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Differentiation of human T-lymphoid leukemia cells into cells that have a suppressor phenotype is induced by phorbol 12-myristate 13-acetate

(OKT and OKM monoclonal antibodies/tumor promoter/phorbol diester/CEM, HSB-2, and HL-60 cells)

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ABSTRACT **Treatment of cultured human T-lymphoid (CEM) leukemia cells with nanomolar concentrations of phorbol 12-myristate 13-acetate (PMA) resulted in a reduction in cell growth and in the acquisition of a surface antigenic pattern that is common to both suppressor and cytotoxic T lymphocytes. This antigenic pattern was detected by OKT monoclonal antibodies. PMA treatment did not cause the expression of a cytotoxic function but rather induced the expression of a suppressor cell marker. This marker was characterized by the ability of the treated CEM cells to suppress [^{3}H]thymidine incorporation into phytohemagglutinin-activated peripheral blood lymphocytes. After 4 days of treatment of CEM cells from either cloned or the parental cell population with 16 nM PMA, 71–98% of the cells expressed reactivity with OKT3 and OKT8 antibodies whereas reactivity with OKT4 and OKT6 was detected in ≤1–8% of the cells. The CEM cells can be divided into five groups based on the antigenic patterns of cells from randomly isolated clones. The cells from four of these groups were characterized by either low or high reactivity with each of the four OKT antibodies. The antigenic pattern of the fifth group resembled that of the parent CEM cells. The acquisition of reactivity with the OKT3 antibody in the CEM cells after PMA treatment was dependent on both time and dose and did not require cell replication. Acquisition of reactivity with OKT3 antibody also occurred after treatment with phorbol 12,13-dibutyrate but not after treatment with phorbol 13-monoacetate, phorbol 12,13-diacetate, or dimethyl sulfoxide. These results indicate that treatment of CEM cells with PMA and related agents can cause the cells to express a phenotype that resembles that of a mature suppressor T lymphocyte.**

Phorbol 12-myristate 13-acetate (PMA) and related phorbol diesters promote tumor formation in mouse skin (1, 2). A number of cellular events, including alterations in differentiation processes, have been attributed to the action of these agents (3). In some human melanoma cells (4) and in myeloid (5–7) and lymphoid leukemia cells (8–10), these agents can induce differentiation markers. In the T-lymphoid leukemia cells, phorbol esters were reported to increase the fraction of cells with receptors for sheep erythrocytes, reduce the level of terminal deoxynucleotidyltransferase activity, and alter cell morphology (9, 10). Changes in these markers, however, are not sufficient to characterize a specific differentiated state—e.g., differentiation into cells with suppressor, cytotoxic, inducer, or helper functions. Recently, Reinherz *et al.* (11) showed that the antigenic pattern of immature human T lymphocytes changes during the maturation process in the thymus. Thus, discrete stages of human T-cell differentiation can be analyzed in normal and leukemia cells by monoclonal antibodies directed against stage-specific surface antigens (12–14).

We report here that tumor-promoting phorbol diesters can induce differentiation of a human T-lymphoid leukemia cell (CEM) into a cell type with an antigenic pattern and functional property that resembles a mature suppressor T lymphocyte.

MATERIALS AND METHODS

Chemicals. PMA, phorbol 12,13-dibutyrate, phorbol 13-monoacetate, and phorbol 12,13-diacetate were supplied by Peter Borchert (University of Minnesota, Minneapolis). Mitomycin C, phytohemagglutinin (PHA), and dimethyl sulfoxide (Me_2SO) were from Sigma, and [^{3}H]thymidine (specific activity, 6.7 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) was from New England Nuclear. The phorbol esters were dissolved in Me_2SO. The final concentration of this solvent in culture medium was 0.1% or less. Me_2SO at these concentrations did not affect cell growth or differentiation.

