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STIMULATION BY INTERFERON OF INDUCTION OF DIFFERENTIATION OF HUMAN PROMYELOCYTIC LEUKEMIA CELLS

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Treatment of human promyelocytic leukemia cells (HL-60) for 6 days with conditioned medium of mouse myeloid leukemic M1 cells which had been treated with phytohemagglutinin resulted in their morphological differentiation into macrophages and induction of ability to reduce nitroblue tetrazolium dye in the cells. Human IFN-α and IFN-β enhanced induction of differentiation of the cells into macrophages by the conditioned medium of M1 cells or 12-O-tetradecanoylphorbol-13-acetate (0.1 ng/ml). IFNs also enhanced induction of differentiation of the cells into granulocytes by retinoic acid.

INTRODUCTION

We previously reported that mouse IFN enhanced induction of differentiation of mouse myeloid leukemic cells (M1) into macrophages by inducers, such as lipopolysaccharide, polyinosinic acid and differentiation stimulating factor in conditioned medium of mouse peritoneal macrophages and mouse embryo cells (1). It was shown that differentiated M1 cells *in vitro* lost both proliferating activity and leukemogenicity (2) and that injection into mice of an inducer of differentiation of the cells or stimulator of *in vivo* production of differentiation stimulating factor prolonged the survival time of mice inoculated with the leukemia cells (3-5). Therefore, stimulation of *in vivo* induction of normal differentiation of myeloid leukemic cells is of potential value in leukemia therapy.

Recently, a human myeloid leukemic cell line (HL-60) was established and shown to be induced to differentiate into granulocytes or macrophages by various inducers (6,7). TPA and conditioned medium of human leucocytes treated with PHA induced differentiation of HL-60 cells into macrophages (8-10), while

Abbreviations: IFN, interferon; CSF, colony stimulating factor; TPA, 12-O-tetradecanoylphorbol-13-acetate; PHA, phytohemagglutinin; NBT, nitroblue tetrazolium; PWM, pokeweed mitogen.

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dimethylsulfoxide and retinoic acid induced differentiation of the cells into granulocytes (7-12).

On the other hand, Verma et al. (13,14) reported that a human leucocyte IFN-α preparation blocked granulopoietic differentiation of human bone marrow cells and that fibroblast IFN-β was less effective than leucocyte IFN-α. In the present study, we examined the effects of purified IFN-α and partially purified IFN-β on induction of differentiation of human promyelocytic leukemia cells into granulocytes and macrophages.

MATERIALS AND METHODS

Cells and cell culture HL-60 cells were maintained in suspension culture in RPMI-1640 medium (GIBCO, Grand Island, USA) supplemented with 10% heat-inactivated fetal calf serum (GIBCO) (6,7). Mouse myeloid leukemic M1 cells were kept in Eagle's minimal essential medium (Nissui Seiyaku Co., Tokyo, Japan) supplemented with double the usual concentrations of amino acids and vitamins and with 10% heat-inactivated calf serum (1,2).

Inducers of cell differentiation Conditioned medium of M1 cells was prepared by incubating 10^6 cells/ml in the modified Eagle's medium mentioned above with 1% (v/v) PHA (Bacto-phytohemagglutinin M, Difco Laboratories, Detroit, USA) and 50 μM 2-mercaptoethanol for 24 hr. TPA and retinoic acid were obtained from Consolidated Midland Co., Brewster, USA, and Sigma Chemical Co., St. Louis, USA, respectively.

Morphological differentiation Morphologic assessment of HL-60 cells was made on smear slide preparations stained with May-Grünwald-Giemsa solution. Differential counts were then performed under a light microscopy on a minimum of 300 cells.

NBT reduction NBT reduction was assayed as reported previously (15). The percentage of cells containing intracellular blue-black formazan deposits was determined by examination of a minimum of 200 cells.

