Phorbol Diester-induced Macrophage Differentiation of Leukemic Blasts from Patients with Human Myelogenous Leukemia

H. P. KOEFFLER, M. BAR-ELI, and M. TERRITO, *Department of Medicine, Division of Hematology-Oncology, University of California School of Medicine, Los Angeles, California 90024; Veterans Administration Medical Center, Sepulveda, California 91343*

ABSTRACT Phorbol esters, including 12-*O*-tetradecanoylphorbol 13-acetate (TPA), induce terminal macrophagelike differentiation of cells from human acute myelogenous leukemia lines. We report that myelogenous leukemia cells obtained from patients undergo macrophagelike differentiation after exposure to TPA. The myeloid leukemic cell cultured with TPA became adherent to charged surfaces with long filamentous pseudopodia; developed positive staining for α-napthyl acetate esterase, increased lysozyme secretion, reduced nitroblue tetrazolium, and acquired the ability to phagocytose candida. Cells from patients with lymphocytic leukemia did not become macrophagelike when cultured with TPA.

INTRODUCTION

Phorbol esters, including 12-*O*-tetradecanoylphorbol 13-acetate (TPA),[1] have profound effects on differentiation of a variety of cells from various species. These agents inhibited spontaneous and induced cell differentiation in avian myoblasts (1), chrondroblasts (2), and ganglion cells (3), as well as murine neuroblastoma (4), adipose (5), and most erythroleukemia cell lines (6, 7). In contrast, TPA enhanced the differentiation mediated by colony-stimulating factors in the cells of a murine myeloid leukemia line (M-l) (8, 9). Studies on human acute myelogenous leukemia cell lines demonstrated that the cells differentiated into macrophagelike cells after exposure to phorbol esters (8, 10–12). This article reports that phorbol esters induced macrophagelike differentiation of leukemic blast cells from patients with myelogenous leukemia.

METHODS

Cell and culture conditions. The 34 leukemic patients that were studied are listed in Table I. 11 patients had acute myeloblastic leukemia, 4 had acute myelomonocytic leukemia, 4 had myeloid blast crisis of chronic myelogenous leukemia (CML), 1 had lymphoid blast crisis of CML, 3 had acute lymphocytic leukemia, 9 had B-type chronic lymphocytic leukemia (CLL), 1 had T-type CLL, and 1 had Sezary syndrome. The patients were morphologically and histochemically classified by criteria developed by the French-American-British cooperative group (13). The cytochemical stains performed on the peripheral blood and bone marrow of the patients included Wright-Giemsa, α-naphthyl acetate esterase (NAE), myeloperoxidase (MPO), Sudan black, ASD-chloroacetate esterase, and periodic acid Schiff (14, 15). Karyotypic studies were performed on the leukemic cells from all patients except those patients with CLL. Lymphocyte marker studies were performed on all cases whose leukemic cells were morphologically poorly differentiated. These studies included E and EA rosettes, fluorescent studies for surface membrane immunoglobulin, enzyme analysis for terminal deoxynucleotidyl transferase, and acute lymphocytic leukemia and T-antigen detection by microcytotoxicity studies (16–21). All patients had high peripheral blood leukemic cell counts. 10–20 ml of heparinized venous blood was drawn from the appropriately informed patients. Mononuclear cells were isolated from blood by buoyant density centrifugation (22), and the nonadherent cells were obtained by twice incubating the cells on plastic for 1 h at 37°C, 5% CO_2 in humidified air and collecting the nonadherent cells (23). The final cell suspensions contained 75% or more leukemic cells as judged by morphology of Wright-Giemsa stained cytocentrifuge preparations (Table I).

The cells were cultured with or without the TPA, in α medium (Flow Laboratories, Inc., Rockville, Md.) containing 20% fetal calf serum and penicillin/streptomycin at 37°C in 5% CO_2 and air in a humidified incubator.

Cell growth and maturation. Cell growth was studied in some leukemic patients. Three million peripheral blood mononuclear cells were placed in petri dishes (Falcon Lab-

Dr. Koeffler is a scholar of the Leukemia Society of America.

Received for publication 9 April 1980 and in revised form 7 July 1980.

[1] *Abbreviations used in this paper*: CLL, chronic lymphocytic leukemia; CML, chronic myelogenous leukemia; MPO, myeloperoxidase; NAE, α-naphthyl acetate esterase; NBT, nitroblue tetrazolium; TPA, 12-*O*-tetradecanoyl phorbol 13-acetate.

