IMMUNE INTERFERON AND LEUKOCYTE-CONDITIONED MEDIUM INDUCE NORMAL AND LEUKEMIC MYELOID CELLS TO DIFFERENTIATE ALONG THE MONOCYTIC PATHWAY

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Human promyelocytic cell lines can be induced to differentiate in vitro along the myeloid or the monocytic pathway depending on the chemical or biological inducers used (1–4). Induction of promyelocytic cell lines along the monocytic pathway can be readily demonstrated by culturing the cells in the presence of medium conditioned by lectin-stimulated leukocytes (5–9). Most cells from human promyelocytic cell lines, such as HL-60 or ML3, cultured in the presence of conditioned medium from phytohemagglutinin-stimulated lymphocytes (PHA-CM),[1] express monocyte-specific antigens, including HLA-DR, and surface receptors and enzymes typical of monocytes (6, 9). The cells acquire morphological characteristics of differentiated monocyte-macrophages and the ability to mediate active phagocytosis and antibody-dependent cytotoxicity (Ab-CMC) (9), but lose the ability to proliferate in culture (7, 9). It has also been shown that PHA-CM can induce at least partial monocytic differentiation in cells freshly obtained from patients with acute myelogenous leukemia (AML) (10).

Lectin-stimulated lymphocytes secrete a variety of soluble factors including colony-stimulating factors (CSF) (11), interferons (IFN) (12, 13), and the T cell growth factor, interleukin 2 (IL-2) (14). The ability of all IFN types to modulate cellular functions, in addition to exerting antiviral activity, has been described, and most of these anticellular effects are inhibitory, with marked suppression of cellular DNA and protein synthesis. However, IFN can also stimulate the functions of the effector cells of nonadaptive immunity, natural killer (NK) cells and

monocyte-macrophages (15, 16), efficiently enhancing nonspecific effector cell activity (17). Human IFNα induces an arrest in granulopoietic maturation of colonies grown in vitro at the level of small clusters (18), and purified mouse IFN enhances differentiation of M1 mouse myeloid leukemic cells to monocyte-macrophages induced by macrophage CM or polyinosinic acid (19). IFNα, IFNβ, and (2'5')oligo-isoadenylate can induce the human macrophage cell line U937, but not the more immature HL-60 cell line, to mediate Ab-CMC (20). IFNγ induces or enhances the expression of Ia antigens on a variety of cell types (21–25) and the expression of the receptor for the Fc fragment of monomeric IgG on myelomonocytic cells (26). We recently observed that human IFNγ can not only induce HLA-DR expression on promyelocytic cell lines, but, like PHA-CM, can also induce expression of the surface antigens, enzymes, and functional activities characteristic of cells differentiating along the monocytic pathway.[2]

In this report, we show that PHA-CM can induce terminal monocytic differentiation in human immature myeloid cells obtained from normal bone marrow or from peripheral blood of patients with chronic myelogenous leukemias (CML) or other myeloproliferative disorders. We also identify the active component of PHA-CM that effects this induction in normal myeloid cells and in cells from acute and chronic myelogenous leukemia patients as IFNγ.

Materials and Methods

Patients. Hematologically normal bone marrow specimens were obtained for diagnostic purposes from donors at the Thomas Jefferson University Hospital, Philadelphia or from normal donors at the Children's Hospital of Philadelphia. Peripheral blood was obtained from eight patients with CML and one with myeloid metaplasia at the Hospital of Jefferson University. Seven out of eight CML patients were Philadelphia chromosome positive. Four of these patients were untreated when tested for the first time, and the others were treated with Myleran (Burroughs Wellcome Co., Research Triangle Park, NC), hydroxyurea, or both. Cells from adult patients with AML were typed according to the French-American-British classification (FAB) at the Hospital of the University of Pennsylvania, Philadelphia, and the identification was confirmed by one of us (J. H.) through analysis of the cells with a large panel of anti-human leukocyte monoclonal antibodies by indirect immunofluorescence (flow cytofluorometry).

Monoclonal Antibodies. Most mouse anti-human leukocyte monoclonal antibodies used in this study were produced and characterized in our laboratory. The IgG2b pan-T cell antibody B36.1 (27) detects the 69,000–71,000 mol wt protein complex and has the same specificity as antibodies OKT1 and L17F12 (anti-Leu-1) (28, 29). Two non–cross-competing IgG2a antibodies, B67.1 and B67.6, react with the 45 K mol wt protein of T cells, presumably identified as the receptor for sheep erythrocytes (E) (27). Antibody B73.1 (27, 30) (IgG1) reacts with a 65–72 K mol wt protein complex present on large granular lymphocytes (LGL) and on neutrophilic polymorphonuclear leukocytes (PMN) that is probably the receptor for the Fc fragment of aggregated immunoglobulin (FcR) (31). The IgM antibodies B44.1 (32), B52.1 (9), and B77.1 specifically react with all peripheral blood monocytes and all α-naphthylacetate esterase (α-NAE)-positive cells in the bone marrow. Antibodies B44.1 and B52.1 cross-compete with each other and with the antimonocyte antibody Mo2 (33) for binding sites on monocytes. Antibody B77.1 does not cross-compete with any of the other antibodies and is presumably directed against a separate antigenic determinant expressed on monocytes (unpublished results). B40.8 and B37.2 (32) are two cross-reacting IgM antibodies of identical specificity: they react with

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[2] Dayton, E. T., B. Perussia, and G. Trinchieri. Immune interferon induces monocytic differentiation in human promyelocytic cell lines. Manuscript submitted for publication.

all normal myeloid cells and with a proportion of myeloid precursor cells in the bone marrow. The subset of cells defined by B40.8 and B37.2 and the subset defined by B44.1, B52.1, and B77.1 in normal peripheral blood and bone marrow leukocytes are nonoverlapping (32). The IgM antibody B13.4 (32, 34) reacts with both mature PMN and monocytes and, within cells of the myeloid lineage, reacts only with metamyelocytes or more mature cells. The IgG2a antibody B33.1 (27, 32) reacts with a nonpolymorphic determinant of the HLA-DR antigen (human Ia-like antigens). The F(ab')$_2$ fragment of B33.1, prepared by pepsin digestion, was used throughout this study. The IgG2a antibody D1-12 (35), kindly provided by Dr. R. Accolla, Ludwig Institute for Cancer Research, Lausanne, Switzerland, is directed to one product of the DR locus in the HLA complex. Monoclonal antibodies Q2/70, Q2/80, Q5/6, and Q5/13 are directed against different subpopulations of human Ia molecules (36) and were kindly provided by Dr. S. Ferrone, Columbia University, New York.

Isolation of Immature Myeloid Cells. Peripheral blood and bone marrow samples were anticoagulated with heparin and layered on a single-step Ficoll/Hypaque (F/H) discontinuous gradient (1.077 ± 0.01 g/ml) to remove mature erythroid and myeloid cells. The mononuclear cells, obtained from the interface of the gradient, were depleted of mature adherent monocytic cells by two cycles of adherence to plastic surfaces (45 min at 37°C). Nonadherent mononuclear cells were depleted of contaminating monocytes, T lymphocytes, and NK cells using indirect rosetting (as described later) and F/H gradient after sensitization of the cell suspension with a mixture of the anti-T B36.1, anti-sheep E receptor B67.1 and B67.6, anti-NK/K cell B73.1, and antimonocyte B44.1 and B52.1. The negative cell population, highly enriched for myeloid cells, was in some experiments purified further. Indirect rosetting with antibody B13.4 and F/H gradient were used to separate the total myeloid cell population from CML patients into a more mature B13.4$^+$ population, composed mostly of metamyelocytes and banded cells, and a more immature B13.4$^-$ population, composed of myelocytes and a minor component of more immature or mature cells. Peripheral blood mononuclear cells from patients with acute leukemia were separated by F/H gradient centrifugation and maintained frozen in liquid nitrogen until induced in culture.

Conditioned Medium. Human peripheral blood mononuclear cells were irradiated (20 Gy) and incubated (10^6/ml) in RPMI 1640 medium supplemented with 1% PHA-M (Wellcome Research Laboratories, Beckenham, Kent, England) and 1% fetal bovine serum (FBS) (Gibco Laboratories, Grand Island, NY) for 72 h at 37°C in 5% CO$_2$ in air. Cell-free supernatant was collected and used as a crude PHA-CM preparation.