IFN preparations IFN-α (5×10^8 IU/mg protein) was kindly donated by Dr. Shin Yonehara, Tokyo Metropolitan Institute of Medical Science. The IFN was produced by Sendai virus stimulation of human Namalwa cells and purified mainly by IFN antibody affinity chromatography. Analysis by SDS-polyacrylamide gel electrophoresis indicated that the IFN was essentially pure (16). IFN-β (2×10^7 IU/mg protein) was kindly donated by Dr. Shigeyasu Kobayashi, Basic Research Laboratories, Toray Industries Inc.. The IFN was prepared by induction of human fibroblasts by poly(I)·poly(C) followed by purification by column chromatography (17). The titers of IFN was determined by measuring the cytopathic effect of vesicular stomatitis virus on FL cells with NIH reference standard IFN-α (Catalog No. G023-901-527) and IFN-β (G023-902-527) and was expressed in International Reference Units (IU).

RESULTS

Induction of differentiation of HL-60 cells by conditioned medium of mouse M1 cells treated with phytohemagglutinin and mercaptoethanol Differentiation of HL-60 cells is induced by conditioned medium of human leucocytes treated

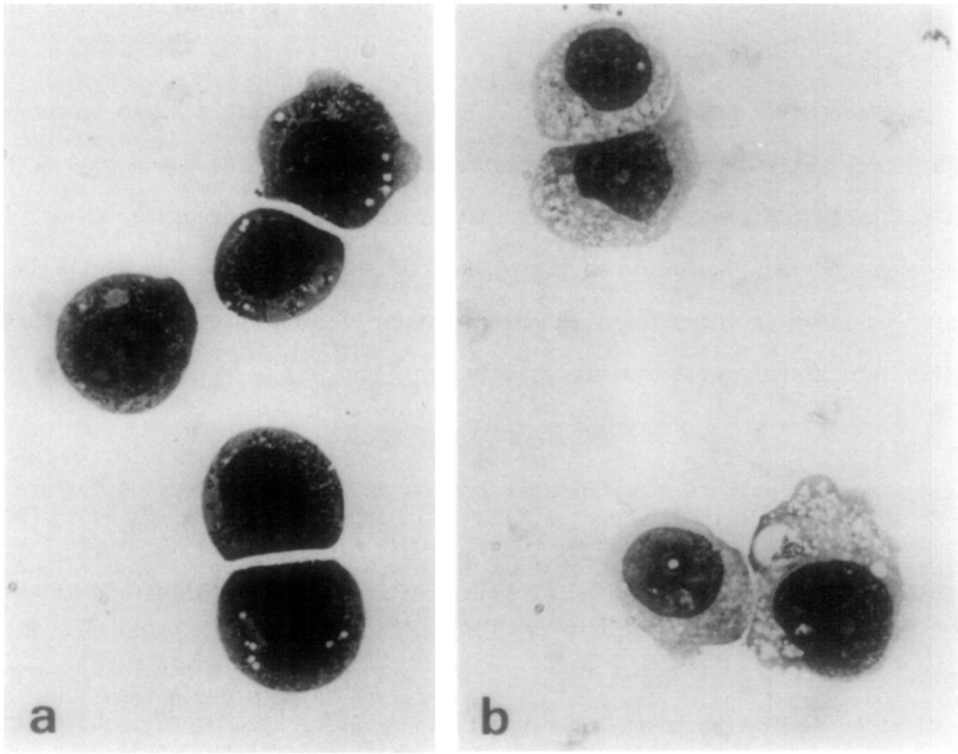

Fig. 1 Induction of morphological differentiation of HL-60 cells. HL-60 cells were cultured without (a) or with (b) 50% conditioned medium of M1 cells treated with PHA and 2-mercaptoethanol. (x 640)