TABLE I
*Effect of TPA on Leukemic Cells**

Patients: Leukemia classification case No.	Leukemic cells in initial suspension	Adhesion		NAE‡		Myeloperoxidase	
		+TPA	−TPA	+ TPA	− TPA	+TPA	−TPA
				%			
Myeloblastic							
1	95§	91	1	99	1	26	99
2	95	83	1	91	1	15	95
3	85	81	1	68	6	6	53
4	95	91	1	65	1	1	23
5	95	88	1	52	ND‖	ND	68
6	95	86	1	99	1	18	99
7	95	89	1	83	1	14	85
8	95	93	1	73	1	11	69
9	95	76	1	ND	ND	ND	ND
10	91	82	1	42	1	19	64
11	90	92	5	55	3	ND	ND
Mean	93±3¶	87±5	1±1	73±20	2±4	14±8	73±25
Myelomonocytic							
12	82	82	3	78	6	8	63
13	95	84	6	91	7	22	94
14	90	95	5	55	6	16	52
15	87	89	8	67	4	4	59
Mean	89±5¶	88±6	6±2	73±15	6±1	13±8	67±19
Blastic CML (Myeloid)							
16	80§	86	1	35	1	12	96
17	76	83	4	24	1	14	92
18	81	90	9	58	4	ND	ND
19	83	93	5	63	3	ND	ND
Mean	80±3¶	88±4	5±4	45±19	2±3	13	94
Blastic CML (Lymphoid)							
20	92	81	6	6	4	2	1
Acute lymphocytic leukemia							
21–23 Mean	97±2¶	32±13	1±1	9±7	1±1	3±4	3±3
CLL (B-type)							
24–32 Mean	97±2¶	30±25	3±2	8±8	1±1	1±1	1±1
Lymphocytic leukemia (T-type)							
33–34 Mean (Range)	98 (97–98)	5 (4–5)	2 (1–2)	1 (0–1)	1 (0–1)	2 (1–2)	1 (0–1)

* Cells cultured with TPA (0.1 μM) for 4 d.
‡ NAE, α-naphthyl acetate esterase-positive cells (diffusely dark brown).
§ Represents percent myeloblasts and promyelocytes as judged by morphology.
‖ ND, not done.
¶ Represents mean±SD.

ware, Div. Becton, Dickinson & Co., Oxnard, Calif.) containing 3 ml of culture medium with or without 0.1 μM TPA. Cultures were harvested on day 4 for viable and differential cell counts. Viable cell counts were performed using trypan blue dye exclusion. Cytocentrifuge preparations were made and the histochemical stains Wright-Giemsa, NAE, ASD-chloroacetate esterase, and MPO were used to help determine cell lineage (14, 15). Morphologic classification of cellular maturation in the granulocytic and monocytic series was as follows: immature cells included only blasts, progranulocytes, and promonocytes; mature cells included myelocytes, metamyelocytes, polymorphonuclear neutrophils, and macrophages.

Adhesion. Adhesion was examined by placing 1×10^6 leukemia cells in 1 ml of culture medium in glass tissue culture chambers (Lab-Tek, Miles Laboratories Inc., Elkhart, Ind.). After incubation for 4 d at 37°C, the slides were washed with warm Dulbecco's phosphate-buffered saline and the nonad-

herent cells collected. The adherent cells were removed by exposure to 0.25% trypsin and collected separately. Viable cells were counted.

Phagocytosis. The method developed for studying phagocytosis has been described in detail (24). Briefly, *Candida albicans* were opsonized in 20% human AB serum. A 5:1 ratio of candida to leukemic cells were incubated at 37°C for 30 min. The cells were stained with Giemsa stain and the percentage of cells completely ingesting one or more yeast determined microscopically. To confirm that the candida had been internalized and had not merely adhered to the cell surface, the method of Delafield and Lehrer (25) was used in some assays. Heat-killed opsonized *Candida albicans* were incubated with the leukemic cells. Using trypan-eosin staining, the candida had to be completely ingested to be protected from taking up the stain. The leukemic cells containing unstained yeast were considered phagocytic. The values obtained with this assay concurred with those using the Giemsa staining technique.

Nitroblue tetrazolium (NBT) reduction. The reduction of NBT was measured qualitatively. 1 ml of cells (2×10^6 cells/ml) was suspended in α medium containing 20% fetal calf serum, NBT (2%, dissolved in Dulbecco's phosphate-buffered saline), and 200 ng freshly diluted TPA. The cell suspension was incubated at 37°C for 20 min; washed three times in cold Dulbecco's phosphate-buffered saline; cytocentrifuged; fixed in methanol; and stained 5 min with safranin (26, 27). The percentage of cells containing intracellular reduced blue-black formazan deposits was determined by light microscopy by differentially counting 200 cells.

Lysozyme activity. The leukemia cells (1×10^6/ml) were cultured for 4 d in 0.1 μM TPA in α medium containing 20% fetal calf serum. The amount of lysozyme in the cells and released into the medium was measured using the dissolution of micrococcus lysodeikitus (28). Parallel cell cultures containing no TPA were studied. The culture medium alone had no detectable lysozyme activity.