IFN Preparation. Purified human IFNγ (10^6 U/mg protein on HEp-2 cells) was obtained from Interferon Sciences, Inc., New Brunswick, NJ. Human recombinant IFNγ (rIFNγ) from E. coli was kindly supplied by Dr. C. G. Sevastopoulos, Genentech, Inc., San Francisco, CA and it has a titer of 5 × 10^7 antiviral U/mg on HeLa cells. Because of the variability of the titer of IFNγ on different cell lines and the lack of an international standard for IFNγ, concentrations of rIFNγ are expressed in ng/ml rather than antiviral units. The concentration used in most experiments, 100 ng/ml, corresponds to ~500 U/ml on HeLa cells. No significant CSF activity was demonstrable in the rIFNγ preparation using either normal bone marrow cells or peripheral blood cells from CML patients. Partially purified IFNα (Leucoferon; 10^6 antiviral U/mg) was obtained from Biotechnologies Inc., Hartford, CT. Recombinant types A and D IFNα from *Escherichia coli* (rIFNA and rIFND) were kindly supplied by Hoffmann-LaRoche, Inc., Nutley, NJ. Crude IFNβ was obtained from Lee Biomolecular Research Lab., Inc., San Diego, CA, and purified IFNβ was kindly supplied by Dr. A. Billiau, (preparation No. 16287; Leuven, Belgium). Rabbit antiserum against human IFNγ (10^4 neutralization U/ml) and sheep antiserum against human IFNα (10^5 neutralization U/ml) were obtained from Interferon Sciences, Inc. Using a colony assay in methyl cellulose with peripheral blood CML target cells, no inhibition of the CSF activity contained in PHA-CM or in GCT medium (Gibco Laboratories) was observed after treatment with anti IFNγ serum.

Culture of Myeloid Cells. Enriched preparations of myeloid cells from patients were cultured (2 × 10^6 cells/ml) in RPMI 1640 medium (Flow Laboratories, Rockville, MD)

supplemented with 10% FBS at 37°C in 5% CO_2 in air. All dilutions of PHA-CM or IFN were prepared in this medium. The absolute number of cells recovered after 5 d of culture was approximately the original input under any of the experimental conditions. Viability, as evaluated by dye exclusion test, was between 75 and 95% and was not significantly different among samples. To avoid nonspecific binding and autofluorescence in immunofluorescence assays, the cells recovered after 5 d of culture were depleted of dead cells by centrifugation over a single-step F/H gradient.

Cell Morphology. Cell morphology was examined on cytocentrifuge preparations (Cytospin centrifuge; Shandon Southern Instrument Inc., Sewickley, PA), stained by the May-Grunwald-Giemsa method. Staining for α-NAE was carried out according to Platt (37).

Electron Microscopy. Cells were fixed in 3% glutaraldehyde (vol/vol) (Fisher Scientific Co., Pittsburgh, PA) in PIPES buffer, pH 7.2 (Sigma Chemical Co., St. Louis, MO), at 4°C for 30–60 min, and postfixated in 1% OsO_4 (Electron Microscopy (EM) Sciences, Fort Washington, PA) in PIPES buffer for 1 h at 20°C. Specimens were stained with uranyl acetate (1% aqueous solution; EM Sciences) for 18 h at 60°C, dehydrated with alcohol, and embedded in Epon (EM Sciences). Sections 50–80 nm thick were cut with a Porter-Blum MT2 ultramicrotome (DuPont Instruments-Sorvall Biomedical Div., DuPont Co., Wilmington, DE) with a diamond knife and were stained with lead citrate. Sections were examined in a Hitachi HU 11-E electron microscope.

Detection of FcR. Ox erythrocytes (E) sensitized with rabbit IgG anti-ox E (EA7S) (Cappel Laboratories, Cochranville, PA) were used as the indicator system for FcR. Cells and E were mixed (1:50) and incubated as pellets for 20 min at 4°C. The proportion of cells forming rosettes with sensitized E was determined by scoring at least 200 cells; only cells with more than five E bound were considered as positive.

Indirect Immunofluorescence Studies. Cells were sequentially incubated with appropriate dilutions of the different monoclonal antibodies and fluorescein isothiocyanate (FITC)-labeled goat F(ab')$_2$ anti-mouse Ig antibody for 30 min at 0°C as described (34). The cells were then washed at 4°C with phosphate-buffered saline (PBS) containing 5% normal human serum. When the populations separated by indirect rosetting were assayed, they were first incubated with biotin-labeled antibodies (38), and then with FITC-avidin (Vector Laboratories, Inc., Burlingame, CA). The proportion of cells reactive with the different antibodies was scored on an Ortho Cytofluorograf 50H (Ortho Diagnostic Systems, Inc., Westwood, MA) connected to a Data General MP/200 microprocessor. The fluorescence signals were assigned into 200 channels by a pulse height analyzer, using a linear scale. The threshold fluorescence channel was used at which 99% of the cell population treated with control supernatant from the parental myeloma and FITC-anti-mouse Ig antibodies or FITC-avidin were negative. Fluorescence intensity, within each experiment, was evaluated by determining the mean fluorescence channel of positive cells.

Separation of Cells Reactive with Monoclonal Antibodies by Indirect Rosetting. Cells sensitized with the monoclonal antibodies were incubated for 1 h at 4°C as pellets with $CrCl_3$-treated sheep E coated with affinity-purified goat F(ab')$_2$ anti-mouse Ig as described (34). Under these conditions, no rosette formation was ever observed on control cells not sensitized with antibody. Rosetting and nonrosetting cells were then separated on a one-step F/H gradient. Contaminating E in the positive fraction were lysed by treatment with hypotonic medium.

Cell-mediated Cytotoxicity Assay. Lytic activity was tested in a 3-h ^{51}Cr-release assay by incubating various numbers of effectors in microtiter plates at 37°C with a constant number (10^4) of ^{51}Cr-labeled P815Y target cells sensitized with a 1/1,500 dilution of a rabbit anti-P815Y serum prepared in our laboratory. Supernatants were harvested and percent specific cytotoxicity was calculated as previously reported (39). One lytic unit 45% (LU) is defined as the number of effector cells required to mediate 45% specific ^{51}Cr-release from 10^4 target cells in the 3-h assay. LU per 10^7 cells were calculated using a modified Van Krogh equation as described (40).

[^{3}H]Thymidine Incorporation. Myeloid cells from CML patients were cultured in flat-bottomed plates with or without 50% PHA-CM or 100 ng/ml rIFNγ. At different times, aliquots from the same sample were pulsed for 1 h with [^{3}H]thymidine ([^{3}H]TdR) (2 μCi/

well, 2 Ci/mmol specific activity; New England Nuclear, Boston, MA). Cells were then washed and cytocentrifuge smears were prepared, fixed in absolute methanol for 30 min, and covered with NTB-2 emulsion (Eastman Kodak Company, Rochester, NY) for a 2-d exposure. After developing, cells were stained with hematoxylin. The proportion of cells incorporating [^{3}H]TdR was scored by counting at least 300 cells.

Results

Effect of PHA-CM and Recombinant IFNγ (rIFNγ) on Cultures of Normal Bone Marrow.   Normal bone marrow cells, prepared as described, were maintained in liquid cultures for 5 d in culture medium supplemented or not with PHA-CM (50% vol/vol) or rIFNγ (50 ng/ml). The cells were then washed through FBS and tested for expression of surface antigens and FcR by indirect immunofluorescence (flow cytofluorometry) and by rosetting with EA7S, respectively. Table I shows results obtained with 11 different bone marrow specimens. Myeloid cells predominate in the cell preparations, as indicated by the large proportion of cells reactive with antibody B40.8. However, expression of the myeloid antigen, as detected by this antibody, did not change significantly as a result of PHA-CM or IFNγ treatment. In all cases, treatment with PHA-CM or rIFNγ consistently determined a marked increase in the percentage of HLA-DR$^+$ cells as detected by antibody B33.1. On day 0, the cell preparations had been depleted of adherent and of almost all B52.1$^+$ cells. After 5 d culture in the absence of inducers, in some of the samples, a low density expression of the monocyte antigen B52.1 was observed in a consistent proportion of the cells. In the presence of PHA-CM or rIFNγ, a significant increase in the proportion of B52.1$^+$ cells was observed in most samples. In all experiments, a several-fold increase in the density of the B52.1 antigen, as judged by the mean fluorescence intensity, was observed (not shown). Thus, after culture with either PHA-CM or rIFNγ, a large proportion of cells simultaneously expressed the B52.1 monocytic antigen, the B40.8 myeloid antigen, and HLA-DR. In the preparations treated with rIFNγ, the percentage of cells expressing FcR was also increased (Table I). The ability of the cell preparation from donor 7 to mediate Ab-CMC against P815Y target cells increased when the cells were cultured in the presence of rIFNγ (40.6% specific ^{51}Cr-release at a 1:1 effector/target ratio) as compared with cells cultured in medium alone (22.7% specific ^{51}Cr-release at a 2:1 ratio).

