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Induction of Differentiation of Human Acute Non-lymphocytic Leukemia Cells in Primary Culture by Inducers of Differentiation of Human Myeloid Leukemia Cell Line HL-60

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Abstract—*Leukemia cells from patients with acute non-lymphocytic leukemia were treated with various inducers of differentiation of the human promyelocytic leukemia cell line HL-60. All cells in 14 specimens tested underwent morphological, functional and histochemical changes after treatment with some inducers of differentiation of HL-60 cells, but the most effective inducer varied for different specimens. These results suggest that treatment with some inducers should be effective for inducing most acute myeloid leukemia cells to differentiate into morphologically and functionally mature granulocytes and macrophages.*

INTRODUCTION

SOME myeloid leukemia cell lines are induced to differentiate *in vitro* into mature granulocytes and macrophages by treatment with various compounds [1–5]. The leukemogenicity of myeloid leukemia M1 cells in syngeneic mice is inversely related to the *in vivo* inducibility of differentiation of the cells [6]. Inducers of differentiation of M1 cells significantly enhanced the survival times of mice inoculated with M1 cells [7]. These results may be significant for leukemia therapy.

A cultured line of cells from a patient with promyelocytic leukemia (HL-60) can be induced to differentiate by various compounds, including 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and retinoic acid [8–13]. Recent reports indicate that TPA or retinoic acid induce differentiation of myeloid leukemia cells obtained directly from the peripheral blood or bone marrow of leukemia patients [14–17]. However, differences in the abilities of various compounds to induce differentiation in different M1 cell clones can be detected in culture [18]. This may also apply to the effectiveness of compounds in inducing differentiation of leukemia cells from different leukemic patients. Therefore it seemed useful to determine which compounds are effective in inducing differentiation of leukemia cells from different individuals. In the present work we tested the effects of inducers of differentiation of HL-60 cells in induction of differentiation of human acute non-lymphocytic leukemia cells in primary culture.

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MATERIALS AND METHODS

Cell source

Cells were obtained from 14 patients with acute nonlymphocytic leukemia before therapy. Diagnosis of leukemias was based on cell morphology and cytochemical staining and leukemias were classified according to the French–American–British Scheme [19].

Cell separation

Heparinized bone marrow (1 ml) was obtained during routine diagnostic procedures. Only specimens containing at least 70% leukemia cells were studied. Cells were diluted with RPMI-1640 medium supplemented with 15% heat-inactivated

fetal calf serum and then overlaid at room temperature on a cushion of 10 ml Ficoll-Hypaque and centrifuged at 800 *g* for 40 min. The leukemic immature bone marrow cells and mononuclear cells were washed twice and suspended with RPMI-1640 medium supplemented with 15% heat-inactivated fetal calf serum, plated in Falcon dishes at $2\text{–}8 \times 10^5$ cells/ml and incubated at 37°C in a humid atmosphere of 5% CO_2 in air.

Compounds used and assay of differentiation-associated properties

The sources of the inducers of HL-60 cell differentiation tested in the present work were as follows: actinomycin D [11, 20], arginase [21] from bovine liver and retinoic acid (all *trans*) from Sigma Chemical Co., St. Louis; dimethyl sulfoxide (DMSO) [8] from Merck, Darmstadt; TPA [9, 10] from Consolidated Midland, Brewster; sodium *n*-butyrate from Wako Pure Chemical Co., Tokyo; aclacinomycin from Sanraku Ocean Co., Tokyo; and behenoyl cytosine arabinoside (bephenoyl araC) from Asahi Kasei Co., Osaka; alkyl-lysophospholipids [(3-octadecyl-2-methoxy) propyl-2-trimethylammonioethyl phosphate (ST-008) and (3-tetradecyloxy-2-methoxy) propyl-2-trimethylammonioethyl phosphate (ST-023)] were synthesized in our laboratories [22].