Chemicals. The phorbol ester TPA was purchased from Consolidated Midland (Brewster, N. Y.). Stock solutions (0.1 mM) of this compound were prepared in acetone (Matheson, Coleman, and Bell, East Rutherford, N. J.) every 3 mo. The concentration of acetone present in the final solution (<0.1%) had no effect when added to the cells. All stock solutions were diluted with α medium and filter-sterilized.

RESULTS

Effect of TPA on growth and morphology of leukemic cells. Net cellular proliferation was measured in myeloid leukemia patients 1–3, 5, 7, 9, 14 (Fig. 1A). Viable cell counts decreased a mean of 30% by the 4th d of TPA (0.1 μM) exposure. Cell numbers in cultures without TPA fell a mean of 17%. This does not represent a statistically significant difference ($P > 0.05$, paired t test).

The patterns of cell maturation in vitro are shown in Fig. 1B. In control cultures not containing TPA, the percentage of immature cells changed very little. At 4 d, 88±8% (±SD) of the control cells were immature. The few differentiating cells were predominantly small cells resembling myelocytes, metamyelocytes, and polymorphonuclear cells. Only rare macrophagelike cells were seen. This pattern of cellular maturation in vitro of myeloid leukemic cells is similar to previous reports (29–31). In contrast, the percentage of blast cells decreased steeply in the TPA cultures. At 4 d immature cells averaged <40% of the population. This represents a statistically significant difference from cultures not containing TPA ($P < 0.005$, paired t test). Almost all the mature cells had the morphologic and histochemical (NAE-positive) features of mature human tissue macrophages (32). The nuclear-cytoplasmic ratio became less than one, nuclear chromatin condensed, 10–15% of cells became multinuclear, cytoplasmic vacuoles became prominent, and >50% of the cells from each patient developed long filamentous pseudopodia.

The leukemic cells from the patients with myeloid blast crisis of CML (patients 16–19) developed similar

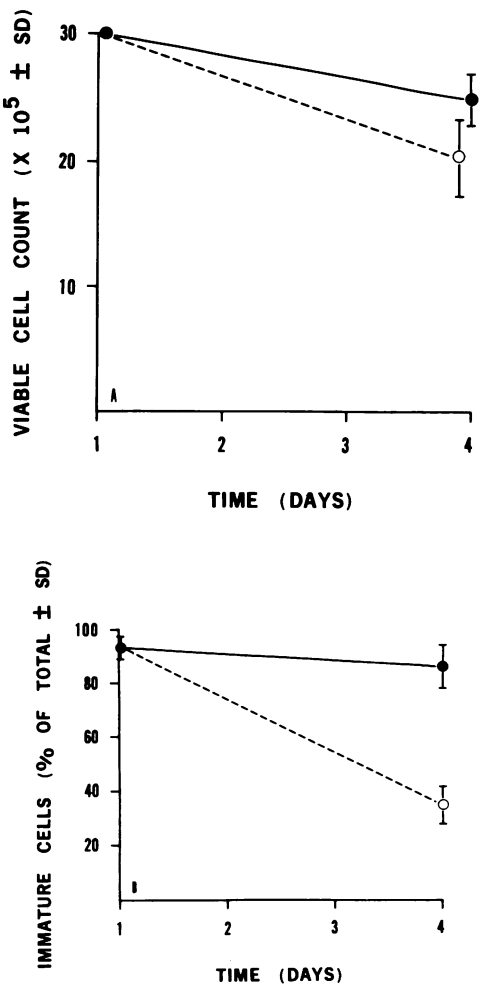

FIGURE 1 (A) Viable cell count as a function of duration of culture. (– – –), mean±SD of cells from seven myeloid leukemic patients cultured with 0.1 μM TPA. (——), control cultures not containing TPA. (B) Percentage of immature cells in culture as a function of time. (– – –), mean±SD of cells from seven myeloid patients cultured with 0.1 μM TPA. (——), same as A.

changes after 4 d culture with 0.1 μM TPA. Cells from the 14 lymphocytic leukemia patients and the one patient with CML in lymphoid blast crisis (patient 20) did not develop the appearance of macrophages after 4 d TPA exposure.

Effect of TPA on adhesion of leukemic cells. Normal human tissue macrophages have the ability to adhere to charged surfaces and develop prominent pseudopodia (24). The leukemia cells from the patients with acute myeloid leukemia and CML in myeloid blast crisis (1–19) became firmly adherent to glass when cultured in the presence of TPA (0.1 μM) (Table I). Within 1 d, >60% of the cells were adherent, and ~85% of the cells were firmly attached to the glass by day 4 of TPA culture. Approximately 40% of the cells developed pseudopodia that were frequently very long (100–200 microns). The percentage of cells that became adherent was very similar in all three myeloid leukemia groups (myeloblastic, myelomonocytic, CML in myeloid blast crisis). Approximately 30% of cells from the patients with lymphocytic leukemia (20–34) became adherent and formed large clumps on the substratum after 4 d in TPA (Table I). In contrast to the myeloid leukemic cells, many of the aggregates of adherent lymphoid cells could be dislodged from the glass surface by shaking vigorously. Less than 10% of cells from the leukemic patients became adherent in cultures containing no TPA (Table I).

