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THE ROLE OF INTERFERON-γ IN INDUCTION OF DIFFERENTIATION OF HUMAN MYELOID LEUKEMIA CELL LINES, ML-1 AND HL-60

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Highly purified natural interferon-γ (IFN-γ) induced differentiation having characteristics that are associated with the human promyelocytic leukemia cell line, HL-60. Monoclonal antibody to INF-γ neutralized its activity. However, the natural IFN-γ had almost no inducing activity in ML-1, a human myeloblastic leukemia cell line. Simialr results were obtained using recombinant IFN-γ. Mitogen stimulated human leukocyte conditioned medium (LCM) induced differentiation of both ML-1 and HL-60 cells. After treatment of LCM with monoclonal antibody to IFN-γ, LCM activity was reduced more than 50% in ML-1 cells, and 80% in HL-60 cells.

Even if IFN-γ was eliminated from LCM by affinity chromatography, the LCM induced differentiation of ML-1 and HL-60 cells, but IFN-γ markedly enhanced the ML-1 cell differentiation induced by IFN-γ free LCM.

The results suggest that leukocytes produce differentiation inducing factor(s) other than IFN-γ, and that IFN-γ is both an inducer and an enhancer of induction of human myelogenous leukemia cells. © 1984 Academic Press, Inc.

The human promyelocytic leukemia cell line, HL-60, and the myeloblastic leukemia cell line, ML-1, can be induced to differentiate into macrophages by protein inducers, differentiation inducing factors (DIFs), in conditioned media of mitogen stimulated human peripheral blood leukocytes (1-4). However, human DIFs have not yet been well characerized.

Tomida et al. reported that human IFN-α or IFN-β alone had no ability to induce differentiation of HL-60 cells, but they did enhance induction of differentiation of the cells into macrophages in the conditioned medium of mouse Ml cells (5).

Human immune interferon, IFN-γ, differs from IFN-α or IFN-β in biological and physicochemical properties (6). These differences have led to interest in possible differences of IFN-γ activity in inducing differentiation of human myeloid leukemia cells. We have also been interested in differences between differentiation inducing activity of DIFs

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and IFN-γ in conditioned medium derived from the culture of mitogen stimulated leukocytes.

In the present study, we examined the effects of highly purified natural IFN-γ on induction of differentiation of human myeloid leukemic cell lines, and compared this activity with DIFs in the conditioned medium.

MATERIALS AND METHODS

RPMI 1640 medium was purchased from Grand Island Biological Co., Grand Island, New York, and fetal bovine serum was obtained from Filtron Pty. Ltd., Victoria, Australia. Pokeweed mitogen, nitroblue tetrazolium dye and the reagents used for the assay of AS-D chloroacetate and α-naphthyl acetate esterase activity were purchased from Sigma Chemical Co. 12-O-Tetradecanoylphorbol-13-acetate (TPA) was purchased from Chemicals for Cancer Research, Inc, Eden Prairie, Minn. Buffy coats from healthy donors were provided by Dr. Y. Shibata, Nihon Seiyaku, Co. IFN-γ was derived from PHA stimulated human leukocyte conditioned medium and purified by affinity chromatography coupling of monoclonal antibody to IFN-γ. Analysis by SDS-polyacrylamide gel electrophoresis indicated that the IFN-γ was essentially pure. Purified IFN-γ (7×10^7 U/mg protein), mouse monoclonal antibody, and IFN-γ free LCM {LCM(-IFN-γ)}, obtained by passing it through affinity chromatography, were kindly donated by Green Cross Co. Recombinant IFN-γ was provided by Shionogi, Co.

Cell Culture: ML-1, established from a patient with human acute myeloblastic leukemia (7), and HL-60, an acute promyelocytic leukemic cell line (8), were maintained as suspension cultures in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum. Incubation was carried out at 37°C in a humidified, 5% CO_2 incubator.

Preparation of conditioned medium: Human peripheral blood mononuclear cells, separated by Ficoll-Hypaque density gradient (1.077 g/ml), were cultured at 2×10^6 cell/ml in RPMI 1640 medium containing 10% heat inactivated serum and 2 μg/ml pokeweed mitogen for 3 days. After removal of cells and debris by centrifugation, the supernatant solution was filtered through a 0.45 μm Millipore filter, and the filtrate stored at -20°C.