Lysozyme activity was determined by the lysoplate method, as described previously [23]. The lysoplate consisted of 0.1% Difco agar in 1/15M sodium phosphate buffer (pH 6.6) containing 0.05M NaCl and 0.5 mg heat-killed bacterial cells of *Micrococcus lysodeikticus* per ml. After 24 hr of incubation at 27°C the diameter of the clear zone was measured. α-Naphthyl acetate esterase and naphthyl AS-D chloroacetate esterase activities were determined cytochemically using a Sigma esterase kit (No. 90) [21]. For assay of phagocytic activity, cells were incubated for 5 hr with a suspension of polystyrene latex particles (2 μl/ml of serum-free culture medium, average diameter 1 μm Dow Chemical Co., Indianapolis). Then the cells were harvested with a rubber policeman and the cell suspensions were vigorously washed 3 times by pipetting with phosphate-buffered saline in order to remove latex particles which were non-specifically associated with the cell surface. One drop of the cell suspension was placed on a glass slide and the number of viable cells which phagocytized more than 5 latex particles was counted under a microscope, and the percentage of phagocytic cells among at least 300 cells was counted. NBT reduction was assayed as reported previously [11, 22]. The percentage of cells containing intracellular blue-black formazan deposits was then determined by examination of a minimum of 300 cells. For morphological examination cell smears were stained with May–Grünwald Giemsa and examined in a light microscope. Morphologically mature cells were classified as follows. Myelocyte: the nucleus has sharply defined chromatin masses, the nucleolus can no longer be seen and the cytoplasm is slightly acidophilic and contains neutrophilic granules. Metamyelocyte: the shape of the nucleus is that of a kidney bean or bent rod, the nucleus has dense chromatin, with numerous dark blocks sharply circumscribed and much more evident than in the myelocyte. Monocyte: the nucleus is large, the chromatin appears pale and has a lace-like or reticular appearance without a compact chromatin block, the cytoplasm is grayish-blue and has fine azurophilic granulations which are occasionally so numerous as to give the cytoplasm a pink look, and cytoplasmic vacuoles are seen. Macrophage: the cell is more than 30 μm in diameter, the nucleus is oval and the cytoplasm is ample. The cytoplasmic borders are irregularly serrated and cytoplasmic vacuoles are present near the cell periphery, reflecting the active pinocytosis and phagocytosis. Most of the TPA-treated leukemia cells adhered to the culture dish or aggregated. The adherent cells were gently removed by a rubber policeman and in some cases the cells were collected with 0.01% trypsin–0.04% EDTA for 5 min with shaking. When the viable cells were not collected by the above method, the culture dish to which the cells adhered were washed with phosphate-buffered saline, fixed and stained with May–Grünwald Giemsa solution.

RESULTS

Acute non-lymphocytic leukemia cells from 14 untreated patients in our hospital were studied (Table 1). During primary culture without any compounds, the cell number usually increased slightly but in some cases it decreased slightly. The viability of untreated cultures was more than 85%, determined by the trypan blue dye exclusion test. In some of the inducers, higher concentrations were cytotoxic and might kill immature cells selectively. Therefore, in the present investigation, the inducers were used in the concentration that gave less than 50% reduction of viable cell number and the results for higher concentrations are omitted. Differentiation of the cells was determined by examining morphological changes, NBT reduction, phagocytosis, lysozyme synthesis and secretion, α-naphthyl acetate esterase and naphthyl AS-D chloroacetate esterase.

Table 1. Induction of morphological differentiation of acute myeloid leukemia cells by inducers of differentiation HL-60 cells

Diagnosis (FAB)	Patient (age, sex)	Morphological differentiation (%)																					
		None		Retinoic acid (4×10^{-7}M)		Actinomycin D (2 ng/ml)		TPA (1 ng/ml)		Arginase (4 U/ml)		DMSO (1%)		Butyrate (1 mM)		ST-023 (6 μg/ml)		ST-008 (1 μg/ml)		Aclacino-mycin (10 ng/ml)		Behenoyl araC (1 μg/ml)	
		Gran	Mono	Gran	Mono	Gran	Mono	Gran	Mono	Gran	Mono	Gran	Mono	Gran	Mono	Gran	Mono	Gran	Mono	Gran	Mono	Gran	Mono
AML	SWS (66, ♀)	4	2	87	0	83	10	8	64	9	36	61	5	59	7	21	36						
(M2)	ES (4, ♀)	5	4			67	4	1	99*	12	34					82	7						
	SS (35, ♂)	7	17	32	43	2	61	1	63	10	54	13	45	8	11	8	16						
APL	HA (31, ♂)	6	0	92	0	32	0	29	36	34	0	0	18	0	68	81	0						
(M3)	NM (36, ♀)	0	0	99	0	48	0	37	10	32	0	36	0	31	0	33	0			34	0	33	0
	TO (41, ♀)	1	0	97	3	33	0	28	51	6	0	2	0	1	0	45	8						
	AO (42, ♀)	4	0	15	3†	40	1	3	4	8	0	12	0	36	3								
AMMoL	RS (10, ♀)	8	7	7	41	58	14	0	99*	13	56												
(M4)	NE (67, ♂)	1	4	11	42	3	64	0	42	5	12									4	43	3	37
	BY (57, ♂)	0	1	0	92	10	87	0	60	0	41					1	32			2	79	1	52
	TN (46, ♂)	0	9	0	54	0	30	0	75	0	22	0	43	0	30	0	56	0	63	0	46	0	47
	KS (65, ♂)	9	17	22	77	31	67	0	100*	12	84	10	88	44	45	2	98						
AMoL	SI (49, ♂)	1	11	7	76	4	68	1	97*	3	48	13	63	11	35	3	78						
	GU (71, ♂)	0	7			0	58	0	89*							0	32						