Effect of PHA-CM and rIFNγ on Cultures of Peripheral Blood Myeloid Cells from CML Patients.   Enriched myeloid cells from CML patients were cultured for 5 d in medium supplemented or not with PHA-CM or rIFNγ. Table II and Fig. 1 report the results of a typical experiment with the cultured cells from donor C.L. On day 0, the majority of the cells expressed myeloid antigens (79.1%) and only a small percentage expressed HLA-DR or surface markers of monocytes and T cells. After 5 d of culture, however, the expression of the two monocyte antigens (B52.1 and B77.1), HLA-DR, FcR, and α-NAE was consistently increased on the cells maintained in the presence of PHA-CM or rIFNγ. The increase of FcR expression was observed after 6–8 h of culture, whereas the other markers of differentiation were increased beginning days 2 and 3 of culture (data not shown). In many cases, the monocyte and HLA-DR antigens were strongly expressed in >50% of the cells cultured for 5 d in the presence of PHA-CM or rIFNγ. The expression of the myeloid antigen recognized by B40.8 did not change signifi-

TABLE I

Effect of PHA-CM and rIFNγ on the Expression of Surface Antigens on Normal Bone Marrow Cells

Exp.	Culture conditions*	HLA-DR antigen (B33.1)[‡]	Monocyte antigen (B52.1)[‡]	Myeloid antigen (B40.8)[‡]	FcR (EA7S)[§]
1	Medium	7.4	54.5	92.2	ND[ǁ]
	PHA-CM	53.2	90.3	91.2	ND
2	Medium	5.4	47.0	88.7	ND
	PHA-CM	20.1	79.0	91.0	ND
3	Medium	3.3	1.9	68.4	ND
	PHA-CM	40.8	75.4	49.4	ND
4	Medium	4.9	74.7	95.5	ND
	PHA-CM	27.0	86.7	72.5	ND
5	Medium	3.9	50.7	75.8	ND
	PHA-CM	41.9	44.4	57.2	ND
6	Medium	17.0	72.7	87.6	14.6
	rIFNγ	36.2	79.1	80.1	56.5
7	Medium	4.6	1.5	85.1	30.4
	rIFNγ	32.8	8.3	80.7	84.9
8	Medium	11.8	11.8	75.3	21.0
	rIFNγ	49.6	35.0	82.7	60.9
9	Medium	5.4	9.0	78.3	48.9
	rIFNγ	43.1	49.4	67.4	83.7
10	Medium	14.9	7.9	70.4	31.4
	rIFNγ	58.7	27.6	57.2	75.4
11	Medium	4.3	11.8	ND	ND
	rIFNγ	43.5	38.3	ND	ND

* Bone marrow cells, prepared as described in text, were cultured for 5 d in culture medium with or without PHA-CM (50%) or rIFNγ (50 ng/ml).
‡ Percentage of the cells that express the antigen detected by the monoclonal antibody as determined in indirect immunofluorescence assay (flow cytofluorometry).
§ Percentage of cells forming rosettes with EA7S.
ǁ Not done.

cantly. A large proportion of the cells were induced to express simultaneously the monocyte antigens (B52.1, B77.1, and HLA-DR) and the myeloid-specific antigen, as judged indirectly by the percentage of cells positive for the various markers. This observation was confirmed in a two-color immunofluorescence assay (data not shown).

The cumulative results with eight CML donors are reported in Table III. Some variability was observed among donors, especially in the ability of the cells

TABLE II

Effect of Culture, PHA-CM, and rIFNγ on the Expression of Various Differentiation Markers on CML Cells

Marker	Day 0*	Day 5*		
		Medium	PHA-CM	rIFNγ
HLA-DR antigen (B33.1)	5.4‡	4.5	61.3	65.2
Monocyte antigen (B52.1)	5.7	23.9	61.8	59.0
Monocyte antigen (B77.1)	ND§	4.0	52.1	30.0
Myeloid antigen (B40.8)	79.1	83.0	82.6	80.9
NK/PMN antigen (B73.1)	2.7	0.8	1.9	0.5
E receptor (B67.1)	3.9	10.1	15.7	9.8
Fc receptor (EA7S)	25.0‖	10.2	33.3	22.4
α-NAE	0.0¶	0.0	17.0	9.0

* Peripheral blood myeloid cells from CML patient C.L. were analyzed on day 0 or after 5-d culture in medium or in the presence of PHA-CM (50%) or rIFNγ (50 ng/ml).
‡ Percentage of cells that express the antigen detected by the monoclonal antibody as determined in indirect immunofluorescence (flow cytofluorometry).
§ Not done.
‖ Percentage of cells forming rosettes with EA7S.
¶ Percentage of cells staining for α-NAE.

to undergo normal differentiation in vitro to mature PMN or to express monocyte antigens spontaneously. However, the cells from three donors (A.B., C.S., and M.S.) each maintained a rather constant behavior upon repeated testing. A portion of cells from donors C.L. and M.S., cultured in the absence of inducer, always expressed B52.1 antigens at low density and, to a lesser extent, HLA-DR antigen. This spontaneous expression was never observed with donor A.B. However, as shown in Fig. 1, even in the cases in which monocyte antigens were expressed spontaneously (donor C.L.), PHA-CM and rIFNγ induced a marked increase in both the percentage of positive cells and the antigenic density. Antibody B67.1 (anti-E receptor) reacted with a small percentage of cells (<10%) only when these had been cultured in PHA-CM.

The proliferative capability of myeloid cells was assessed to exclude the possibility that a small number of monocytes, or monocyte precursors, selectively surviving in culture, could overgrow the other myeloid cells. Myeloid cells from three CML patients were tested for [³H]TdR incorporation during the 5-d culture period (Table IV). The number of cells present at the end of the culture was not significantly different from the number of cells originally seeded. The proportion of [³H]TdR⁺ cells, as evaluated by autoradiography, was 16–31% on the first day of culture under any of the experimental conditions; it decreased on day 2, and only ∼1–10% of the cells were able to incorporate [³H]TdR under any condition at the end of the culture period. We evaluated whether irradiated cells (10 Gy) could be induced to differentiate by PHA-CM or rIFNγ. Although the results were difficult to interpret due to cell death and low recovery (from 25 to 40% after 5-d cultures), it was observed that the irradiation dose used completely prevented [³H]TdR incorporation in the cells, without affecting monocyte differentiation, as evaluated by the induction of both HLA-DR and
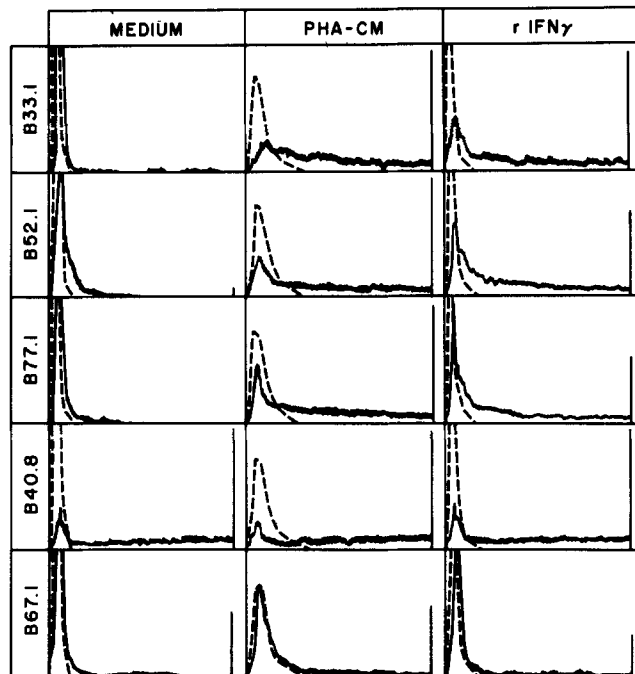
FIGURE 1. Effect of PHA-CM or rIFNγ on the expression of surface antigens on CML cells. Peripheral blood mononuclear cells from CML patient C.L. were enriched for myeloid cells as described in text and cultured for 5 d in culture medium with or without 50% PHA-CM or 50 ng/ml of rIFNγ. Cells were then washed, stained by indirect immunofluorescence with the indicated monoclonal antibodies, and analyzed with the cytofluorometer. In the histograms, the x axis represents the intensity of fluorescence, and the y axis, the number of cells. The broken line represents the histogram obtained when only the second FITC-labeled reagent was added; the continuous line represents the histogram obtained when the monoclonal antibodies have been added.

monocyte-specific antigens (not shown).