Effect of TPA on enzymes present in leukemic cells. An enzyme marker of macrophages is α-naphthyl acetate esterase (14). Histochemically this activity appears as diffuse dark brown staining. Normal human lymphocytes have between one and two small brown dots with histochemical staining for NAE. A mean of 73±18% (±SD) of the cells from the acute myeloblastic and myelomonocytic leukemia patients stained strongly with a diffuse pattern for NAE after 4 d of culture in 0.1 μM TPA (Table I, Fig. 2). An average of 45±19% of the cells from the patients with myeloid blast crisis of CML developed prominent esterase activity. The esterase brown dots of the cells from the lymphocytic leukemia patients (20–34) became approximately two times larger and darker after TPA exposure, and a mean of 7±7% of the cells became diffusely dark brown (Table I). Less than 8% of the leukemic cells contained diffuse esterase activity after 4 d in cultures containing no TPA (Table I).

The esterase activity of macrophages, but not lymphocytes, is normally inhibitable by NaF. We found that NaF inhibited the NAE activity of the leukemic cells from the patients with myeloid leukemia after the cells had been exposed to 0.1 μM TPA for 4 d. In contrast, the increased esterase activity of the TPA-cultured acute and CLL cells was not significantly decreased by NaF.

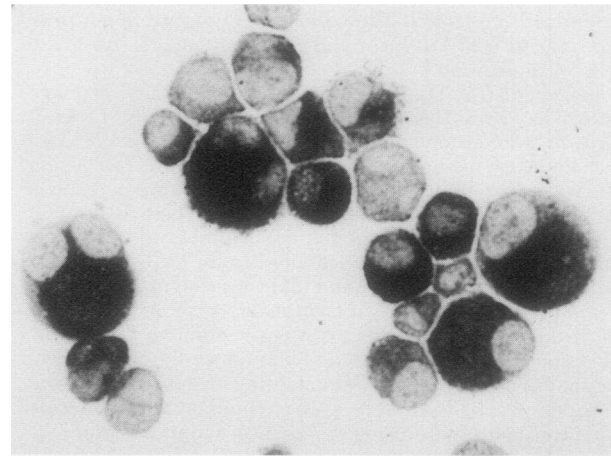

FIGURE 2 Human acute myelogenous leukemia cells stained positively for α-naphthyl acetate esterase after exposure to TPA (0.1 μM) for 4 d.

With normal human mononuclear phagocyte development, MPO is lost as the cell differentiates into a macrophage (33). Granulocytes are rich in MPO. A mean of 73±22% (±SD) of the cells from the patients with acute myeloid leukemia and CML in myeloid blast crisis contained abundant MPO in cultures containing no TPA for 4 d (Table I). In contrast, only 13±7.2% of the leukemic cells from these patients retained MPO activity after TPA (0.1 μM) exposure for 4 d. The cells from the patients with lymphocytic leukemia and CML in lymphoid blast crisis had <2% MPO positive cells before and after TPA exposure (Table I).

Lysozyme is abundant in human macrophages (34, 35). The cells from patients with myeloid leukemia cultured with TPA (0.1 μM) for 4 d had a mean 2.8-fold increase in lysozyme secretion as compared with control dishes not containing TPA (Table II). The mean secreted lysozyme activity was 1.52±0.80 $\mu g/10^6$ cells in cultures not containing TPA and 3.85±5.2 $\mu g/10^6$ cells in cultures containing TPA. Although TPA increased secretion of lysozyme in each myeloid leukemic culture, the results varied greatly and were not significantly different between the control and TPA-containing cultures. Cellular lysozyme was measured to insure that TPA was not merely promoting a degranulation of preformed lysozyme into the culture medium. Mean intracellular lysozyme did not significantly change (Table II). In the myeloid leukemic cultures, the mean intracellular lysozyme was 0.509 ±0.673 $\mu g/10^6$ cells in control dishes and 0.593±0.644 $\mu g/10^6$ cells in dishes containing TPA. The cells from the patients with lymphocytic leukemia and lymphoid blast crisis of CML had low levels of secreted and intracellular lysozyme activity both in control and TPA-containing cultures (Table II).