Cells and Culture Conditions. The human T-lymphoid cell lines CEM (CCL 119) and HSB-2 (CCL 120.1) were obtained from the American Type Culture Collection (Rockville, MD). The human promyelocytic HL-60 leukemia cells were supplied by R. Gallo (National Cancer Institute, Bethesda, MD). The cells were inoculated into tissue culture plastic Petri dishes and cultured in growth medium [RPMI 1640 (Flow Laboratory)/20% fetal bovine serum supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), and Fungizone (0.25 μg/ml) (GIBCO)] in humidified 5% CO_2/95% air at 37°C. This complete growth medium was used in all experiments. Cell numbers were determined after exclusion of trypan blue-stained cells. For the different experiments, 1.5×10^6 cells in logarithmic growth phase were seeded in 5 ml of growth medium in 60-mm Petri dishes, and incubation with the phorbol esters was initiated the same day and continued for 1–6 days. Cloning of the CEM cells was carried out as follows: Individual colonies (≈100 cells per colony) were isolated from 60-mm Petri dishes inoculated 3 weeks earlier with 400 cells in 5 ml of growth medium/0.8% methyl cellulose. The colonies were transferred to wells of microtiter plates (Falcon 3072; Becton Dickinson Labware, Oxnard, CA) containing growth medium. Confluent cells were thereafter transferred into 5 ml of growth medium in 60-mm Petri dishes and designated as CEM-CL1, etc.

Monoclonal Antibodies and Their Reactivity with the Leukemia Cells. Murine monoclonal antibodies (IgG) against human T-cell antigens were obtained from Ortho Pharmaceutical

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Abbreviations: Me_2SO, dimethyl sulfoxide; PBLs, peripheral blood lymphocytes; PHA, phytohemagglutinin; PMA, phorbol 12-myristate 13-acetate.

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(Raritan, NJ). Reactivity with the monoclonal antibodies OKT3, OKT4, OKT6, and OKT8 is characteristic of peripheral T lymphocytes, inducer/helper T lymphocytes, "common" thymocytes, and suppressor/cytotoxic T lymphocytes, respectively (11–14), whereas reactivity with OKM1 is a characteristic marker of monocytes and granulocytes (15).

Determination of the reactivity of each antibody with the cells was carried out as follows: after two washes with ice-cold phosphate-buffered saline (buffer), 10^6 cells in 200 μl of buffer were incubated with the reconstituted monoclonal antibodies (final dilution, 1:40 with buffer) for 30 min at 4°C. After this incubation, the cells were washed twice with ice-cold buffer and the reactivity of the primary antiserum was developed with a fluorescein isothiocyanate-conjugated goat anti-mouse IgG (heavy and light chain) or $F(ab')_2$ fragment-specific goat anti-mouse IgG (Cappel Laboratories, West Chester, PA) for 30 min at 4°C. The cells were then given two final washings in buffer, suspended in 20 μl of glycerol, and examined in a Leitz fluorescent microscope.

In addition, reactivity of OKT3 monoclonal antibody with CEM cells was analyzed by the antibody/complement-mediated lympholysis technique described by Reinherz *et al.* (11). Lympholysis was determined by counting the percent of cells that stained with trypan blue.

Suppressor Assay. For the suppressor assay (16–18), we used CEM and CEM-CL3 cells that had previously been incubated for 3 days with growth medium alone or growth medium/16 nM PMA. The cells were washed twice with fresh medium, suspended at 10^6/ml, and treated for 30 min with mitomycin C at 25 μg/ml. Following this treatment, the cells were washed three times with fresh medium.

The human peripheral blood lymphocytes (PBLs) were obtained from fresh heparinized blood from healthy volunteers. After Ficoll-Histopaque (Sigma) density-gradient centrifugation, the PBLs were harvested from the interface, washed three times with fresh growth medium, and diluted to a concentration of 10^6/ml of growth medium. The PBLs were isolated and used on the same day as the CEM and CEM-CL3 cells were treated with mitomycin C.

The assay was carried out in 96-well microtiter plates (Falcon). Each well was inoculated with 0.1 ml of growth medium alone or a cell suspension containing 10^5 mitomycin C-treated CEM or CEM-CL3 cells. In addition, the wells were inoculated with 0.1 ml of either growth medium or a cell suspension containing 10^5 PBLs and PHA at a final concentration of 1 μg/ml of growth medium (16). A group of wells, without CEM or CEM-CL3 cells, that had been inoculated with PBLs in the absence or presence of PHA were treated with a final concentration of 1.6 nM PMA. The microtiter plates with the various cell combinations were incubated for 4 days, after which each well received 10 μl of [^{3}H]thymidine (50 μCi/ml). After 16–18 hr of incubation with [^{3}H]thymidine, the cells were collected on a glass fiber filter paper by means of an automated sample harvester (Mash II, Microbiological Associates). The filter paper with the cellular material was dried and the radioactivity was determined by liquid scintillation counting. Each experimental point is based on four replicates (four independent wells). Results are given as mean ± SD.