Cell growth inhibition and differentiation associated characteristics were assayed by adding 2 ml of RPMI 1640 medium containing 6×10^5 cells, 10% FBS and the test materials at the desired concentrations. Differentiation was monitored after 3 days by determining the appearance and accrual of various cellular markers normally associated with the maturation of the granulocytic and monocytic elements. Cell growth was also determined with a hematocytometer, and viability was estimated by by trypan blue dye exclusion.

NBT reducing ability: Following incubation, 3×10^5 cells were suspended in 0.2 ml of RPMI 1640 medium containing 10% FBS, 0.1% NBT and 30 ng TPA for 20 minutes at 37°C, the reaction was stopped by cooling in ice water. A drop of cell suspension was placed on microscope slides and the percentage of cells containing blue-black formazan deposits was determined by counting at least 200 cells (9).

Phagocytic activity: Phagocytic activity in the cells was determined by the method of Baker's yeast digestion (10).

Esterase activity: The cytochemical stains for chloroacetate esterase and α-naphthyl acetate esterase activity were performed as previously described (11).

Morphological differentiation: After staining with May-Grünward-Giemsa, differential counts were made with a light microscope.

RESULTS

Dose dependent effect of IFN-γ: As shown in Fig. 1, highly purified natural IFN-γ induced NBT reducing activity dose dependently, at least up to 10,000 U/ml in HL-60 cells, but it had almost no inducing activity in ML-1 cells. The phagocytic activity displayed similar results. However IFN-γ (1,000 U/ml) inhibited growth of both ML-1 and HL-60 cells approximately 30%.

Neutralization of IFN-γ and LCM differentiation inducing activity by monoclonal antibody to IFN-γ: 10% Pokeweed mitogen stimulated LCM, 1,200 U/ml IFN titer, induced NBT reducing activity in both ML-1 and HL-60 cells. Treatment of IFN-γ preparation or LCM with mouse monoclonal antibody reduced LCM activity more than 50% in ML-1 cells and 80% in HL-60 cells, and neutralized IFN-γ activity (Table 1).

Combination effect of IFN-γ and other factors: IFN-γ free LCM {LCM(-IFN-γ)} induced NBT reducing activity in both ML-1 and HL-60 cells. IFN-γ markedly enhanced the NBT reduction of ML-1 cells induced by LCM(-IFN-γ) (Figure 2).

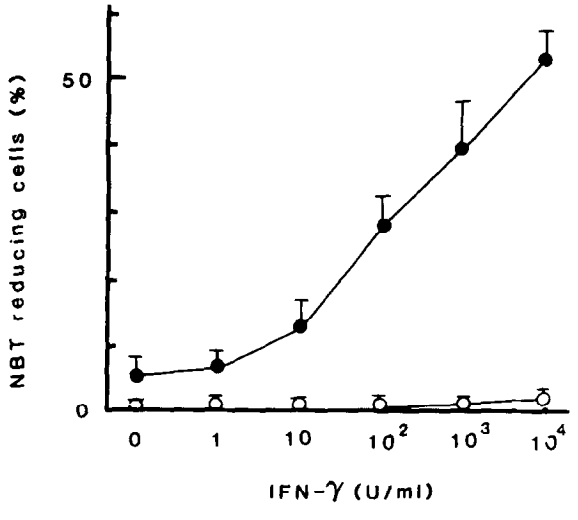

Fig. 1. Dose dependence of induction of NBT reducing activity on IFN-γ in human myeloid leukemia cell lines. ML-1 and HL-60 cells cultured for 3 days with IFN-γ at indicated concentration, and NBT reducing activity assayed: ML-1 (○), HL-60 (●). Mean value ± S.E. shown for 6 determinations.

Table 1

Neutralization of differentiation inducing activity of LCM by monoclonal antibody to IFN-γ

Preparation	NBT reducing cells (%)	
	ML-1	HL-60
control	3.9 ± 1.0	2.5 ± 0.7
IFN-γ 1,000 U/ml	6.7 ± 1.9	35.9 ± 12.7
Mo-Ab 1,000 U/ml	3.3 ± 0.9	0.5 ± 0.2
LCM 10%	37.6 ± 4.7	66.8 ± 4.7
IFN-γ 1,000 U/ml + Mo-Ab 1,000 NU/ml	0.9 ± 0.3	5.4 ± 2.2
LCM 10% + Mo-Ab 1,000 NU/ml	19.6 ± 4.3	18.7 ± 3.6

To neutralize IFN-γ activity, each samples incubated at 37°C for 1 hr with monoclonal antibody to IFN-γ. NBT reducing activity assayed after 3 days incubation of cells in presence of test materials. All values: averages of 6 determinations ± S.E.