Cells were cultured with the indicated concentration of the compound for 6 days except some treatments. Gran: myelocytes and mature granulocytes; mono: monocytes and macrophages.
*Cells were treated for 3 days.
†Cells were treated for 7 days.

Table 2. Induction of differentiation of APL (TO) cells by inducers of HL-60 cell differentiation

Treatment*		Cell No. (× 10^5/ml)	Morphological changes (%)					Lysozyme† activity (U/10^7 cells)	NBT reduction (%)	α-Naphthyl acetate esterase (%)
			Myeloblasts, promyelocytes	Myelocytes	Metamyelocytes	Mature granulocytes	Monocytes and macrophages			
None		3.8	99	1	0	0	0	9.3	0	1
Retinoic acid:	10^{-8}M	3.7	0	4	92	3	1	9.8	81	0
	10^{-7}M	3.5	0	2	95	2	1	9.7	79	2
Actinomycin D:	0.5 ng/ml	3.3	98	2	0	0	0	12.9	0	1
	1.0 ng/ml	2.8	85	15	0	0	0	14.5	0	6
	2.0 ng/ml	2.6	67	22	11	0	0	16.0	0	10
TPA:	0.2 ng/ml	4.0	77	23	0	0	0	11.1	0	0
	0.5 ng/ml	3.7	12	18	6	1	63	14.1	0	4
	1.0 ng/ml	3.4	21	24	4	0	51	11.7	0	11
Arginase:	6 U/ml	3.3	93	6	0	0	0	9.6	0	15
	8 U/ml	3.2	97	3	0	0	0	10.3	0	18
DMSO:	1%(v/v)	3.4	98	2	0	0	0	11.0	0	6
Butyrate:	1 mM	3.1	99	1	0	0	0	9.7	0	41
ST-023:	4 μg/ml	2.9	50	44	2	0	4	11.3	0	2
	6 μg/ml	2.8	47	36	6	3	8	10.5	0	6

*Cells were cultured with various compounds for 6 days.

†One unit of lysozyme activity was defined as equivalent to that of 1 μg of hen egg white lysozyme.