RESULTS

Reactivity of CEM Cells with OKT3 Monoclonal Antibody After Treatment with Phorbol Esters. During a period of 6 days in culture, 10–20% of the CEM cells exhibited reactivity with the OKT3 monoclonal antibody, which detects a surface antigen specific to human peripheral T lymphocytes (19). Treatment of these cells for more than 24 hr with 16 nM PMA resulted in a reduction in cell growth and an increase in the percentage of cells that reacted with the OKT3 antibody. After the third day of treatment with PMA, >80% of the cells had acquired reactivity with the antibody as detected by either immunofluorescence or the antibody/complement-mediated lympholysis technique (11) (Fig. 1). Because of the ease of the assay, in all subsequent experiments, we used immunofluorescence as the marker for reactivity with the antibodies. Treatment of CEM cells with different doses of PMA showed that, up to 16 nM PMA, the percentage of cells that reacted with OKT3 antibody increased in a dose-dependent manner. Higher doses of PMA were less effective than the 16 nM dose (Fig. 2).

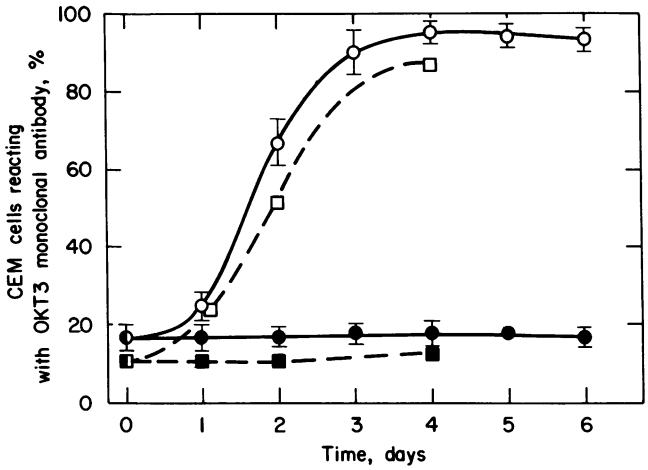

FIG. 1. Reactivity of CEM cells with OKT3 monoclonal antibody at different times after initiation of treatment with 16 nM PMA. ●, ■, Untreated; ○, □, PMA treated. Reactivity was determined by counting the percentage of cells that exhibited immunofluorescence (●, ○) or by counting the fraction of cells that stained with trypan blue after antibody/complement-mediated lympholysis (■, □). Immunofluorescence results are given as mean ± SD of three experiments, each including two independent determinations. Each determination involved analysis of 200 cells.

In one set of experiments, cells were treated with 16 nM PMA for 2, 6, 12, 24, and 96 hr. After this exposure, they were washed with three 25-ml portions of fresh medium and incubation was continued to a total of 96 hr, including the time of PMA treatment. At the end of 96 hr, reaction with OKT3 an-

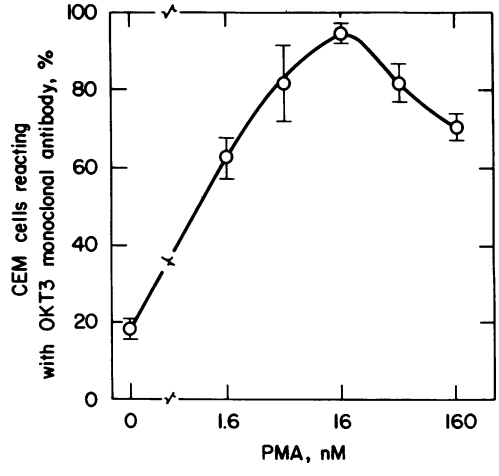

FIG. 2. Reactivity of CEM cells with OKT3 monoclonal antibody after 4 days of treatment with different concentrations of PMA. Results are given as mean ± SD of three experiments, each including two independent determinations. Each determination involved analysis of 200 cells.

tibody showed that a 12-hr induction time with PMA was required for 80% reactivity. Incubation of CEM cells for only 2 hr did not change the percentage of fluorescent cells. The acquisition of reactivity with the OKT3 antibody after PMA treatment does not require cell replication because inhibition of such replication by x-irradiation or mitomycin C did not significantly alter the percentage of cells that reacted with the antibodies (data not shown).

