

## IMMUNE INTERFERON INDUCES THE RECEPTOR FOR MONOMERIC IgG1 ON HUMAN MONOCYTIC AND MYELOID CELLS\*

By BICE PERUSSIA, ELAHE TALIEH DAYTON, ROBERT LAZARUS,  
VIRGINIA FANNING, AND GIORGIO TRINCHIERI

*From The Wistar Institute of Anatomy and Biology, Philadelphia, Pennsylvania 19104*

Receptors for the Fc domain of IgG (FcR)<sup>1</sup> are present on the membranes of cells of the myeloid and monocytic lineages (1) as well as on other unrelated cell types (2–4). Myelomonocytic cells interact with IgG antibody-sensitized cells or pathogenic organisms through their FcR: this interaction results in the activation of cellular defense mechanisms, such as antibody-dependent cell-mediated cytotoxicity (Ab-CMC), phagocytosis, and the extracellular release of lysosomal enzymes (5, 6). FcR are first detected at the promyelocyte stage of myeloid differentiation, and their expression increases at later stages of maturation (3, 7).

An FcR that binds human monomeric immunoglobulins of the IgG1 class (8–12) has been purified by immunoabsorption to human IgG1 from both freshly isolated human monocytes and the macrophage cell line U937 (12). This 73,000-dalton molecule is not detectable on normal peripheral blood polymorphonuclear cells (PMN) using the same technique. Two antibodies, 3G8 (13) and B73.1 (14, 15), precipitate a molecule of 50,000–70,000 daltons from PMN and from natural killer/killer cells respectively: both antibodies inhibit binding of immune complexes to PMN (13–15). Antibody B73.1 also induces modulation of FcR on natural killer cells and inhibits antibody-dependent cytotoxicity (Ab-CMC) mediated by killer cells and PMN probably by reaction with the FcR itself or a closely related structure (14). Binding of immune complexes to FcR of both monocytes and PMN is preferentially inhibited by human IgG1 and IgG3 (11, 16). The available data suggest that, as in the mouse, there are at least two human FcR for IgG; the human monocyte FcR is analogous to the mouse macrophage FcR1 (trypsin-sensitive, specifically binding to monomeric mouse IgG2a) (17, 18), whereas the PMN and natural killer cell FcR correspond structurally and in binding properties to the mouse macrophage FcR2 (trypsin-

\* Supported in part by U.S. Public Health Service Grants CA10815, CA20833, and CA32898.

<sup>1</sup> *Abbreviations used in this paper:* ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia;  $\alpha$ -NAE,  $\alpha$ -naphthyl acetate esterase; CML, chronic myelogenous leukemia; EA, IgG antibody-sensitized erythrocytes; EDTA, ethylenediamine tetracetic acid; FBS, fetal bovine serum; FcR, receptor for the Fc domain of immunoglobulin G; F/H, Ficoll/Hypaque; HAU, hemagglutinating units; HK, A/Hong Kong/107/68 influenza virus; HK-SPN, supernatant fluid from HK-infected lymphocytes; IFN, interferon; MOI, multiplicity of infection; NRS, normal rabbit serum; PBL, peripheral blood lymphocytes; pfu, plaque-forming units; PMN, polymorphonuclear leukocytes; r, recombinant; VSV, vesicular stomatitis virus.

resistant, preferentially binding complexed mouse IgG1 and IgG2b) (17, 18). Human monocytes cultured in vitro might bear both types of receptors, based on the reactivity of antibody 3G8 with ~20% of cultured human monocytes and its ability to inhibit phagocytosis of E-IgG1 complexes (13) by these cells. A trypsin-sensitive receptor, specific for aggregated mouse IgG3, has been described on mouse macrophages (FcR3) (19): a corresponding receptor on human cells has not been demonstrated.

