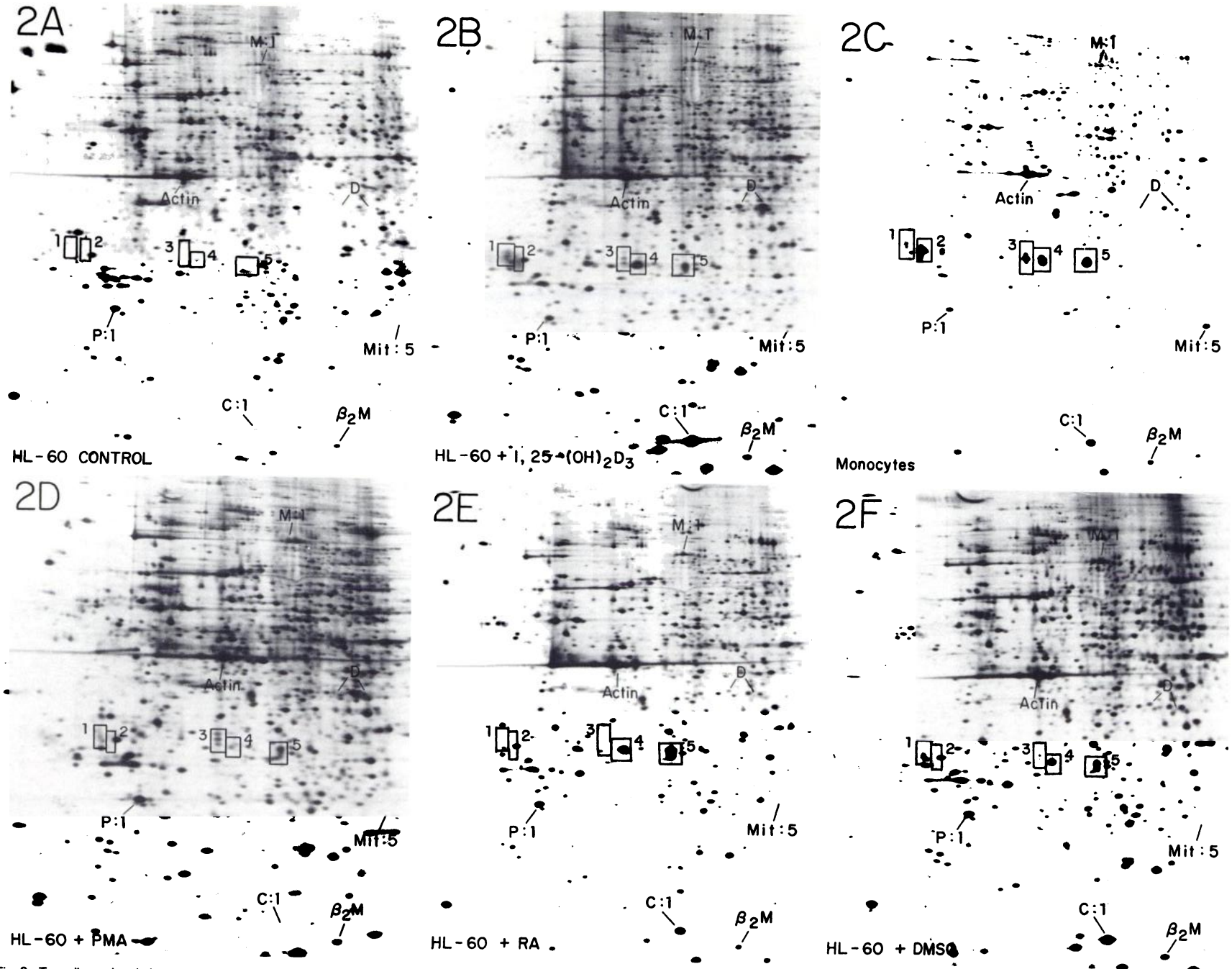

Fig. 2. Two-dimensional electrophoretic patterns of cells labeled with [^{35}S]methionine. *A*, control HL-60 cells; *B*, HL-60 cells treated with 10^{-6} M 1,25-$(OH)_2D_3$; *C*, monocytes from human peripheral blood; *D*, HL-60 cells treated with 5×10^{-8} M PMA; *E*, HL-60 cells treated with 3×10^{-8} M RA; *F*, HL-60 cells treated with 1.5% DMSO. *Rectangles* (labeled *1* to *5*), series of monocyte marker proteins; *P:1*, protein specifically induced by PMA treatment; *D*, 2 proteins induced by 1,25-$(OH)_2D_3$; *M:1*, protein expressed at high levels in macrophages; *Mit:5*, mitochondrial protein characteristic of monocytes and granulocytes; *C:1*, cytoplasmic protein marker of monocytes and granulocytes but in much greater abundance in the latter. Actin and β_2-microglobulin (β_2M) are indicated for reference. The acid end of the focusing dimension is to the *left* and the high apparent molecular weight is at the *top*.