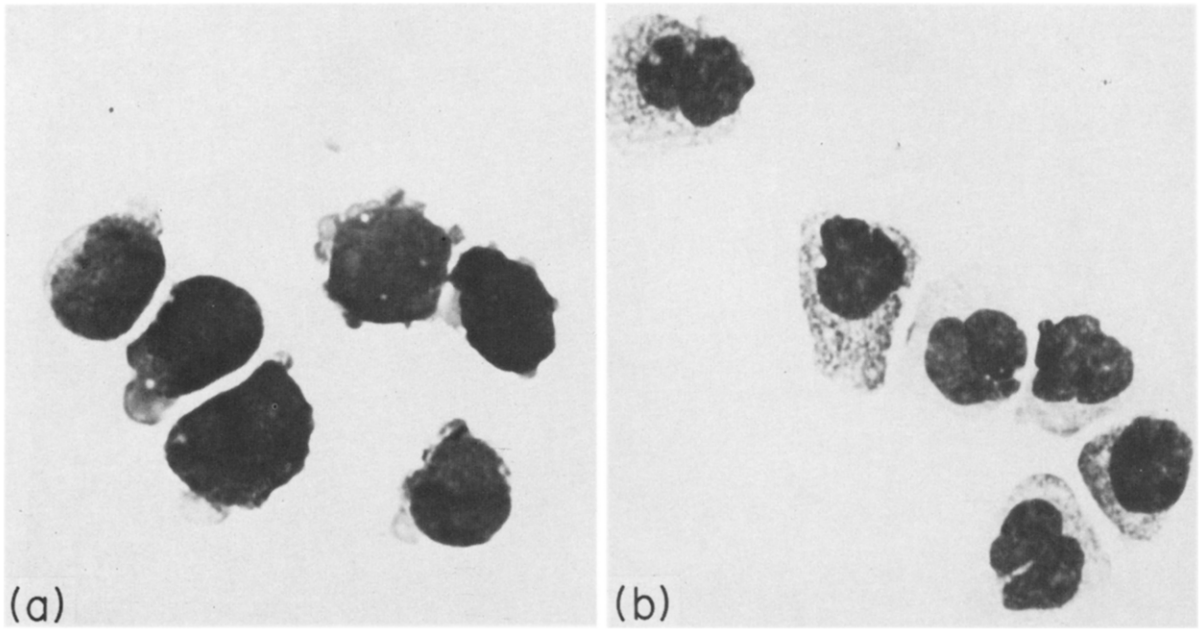

Fig. 1. Morphological maturation of leukemic cells from an APL (NM) patient. Cells were cultured with (b) or without (a) 10^{-7}M *retinoic acid for* 6 days (× 675).

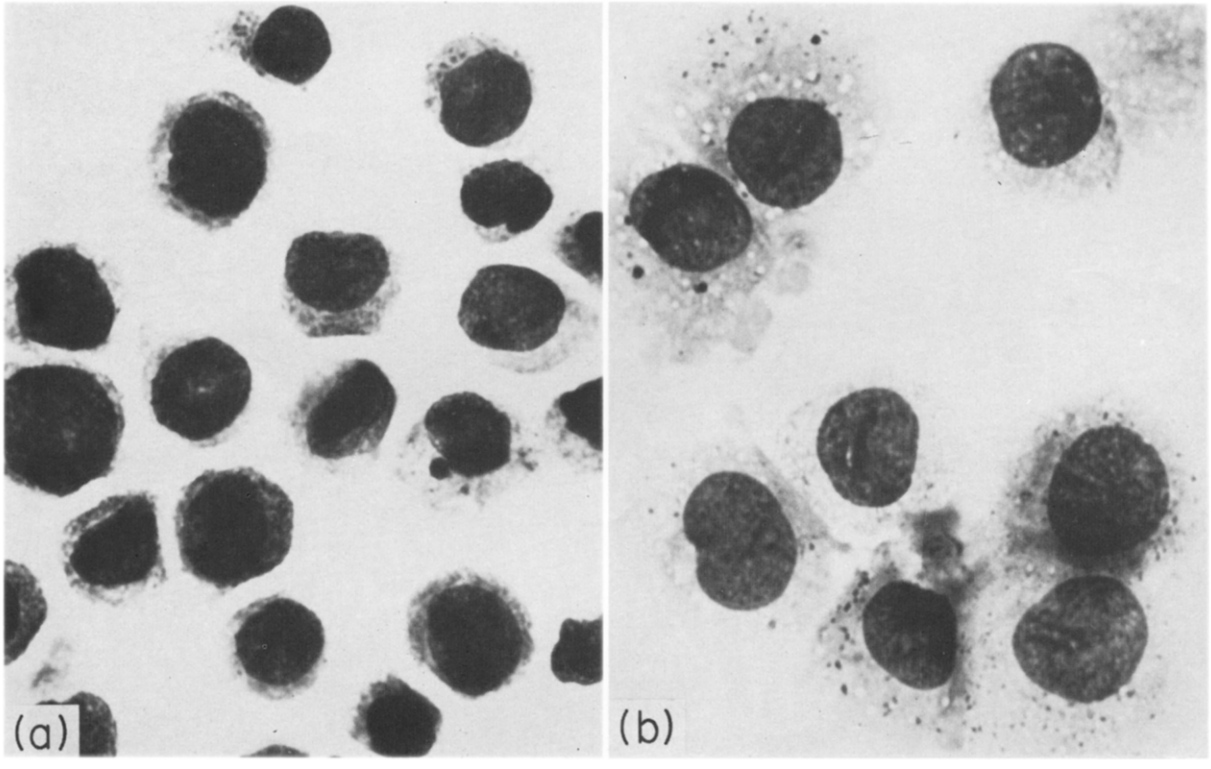

Fig. 2. Morphological maturation of leukemic cells from an AMMol(TN) patient. Cells were cultured with (b) or without (a) 0.5 ng/ml *TPA for* 4 days (× 675).