Effect of Different Types of IFN on the Expression of Monocyte Differentiation Antigens on Myeloid Cells. Various doses of PHA-CM, rIFNγ, and other IFN types were tested on CML cells in culture (Table V and Fig. 2). PHA-CM, purified IFNγ (Table V), and rIFNγ (Fig. 2) induced a dose-dependent increase in the percentage of both HLA-DR- and monocyte antigen-positive cells. The effect induced by rIFNγ was significant at concentrations as low as 1 ng/ml. Purified IFNγ had a similar effect at approximately equivalent antiviral activity. No significant effect was observed with up to 10^3 antiviral U/ml of rIFNA (Table V), rIFND, partially purified IFNα, crude and purified IFNβ (results not shown).

Induction of Ability to Mediate Ab-CMC in CML Cells Treated with PHA-CM or rIFNγ. The cytotoxic ability of enriched myeloid cell preparations from CML patients was usually low or absent. After 5 d of culture with PHA-CM or rIFNγ, the cells became highly effective in mediating lysis of IgG antibody-sensitized P815Y cells in a 3-h lytic assay (Fig. 3). rIFNA was unable to induce cytotoxicity. In the absence of antibodies directed to the target, no spontaneous cytotoxicity was observed against various target cell lines (not shown). Induction or strong increases in Ab-CMC, after culture in PHA-CM or rIFNγ, was observed in all

TABLE III

Effect of PHA-CM on the Differentiation of Cells from Patients with Chronic Myeloproliferative Disorders

	Patients*								
	N.S.	J.R.	R.W.	F.M.	J.B.	A.B. (6)	C.L. (9)	M.S. (3)	E.H.
Age (years)	64	63	56	67	75	76	31	44	68
Philadelphia chromosome	+	+	+	+	+	+	+	–	–
Myeloid antigen (B40.8)									
Day 0, medium	ND‡	78.1§	ND	80.2	71.7	77.6 ± 15.9	78.8 ± 10.3	35.6 ± 12.3	40.5
Day 5, medium	5.6	88.6	69.2	82.4	28.4	78.0 ± 10.9	75.8 ± 10.5	48.2 ± 22.9	60.8
Day 5, PHA-CM	72.9	88.8	73.1	85.4	52.1	76.3 ± 7.4	67.6 ± 12.9	36.7 ± 12.0	52.0
Monocyte antigen (B52.1)									
Day 0, medium	ND	3.0	ND	3.1	12.0	3.8 ± 3.0	14.9 ± 13.2	9.7 ± 3.0	9.8
Day 5, medium	17.9	9.1	49.2	7.8	7.0	14.2 ± 11.2	38.9 ± 23.9	32.4 ± 22.2	4.0
Day 5, PHA-CM	44.4	60.2	51.9	57.9	63.3	55.9 ± 18.3	62.2 ± 11.6	58.2 ± 33.4	28.4
HLA-DR (B33.1)									
Day 0, medium	ND	5.7	ND	4.7	2.7	10.5 ± 5.6	9.0 ± 5.3	8.9 ± 4.4	9.5
Day 5, medium	10.2	4.0	18.2	8.9	36.1	7.4 ± 4.9	22.9 ± 23.2	23.6 ± 20.2	7.6
Day 5, PHA-CM	30.0	40.0	44.7	36.7	88.2	39.2 ± 8.2	58.3 ± 18.5	60.6 ± 14.1	69.3
Ab-CMC									
Day 0, medium	ND	<0.1§	<0.1	9.3	0.1	1.5 ± 2.7	5.3 ± 9.2	ND	ND
Day 5, medium	20.6	8.4	<0.1	9.9	<0.1	11.4 ± 15.8	77.6 ± 83.3	ND	ND
Day 5, PHA-CM	47.4	125.6	50.4	33.3	9.0	52.7 ± 57.2	141.3 ± 97.3	ND	ND

* Diagnosis for all patients was CML in chronic phase, except for patient E.H., whose diagnosis was myeloid metaplasia. The number of experiments performed on the cells from each patient is given in parentheses.
‡ Not done.
§ Percentage of cells that express the antigen detected by the monoclonal antibody, as determined in indirect immunofluorescence (flow cytofluorometry). Data are given as mean ± SD when at least three experiments were performed on the cells of that donor.
∥ LU 45%/10⁷ cells against IgG antibody-sensitized P815Y target cells in a 3-h ⁵¹Cr-release assay.

the cell preparations tested (Table III). The cultured cells from patient C.L., which were positive for monocyte-specific antigens in the absence of inducer, were also able to mediate a low but signficant level of cytotoxicity under these conditions.

Analysis of Cultured Myeloid Populations at Different Stages of Differentiation. The preparations of myeloid cells, separated from the peripheral blood of CML patients as described above, comprised a mixture of different morphological cell types with some variability among donors (e.g., in the experiment described in Table V, the cell preparation was composed of 6% blasts, 6% promyelocytes, 22% myelocytes, 44% metamyelocytes, 22% band and segmented neutrophils). When the cells were maintained in culture medium for 5 d, a progressive differentiation toward mature PMN was always observed (3% myelocytes, 6% metamyelocytes, 91% band and segmented neutrophils). However, myeloid maturation was blocked in most cells after 5 d of culture in PHA-CM or rIFNγ: nuclei remained round or kidney-shaped; the cells had fewer granules and a more irregular shape than the cells maintained in culture medium. A small proportion of large irregular cells had the phagosomes and very strong α-NAE activity characteristic of macrophages. All the cells cultured in normal medium remained in suspension, whereas a variable proportion of cells cultured with PHA-CM or rIFNγ were adherent and spread out on the plastic surface of the culture flasks.

To identify the cells that were able to respond to the differentiation inducers, cell preparations at different stages of differentiation were purified on the basis

TABLE IV

Proliferative Capability of Immature CML Cells in Culture

Exp.	Treatment*	Days in culture					
		1		2		5	
		Number of cells	[³H]TdR	Number of cells	[³H]TdR	Number of cells	[³H]TdR
			%		%		%
1	Medium	6.5‡	15.7§	6.6	13.9	ND∥	ND
	PHA-CM	6.5	31.0	6.6	14.7	6.3	9.0
	IFNγ	6.5	20.3	5.5	19.7	5.7	7.4
2	Medium	4.8	16.7	5.7	13.3	7.5	8.2
	PHA-CM	4.5	26.7	6.3	24.9	9.6	8.7
	IFNγ	4.5	16.4	5.4	18.3	7.4	7.9
3	Medium	20.0	25.5	18.5	12.5	17.0	10.9
	PHA-CM	18.7	28.5	18.6	13.4	16.0	5.4
	IFNγ	19.6	19.3	17.9	12.0	9.5	1.0

* Immature myeloid cells from three CML patients were kept for 5 d in culture in RPMI-10% FBS medium or in medium containing PHA-CM (50%) or purified IFNγ. The cells were seeded on day 0 at 7×10^5/ml (exp. 1), 5×10^5/ml (exp. 2), and 20×10^5/ml (exp. 3). Each day of culture, cells were pulsed for 1 h with [³H]TdR and centrifuge smears were prepared for autoradiography. After 3 d, the cells in PHA-CM- and IFNγ-treated cultures were ~50% adherent.
‡ Cells $\times$ ml^{-1} $\times$ 10^{-5}.
§ Percentage of cells incorporating [³H]TdR.
∥ Not done.

of the expression of the antigen recognized by antibody B13.4 (anti-PMN, monocytes, and mature myeloid cells) and separately cultured. Fig. 4 shows the morphology of the cells in a typical experiment and Table V, the analysis of the differentiation markers. The B13.4⁻ cell preparations in all donors were composed mostly of myelocytes (4% blasts, 8% promyelocytes, 76% myelocytes, 12% metamyelocytes) (Fig. 4A). The B13.4⁺ cell preparations were composed almost exclusively of metamyelocytes and band cells, with few mature PMN (45% metamyelocytes, 55% band and segmented neutrophils) (Fig. 4D). Upon culture in normal medium, both populations continued maturation to PMN, as judged morphologically on day 5 and by the expression of the B13.4 antigen on the negative cell preparations (not shown). Almost all B13.4⁺ cells became mature PMN (Fig. 4E), whereas some B13.4⁻ cells remained more immature (mixture of metamyelocytes, bands, and mature PMN) (Fig. 4B). Dramatic morphological changes occurred in both populations when cells were cultured in the presence of PHA-CM (not shown) or rIFNγ (Fig. 4C and F). In both cases, treatment with rIFNγ induced the appearance of cells with monocyte-macrophage characteristics and prevented myeloid differentiation of the cells.