An increase in reactivity of the CEM cells with OKT3 antibody was also observed after treatment with phorbol 12,13-dibutyrate (Table 1), a phorbol diester that is less effective than PMA in promoting skin tumors in mice (1). Two other phorbol esters, phorbol 13-monoacetate and phorbol 12,13-diacetate, which are inactive as tumor promoters (1), were also inactive in inducing reactivity with this antibody (Table 1).

Reactivity of CEM Cells with OKT4, OKT6, OKT8, and OKM1 Monoclonal Antibodies After Treatment with PMA. To determine whether treatment of the CEM cells with PMA can also cause changes in the surface antigens that are associated with either the inducer/helper or the cytotoxic/suppressor phenotype in human T lymphocytes, we tested the reactivity of untreated and PMA-treated cells with OKT4, OKT6, and OKT8 monoclonal antibodies (19). In the untreated CEM cells, 40–50% exhibited reactivity with all three monoclonal antibodies. Treatment of the cells with 16 nM PMA for more than 2 days resulted in a reduction in reactivity with OKT4 and OKT6 antibodies. Reactivity with these antibodies was detected in <5% of the cells, whereas the reactivity with OKT8 was increased and could be detected in ≈80% of the cells (Fig. 3). The resulting antigenic profile was the same as that found in both suppressor and cytotoxic T lymphocytes (11), suggesting that PMA induced in the CEM cells a differentiated state having a suppressor/cytotoxic T-lymphocyte-like phenotype.

To analyze further the specificity of PMA in inducing a suppressor/cytotoxic-like cell differentiation in CEM cells, we included in our studies two additional cell types, the T-lymphoid HSB-2 and the promyelocytic HL-60 leukemia cells. For comparison, we also tested Me_2SO, which, like PMA, can induce terminal differentiation in HL-60 cells (5–7, 20, 21). In the HL-60 cells, both PMA and Me_2SO increased in a dose-dependent manner the percentage of cells that reacted with the OKM1 antibody, which characterizes mature myeloid cells (15). None of these treatments caused detectable reactivity with OKT3, OKT6, or OKT8 antibodies. However, >95% of the HL-60 cells exhibited a weak reactivity with the OKT4 antibody and, as in the case of CEM cells, this reactivity was reduced after treatment with PMA but not with Me_2SO (Table 2). Neither untreated nor PMA-treated CEM cells exhibit reactivity with the OKM1 antibody (Table 2) nor did untreated or treated HSB-2 cells exhibit reactivity with OKT antibodies.

Table 1. Acquisition of reactivity of CEM cells with OKT3 monoclonal antibody after treatment with different phorbol esters

Inducer	Cells, no. $\times 10^6$/ml	% cells reacting with OKT3 monoclonal antibody
None	3.6	16 ± 5
PMA	1.5	92 ± 4
Phorbol 12,13-dibutyrate	1.8	76 ± 3
Phorbol 13-monoacetate	3.6	26 ± 5
Phorbol 12,13-diacetate	3.7	18 ± 2

Reactivity with the OKT3 antibody and cell numbers were determined 4 days after treatment at 0.3 × 10^6 CEM cells/ml with 16 nM phorbol ester. Reactivities are mean ± SD.

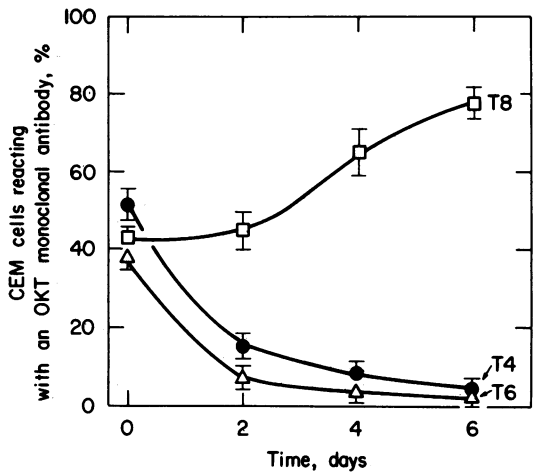

FIG. 3. Reactivity of CEM cells with OKT4 (●), OKT6 (△), and OKT8 (□) monoclonal antibodies at different times after treatment with 16 nM PMA. Untreated cells exhibited similar reactivities with the various OKT antibodies, 47–52% in the case of OKT4 antibody, 35–40% in the case of OKT6 antibody, and 40–45% in the case of OKT8 antibody. Results are given as mean ± SD of three separate experiments, each including two independent determinations. Each determination involved analysis of 200 cells.