IFN-γ and LCM(-IFNγ) treated cells were classified as macrophage-like by morphology and lineage-specific α-naphthyl acetate esterase stain.

We obtained similar results by using recombinant IFN-γ.

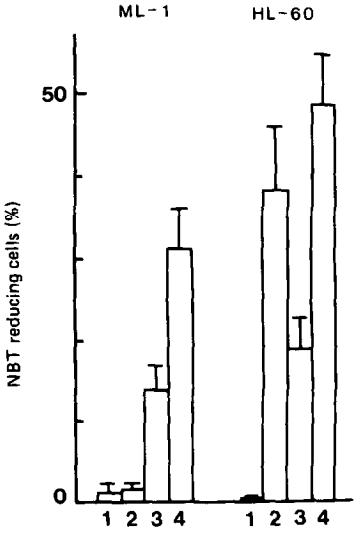

Fig. 2. Combination effects of IFN-γ and LCM(-IFN-γ) on induction of NBT reducing activity in human myeloid leukemia cell lines. NBT reducing activity assayed after 3 days incubation of cells in presence of test materials. Values: averages of 6 determination ± S.E. 1) control. 2) IFN titer:1,000 U/ml. 3) LCM(-IFN-γ) prepared by coupling monoclonal antibody to IFN-γ by affinity chromatography; IFN titer less than 1 U/ml. 4) IFN-γ 1,000 U/ml + LCM(-IFN-γ).

DISCUSSION

The present study demonstrated that highly purified IFN-γ induced characteristics associated with differentiation of the human myeloid leukemia cell line, HL-60, but was almost ineffective on ML-1, a line of human myeloblastic leukemia cells. These results were repeated using recombinant instead of natural IFN-γ, and both were confirmed by neutralizing IFN-γ activity with monoclonal antibody to IFN-γ. Henmi recently observed differentiation of HL-60 cells induced by recombinant IFN-γ (personal communication). Our data suggest that IFN-γ can induce the whole range of differentiation properties that are associated with HL-60. Although the morphological and non-specific esterase activity changes were not as dramatic as those of TPA treated cells (12,13), they did reveal characteristics of monocytes/macrophages.

Most of the activity in LCM that induced NBT reducing activity in HL-60 cells was identified with IFN-γ, since anti-IFN-γ monoclonal antibody neutralized the inducing activity. On the other hand IFN-γ, which alone is ineffective on ML-1, potentiated the differentiation inducing effects of DIF(s) on ML-1. Neutralization of IFN-γ activity in the LCM with monoclonal antibody reduced LCM activity more than 50% in ML-1, so IFN-γ also has a great effect on induction of differentiation of ML-1 cells in LCM.

Ralph, P. et al. recently reported that highly purified IFN-γ induced expression of Fc receptors of U-937, a human monoblastic leukemia line (14). In our data IFN-γ alone was less effective in inducing NBT reducing activity in U-937 despite its potency in inducing Fc receptor activity (Takei, M., unpublished data). The disparity may imply that the nature of the differentiation path depends, in part, on the stage at which maturation progression of the leukemic cells is blocked.

Recently, mitogen stimulated human leukocytes were found to release DIFs, polypeptide factors that induce HL-60 or ML-1 to mature with

characteristics of monocytes/macrophages (1-4). However, the DIFs in LCM have not yet been well characterized.

Since DIF(s) and IFN-γ show similar heat stability and mobility on gel chromatography, it is not yet clear whether DIF(s) can be distinguished from IFN-γ, but our data that IFN-γ free LCM induces differntiation of ML-1 cells suggests that leukocytes produce DIF(s) other than IFN-γ. Purification of DIFs is currently under way in our laboratory.

A number of papers show that IFN activates monocytes or macrophages (15-18). Our findings suggest that IFN-γ also induces or potentiates differentiation of precursor cells of monocytes and/or granulocytes.

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