TABLE II
Lysozyme Changes after TPA Exposure of Leukemic Cells

Patients Leukemia classification case No.	Lysozyme secretion		Cellular lysozyme	
	−TPA	+TPA	−TPA	+TPA
	μg/10⁶ cells per ml			
Myeloblastic				
4	0.70	1.37	0.23	0.56
5	1.74	2.22	0.39	0.56
9	1.86	1.99	0.38	0.31
10	0.68	1.34	0.031	0.10
11	1.65	2.38	2.07	0.69
Myelomonocytic				
15	2.03	6.76	0.114	0.31
Blast CML (myeloid)				
18	0.62	1.76	0.099	0.12
19	2.90	16.80	0.77	2.10
Mean	1.52±0.80	3.85±5.2	0.51±0.67	0.59±0.64
Blast CML (lymphoid)				
20	0.89	0.83	0.12	0.062
Acute lymphocytic leukemia				
21	0.058	0.041	0.054	0.003
23	0.17	0.25	0.061	0.065
CLL				
26	0.45	0.95	0.099	0.040
30	0.86	0.32	0.17	0.19
31	0.06	0.042	0.162	0.021
Mean	0.41±0.38	0.41±0.39	0.11±0.05	0.06±0.01

Effect of TPA on NBT reduction of leukemic cells. Reduction of NBT is dependent on an intact respiratory burst with production of hydrogen peroxide (H_2O_2) and superoxide (O_2^-) (36). Macrophages and granulocytes are able to reduce NBT but their less mature progenitors cannot. We cultured the cells from nine myeloid leukemic patients with TPA (0.1 μM) for 4 d and examined the ability of the cells to reduce NBT (Fig. 3). A mean of 32±23 percent (±SD) of the TPA-cultured cells reduced NBT. An average of 13±15 percent of the cells cultured without TPA reduced NBT. This represents a statistically significant difference between cultures with and without TPA ($P < 0.005$, paired t test). Patients 3, 17, and 18 had a large number of NBT positive cells without TPA exposure. This probably reflects an increased number of myelocytes and more mature granulocytic forms in the leukemic cell suspension. Less than 10% of the cells in the lymphocytic leukemic cultures with or without TPA were able to reduce NBT (data not shown).

Effect of TPA on phagocytosis by leukemic cells. A valuable function of normal mononuclear phagocytes is ingestion of foreign material (24). The leukemic blast cells from the patients with myeloblastic and myelomonocytic leukemia and myeloid blastic CML developed the ability to phagocytose Candida albicans after exposure to 0.1 μM TPA in liquid culture (Figs. 4 and 5). A mean of 35±16% (±SD) of the cells from the patients with myeloid leukemia ingested one or more

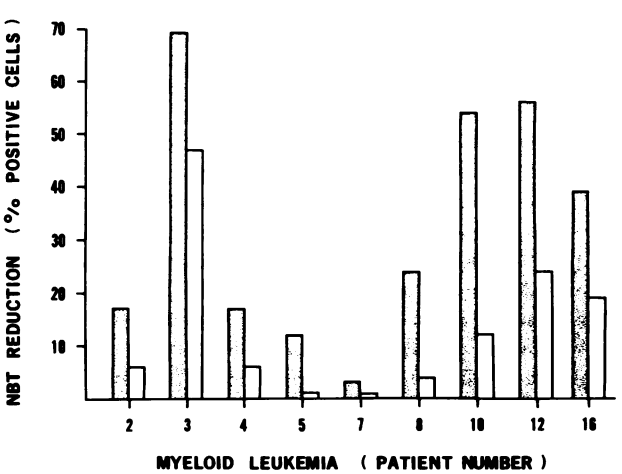

FIGURE 3 Effect of TPA on NBT reduction of myeloid leukemic cells. Stippled boxes represent cells cultured with 0.1 μM TPA for 4 d. Clear boxes represent cells cultured without TPA.

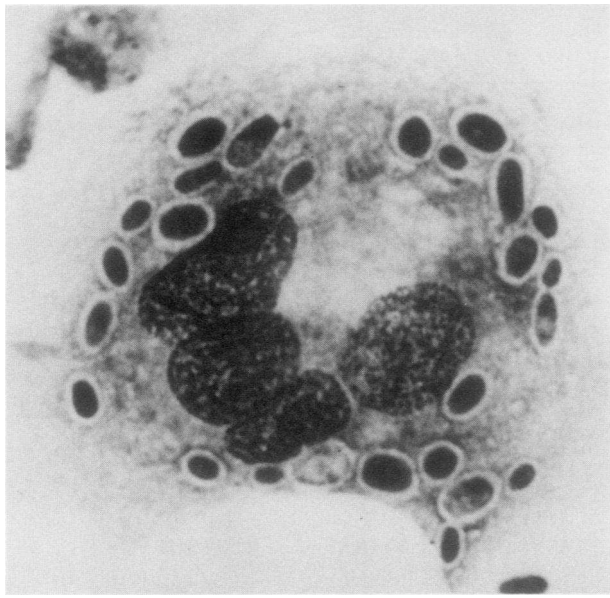

FIGURE 4 Human multinuclear acute myelogenous leukemia cell phagocytosing Candida after exposure to TPA (0.1 μM) for 4 d.