In the total myeloid cell preparation and in both the separated populations, PHA-CM and rIFNγ, but not rIFNA, induced HLA-DR antigen, B52.1 and B77.1 monocyte antigens, FcR, and α-NAE activity (Table VI). The modest induction by rIFNγ of monocyte antigen on B13.4⁻ cells shown in the experiment of Table VI was unusual, insofar as in the other four experiments (see, e.g., Fig.

TABLE V
Effect of Different Types of Inducers on the Expression of Surface Antigens on CML Cells

Inducer*		HLA-DR (B33.1)	Monocyte antigen (B52.1)
None		15.0 (157)[‡]	9.4 (44)
PHA-CM	50%	75.2 (149)	23.7 (106)
PHA-CM	10%	60.1 (140)	21.7 (70)
PHA-CM	2%	23.3 (154)	14.4 (106)
Purified IFNγ	1,000 U/ml	81.6 (179)	34.3 (71)
Purified IFNγ	100 U/ml	55.6 (126)	16.5 (86)
Purified IFNγ	10 U/ml	21.1 (141)	6.1 (68)
rIFNγ	100 ng/ml	85.5 (157)	47.6 (64)
rIFNA	1,000 U/ml	23.5 (130)	7.6 (53)
rIFNA	100 U/ml	18.6 (152)	10.0 (38)

* Peripheral blood myeloid cells from CML patient C.L. were cultured for 5 d in the presence of the indicated concentration of inducers.
‡ Percentage of cells expressing the antigen detected by the monoclonal antibody, as determined by indirect immunofluorescence (flow cytofluorometry). Values in parentheses represent the fluorescence intensity of the positive cells (mean fluorescence channel).

2), monocyte antigens were induced by rIFNγ in a large proportion of B13.4⁻ cells. A small proportion of T cells, as defined by reactivity with antibody B67.1, was detected in the cultures treated with the PHA-CM, presumably due to the presence of IL-2 in this medium. In the absence of PHA-CM or rIFNγ, only the B13.4⁺ cells, in all donors tested, mediated significant levels of Ab-CMC. In this population, PHA-CM or rIFNγ induced only a small increase of cytotoxic ability, and, in some experiments, no increase at all. PHA-CM or rIFNγ consistently induced an increase in Ab-CMC on IgG antibody-sensitized target cells in the originally B13.4⁻ cell population. Again, rIFNA was unable to induce any increase in cytotoxicity.

Induction of HLA-DR Antigens on Myeloid Cells by PHA-CM or rIFNγ.   Because of the complexity of the products of the human Ia genes, some of which probably reside at the HLA-DR locus and others at closely linked loci, six monoclonal antibodies directed against different subpopulations of human Ia antigens were used to detect HLA-DR antigens on the cells after culture. As shown in Table VII, all six antibodies reacted with a large proportion of myeloid cells from CML patients after culture with PHA-CM or rIFNγ. None of the antibodies reacted with a significant proportion of the cells at the onset of the cultures. The strong reactivity of all six anti-Ia monoclonal antibodies tested, which detect different subpopulations of Ia molecules (35, 36), with the majority of induced cells, indicated that the various subpopulations of human Ia were induced. The reactivity of the cells with antibody D1-12 (35) indicated that at least one of these subpopulations is encoded by the DR locus (41) of the HLA region.
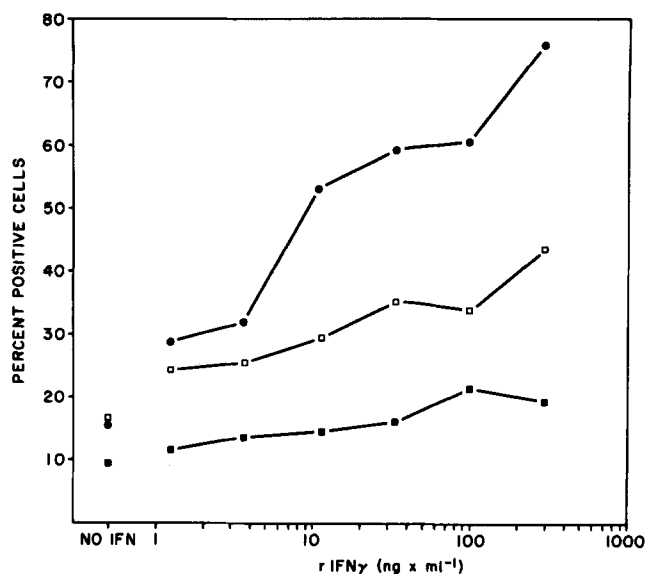
FIGURE 2. Effect of different doses of rIFNγ on the expression of surface antigens on CML cells. B13.4⁻ peripheral blood myeloid cells from patient M.S. were incubated for 5 d in the presence of rIFNγ at the indicated concentrations and then tested by indirect immunofluorescence for the expression of the antigen recognized by different monoclonal antibodies. (●) antibody B33.1 (anti-HLA-DR); (□) antibody B52.1 (monocyte-specific); and (■) antibody B77.1 (monocyte-specific).
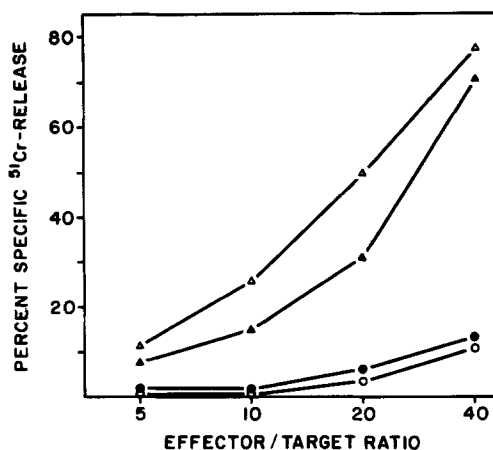

FIGURE 3. Induction of antibody-dependent cytotoxic ability in CML cells by PHA-CM or rIFNγ. Peripheral blood myeloid cells from patient A.B. were cultured for 5 days in the presence of culture medium, PHA-CM (50%), rIFNγ (50 ng/ml) or rIFNA (10⁵ antiviral U/ml). The cells were then tested as effectors against antibody-sensitized murine P815Y target cells as described. (●) culture medium; (△) PHA-CM; (▲) rIFNγ; (○) rIFNA.

