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INDUCTION OF DIFFERENTIATION OF CULTURED HUMAN PROMYELOCYTIC LEUKEMIA CELLS BY RETINOIDS

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SUMMARY

Human promyelocytic leukemia cells (HL-60) were induced to phagocytize, reduce NBT dye(nitroblue tetrazolium), and change into forms that were morphologically similar to mature granulocytes by retinoic acid and related retinoids, but not by the pyridyl analog of retinoic acid. Induction of differentiation could be detected after 4 days of treatment of the cells with retinoic acid at as low a dose as 4×10^{-8} M. Thus, retinoids may be used in studies on the control of cell differentiation and malignancy of human myeloid leukemia cells.

INTRODUCTION

The effects of natural metabolites and synthetic analogs of vitamin A (retinoids) on differentiation of epithelial cells and other types have been studied in organ culture and cell culture systems (1). Retinoic acid induced differentiation of mouse F9 embryonal carcinoma cells into endodermal or fibroblastic cells (2). Recently, retinoids were found to induce lysosomal enzyme activities in mouse myeloid leukemia Ml cells, but to inhibit induction of other differentiation-associated properties of Ml cells, such as phagocytosis, morphological changes, and migration (3). However, the mechanisms of the effect of retinoids on cell differentiation are unknown.

It seemed important to determine whether retinoids can also affect cell differentiation of human cells, since the compounds are used clinically for treatment of cancer and other diseases (1,4). The present studies were undertaken to examine the

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response of human promyelocytic leukemia cells (HL-60) to retinoids. HL-60 cells were used because they display distinct biological and morphological commitments towards myeloid differentiation and can be efficiently induced to differentiate into mature granulocytes and macrophages by the polar planar compounds, actinomycin D, hypoxanthine, phorbol ester, and tunicamycin (5-12). The present work showed that retinoids induced functional and morphological differentiation of human HL-60 cells.

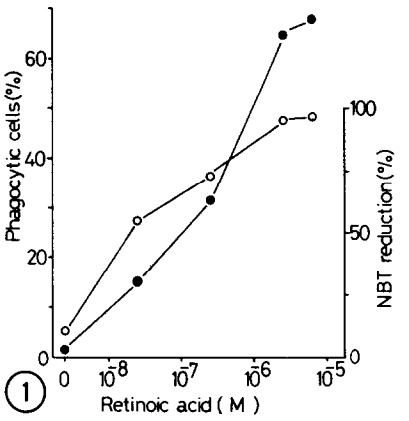
MATERIALS AND METHODS

Cell line and cell culture; The HL-60 cell line was generously provided by Dr. R.C. Gallo(National Institute of Health, USA) and was maintained in suspension culture in RPMI-1640 medium (GIBCO, Grand Island, USA) supplemented with 20% fetal calf serum (GIBCO) as previously described (5). Retinoic acid (all-trans: vitamin A acid), retinol (all-trans: vitamin A alcohol), retinal (all-trans: vitamin A aldehyde), and retinyl acetate (all-trans: vitamin A acetate) were supplied by Sigma Chemical Co., St Louis, USA. The pyridyl analog of retinoic acid was prepared by Hoffman-La Roche Inc. Ro10-9359 [ethyl all-trans-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraenoate] was supplied by Nippon Roche Inc., Kamakura, Japan. Stock retinoid solutions were prepared in absolute ethanol.

Assay of the properties of differentiated cells; For assay of phagocytic activity, cells were incubated for 5 h with a suspension of polystyrene latex particles (2 µl/ml of serum-free culture medium, average diameter, 1 µm, Dow Chemical Co., Indianapolis, USA). Then the cells were washed 3 times with phosphate-buffered saline, and the percentage of phagocytic cells among at least 300 viable cells was counted. NBT reduction was assayed as reported previously (7). The percentage of cells containing intracellular blue-black formazan deposits was then determined by examination of a minimum of 300 cells. The percentages of cells that were morphologically similar to granulocytes and macrophages were determined in smears treated with May-Grünwald-Giemsa stain. Lysozyme activity was assayed as reported previously (3).

RESULTS AND DISCUSSION

In this study we examined the ability of retinoic acid to induce phagocytosis and the reduction of NBT dye by HL-60 cells, which are typical functional markers of differentiation of these cells. Results showed that these inductions by retinoic acid



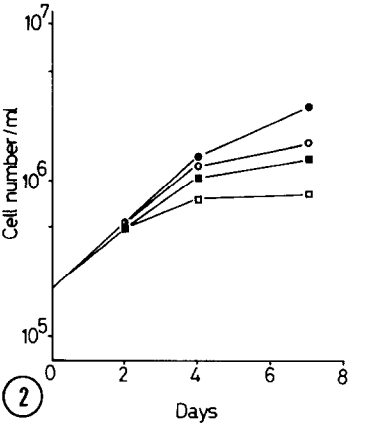


Fig. 1. Effects of retinoic acid on inductions of phagocytic activity(●) and the capacity to reduce NBT dye(O) in HL-60 cells. HL-60 cells were incubated with or without various concentrations of retinoic acid for 5 days.