Candida after culture in TPA for 4 d. An average of 9±9% of the cells phagocytosed Candida when cultured in the absence of TPA (Fig. 5). There was a statistically significant difference between cultures with and without TPA ($P < 0.005$, paired t test). Less than 5% of the cells from the lymphocytic leukemia patients were phagocytic after TPA exposure (data not shown).

DISCUSSION

Human myeloid leukemia cells changed dramatically after TPA exposure. The observed alterations were characteristic of terminally differentiated macrophage-like cells. Morphologically the TPA-induced cells developed one or more vesicular nuclei, condensed nuclear chromatin, and long cytoplasmic pseudopodia. Most of the TPA-treated acute and chronic myeloid leukemic blast cells adhered to the glass substratum. The cells from several of the lymphocytic leukemia patients became adherent and clumped on glass after TPA exposure. This has been noted previously in several human lymphocyte lines grown with TPA (37).

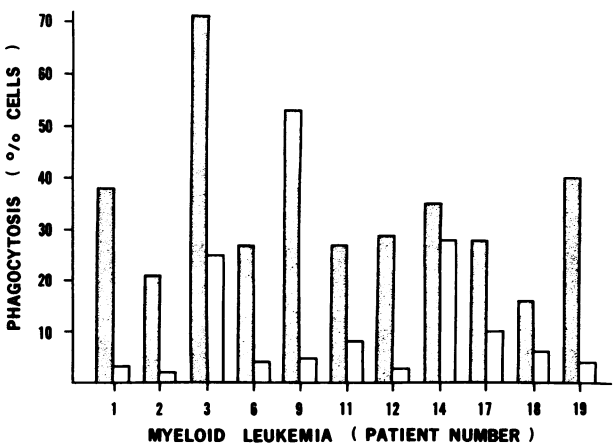

FIGURE 5 Effect of TPA on phagocytosis by myeloid leukemic cells. Stippled boxes represent cells cultured with TPA (0.1 μM) for 4 d. Clear boxes represent cells cultured without TPA.

The production of hydrolytic enzymes is important in the antimicrobial function of mononuclear phagocytes. These enzymes are within the lysosomal cell fraction and include α-naphthyl acetate esterase (34, 35). The NAE enzyme is present in large amounts in macrophages but not in myeloblastic and myelomonocytic leukemia cells or mature granulocytes. In our study, the acute and chronic myeloid leukemia blast cells focally increased their NAE activity after TPA exposure and <10% of the cells became strongly positive. With normal maturation, the human tissue macrophages lose MPO (33). We found that many MPO-positive acute and chronic myeloid leukemic blast cells lost their MPO activity after TPA exposure. The synthesis of lysozyme increases as normal human promonocytes and monocytes differentiate into macrophages. We found that TPA stimulated a mean 2.5-fold increase in lysozyme secretion in cultures containing acute or chronic myeloid leukemia cells as compared with cultures containing no TPA. Intracellular lysozyme activity did not change suggesting that the increased lysozyme secretion did not represent TPA-induced degranulation. Lymphocytic leukemia cells exposed to TPA did not increase their secretion or intracellular content of lysozyme.

An important factor in bacterial killing is the development of the oxygen burst with H_2O_2 and O_2^- production (36). Approximately 30% of the myeloid leukemic cells cultured with TPA developed the oxygen burst, as shown by NBT reduction. Results, however, varied considerably between different patients. A mean 13% of the cells from myeloid leukemic patients not cultured with TPA reduced NBT.

A vital function of normal human mononuclear phagocytes is the ingestion of foreign material (24, 38). Approximately 35% of the TPA-stimulated cells from the acute myeloid and blastic CML patients developed the ability to phagocytose Candida. Less than 10% of the cells from the myeloid leukemic patients not cultured with TPA were phagocytic. Likewise, the TPA-exposed lymphocytic leukemic cells were unable to phagocytose.

Several conclusions emerge from this study. Our investigation provides evidence that myelogenous leukemia cells that are morphologically at the myeloblast stage of maturation are not irreversibly committed to the granulocytic pathway of maturation, but can be in-

duced to develop into macrophagelike cells. Also the capacity of the leukemic cells to differentiate in the presence of TPA implies the genetic information for terminal macrophage differentiation is present and capable of expression in these cells.