with PHA (9), PWM (18) or 0.5 mM 2-mercaptoethanol (19). Conditioned medium of human leucocytes treated with mitogens for T-lymphocytes is likely to contain human IFN-γ. We examined conditioned medium of mouse myeloid leukemic M1 cells for ability to induce differentiation of HL-60 cells, since leukemic M1 cells are reported to be similar to human leucocytes in producing colony-inducing activity for human T-lymphocytes (20). As shown in Fig. 1 and Table 1, conditioned medium of M1 cells treated with PHA and 2-mercaptoethanol for 24 hr induced morphological differentiation of HL-60 cells into monocytes or macrophages and capacity to reduce NBT dye in the cells. PHA plus conditioned medium collected from M1 cells after 24 hr culture without PHA and 2-mercaptoethanol did not induce differentiation of HL-60 cells.

Table 1. Induction of differentiation of HL-60 cells by conditioned medium of Ml cells

Conditioned medium	Cell type (%)			NBT reduction (%)	No. of cells ($\times 10^{-6}$/ml)
	Promyelocytes	Myelocytes	Monocytes & macrophages		
None	90	10	0	4.7	2.56
25% (v/v)	74	0	26	10.0	1.92
38% (v/v)	15	2	83	19.5	0.88
50% (v/v)	1	0	99	47.5	0.54

HL-60 cells (2×10^5 cells/ml) were cultured for 6 days with conditioned medium of Ml cells treated with PHA and 2-mercaptoethanol.

Stimulation by IFNs of induction of differentiation of HL-60 cells by conditioned medium of Ml cells We examined the effects of IFN-α and IFN-β on growth and differentiation of HL-60 cells. Treatment of the cells with IFNs alone for 6 days did not inhibit growth of the cells (Fig. 2) or induce their differentiation. IFNs, however, enhanced inhibition of growth of HL-60 cells and induction of differentiation of the cells into monocytes and macrophages by a suboptimal concentration of conditioned medium of Ml cells (Fig. 3). Both IFN-α and IFN-β were effective at concentrations of more than 500 IU/ml.

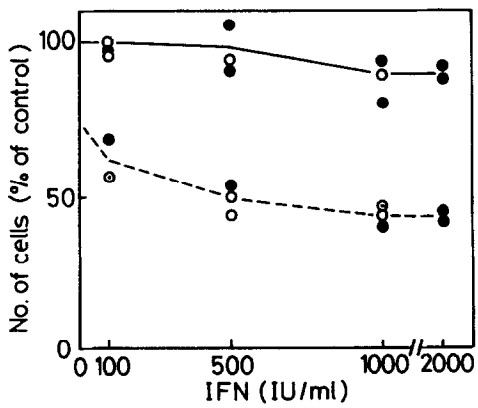

Fig. 2 Effects of IFNs on growth of HL-60 cells. HL-60 cells (2×10^5 cells/ml) were incubated with different concentrations of IFN-α (○) or IFN-β (●) for 6 days in the absence (—) or presence (---) of 25% conditioned medium of Ml cells.

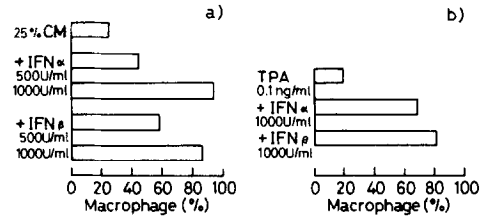

Fig. 3 Stimulation by IFNs of induction of morphological differentiation of HL-60 cells into macrophages. HL-60 cells were incubated with IFNs for 6 days in the presence of 25% conditioned medium (CM) of Ml cells (a), or for 3 days in the presence of 0.1 ng/ml of TPA (b).

Stimulation by IFNs of induction of differentiation of HL-60 cells into macrophages by TPA Although phorbol ester TPA is a potent inducer of differentiation of HL-60 cells into macrophages (8-10), after treatment of the cells with a low concentration of TPA (0.1 ng/ml) for 3 days, most of the cells were promyelocytes. However, on simultaneous treatment of the cells with TPA and IFNs, over 60% of the cells differentiated into macrophages (Fig. 3).