Naturally occurring soluble factors can modulate the activity and the expression of surface receptors of lymphocytes, monocytes, or granulocytes. Supernatant fluids from concanavalin-A treated spleen cells stimulate phagocytosis of opsonized erythrocytes by mouse macrophages (20), and mixed lymphocyte culture supernatant fluids induce maturation and FcR appearance in a murine macrophage line (21). The expression of the murine macrophage FcR for monomeric IgG2a, but not that of the FcR for aggregated IgG2b, has been recently shown to be enhanced following infection with BCG in vitro, thus suggesting that two of the mouse macrophage FcR types can be regulated independently (22). Medium, conditioned by human mixed leukocyte cultures, enhances Ab-CMC and formation of rosettes with IgG-sensitized erythrocyte (EA7S) by the human macrophage cell line U-937 (23, 24). Supernatant fluid of mixed cultures of human lymphocytes and the Burkitt lymphoma-derived cell line Daudi induces increased expression of receptors for human IgG1 on the human promyelocytic cell line HL-60 (25). We have recently shown that medium conditioned by PHA-stimulated human lymphocytes induces both FcR expression and ability to mediate Ab-CMC in HL-60 and other promyelocytic cell lines (26). Crude interferon (IFN) preparations have also been reported to induce a transient enhancement of FcR expression on human lymphocytes and on lymphoid B cell lines (27, 28).

In this report, we show that human peripheral blood lymphocytes infected with different types of viruses or cultured with certain cell lines produce a factor that enhances or induces the expression of FcR on human peripheral blood monocytes, mature PMN, immature myeloid cells and in vitro established myeloid and monocytic cell lines. We identify this factor as immune interferon (IFN $\gamma$ ) and show that preparations of purified or recombinant IFN $\gamma$  (rIFN $\gamma$ ), but not similar preparations of IFN $\alpha$  or  $\beta$ , can induce FcR expression. The FcR induced by IFN $\gamma$ , like the FcR present on normal mature monocytes but not on freshly obtained mature PMN, binds monomeric human IgG1 with high affinity.

### Materials and Methods

*Cell Lines.* The human myeloid (HL-60 (29), ML1, ML2, ML3 (30), KG1), monocytic (U937), erythromyeloid (K562), B-lymphoblastoid (Daudi, Raji, 8866), T-lymphoblastoid (HSB-2, MOLT/4 and Jurkat), and rhabdomyosarcoma (RDMC) cell lines, the human fetal skin (HF-28), mouse (LF2) and bovine (CB2) fibroblast strains were grown in our laboratory in RPMI 1640 medium (Flow Laboratories, Rockville, MD) supplemented with 10% fetal bovine serum (FBS; Gibco Laboratories, Grand Island, NY). B cell lines BC10R and the human melanoma cell line SK-Mel 23 were kindly provided by Dr. E. De Freitas and Dr. M. Herlyn (The Wistar Institute).

*Viruses.* Influenza viruses A/Hong Kong/107/68 (H3N2) (HK), A/PR/8/34 (HON1) (PR8), and A/Japan/305/57 (H2N2) (JAP) were grown in embryonated chicken eggs, and had titers of 850 hemagglutinating units (HAU)/ml, 1800 HAU/ml, and 2,000

HAU/ml, respectively (allantoic fluid); Herpes simplex virus type 1 (HSV-1) was grown in Vero cells and had a titer of  $10^8$  plaque-forming units (pfu)/ml; the 73-T strain of Newcastle disease virus (NDV) (allantoic fluid) had a titer of  $3.3 \times 10^9$  pfu/ml; Sendai virus had a titer of 512 HAU/ml; vesicular stomatitis virus (VSV) was grown in RDMC cells and had a titer of  $10^8$  pfu/ml.

*Peripheral Blood and Bone Marrow Cell Preparations.* Venous peripheral blood samples were obtained under informed consent from healthy volunteers or from patients with myeloproliferative diseases or leukemias. Included were samples from seven patients in the chronic phase of Philadelphia chromosome-positive chronic myelogenous leukemia (CML), from eight cases of acute myeloid leukemia (AML) at different stages according to the FAB classification (31) and from four patients with acute lymphatic leukemias (ALL), two of B- and two of T-cell origin, as diagnosed on the basis of their reaction with a panel of anti-lymphocyte antibodies. Blood specimens were kindly provided by Dr. P. Thiagarajan (Jefferson Medical College) and Dr. J. Hoxie (University of Pennsylvania). Hematologically normal bone marrow specimens, obtained for diagnostic purposes at the Thomas Jefferson University Hospital, were kindly provided by Dr. P. Thiagarajan and Dr. J. Caro (Jefferson Medical College). The procedures used to prepare peripheral blood mononuclear cells, lymphocytes (PBL), monocytes (density  $< 1.077$  g/ml), and PMN (density  $> 1.077$  g/ml) have been described in detail (11). Monocyte preparations were  $>75\%$  positive for  $\alpha$ -naphthyl acetate esterase ( $\alpha$ -NAE) and PMN preparations contained no  $\alpha$ -NAE positive cells. Mononuclear nonadherent cells obtained from normal bone marrow and myeloid leukemia patients were mostly myeloid (from myelocytes to banded granulocytes) as judged by morphological examination of Giemsa-stained smears and enzyme cytochemistry.