We and others have shown that TPA and its analogs induced cells from various human myelogenous leukemia cell lines (HL-60, KG-1, ML-3) to differentiate into macrophagelike cells (8, 10–12). The TPA-treated cells became adherent, developed long pseudopodia, and stained strongly for NAE. The TPA-induced cells from the myeloid leukemic lines developed Fc-IgG receptors, phagocytosed and killed microorganisms, reduced NBT, and increased 2- to 20-fold lysozyme secretion and enzyme activity for β-glucuronidase and acid phosphatase. Cells from lymphocytic lines did not become macrophagelike after TPA exposure. One report suggested that HL-60 matured to granulocytic cells after TPA-exposed (39). The majority of the TPA studies with leukemic cell lines and the present study using leukemic cells from patients demonstrate that TPA (0.1 μM–10 nM) can induce myeloid, but not lymphoid, leukemic blast cells to terminally differentiate into macrophagelike cells. After our paper was submitted for publication, another report with similar data was published by Pegoraro et al. (40). The study showed that TPA was able to induce formation of macrophagelike cells from leukemic blasts obtained from the blood of patients with acute myeloid leukemia but not from patients with acute lymphocytic or undifferentiated leukemia. Taken together, the data suggest that the TPA exposure of leukemic cells may aid in the classification of blast cells as either myeloid or lymphoid in difficult cases.

ACKNOWLEDGMENTS

We thank Leslie Lowe and Deanna Ruttenberg for excellent technical assistance, Fred Dorey for superb statistical analysis, and Janice Furst for her assistance in the preparation of this manuscript.

This work was supported by U. S. Public Health Service grant CA 26038, CA 15688, the Medical Research Service of the Veterans Administration Medical Center, Brown and Williamson, Philip Morris, R. J. Reynolds, and the United States Tobacco Company.

REFERENCES

1. Cohen, R., M. Pacifici, N. Rubenstein, J. Biehl, and H. Holtzer. 1977. Effect of a tumor promoter on myogenesis. *Nature (Lond.).* **226:** 538–540.
2. Pacifici, M., and H. Holtzer. 1977. Effects of a tumor-promoting agents on chondrogenesis. *Am. J. Anat.* **150:** 207–212.
3. Ishii, D. N. 1978. Effect of tumor promoters on the response of cultured embryonic chick ganglia to nerve growth factor. *Cancer Res.* **38:** 3866–3893.
4. Ishii, D., E. Fibach, H. Yamasaki, and I. B. Weinstein. 1978. Tumor promoters inhibit morphological differentiation in cultured mouse neuroblastoma cells. *Science (Wash. D. C.).* **200:** 556–559.
5. Diamond, L., T. A. O'Brien, and G. Rovera. Inhibition of adipose conversion of 3T3 fibroblasts by tumor promoters. *Nature (Lond.).* **269:** 247–248.
6. Rovera, G., T. A. O'Brien, and L. Diamond. 1977. Tumor promoters inhibit spontaneous differentiation of Friend erythroleukemia cells in culture. *Proc. Natl. Acad. Sci. U. S. A.* **74:** 2894–2898.
7. Yamasaki, H., E. Fibach, U. Nudel, I. B. Weinstein, R. A. Rifkind, and P. A. Marks. 1977. Tumor promoters inhibit spontaneous and induced differentiation of murine erythroleukemia cells in culture. *Proc. Natl. Acad. Sci. U. S. A.* **74:** 3451–3455.
8. Lotem, J., and L. Sachs. 1979. Regulation of normal differentiation in mouse and human myeloid leukemic cells by phorbol esters and the mechanism of tumor promotion. *Proc. Natl. Acad. Sci. U. S. A.* **76:** 5158–5162.
9. Nakayasu, M., M. Shoji, N. Aoki, S. Sato, M. Miwa, and T. Sugimura. 1979. Enhancing the effect of phorbol esters on induction of differentiation of mouse myeloid leukemia cells by human urinary protein and lipopolysaccharide. *Cancer Res.* **39:** 4668–4672.
10. Rovera, G., T. A. O'Brien, and L. Diamond. 1979. Induction of differentiation in human promyelocytic leukemia cells by tumor promoters. *Science (Wash. D. C.).* **204:** 868–870.
11. Rovera, G., D. Santoli, and C. Damksy. 1979. Human promyelocytic leukemia cells in culture differentiate into macrophage-like cells when treated with a phorbol diester. *Proc. Natl. Acad. Sci. U. S. A.* **76:** 2779–2789.
12. Koeffler, H. P., M. Bar-Eli, and M. Territo. 1979. Heterogeneity of human myeloid leukemia cell response to phorbol diester. *Blood.* **54:** 174a.
13. Bennett, J. M., D. Catovsky, M. T. Daniel, G. Flandrin, D. A. G. Galton, H. R. Gralnick, and C. Sultan. 1976. Proposals for the classification of the acute leukemias. *Br. J. Haematol.* **33:** 451–458.
14. Yam, L. T., C. Y. Li, and W. H. Crosby. 1971. Cytochemical identification of monocytes and granulocytes. *Am. J. Clin. Pathol.* **55:** 283–290.
15. Willcox, M. B., D. W. Golde, and M. J. Cline. 1976. Cytochemical reactions of human hematopoietic cells in liquid culture. *J. Histochem. Cytochem.* **24:** 979–983.
16. Portaro, J. K., G. I. Glick, and J. Zighelboim. 1978. Population immunology and immune cell parameters. *Clin. Immunol. Immunopathol.* **11:** 339–345.
17. Natvig, J. B., P. Perlmann, and H. Wigzell., eds. 1976. Lymphocytes: Isolation, Fractionation, and Characterization. University Park Press, Baltimore.
18. Winchester, R. J., and S. M. Fu. 1976. Lymphocyte surface membrane immunoglobulin. *Scand. J. Immunol.* **5:** 745–757.
19. Terasaki, P. I., and J. D. McClelland. 1964. Microdroplet assay of human serum cytotoxins. *Nature (Lond.).* **204:** 998–1000.
20. Billing, R., J. Minowada, M. J. Cline, B. Clark, and K. Lee. 1978. *J. Natl. Cancer Inst.* **61:** 423–429.
21. Billing, R. J., W. Clark, and P. I. Terasaki. 1979. Heteroantiserum against a subpopulation of human T lymphocytes. *Clin. Immunol. Immunopathol.* **13:** 85–91.
22. Boyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.* **21**(Suppl. 97): 77–89.
23. Messner, M. A., J. E. Till, and E. A. McCulloch. 1973. Interacting cell populations affecting granulopoietic colony formation by normal and leukemic marrow cells. *Blood.* **42:** 701–710.