Acute promyelocytic leukemia (M3) cells

Table 1 lists the leukemic cells obtained from leukemia patients. TO cells were taken from a woman with acute promyelocytic leukemia (APL). The cells were incubated for 3–7 days in suspension culture with various inducers of differentiation of HL-60 cells (Table 2). Morphological differentiation of TO cells to mature granulocytes was induced by retinoic acid (Fig. 1), and to monocytes and macrophages by TPA, as in the case of HL-60 cells. Actinomycin D and *O*-alkyl-lysophospholipid (ST-023) induce some morphological changes, but the other inducers tested did not induce morphological differentiation of TO cells. Butyrate, TPA, actinomycin D and arginase induced α-naphthyl acetate esterase activity, and lysozyme synthesis and secretion were enhanced by actinomycin D or TPA. NM cells were induced to differentiate into granulocytes by all the compounds tested, with retinoic acid being the most effective inducer. HA cells were induced to differentiate into granulocytes by retinoic acid, ST-023, actinomycin D and arginase, and to differentiate functionally and morphologically into monocytes and macrophages by TPA and butyrate. AO cells from an acute promyelocytic leukemia patient were induced to differentiate into mature granulocytes by actinomycin D and butyrate. When the cells were cultured with 2 ng/ml of actinomycin D for 6 days, 38% of the cells became morphologically myelocytic and 7% became more mature granulocytes, whereas only 4% of the cells in the control culture were myelocytic. Similar results were obtained when the cells were treated with 1–1.5 mM butyrate. Retinoic acid was a potent inducer of other acute promyelocytic leukemia cells (Table 1), but even at a high concentration (4×10^{-6}M) did not induce morphological or functional differentiation of AO cells.

Acute myelomonocytic leukemia (M4) cells

TN cells were taken from a man with acute myelomonocytic leukemia (AMMoL). All the compounds tested induced morphological differentiation of cells into monocytes and macrophages (Table 3), TPA being the most effective (Fig. 2). TN cells were not induced to undergo granulocytic differentiation by any of the compounds, judging by their morphological appearance and naphthyl AS-D chloroacetate esterase activity. BY cells, like TN cells, were induced to undergo differentiation into monocytes and macrophages by all the compounds tested. NE cells were induced to differentiate functionally and morphologically into monocytes and macrophages by TPA, ST-023, aclacinomycin and behenoyl araC, and into granulocytes by actinomycin D. Arginase caused little morphological differentiation, although it enhanced α-naphthyl acetate esterase and NBT reduction.

Most of the freshly isolated KS cells were morphologically blastic and promonocytic, and were weakly positive for α-naphthyl acetate esterase. However, about 30% of the cells cultured for several days without any inducer were promyelocytic. The cells had large azurophilic granules, which are typical of promyelocytes. The cells were induced to differentiate functionally and morphologically into monocytes and macrophages by TPA, ST-023, DMSO and arginase. Butyrate, actinomycin D and retinoic acid induced morphological differentiation into both granulocytes and macrophages. Butyrate induced lysozyme and α-naphthyl acetate esterase activities in KS cells, but caused little morphological differentiation (Table 4). Thus AMMoL cells were induced to differentiate into mature granulocytes or macrophages by some of the inducers, although the most effective inducer varied for different leukemia cells (Table 1).

Acute monocytic leukemia (M5) cells

SI and GU cells were induced to differentiate functionally and morphologically into monocytes and macrophages by all the compounds tested, TPA being the most effective.

Acute myeloblastic leukemia (M2) cells

SS cells differentiated spontaneously into monocytes in suspension culture without any inducer. Similar spontaneous differentiation of fresh leukemia cells in short-term culture has been described [24]. After culture of SS cells for 6 days, 17% of the cells were morphologically monocytes. These cells contained α-naphthyl acetate esterase and NBT reduction activities. Some inducers enhanced the terminal differentiation of the cells. SWS and ES cells were induced to differentiate functionally and morphologically into mature granulocytes or macrophages by some of the inducers of HL-60 cells.

DISCUSSION

Recent reports show that *in vitro* treatment of human acute and chronic myeloid leukemia cells with TPA or retinoic acid induces morphological and functional differentiation. TPA drives the cells in the direction of expressing the characteristics of monocytes and macrophages [14–16]. APL cells are induced to differentiate into mature granulocytes by retinoic acid, but other types of leukemia cells are not [17]. In this work we studied the effects of *in vitro* treatment of human acute nonlymphocytic leukemia cells with several inducers of differentiation of HL-60 cells.