Nonadherent mononuclear cells from one CML patient were separated into mature (B13.4 [+]), and immature (B13.4 [-]) cells by an indirect rosetting procedure using erythrocytes coated by the  $\text{CrCl}_3$  method with goat  $\text{F(ab')}_2$  anti-mouse Ig, as previously described (15). Monoclonal antibody B13.4 (IgM) reacts with mature cells of the myeloid and monocytic lineage, bands and metamyelocytes (32).

*FcR-Inducing Supernatant Fluids and Interferon (IFN) Preparations.* PBL ( $10^7$  cells/ml) were incubated for 18 h at  $37^\circ\text{C}$  in RPMI-10% FBS in the presence of the inducer cell line ( $10^6$  cells/ml) or with different viruses. Cells were infected with NDV and HSV-1 at a multiplicity of infection (MOI) of 10; the other virus preparations were used at a 1:10 final dilution. Cell culture media were harvested, centrifuged, and kept frozen at  $-80^\circ\text{C}$  until tested. Supernatant fluids (500 ml, concentrated 200 times by vacuum dialysis) were fractionated by gel filtration on a Sephadex G100 column (bed volume, 480 ml; void volume, 185 ml) (Pharmacia Fine Chemicals, Uppsala, Sweden). Fractions of 9 ml were collected, dialyzed against medium, and sterilized by filtration.

Several IFN preparations were used: purified human  $\text{IFN}\gamma$  ( $10^6$  U/ml on Hep-2 cells) was obtained from Interferon Sciences, Inc. (New Brunswick, NJ). Human r $\text{IFN}\gamma$ , purified to homogeneity from *E. coli*, was kindly supplied by Dr. C. G. Sevastopoulos (Genentech Inc., San Francisco, CA) and it has a titer of  $\sim 10^7$  antiviral U/mg protein on HeLa cells. Because of the variability of the titer of  $\text{IFN}\gamma$  on different cell lines and the lack of an international standard for  $\text{IFN}\gamma$ , concentrations of r $\text{IFN}\gamma$  are expressed in ng/ml rather than antiviral units (the concentration of 100 ng/ml, most frequently used in these experiments, corresponds to  $\sim 500$  U/ml on HeLa cells). Partially purified  $\text{IFN}\alpha$  (Leucoferon,  $10^6$  antiviral units/ml) was obtained from Biotechnologies Inc. (Hartford, CT). Recombinant types A and D  $\text{IFN}\alpha$  from *E. coli* were kindly supplied by Hoffmann-LaRoche Inc. (Nutley, NJ). Samples of purified  $\text{IFN}\beta$  (Prep. 16287) were kindly supplied by Dr. A. Billiau (Leuven, Belgium). Crude  $\text{IFN}\beta$  was obtained from Lee Biomolecular Research Lab., Inc. (San Diego, CA).

*Immunoglobulin Preparations.* Human IgG1 was purified from the serum of a patient affected with multiple myeloma (provided by Dr. P. L. Meroni, Istituto di Clinica Medica II, Universita' di Milano, Italy). After labeling with  $^{125}\text{I}$  by the chloramine T method (33), monomeric IgG1 was purified on a Sepharose 6B column (Pharmacia) and used immediately for binding assays. Supernatants from hybrid cell lines produced in our laboratory,

containing antibodies specific for cells other than myeloid or monocytic, were used as a source of monoclonal mouse immunoglobulins. The IgG3 monoclonal antibodies were kindly provided by Dr. M. Herlyn (The Wistar Institute, Philadelphia, PA). All hybrid cell cultures were established with a nonsecretor myeloma cell line and contain only one monoclonal immunoglobulin.