24. Territo, M. C., and M. J. Cline. 1977. Monocyte function in man. *J. Immunol.* **118:** 187–192.
25. Delafield, J. P., and R. I. Lehrer. 1977. A simple microscopic method for identifying and quantitating phagocytic cells in vitro. *J. Immunol. Methods.* **18:** 377–379.
26. Segal, A. W. 1974. Nitroblue-tetrazolium tests. *Lancet.* **II:** 1248–1252.
27. Baehner, R. L., L. A. Boxer, and J. Davis. 1976. The biochemical basis of nitroblue-tetrazolium reduction in normal and chronic granulomatous disease polymorphonuclear leukocytes. *Blood.* **48:** 309–313.
28. Solsted, M. E., and R. J. Martinez. 1978. Lysozyme: primary bactericidin in human plasma serum active against Bacillus subtilis. *Infect. Immun.* **20:** 782–791.
29. Golde, D. W., and M. J. Cline. 1973. Human preleukemia. Identification of a maturation defect in vitro. *N. Engl. J. Med.* **288:** 1083–1086.
30. Morley, A., and D. Higgs. 1974. Abnormal differentiation of leukemic cells in vitro. *Cancer (Phila.).* **33:** 716–720.
31. Golde, D. W., and L. A. Byers. 1975. Proliferation and maturation of human leukemia cells in liquid culture. *In* Leukemogenesis. Y. Ito, and R. M. Dutcher, editors. University of Tokyo Press, Tokyo. 243–250.
32. Lessin, L. S., M. Bessis, and S. D. Douglas. 1977. Morphology of monocytes and macrophages. *In* Hematology. E. Beutler, A. J. Erslev, and R. W. Rundles, editors. McGraw Hill, Inc., New York, 2nd edition. 851–860.
33. Stossel, T. P. 1974. Phagocytosis. *N. Engl. J. Med.* **290:** 717–723, 774–780.
34. Cohn, Z. A., and B. Benson. 1965. The differentiation of mononuclear phagocytes: morphology, cytochemistry and biochemistry. *J. Exp. Med.* **121:** 153–170.
35. Bennett, W. E., and Z. A. Cohn. 1966. The isolation and selected properties of blood monocytes. *J. Exp. Med.* **123:** 145–159.
36. Babior, B. M. 1978. Oxygen-dependent microbial killing by phagocytes. *N. Engl. J. Med.* **298:** 659–668, 721–725.
37. Castagna, M., and C. Rochette-Egley. 1979. Tumour-promoting phorbol diester induces substrate-adhesion and growth inhibition by lymphoblastoid cells. *Cancer Lett.* **6:** 227–234.
38. Cline, M. J., and R. I. Lehrer. 1968. Phagocytosis by human monocytes. *Blood.* **32:** 432–436.
39. Huberman, E., and M. F. Callahan. 1979. Induction of terminal differentiation in human promyelocytic leukemia cells by tumor-promoting agents. *Proc. Natl. Acad. Sci. U. S. A.* **76:** 1293–1297.
40. Pegoraro, L., J. Abrahm, R. A. Cooper, A. Levis, B. Lange, P. Meo, and G. Rovera. 1980. Differentiation of human leukemias in response to 12-O-tetradecanoylphorbol-13-acetate in vitro. *Blood.* **55:** 859–862.