Effect of rIFNγ on Cultures of Acute Leukemia Cells.   Cells from six AML and from three acute lymphocytic leukemia (ALL) patients were cultured in normal medium or in the presence of rIFNγ, using a protocol similar to that for CML cells. The results of the antigenic analysis are reported in Table VIII. In all but
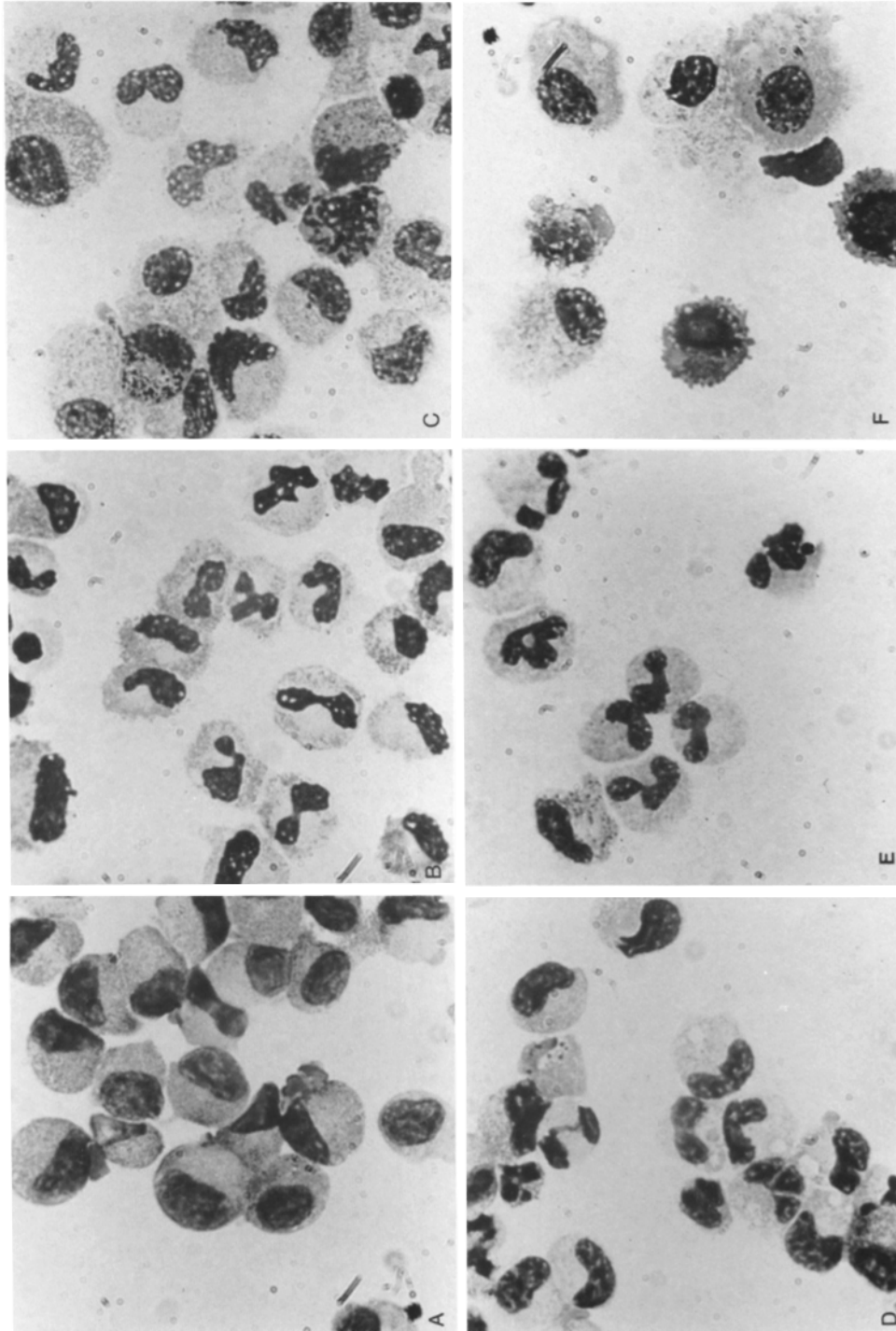
FIGURE 4. Micrographs (× 440) of CML peripheral blood cells separated with monoclonal antibody B13.4 and incubated for 5 d in culture medium with or without 50 ng/ml of rIFNγ. (A) B13.4⁻ cells, day 0; (B) B13.4⁻ cells, culture medium, day 5; (C) B13.4⁻ cells, rIFNγ, day 5; (D) B13.4⁺ cells, day 0; (E) B13.4⁺ cells, culture medium, day 5; (F) B13.4⁺ cells, rIFNγ, day 5.

TABLE VI

Effect of PHA-CM and rIFNγ on Myeloid Cells at Different Stages of Maturation

Cell prepara-tion*	Treatment‡	B40.8	B33.1	B52.1	B77.1	B67.1	FcR	α-NAE	Ab-CMC
Total	Medium	84.6§	7.5	5.8	1.4	4.6	9.0§	0.0¶	5.7**
	PHA-CM	86.3	53.7	54.1	45.1	9.9	60.0	17.0	31.0
	rIFNγ	86.2	46.4	23.3	11.4	2.9	68.0	9.0	43.8
B13.4⁺	Medium	89.8	3.8	6.2	3.6	0.8	38.0	0.0	21.0
	PHA-CM	71.0	49.9	48.1	37.8	4.8	74.0	48.0	34.7
	rIFNγ	71.7	48.9	46.8	27.2	6.0	72.0	38.0	ND‡‡
	rIFNA	84.6	1.5	4.2	1.6	0.4	26.0	1.0	17.2
B13.4⁻	Medium	85.2	8.8	0.7	1.1	1.7	22.0	0.0	10.8
	PHA-CM	85.4	42.3	49.2	32.5	8.3	61.0	16.0	50.2
	rIFNγ	85.0	34.2	9.4	3.7	7.1	59.0	17.0	39.8
	rIFNA	79.2	6.4	2.9	0.4	0.3	34.0	1.0	7.5

* Peripheral blood myeloid cells from CML patient A.B. were cultured either unseparated (total) or after separation into B13.4⁺ and B13.4⁻ cells.

‡ Cells were cultured for 5 d in culture medium or in the presence of PHA-CM (50%), rIFNγ (50 ng/ml), or rIFNA (1,000 U/ml).

§ Percentage of cells expressing the antigen detected by the indicated antibody, as determined in indirect immunofluorescence (flow cytofluorometry) assay.

∥ Percentage of cells forming rosettes with EA7S.

¶ Percentage of cells positive for α-NAE.

** LU 45%/10⁷ cells.

‡‡ Not done.

TABLE VII

Reactivity of Different Anti-HLA-DR Monoclonal Antibodies with CML Cells Cultured in the Presence of PHA-CM or rIFNγ

Antibody	Culture conditions*		
	Medium	PHA-CM	rIFNγ
B33.1	14.0‡	46.3	45.5
D1-12	15.6	66.9	66.4
Q2/70	18.9	68.8	72.3
Q2/80	5.6	58.9	61.7
Q5/6	11.2	67.2	68.2
Q5/13	17.9	75.1	82.7

* Myeloid cells (B13.4⁻) from the peripheral blood of CML patient M.S. were incubated for 5 d in culture medium or in the presence of PHA-CM (50%) or rIFNγ (50 ng/ml).

‡ Percentage of cells expressing the antigen detected by the monoclonal antibody, as determined in indirect immunofluorescence (flow cytofluorometry).

one of the AML preparations, both the intensity of antigen expression and the percentage of cells positive with anti-HLA-DR antibodies increased. In some cases, a moderate increase in the proportion of the B52.1 monocyte antigen-positive cells, and, in most cases of AML, an increased expression of FcR, were also observed. No significant increases in the expression of HLA-DR, B52.1 antigen, FcR, or α-NAE activity were observed with the cells from the three ALL cases. Fig. 5 depicts the flow cytofluorometry analysis of the expression of HLA-DR and B52.1 antigens in two of the AML cases. The M2 leukemia represents an example of a case in which HLA-DR antigens were expressed on

TABLE VIII

Effect of rIFNγ on the Expression of Differentiation Markers in Cells from Patients with Acute Leukemia

Diagnosis	HLA-DR (B33.1)*			Monocyte Ag (B52.1)*			FcR (EA7S)[‡]			α-NAE[§]		
	Day 0	Day 5		Day 0	Day 5		Day 0	Day 5		Day 0	Day 5	
		Medium	rIFNγ		Medium	rIFNγ		Medium	rIFNγ		Medium	rIFNγ
AML (M1)	60.9	63.7	68.3	2.0	4.7	17.7	0.0	28.0	65.0	8.6	14.0	73.0
AML (M1)	36.9	45.5	78.8	0.6	3.6	9.6	0.0	0.0	9.2	ND[∥]	1.0	8.0
AML (M2)	25.9	62.2	92.0	2.3	6.4	16.0	14.0	6.0	10.5	ND	6.0	76.0
AML (M3)	9.2	2.4	53.2	1.6	0.6	22.8	2.0	0.0	37.5	7.5	0.0	83.0
AML (M4)	70.3	68.5	85.3	8.2	17.8	16.2	7.0	6.9	54.9	20.0	5.0	39.0
AML (M4)	56.8	86.6	68.8	23.9	15.6	28.2	32.0	22.6	42.2	95.0	18.5	48.0
ALL (pre-B)	90.1	86.8	95.0	5.1	ND	ND	1.0	20.6	35.9	0.0	0.0	0.0
ALL (B)	84.3	41.0	25.3	3.6	ND	ND	1.0	1.9	3.0	0.0	0.0	0.0
ALL (T)	0.4	4.1	4.3	0.5	2.9	3.2	0.0	1.7	2.0	0.0	0.0	0.0