Table 3. Induction of differentiation of AMMoL (TN) cells by various inducers

Treatment*		Cell number ($\times 10^5$/ml)	Morphological changes (%)					Phagocytosis (%)	α-Naphthyl acetate esterase (%)	Naphtyl AS-D chloroacetate esterase (%)
			Myeloblasts	Promyelocytes	Promonocytes†	Monocytes	Macrophages			
None		5.9	11	49	31	9	0	4	1	2
Retinoic acid:	10^{-6}M	3.9	0	6	40	48	6	12	2	5
Actinomycin D:	0.5 ng/ml	4.3	9	36	48	7	0	6	1	3
	1.0 ng/ml	3.4	8	31	55	6	0	7	0	2
	2.0 ng/ml	3.0	5	24	41	28	2	36	2	4
TPA:	0.2 ng/ml	4.6	4	30	58	8	0	7	4	
	0.5 ng/ml	3.9	0	33	11	7	49	86	7	
	1.0 ng/ml	3.2	0	6	19	5	70	95	14	
	2.0 ng/ml	3.1	0	4	11	15	68	95	29	
Arginase:	4 U/ml	3.9	2	16	60	21	1	10	5	
	6 U/ml	3.4	1	7	57	33	2	14	9	
DMSO:	0.5% (v/v)	4.5	1	10	68	21	0	21	1	
	1.0% (v/v)	4.2	0	2	55	43	0	32	2	
	1.5% (v/v)	3.9	0	0	42	58	0	37	2	3
Butyrate:	0.5 mM	5.2	0	2	80	18	0	11	3	
	1.0 mM	4.9	0	1	69	28	2	15	6	
	2.0 mM	4.2	1	0	85	14	0	7	13	
ST-023:	4 μg/ml	4.1	0	1	43	56	0	16	2	2
	6 μg/ml	3.5	0	0	44	51	5	41	2	5
	8 μg/ml	3.3	0	3	52	34	11	38	3	3
ST-008:	1 μg/ml	3.9	0	0	37	48	15	29	1	
Aclacinomycin:	5 ng/ml	4.0	0	1	61	33	5	18	1	
	10 ng/ml	3.6	0	1	53	37	9	23	2	
	20 ng/ml	3.2	0	2	64	34	0	14	1	
Behenoyl araC:	200 ng/ml	4.2	0	23	45	29	3	13	3	
	500 ng/ml	3.4	0	10	43	37	10	16	4	

*See legend to Table 2.

†Promonocytes were defined as cells with reddish granules, which were numerous in monocytes, but with more delicate and lacy chromatin than that of monocytes.

Table 4. Induction of differentiation of AMMoL (KS) cells by various inducers

Treatment*	Cell number ($\times 10^5$/ml)	Morphological changes (%)						Lysozyme activity (U/10^7 cells)	NBT reduction (%)	α-Naphthyl acetate esterase† (%)
		Myeloblasts, promyelocytes	Myelocytes	Metamyelocytes	Mature granulocytes	Monocytes	Macrophages			
None (before culture)	6.0	91	3	1	0	5	0		0	0
None	6.1	74	9	0	0	17	0	13	29	4
Retinoic acid: 4×10^{-6}M	5.7	1	18	1	3	39	38	14	32	7
Actinomycin D: 0.5 ng/ml	5.5	0	23	2	1	71	3	24	49	8
1.0 ng/ml	5.2	2	19	4	8	62	5	26	58	15
2.0 ng/ml	4.9	1	25	2	5	52	15	23	51	56
TPA: 0.2 ng/ml	5.8	0	0	0	0	8	92	17	52	39
0.5 ng/ml	5.1	0	0	0	0	5	95	20	56	84
2.0 ng/ml	4.7	0	0	0	0	11	89	11	43	94
Arginase: 4 U/ml	5.4	4	6	0	6	60	24	13	30	99
6 U/ml	5.2	5	2	0	0	42	51	13	34	99
8 U/ml	5.2	8	10	0	1	45	36	13	42	96
DMSO: 1.0% (v/v)	5.7	2	6	2	2	33	55	18	29	68
1.5% (v/v)	5.2	10	8	0	4	58	20	15	46	56
Butyrate: 1.0 mM	5.9	11	40	2	2	43	2	32	38	99
1.5 mM	5.3	15	39	4	5	31	6	30	42	98
ST-023: 6 μg/ml	4.5	0	2	0	0	2	96	15	32	28

*See legend to Table 2.