Stimulation by IFNs of induction of differentiation of HL-60 cells into granulocytes by retinoic acid Retinoic acid, an analog of vitamin A, induced differentiation of HL-60 cells into mature granulocytes (metamyelocytes, banded neutrophils and segmented neutrophils) dose- and time- dependently as reported previously (11,12). Simultaneous treatment of the cells with IFNs and retinoic acid decreased the concentration of retinoic acid and the incubation time necessary to induce differentiation of the cells (Table 2).

DISCUSSION

We showed that IFN alone did not induce differentiation of human myeloid leukemic HL-60 cells but enhanced induction of differentiation by inducers, such as retinoic acid, TPA and conditioned medium of Ml cells treated with PHA. These findings are compatible with our previous findings with mouse myeloid leukemic Ml cells (1), although the effective inducers for differentiation of human HL-60 cells were different from those for mouse Ml cells.

It has been reported that conditioned medium of human leucocytes induces differentiation of HL-60 cells (9,18,19). We found that conditioned medium of

Table 2. Effect of IFNs on induction of morphological differentiation of HL-60 cells by retinoic acid

Addition	Days	Cell type (%)			
		Promyelo-cytes	Myelo-cytes	Metamyelo-cytes	Banded & segmented neutrophils
None	5	95	5	0	0
Retinoic acid (4×10^{-8}M)	4	20	35	29	16
	5	2	14	57	27
+ IFN-α*	4	14	6	20	60
	5	15	1	27	69
+ IFN-β*	4	19	25	5	51
	5	15	1	37	47
Retinoic acid (4×10^{-7}M)	4	2	1	71	26
	5	0	1	45	54
+ IFN-α*	4	4	2	23	71
	5	0	0	21	79
+ IFN-β*	4	8	6	31	55
	5	1	1	30	68

*HL-60 cells were cultured with retinoic acid plus IFNs (1000 IU/ml) for the indicated times.

mouse myeloid leukemic cells also induced differentiation of human HL-60 cells. The factors in the conditioned medium responsible for induction of differentiation of the cells have not been characterized. The differentiation-inducing activity in the conditioned medium seems to be different from CSF that stimulates growth and differentiation of normal precursor cells of macrophages and granulocytes. Ruscetti et al. (21) reported that various CSF preparations stimulated colony formation of HL-60 cells in agar culture but did not induce differentiation of the cells. Furthermore, Olsson and Olofsson (18) reported that differentiation inducing factor is distinct from CSF present in pokeweed mitogens-stimulated lymphocyte conditioned medium, as judged by its mobility on gel chromatography.

IFNs are known to inhibit colony formation of granulocyte-macrophage progenitor cells stimulated by CSF (22-24). However, Verma et al. (13,14) pro-

posed that the main effect of IFN on granulopoiesis was not inhibition of growth of immature granulocyte-macrophage progenitor cells but block of differentiation beyond the myelocyte level and that the action of IFN is tissue-specific; fibroblast IFN-β was less effective than leucocyte IFN-α. We examined the effects of highly purified IFN-α and partially purified IFN-β on induction by retinoic acid of differentiation of leukemic cell line into granulocytes, and found that the IFN-α and IFN-β preparations enhanced induction of differentiation of the cells similarly. This discrepancy between our results and those of Verma et al. may be mainly due to a difference in the cells used; leukemic cells and normal cells. However, it is necessary to examine the effects of purified IFNs on growth and differentiation of normal bone marrow cells, since Verma et al. used crude IFN-α ($3x10^6$ IU/4 mg of protein).

Details of the mechanisms of action of IFNs on differentiation of bone marrow cells into macrophages remain to be examined, although a number of papers showed that IFN preparations activate monocytes or macrophages (25,27). Our finding that IFNs enhanced induction of differentiation of mouse and human myeloid leukemic cells into macrophages by various inducers, suggests that IFNs also enhance differentiation or maturation of normal precursor cells of macrophages.

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