* Peripheral blood cells from the patients were analyzed at day 0 or after 5-d incubation in culture medium with or without rIFNγ (50 ng/ml). Data are percentages of cells expressing the antigen detected by the monoclonal antibody, as analyzed in indirect immunofluorescence (flow cytofluorometry).
[‡] Percentage of cells forming rosettes with EA7S.
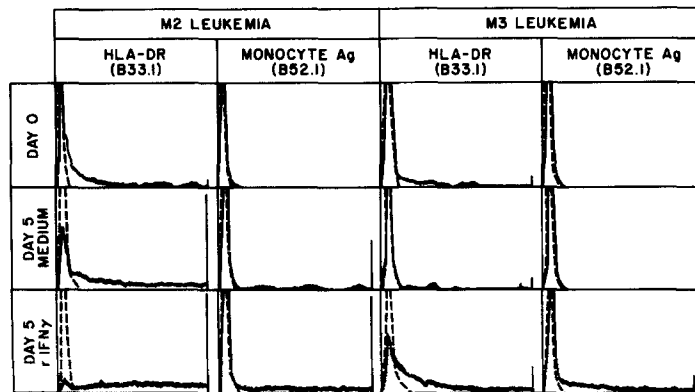[§] Percentage of cells staining for α-NAE.
[∥] Not done.

FIGURE 5.   Effect of rIFNγ on the expression of HLA-DR and monocyte surface antigens on AML cells. Peripheral blood cells from two patients were maintained in culture medium or in the presence of 50 ng/ml of rIFNγ for 5 d and then analyzed by indirect immunofluorescence with antibodies B33.1 and B52.1. In the histograms, the x axis represents the intensity of fluorescence, and the y axis, the number of cells. The broken line represents the histogram obtained when only the second FITC-labeled reagent was added, and the continuous line represents the histogram obtained when the monoclonal antibodies were added.

a large proportion of cells at the beginning of the culture, but in which rIFNγ induced a strong increase in both the density of the antigen and the number of positive cells. The promyelocyte leukemia (M3) represents a case of HLA-DR-negative, B52.1-negative cells that can be induced by rIFNγ to express both HLA-DR and B52.1 antigens, similar to the observations made in human promyelocytic cell lines (9), normal bone marrow cells, and CML cells.

Inhibition of Induction of CML Cell Differentiation by Anti-IFN Serum.   The induction of HLA-DR⁺ cells by either rIFNγ or PHA-CM in immature (B13.4⁻) cells from CML patients was blocked when rabbit anti-human IFNγ was present

TABLE IX

Effect of Anti-IFNγ on PHA-CM and rIFNγ-dependent Induction of CML Differentiation

Culture conditions*	HLA-DR‡ (B33.1)	Monocyte antigen (B52.1)	Myeloid antigen (B40.8)	FcR§ (EA7S)
Medium	7.0 (138)	9.5 (64)	77.2 (195)	38.9
PHA-CM	40.4 (128)	51.4 (115)	81.5 (195)	77.3
PHA-CM + NRS	37.5 (109)	58.2 (117)	70.6 (192)	78.0
PHA-CM + rabbit anti-IFNγ	12.2 (152)	44.9 (87)	80.3 (191)	46.4
rIFNγ + NRS	21.6 (132)	22.7 (108)	71.9 (193)	84.2
rIFNγ + rabbit anti-IFNγ	12.0 (137)	9.4 (86)	77.8 (193)	13.5

* B13.4⁻ myeloid cells from patient A.B. were cultured for 5 d (2 × 10⁶ cells/ml) in medium with or without PHA-CM (50%) or rIFNγ (50 ng/ml) in the presence or absence of rabbit anti-human IFNγ (1:40 dilution, corresponding to 250 neutralizing U/ml) or normal rabbit serum (NRS) (1:40 dilution).
‡ Percentage of cells expressing the antigen detected by the indicated monoclonal antibody, as determined in indirect immunofluorescence (flow cytofluorometry). The mean fluorescence intensity, in arbitrary units ranging from 1 to 200, is given in parentheses.
§ Percentage of cells forming rosettes with EA7S.

during the 5-d culture period (Table IX). The percentage of cells induced to express monocyte antigen B52.1 by rIFNγ or by PHA-CM was reduced, especially when rIFNγ was the inducer. In both cases, the density of the antigen, as judged by the intensity of fluorescence, was also much lower than that observed in the respective induced cells cultured in the presence of normal rabbit serum (NRS). No significant change in the proportion of B40.8⁺ cells was observed. Noninduced cells and cells induced by rIFNγ or PHA-CM in the presence of rabbit anti-IFNγ were nonadherent; by contrast, a large proportion of the cells in rIFNγ- or PHA-CM-induced cultures in the presence or absence of NRS were adherent.

Discussion

The block in differentiation of leukemia cells or cell lines derived from them often can be overcome in vitro by the use of synthetic or naturally occurring inducers. Though the characteristics of the process of differentiation delineated in these systems are thought to approximate those of normal differentiation (42, 43), they might only represent an aberration linked to mutations in the cell line, as a function of their leukemic origin or of a long in vitro culture history. Nevertheless, established cell lines provide an unlimited number of homogeneous cells and, as a consequence, the possibility of defining optimal experimental conditions for the induction of differentiation, and of identifying the most reliable markers for an analysis of cell maturation. By monitoring those surface markers, surface receptors, enzymatic activities, and cellular functions shown in our previous studies to be the most relevant for myeloid differentiation (9, 32, 34, 44), we now show that both PHA-CM and IFNγ induce normal or leukemic immature human myeloid cells to differentiate along the monocytic pathway.

Chiao et al. (10) reported that PHA-CM induces maturation of AML cells. Using rIFNγ, we were able to induce at least some of the markers of monocytic differentiation in six cases of AML analyzed; in one case of promyelocytic

leukemia (M3), the induced pattern of differentiation of surface markers and α-NAE activity was indistinguishable from that observed with promyelocytic cell lines (9). We were able to induce analogous monocytic differentiation of myeloid cells from normal bone marrows and CML patients, using this experimental protocol. CML peripheral blood contains a large number of immature myeloid cells that, despite their clonal origin and chromosomal anomalies, undergo maturation to terminal cells (monocytes and PMN) both in vivo and in vitro. Immature myeloid cells cultured in the presence of PHA-CM or IFNγ acquired characteristics of monocyte-macrophages, as evidenced by (a) the morphological resemblance of most cells to monocyte-macrophages without myeloid maturation and segmentation of the nuclei; (b) the induction of several surface and enzymatic markers of monocytes in the majority of the cells; (c) development of phagocytic ability for EA7S in most of the cells (not shown); and (d) the ability to mediate high levels of Ab-CMC. We have shown previously (9) that a subpopulation of HL-60 cells, induced to undergo maturation and incapable of further proliferation, simultaneously expresses all of these differentiation markers; a second subpopulation, unresponsive to the differentiation inducer, maintains proliferative ability and expresses none of the differentiation markers. The expression of all the differentiation markers by the majority of cells in the present experiments suggests that at least a portion of the cells simultaneously expresses the markers characteristic of normal monocytes. In a few initial experiments, we observed T cell growth induced by PHA-CM containing large quantities of IL 2; careful elimination of contaminant T cells, as confirmed by analysis for T cell markers both before and after culture, avoided this potentially confounding factor.