†Most untreated cells gave a weakly positive reaction, but only those giving a strongly positive reaction were counted.

Consistent with previous findings, TPA treatment of cells from patients with acute nonlymphocytic leukemia, especially those with AML(M2), AMMoL and AMoL, induced functional and morphological differentiation of leukemia cells into monocytes and macrophages. Retinoic acid induced cells from three patients with APL to differentiate functionally and morphologically into mature granulocytes, but had no effect on cells from one patient with APL. It also induced cells from some patients with AMMoL and AMoL to differentiate into monocytes and macrophages, as judged by the morphological appearance and α-naphthyl acetate esterase and naphthol AS-D chloroacetate esterase activities of the cells. These results suggest that retinoic acid may be useful as a differentiation-inducing agent in the treatment of some patients with AMMoL and AMoL and also some with AML(M2) and APL. Differentiation of leukemia cells from all the patients tested was induced by some of the inducers of differentiation of HL-60 cells, and in 12 of 14 cases more than 50% of the treated cells displayed the characteristics of more-differentiated cells. Of the compounds we tested, actinomycin D was effective on all the leukemia cells, although in some cases TPA or retinoic acid was the more effective. These results suggest that treatment of acute nonlymphocytic leukemia cells with some inducers of differentiation of HL-60 cells including actinomycin D may induce functional and morphological differentiation of most of the leukemia cells into mature granulocytes and macrophages.

In differentiation of some myeloid leukemia cell lines, the differentiation-associated properties are under separate controls [25–27]. The present results also indicate that functional differentiation is not always accompanied by morphological differentiation. Table 2 shows that TO cells are induced to differentiate morphologically into 63% monocytes and macrophages by TPA, but functionally there is no change in NBT reduction or α-naphthyl acetate esterase level. However, most of morphologically differentiated cells expressed functional changes. Then morphological evaluation may be more subjective than the functional test in the differentiation of fresh leukemia cells.

We previously showed that inducers of cell differentiation significantly enhanced the survival times of mice inoculated with myeloid leukemia M1 cells [7]. From these findings in animals, we suggested that treatment of human leukemia *in vivo* with inducers of terminal differentiation could be a new approach to control of leukemia. The previous and present results indicate that human acute myelogenous leukemia cells are induced to differentiate into mature granulocytes and macrophages by some of the inducers of differentiation of HL-60 cells. In order to develop adequate leukemia therapy based on induction of differentiation, we must identify suitable drugs able to induce 100% of the population to differentiate, because if any leukemia cells survive at all, the disease cannot be cured. Unfortunately, none of the inducers tested cause 100% of the cells to differentiate. Moreover, some inducers of differentiation are toxic and cannot be used *in vivo*. The solution of these problems should be required in the development of new methods of leukemia therapy.

REFERENCES

1. COLLINS SJ, GALLO RC, GALLAGHER RE. Continuous growth and differentiation of human myeloid leukaemic cells in suspension culture. *Nature, Lond* 1977, **270**, 347–349.
2. ICHIKAWA Y. Differentiation of a cell line of myeloid leukemia. *J Cell Physiol* 1969, **74**, 223–234.
3. ICHIKAWA Y, MAEDA M, HORIUCHI M. *In vitro* differentiation of Rauscher-virus-induced myeloid leukemia cells. *Int J Cancer* 1976, **17**, 789–797.
4. HOZUMI M, HONMA Y, TOMIDA M *et al.* Induction of differentiation of myeloid leukemia cells with various chemicals. *Acta Haematol Jap* 1979, **42**, 941–952.
5. SACHS L. Control of normal differentiation and the phenotypic reversion of malignancy in myeloid leukemia. *Nature, Lond* 1978, **274**, 535–539.
6. HONMA Y, KASUKABE T, HOZUMI M. Relationship between leukemogenicity and *in vivo* inducibility of normal differentiation in mouse myeloid leukemia cells. *J Natl Cancer Inst* 1978, **61**, 837–841.
7. HONMA Y, KASUKABE T, OKABE J, HOZUMI M. Prolongation of survival time of mice inoculated with myeloid leukemia cells by induction of normal differentiation. *Cancer Res* 1979, **39**, 3167–3171.
8. COLLINS SJ, RUSCETTI FW, GALLAGHER RE, GALLO RC. Terminal differentiation of human promyelocytic leukemia cells induced by dimethylsulfoxide and other polar compounds. *Proc Natl Acad Sci USA* 1978, **75**, 2458–2462.