*IFN Tests.* Supernatant fluids were tested for antiviral activity by inhibition of the cytopathic effect of VSV on both bovine (CB2) and human (HF-28) fibroblasts (34). Human IFN $\alpha$  has antiviral activity on both bovine and human fibroblasts, whereas human IFN $\gamma$  and  $\beta$  are active only on the human cells (34). Antiviral units are expressed as the reciprocal of the dilution inhibiting 50% of the cytopathic effect and are equivalent to 1 Reference Unit of the NIH Human reference IFN G-023-901-527.

*Induction of HLA-DR on Human Melanoma Cell Lines.* Melanoma cells SK-Mel 23, which express HLA-DR antigens at low density, were seeded in flat-bottom microtiter plates ( $2.0 \times 10^4$  cells/well). After an 18-h incubation at 37°C in RPMI 1640-10% FBS, the medium was replaced with 200  $\mu$ l of the appropriate dilution of supernatant fluid or IFN in triplicate; control wells contained 200  $\mu$ l of medium. After a 48-h incubation at 37°C, the presence of HLA-DR antigens on the cell membrane was determined in a direct radioimmunoassay using the IgG2a monoclonal antibody B33.1 directed against a non-polymorphic determinant of the HLA-DR molecules (14) ( $^{125}$ I-F(ab') $_2$  fragment; 50  $\mu$ l/well of a preparation of 10  $\mu$ g/ml). After a 30-min incubation on ice, cells were washed four times, removed by trypsinization and the cell-associated radioactivity measured in a gamma scintillation counter.

*Induction of FcR.* Cells were cultured at 37°C in the presence of different concentrations of FcR-inducing supernatant fluid obtained from HK-infected lymphocytes (HK-SPN) or of the different IFN types. Cell lines were incubated at a density of  $3 \times 10^5$ /ml; nonadherent peripheral blood cells, PBL, early myeloid cells and PMN, at  $2 \times 10^6$ /ml; adherent monocytes were incubated with the different inducers directly in the flasks to which they adhered. Immediately before testing, the adherent cells were gently scraped off the flasks in the presence of ethylenediaminetetraacetic acid (EDTA, 0.15 M) using a rubber policeman. After culture, the cells were washed three times, incubated for 2 h at 37°C in medium without FBS to allow detachment of IgG possibly absorbed from the serum. When indicated, rabbit anti-human IFN $\gamma$  (final concentration, 250 neutralizing U/ml, i.e. 1:40 dilution), sheep anti-human IFN $\alpha$  ( $10^3$  neutralizing U/ml, i.e. 1:100 dilution) or normal rabbit serum (NRS) at a 1:40 final dilution was added during the incubation with the inducer. The anti-human IFN sera were obtained from Interferon Sciences, Inc.

*Detection of FcR.* Either  $^{125}$ I-labeled IgG1 binding or formation of rosettes with rabbit IgG (7S) antibody-sensitized ox erythrocytes (EA7S) was used:

(a) *Binding Assay with Monomeric Human IgG1.* Cell suspensions (25  $\mu$ l, usually 20 or  $40 \times 10^6$  cells/ml) were mixed with 25  $\mu$ l of serially diluted human monomeric  $^{125}$ I-labeled IgG1 in phosphate-buffered saline (PBS) with 1% gelatin, 0.1% NaN $_3$  in a 96-well round-bottom microtiter plate. All assays were performed in triplicate. After a 2-h incubation at 0°C, the cells were collected, overlaid on FBS in Eppendorf microtest tubes, and centrifuged for 30 s in an Eppendorf microfuge (Model S412, Brinkmann Instruments, Inc. Westbury, NY). The tubes were immediately frozen in dry ice and the pellet-associated radioactivity, in the resected tips of the tubes, was determined in a gamma scintillation counter.  $^{125}$ I-labeled IgG1 binding in buffer containing the same human myeloma serum from which the IgG1 was purified (5%, vol/vol) was measured in a separate set of tubes and subtracted from the experimental values, in order to measure only saturable binding. The data are plotted according to Scatchard (35) as  $r$  (number of molecules bound per cells) vs.  $r/A-x$ , where  $A$  is the initial molar concentration of IgG1 and  $x$ , the concentration of the cell-bound antibody at equilibrium.