IFNγ induces high levels of Ia antigen on the Ia-negative immature myeloid cells during induction of these cells along the monocytic pathway. The earliest observable effect of IFNγ is the induction of FcR for monomeric human IgG1 within 8–12 h of incubation (26) followed 1–2 d later by the expression of Ia antigens and, finally, the other markers. The reaction of the antibody with the differentiated cells is not due to FcR binding, because: (a) the expression of differentiating antigen is not detected during the first 24–48 h of incubation, when FcR are already fully expressed; (b) F(ab')$_2$ fragments of both B33.1 and goat anti-mouse Ig antibody were used; (c) the antimonocyte antibodies were all IgM that do not bind to the FcR induced by IFNγ; (d) the presence of 5% human serum used during cell incubation and washing competitively blocked the FcR; and (e) control irrelevant monoclonal antibodies did not bind to the cells.

Analogous to our findings with promyelocytic lines (9), the majority of the cells in the preparations from normal bone marrows and the peripheral blood of CML patients expressed the myeloid antigen recognized by antibody B40.8. Induction of monocyte markers was not accompanied by significant reduction in the expression of this antigen. One possible interpretation of these findings is that promyelocytes have already acquired antigenic, enzymatic, and morphological properties of the granulocyte lineage toward which they are differentiating, but are not yet irreversibly committed to that lineage and thus can be redirected toward the monocytic lineage (45). Cells with intermediate enzymatic characteristics of granulocytes and monocytes can be identified in normal bone marrow (46), and cells from AML (M4) patients often display surface markers of both

granulocytes and monocytes (45, and our unpublished observation). Our results with CML cells suggest that metamyelocytes and band neutrophils can still be induced to coordinately express a series of differentiation markers characteristic of normal monocytes, although the morphological, antigenic, and enzymatic properties identify the induced cells more as intermediate myelomonocytic cells than as fully differentiated monocytes (47).

The analogy between the induction by PHA-CM and IFNγ of differentiation markers on myeloid cell lines and on the culture of normal or leukemic myeloid cells and the persistence in the differentiated monocytoid cells of myeloid antigens never expressed on normal monocytes, suggests that the cells with monocyte characteristics are derived from the myeloid cells. The possibility that monocytes or immature monocytes selectively survive in the presence of PHA-CM or IFNγ is excluded by the cell purification procedures used, which eliminated all monocytes and α-NAE-positive cells, and by the limited extent of cell death in the cultures. However, a very small number of monocyte precursors could escape the cell separation procedures and overgrow the myeloid cells in the presence of PHA-CM or IFNγ. A series of experimental data makes this possibility unlikely. Although crude PHA-CM contains CSF, no significant colony formation in methylcellulose is induced by the presence of rIFNγ using as target cells either normal bone marrow cells or peripheral blood CML cells. In the cultures of CML cells, the number of cells able to incorporate [^{3}H]TdR rapidly decreased after the first 1 or 2 d of culture, indicating that the number of possibly replicating cells was too low to allow the overgrowing of the culture by a small number of monocyte precursors. Finally, experiments of γ irradiation of the cells, although troubled by a decreased cell viability, showed induction of monocyte differentiation in CML cells in the absence of cell replication.

Mature PMN from normal peripheral blood survive only 1 or 2 d in our culture conditions and PHA-CM or rIFNγ were unable to induce them to express HLA-DR or monocyte antigens. However, we have reported (26) that even a brief incubation of mature PMN with IFNγ results in the expression on these cells of an FcR for human monomeric IgG1, with affinity and specificity analogous to that expressed by monocytes. As previously observed with HL-60 and other promyelocytic cell lines (9), induction of FcR is necessary but not sufficient to confer cytotoxic ability to the cells. Apparently, the ability to mediate Ab-CMC requires more complete maturation of the cells than does expression of the other monocytic markers. The ability to lyse IgG-sensitized P815Y cells, however, is shared by monocytes, PMN and LGL (27). The observation that myeloid maturation of CML can occur spontaneously might explain the occasional generation of antibody-dependent cytotoxic ability in the absence of inducers seen in preparations of more mature myeloid cells. In some experiments, PHA-CM or IFNγ decreased this activity, probably by blocking myeloid maturation without inducing complete monocytic differentiation. It is unlikely that PHA-CM and IFNγ act by enhancing the cytotoxic ability of a few contaminant LGL, rather than by inducing this ability in myeloid cells, because: (a) IFN are potent activators of the spontaneous cytotoxicity of LGL, but are almost completely ineffective in enhancing Ab-CMC (15, 17, 48); (b) no spontaneous cytotoxic activity against target cells susceptible to lysis mediated by LGL was

ever mediated by the cell preparations in culture; (c) LGL were removed from our cell preparations by using antibody B73.1, directed against an antigen present on all LGL, and antibody B67.1, which reacts with the majority of them (30, 49); and (d) after culture in the presence of IFNγ, no significant number of B73.1+ or B67.1+ cells were observed.

We observed that purified or recombinant IFNγ closely mimics the effect of PHA-CM in inducing monocytic differentiation in myeloid cells. PHA-CM contains, in addition to IFNγ (12), various other lymphokines, including CSF. In addition to their ability to induce differentiation by affecting proliferation, CSF can also act by influencing RNA and protein synthesis in mature postmitotic neutrophilic granulocytes (50, 51). Olsson et al. (52) reported that concanavalin A-induced CM contained two molecular species of differentiation-inducing factors: one is a 25,000 D species that coelutes with the colony-stimulating activity, and the other, a 40,000 D species not associated with this activity. The factor present in our PHA-CM that induces differentiation in HL-60 cells is destroyed at pH 2.0 or at 56°C, has a molecular weight of 40,000–50,000, elutes, by gel filtration, in the same fractions as IFNγ activity, and is inactivated by an antiserum against IFNγ.[2] We were able to inhibit the rIFNγ-induced monocytic differentiation of immature myeloid cells by adding anti-IFNγ serum at the beginning of the culture. When the same antiserum, in which no activity against CSF was demonstrable, was added to PHA-CM-induced cultures, HLA-DR and FcR expression was blocked and a much reduced proportion of the induced cells expressed low levels of the B52.1 antigen. Our data, therefore, show that IFNγ is the predominant type of differentiation-inducing factor contained in the PHA-CM. The inability of the rIFNγ preparation to induce colony formation in normal bone marrow or peripheral blood CML cells suggests that CSF is not primarily involved in the differentiation induced by IFNγ. However, the possibility that the differentiation-inducing ability of PHA-CM remaining after treatment with anti-IFNγ serum resides in CSF requires further investigation.

The effect of IFNγ on the differentiation of myeloid cells to HLA-DR-positive mature "monocytoid" cells that we describe has biological relevance for both the adaptive and nonadaptive arms of the immune system and for the regulation of hematopoiesis. Monocyte-macrophages can produce and secrete a series of substances with regulatory effects on both the hematopoietic and immune systems. Ia-positive macrophages and precursor cells play a complex regulatory role in the control of proliferation and maturation of both normal and leukemic myeloid cells (53). Moreover, the Ia-positive cells might be able to function as accessory or antigen-presenting cells. IFNα, the type of IFN commonly used in clinical trials, not only lacks the ability to mediate most of these regulatory effects, but also exerts an inhibitory and antiproliferative effect on myelopoiesis (18). The specific regulatory functions of IFNγ on myelomonocytic differentiation should be kept in mind when interpreting observations made during future therapeutic trials of rIFNγ.

Summary

Conditioned medium from phytohemagglutinin-stimulated human leukocytes contains a factor that can induce promyelocytic cell lines and certain acute

myelogenous leukemia cells to differentiate along the monocytic pathway. In this report, we show that immature myeloid cells from normal bone marrow or the peripheral blood of patients with chronic myelogenous leukemia can be induced to differentiate to monocyte-like cells by immune γ interferon (IFNγ). We have identified IFNγ as the predominant differentiation factor contained in the conditioned medium. Purified or recombinant IFNγ, but not various preparations of IFNα or β, can induce monocytic differentiation in myeloid cells. In cultures containing conditioned medium, the cells fail to continue myeloid maturation, and are induced to express monocyte markers and functions, such as monocyte-specific surface antigens, HLA-DR antigens, Fc receptors for monomeric immunoglobulins, nonspecific esterase, and the ability to mediate antibody-dependent, cell-mediated cytotoxicity. Even myeloid cells as mature as metamyelocytes or band cells can be induced by IFNγ to undergo monocyte differentiation, but monocyte-specific or HLA-DR antigens are not induced in mature neutrophils. These findings reveal a previously unknown, specific function of human IFNγ and offer new insights to the regulation of monocyte recruitment and differentiation during a virus infection or immune response.

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