These results thus indicate that a suppressor/cytotoxic-like phenotype, characterized by changes in the reactivity with OKT antibodies, can be induced in CEM cells after treatment with PMA but not with Me_2SO and that reactivity with OKT4 antibody is reduced during PMA-induced cell differentiation in both CEM and HL-60 cells. Furthermore, reactivity with OKM1 antibody, which is specific to mature myeloid cells (15), can be detected in HL-60 cells after treatment with either PMA or Me_2SO, which induces in these cells a macrophage and granulocyte-like cell differentiation, respectively (6, 20, 21).

Reactivity with OKT Monoclonal Antibodies After PMA Treatment of Cells Derived from CEM Clones. Independent cell clones were isolated from the CEM cells and their reactivities with the four OKT monoclonal antibodies were analyzed to determine whether the reactivity with the OKT antibodies in the untreated parent CEM cells is due to the presence of a

Table 2. Reactivity of CEM and HL-60 cells with monoclonal antibodies after treatment with Me_2SO or PMA

Inducer	% cells reacting with monoclonal antibody				
	OKT3	OKT4	OKT6	OKT8	OKM1
	CEM cells				
None	15	52	38	42	<1
PMA (16 nM)	96	8	3	76	<1
Me_2SO					
1.0%	15	49	NT	41	<1
1.5%	21	56	NT	39	<1
	HL-60 cells				
None	<1	97	<1	<1	1
PMA					
1.6 nM	<1	13	<1	<1	34
16 nM	<1	3	<1	<1	95
Me_2SO					
1.0%	<1	97	<1	<1	14
1.5%	<1	95	<1	<1	68

Cells were analyzed after 4 days of treatment at 0.3 × 10^6 cells/ml with various inducers. At the time of analysis, the cell densities of the untreated CEM and HL-60 cells were 3.4 and 2.3 × 10^6 cells/ml, respectively. The cell densities of the treated cells, which decreased in a dose-dependent manner, were 1.8–1.0 × 10^6 cells/ml for the CEM cells, and 2.1–0.2 × 10^6 cells/ml for the HL-60 cells. NT, not tested.

mixed cell population in which different subpopulations exhibit different antigenic patterns. The results indicate that, indeed, the parent CEM cells contain a number of subpopulations that can be divided into five groups based on the reactivities of the different cell clones with the OKT antibodies (Table 3). In three of these groups (1, 2, and 3), <3% of the untreated cells in a clone exhibited reactivity with the OKT3 antibody whereas >80% of the cells in such a clone were reactive with OKT4 antibody. Treatment of cells from the different clones with PMA resulted in the acquisition of reactivity with OKT3 antibody in >80% of the cells and in a loss of reactivity with OKT4 antibody such that reactivity could be detected in <9% of the cells. Untreated and PMA-treated cells from groups 1, 2, and 4 do not exhibit reactivity with the OKT6 antibody; untreated cells from groups 3 and 5 do exhibit reactivity with the OKT6 antibody and, as in the case of the parent CEM cells, this reactivity is reduced on PMA treatment so that it can be detected in <5% of the cells. Group 1 is characterized by the fact that >75% of cells react with OKT8 antibody without PMA treatment (Table 3). The reactivity of the untreated cells from group 5 with the OKT antibodies resembles in part that of the parent CEM cells, thus raising the possibility that clones 1 and 6 were derived from more than one cell.

These results suggest that the CEM cell line is composed of a mixed cell population in which each subpopulation expresses different antigenic patterns and that each subpopulation may represent a different stage in the differentiation process. The results also suggest that the expression of the different antigenic determinants is not necessarily under a similar control. Treatment of these different clones, as well as the parent uncloned CEM cell line, with PMA yields an antigenic pattern characteristic of the pattern of suppressor or cytotoxic T lymphocytes.

Induction of a Suppressor Functional Property in CEM and CEM-CL3 Cells Previously Treated with PMA. To characterize further the differentiated state of the CEM and CEM-CL3 cells, we analyzed their ability to exhibit cytotoxic or suppressor cell functions. In the cytotoxicity assays, cocultivation of CEM or CEM-CL3 cells, either untreated or previously treated with 16 nM PMA for 4 days, with target cells (Chinese hamster V79, HL-60, or human P3 teratoma cells) for 1 and 4 days did not reduce the cloning efficiency of the V79 or P3 cells nor was there a detectable increase in the release of ^{51}Cr or [^{3}H]thymidine from prelabeled HL-60 or P3 cells (data not shown).