9. ROVERA G, O'BRIEN TG, DIAMOND L. Induction of differentiation in human promyelocytic leukemia cells by tumor promoters. *Science* 1979, **204**, 868–870.
10. LOTEM J, SACHS L. Regulation of normal differentiation in mouse and human myeloid leukemic cells by phorbol esters and the mechanism of tumor promotion. *Proc Natl Acad Sci USA* 1979, **76**, 5158–5162.
11. COLLINS SJ, BODNER A, TING R, GALLO RC. Induction of morphological and functional differentiation of human promyelocytic leukemia cells (HL-60) by compounds which induce differentiation of murine leukemia cells. *Int J Cancer* 1980, **25**, 213–218.
12. BREITMAN TR, SELONICK SE, COLLINS SJ. Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. *Proc Natl Acad Sci USA* 1980, **77**, 2936–2940.
13. HONMA Y, TAKENAGA K, KASUKABE T, HOZUMI M. Induction of differentiation of cultured human promyelocytic leukemia cells by retinoids. *Biochem Biophys Res Commun* 1980, **95**, 507–512.
14. PEGORARO L, ABRAHM J, COOPER RA *et al.* Differentiation of human leukemias in response to 12-*O*-tetradecanoylphorbol-13-acetate *in vitro*. *Blood* 1980, **55**, 859–862.
15. KOEFFLER HP, BAR-ELI M, TERRITO M. Phorbol diester-induced macrophage differentiation of leukemic blasts from patient with human myelogenous leukemia. *J Clin Invest* 1980, **66**, 1101–1108.
16. FIBACH E, RACHMILEWITZ EA. Tumour promoters induce macrophage differentiation in human myeloid cells from patients with acute and chronic myelogenous leukaemia. *Br J Haematol* 1981, **47**, 203–210.
17. BREITMAN TR, COLLINS SJ, KEENE BR. Terminal differentiation of human promyelocytic leukemic cells in primary culture in response to retinoic acid. *Blood* 1981, **57**, 1000–1004.
18. HONMA Y, KASUKABE T, OKABE J, HOZUMI M. Glucocorticoid binding and mechanism of resistance in some clones of mouse myeloid leukemic cells resistant to induction of differentiation by dexamethasone. *J Cell Physiol* 1977, **93**, 227–236.
19. BENNETT JM, CATOVSKY D, DANIEL M-T *et al.* Proposals for the classification of the acute leukemias. *Br J Haematol* 1976, **33**, 451–458.
20. LOTEM J, SACHS L. Potential pre-screening for therapeutic agents that induce differentiation in human myeloid leukemia cells. *Int J Cancer* 1980, **25**, 561–564.
21. HONMA Y, FUJITA Y, OKABE-KADO J, KASUKABE T, HOZUMI M. Induction of differentiation of human promyelocytic leukemia cells (HL-60) by arginase. *Cancer Lett* 1980, **10**, 287–292.
22. HONMA Y, KASUKABE T, HOZUMI M, TSUSHIMA S, NOMURA H. Induction of differentiation of cultured human and mouse myeloid leukemia cells by alkyl-lysphospholipids. *Cancer Res* 1981, **41**, 3211–3216.
23. KASUKABE T, HONMA Y, HOZUMI M. Characterization of lysozyme synthesized by differentiated mouse myeloid leukemia cells. *Biochim Biophys Acta* 1979, **586**, 615–623.
24. PALU' G, POWLES R, SELBY P, SUMMERSGILL BM, ALEXANDER P. Patterns of maturation in short-term culture of human acute myeloid leukaemic cells. *Br J Cancer* 1979, **40**, 719–730.
25. NEWBURGER PE, BAKER RD, HANSEN SL, DUNCAN RA, GREENBERGER JS. Functionally deficient differentiation of HL-60 promyelocytic leukemia cells induced by phorbol myristate acetate. *Cancer Res* 1981, **41**, 1861–1865.
26. LOTEM J, SACHS L. Genetic dissection of the control of normal differentiation in myeloid leukemic cells. *Proc Natl Acad Sci USA* 1977, **74**, 5554–5558.
27. HONMA Y, KASUKABE T, HOZUMI M. Induction of lysozyme activity by adenosine 3′:5′ cyclic monophosphate in culture mouse myeloid leukemic cells. *Biochem Biophys Res Commun* 1978, **82**, 1246–1250.