Fig. 2. Effect of retinoic acid on growth of HL-60 cells. HL-60 cells were cultured without (●) or with 4 X 10^{-8} M(O), 4 X 10^{-7} M(■), or 4 X 10^{-6} M(□) retinoic acid.

were both dose-dependent, and were maximal with 5-10 X 10^{-6}M retinoic acid (Fig. 1). Growth of HL-60 cells was inhibited when the cells were cultured in the presence of retinoic acid; even at concentrations as low as 4 X 10^{-8}M (Fig. 2). However, no cytotoxic effects on the cells were observed during induction of cell differentiation, indicating that retinoic acid did not selectively kill immature HL-60 cells, but induced differentiation to mature cells.

Previous reports showed that HL-60 cells were induced to differentiate into macrophages by incubation with 12-O-tetradecanoylphorbol-13-acetate, and into granulocytes by treatment with actinomycin D or dimethylsulfoxide (5-12). Retinoic acid induced HL-60 cells to differentiate morphologically into granulocytes (Table 1, Fig. 3).

We next examined the effects of various retinoids on induction of differentiation of HL-60 cells. Phagocytosis, NBT reduction and morphological changes were induced by treating the

Table 1. Induction of morphological differentiation of HL-60 cells by retinoic acid

Days after treatment	Cell type, %		
	Myeloblasts & Promyelocytes	Myelocytes & Metamyelocytes	Banded and segmented neutrophils
0	94.9	4.1	1.0
4	56.5	34.1	9.4
6	11.9	41.7	45.4
7	8.2	41.3	50.5

HL-60 cells were cultured with 4 X 10^{-6}M retinoic acid.

cells with 4 X 10^{-6}M retinoic acid, retinal, retinol, retinyl acetate or Ro10-9359, but not the pyridyl analog of retinoic acid. Among these retinoids, retinoic acid caused greatest induction of differentiation of HL-60 cells (Table 2).

Untreated HL-60 cells had considerable lysozyme activity (equivalent to 40-50 μg of egg white lysozyme/10^7 cells) and this activity was not significantly enhanced during culture of

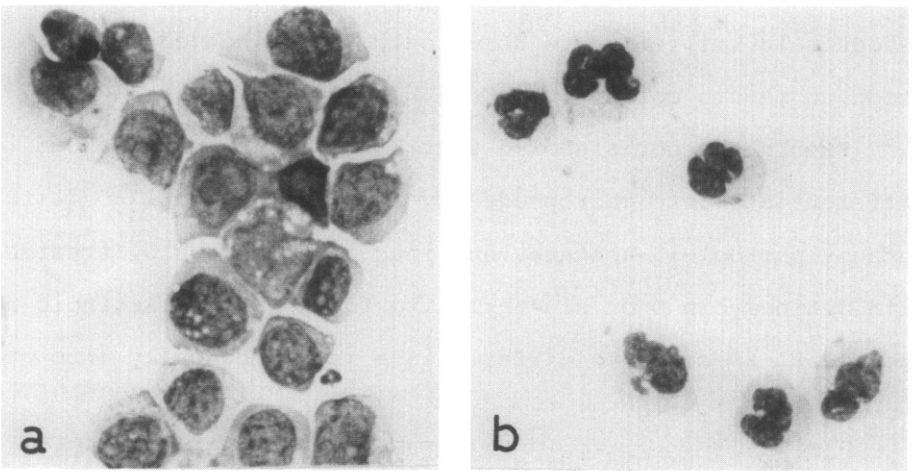


Fig. 3. Morphological myeloid differentiation in retinoid-treated HL-60 cells. HL-60 cells were cultured without (a) or with (b) 4 X 10^{-6} M retinoic acid for 6 days. (X 640)

Table 2. Effect of various retinoids on differentiation of HL-60 cells

Retinoid (4×10^{-6}M)	Phagocytosis (%)	NBT reduction (%)	Mature myeloid cells (%)
None	1.2	13.5	6.5
Retinoic acid	42.7	94.9	88.3
Pyridyl analog of retinoic acid	0.9	13.2	8.3
Retinal	21.9	70.6	54.8
Retinol	7.4	45.3	34.0
Retinyl acetate	2.4	29.0	29.6
Ro10-9359	7.5	22.1	32.4

HL-60 cells were cultured with the indicated concentration of retinoid for 6 days.
Mature myeloid cells; myelocytes, metamyelocytes, banded and segmented neutrophils.

the cells with various concentrations of retinoic acid (data not shown).

These results show that retinoids induced terminal differentiation of human HL-60 cells, although they have been found to inhibit morphological and functional differentiation of mouse myeloid leukemia Ml cells (3). Thus human leukemia cells respond differently from mouse leukemia cells to retinoids. Intracellular proteins that specifically bind retinoic acid have been demonstrated in many tissues and in experimental tumors including leukemia cells (1). The biochemical processes resulting from binding of retinoids to their binding proteins in Ml cells and HL-60 cells should provide information on the reason why retinoids have different effects in mouse Ml cells and human HL-60 cells.

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