In the suppressor assay, however, PMA-treated CEM and CEM-CL3 cells were able to exhibit a suppressor effect on PHA-activated PBLs (18) (Table 4). Cocultivation of PMA-treated CEM or CEM-CL3 cells with PHA-activated PBLs resulted in a 50% reduction in the incorporation of [^{3}H]thymidine into the activated cells as compared with the corresponding controls. The untreated CEM cells also exhibited some degree of suppression, yielding a 30% reduction in [^{3}H]thymidine incorporation into cocultivated PHA-activated PBLs. This may be due to the presence of a fraction of spontaneously differentiated cells in the parental CEM cell line (Table 2). Pretreatment of the CEM or CEM-CL3 cells with PMA made these cells less susceptible to mitomycin C inhibition of [^{3}H]thymidine incorporation than cells that had not been treated with PMA. Furthermore, as described (22, 23), treatment of PBLs with PMA not only does not suppress [^{3}H]thymidine incorporation but rather stimulates it.

In summary, we conclude that treatment of CEM cells with PMA causes the expression of an antigenic pattern that is characteristic of suppressor or cytotoxic T lymphocytes. This treatment, however, did not cause the acquisition of a cytotoxic phenotype but rather the expression of a functional marker of a T-suppressor cell that is defined by the ability of the treated cells to suppress thymidine incorporation into PHA-activated PBLs. The latter finding suggests that these CEM cells can be converted into a highly differentiated cell that resembles a normal suppressor T lymphocyte.

Table 3. Reactivities of CEM cell clones with OKT monoclonal antibodies after treatment with PMA

Group	Clone	PMA	% cells reacting with monoclonal antibody			
			OKT3	OKT4	OKT6	OKT8
1	7	−	≤1	98	≤1	95
		+	81	8	≤1	98
	10	−	≤1	98	≤1	76
		+	94	3	≤1	92
2	3	−	≤1	98	≤1	≤1
		+	96	≤1	≤1	92
	9	−	≤1	96	2	2
		+	88	5	≤1	73
3	2	−	2	81	93	8
		+	89	4	2	72
4	12	−	41	54	≤1	9
		+	93	3	≤1	62
5	1	−	11	44	86	44
		+	88	2	4	71
	6	−	13	53	57	53
		+	94	7	≤1	71

Reactivities of the cell clones with the monoclonal antibodies were tested after 4 days of treatment of 0.3×10^6 cells/ml with 16 nM PMA. Two hundred cells were analyzed for each experimental point. None of the clones exhibited reactivity with OKM1 antibody. At the time of analysis, the cell densities of the untreated cells were $2.0–3.0 \times 10^6$ cells/ml and those of the PMA-treated cells were $0.8–1.7 \times 10^6$ cells/ml.

DISCUSSION

The transformation of normal hematopoietic cells into leukemia cells involves specific cellular blocks in the normal differentiation processes. This is evident from various studies that show that some natural or synthetic chemical agents may cause leukemia cells to overcome such blocks and to proceed into a differentiated state in which they exhibit "normal" cell characteristics (24). Phorbol diesters and related chemicals that require specific receptors for their biological activity (25–27) belong to this class of agents because they are able to induce the expres-

Table 4. Suppression of [^{3}H]thymidine incorporation in PHA-activated PBLs cocultivated with either CEM or CEM-CL3 cells

Cell type	Pretreatment	[^{3}H]Thymidine incorporation,* cpm × 10^{-3} per well	
		Absence	Presence
None	Untreated	0	153 ± 23
	Untreated†	0	162 ± 18
CEM	Untreated	0.7 ± 0.1	107 ± 14
	16 nM PMA	5.1 ± 0.3	55 ± 12
CEM-CL3	Untreated	0.2 ± 0.1	152 ± 18
	16 nM PMA	9.3 ± 0.7	78 ± 12

* [^{3}H]Thymidine incorporation into control cells and cells pretreated with 16 nM PMA was measured in the absence and presence of PHA-activated PBLs. Results are mean ± SD.

† PMA at a final concentration of 0.3 nM was added to the wells with the PHA-activated PBLs. PMA at this concentration increased [^{3}H]thymidine incorporation in PBLs (not activated by PHA) from $1.0 \pm 0.2 \times 10^3$ cpm per well to $80 \pm 10 \times 10^3$ cpm per well.

sion of a differentiated state in some human myeloid and lymphoid leukemia cells (5–10, 14, 20, 21).

In this paper, we describe the differentiation of human T-lymphoid (CEM) leukemia cells into cells having a phenotype that resembles that of a suppressor T lymphocyte. This was accomplished by treating the CEM cells with a low concentration (16 nM) of PMA. The new phenotype was characterized in part by the appearance of a specific antigenic pattern. More specifically, PMA caused the cells to exhibit increased reactivity with OKT3 and OKT8 monoclonal antibodies, which characterize both mature suppressor and cytotoxic T lymphocytes. PMA also reduced the reactivity of the treated cells with OKT4 monoclonal antibody, which detects inducer/helper T lymphocytes, and with OKT6 antibody, which detects immature "common" thymocytes (11, 12, 19). In addition, the treated CEM cells, in common with mature suppressor T lymphocytes, were able to suppress [^{3}H]thymidine incorporation in PHA-activated PBLs (18) but, unlike cytotoxic lymphocytes, were unable to induce a cytotoxic response in a number of human and rodent target cells. The differentiation into the suppressor T-lymphocyte-like phenotype, which is a highly differentiated state, suggests that the block involved in the malignant transformation of the CEM cells was at an advanced stage in the differentiation process. In comparison, the detection after PMA treatment of nonspecific T-lymphocyte markers and the absence of specific markers in a number of other T-lymphoid leukemias suggest that the block in these cases was at an earlier step in the differentiation process (9, 10, 14). The differentiated state *in vitro* after PMA treatment may thus be helpful in determining the specific blocks in T-lymphoid leukemia cells and perhaps be indicative of the response of such cells to therapy. In addition, T-lymphoid leukemia cells differentiating after PMA treatment may also be helpful in identifying the cellular and biochemical events that are involved in the differentiation process and allow the isolation and characterization of specific differentiation cell products [e.g., human suppressor factors (28, 29)].

Analysis of the reactivities with the OKT antibodies in the untreated and PMA-treated CEM cell clones can help in suggesting the acquisition sequence of the different antigenic determinants that characterize the suppressor T lymphocyte. From these studies and those of Reinherz and co-workers (11, 12, 19), we suggest that, in human T-lymphoblastoid cells, the acquisition of a suppressor phenotype involves the following steps: (*i*) $T3^-T4^+T6^+T8^-$ (represented by group 3) → (*ii*) $T3^-T4^+T6^-T8^-$ (represented by group 2) → (*iii*) $T3^-T4^+T6^-T8^+$ (represented by group 1) → (*iv*) $T3^+T4^-T6^-T8^+$, which is the differentiated state. Our unpublished results, which show that treatment of CEM-CL3 cells with 1–10 μM 5-azacytidine (30) causes an induction in reactivity with OKT8 antibody without affecting reactivity with the other three OKT antibodies, are in agreement with this suggestion.

In the present studies, we have also found that myeloid HL-60 cells exhibit reactivity with the T-lymphoid-specific OKT4 antibody. PMA, which induces a macrophage phenotype in HL-60 cells (6, 7, 21), reduces the reactivity with this antibody, as it also does in the case of CEM cells. Me_2SO, which induces a granulocytic phenotype in HL-60 cells (20, 21), does not affect this reactivity. However, both of these agents induce reactivity in HL-60 cells with the OKM1 antibody that is characteristic of both normal monocytic and granulocytic cells (15). These studies thus suggest that reactivity with OKT and OKM1 monoclonal antibodies can be useful in determining differentiation characteristics in both HL-60 and CEM cells and that the presence of reactivity with OKT4 antibody is associated with responsiveness of these cells to PMA.

Based on the present studies, we also speculate that tumor promotion with PMA and related agents in the mouse skin may, among other things, involve suppression of the immunity against "initiator"-induced papillomas and carcinomas. Such suppression could result from an increase in the number of mature suppressor T lymphocytes in a PMA-treated animal (1–3). In this connection, suppressor cells were implicated in the induction of mouse skin tumors by UV irradiation (31). Thus, the control of differentiation of specific cell types, including those that mature into suppressor T lymphocytes, can play an important role in the carcinogenesis process.

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