(b) *EA7S Rosetting.* The EA7S indicator system and the rosetting assay have been described in detail (15). Incubation of the cells with EA7S was carried out for 30 min on ice to prevent phagocytosis. Cells binding  $>5$ EA7S were considered positive. At least 200 viable cells, as judged by erythrosin B dye exclusion, were counted in optical microscopy.

In several experiments, the cell lines were incubated with the inducer in the presence of cycloheximide (Sigma Chemical Co., St. Louis, MO) or actinomycin D (Calbiochem-Behring Corp., San Diego, CA) in concentrations ranging from 100 to 25  $\mu\text{g}/\text{ml}$  and 2 to 0.5  $\mu\text{g}/\text{ml}$ , respectively.

## Results

*Supernatant Fluids from Virus-infected Cells Induce Expression of FcR on Cells of Myeloid Origin.* Incubation of the HL-60 human promyelocytic cells with medium containing 10% (vol/vol) supernatant fluid from lymphocytes infected with the HK strain of influenza virus (HK-SPN) induced a significant increase in the percentage of EA7S rosette-forming cells, from 5–15% in the untreated ones (Fig. 1, A) up to 100% in the treated ones (Fig. 1, B). The number of erythrocytes bound per HK-SPN-treated cell was much higher than that bound per untreated ones, and the rosettes were more stable. The FcR-inducing factor was present in

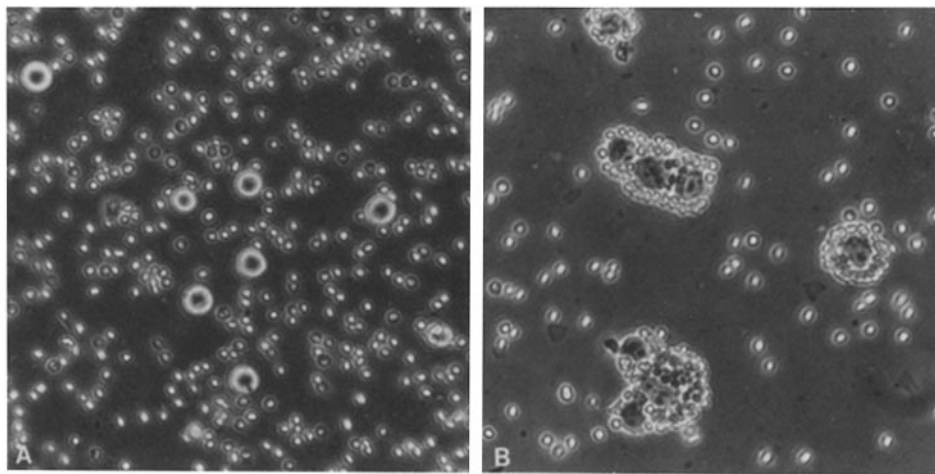


FIGURE 1. Phase-contrast light microscopy observation of HL-60 cells forming EA7S rosettes after an 18-h incubation at 37°C in the absence (A) or presence (B) of HK-SPN.

TABLE I  
Ability of Viruses and Cell Lines to Induce Peripheral Blood Mononuclear Cells to Produce FcR Enhancing Activity

Responder cells <sup>‡</sup>	Inducer*								
	None	HK	PR8	JAP	HSV-1	NDV	Sendai	RDMC	LF2
Mononuclear cells	7.4 <sup>§</sup>	ND <sup>†</sup>	72.9	ND	ND	ND	ND	7.4	50.6
Nonadherent cells	6.7	100.0	80.7	85.1	87.5	100.0	100.0	90.3	92.6
Adherent cells	6.9	ND	16.7	ND	ND	ND	ND	28.5	48.2

\* The inducers were incubated for 24 h with each of the three responder populations. Cell-free supernatant fluid was collected and tested for induction of FcR on HL-60 cells.

<sup>‡</sup> Peripheral blood mononuclear cells were further separated into plastic adherent and nonadherent populations. Monocytes (evaluated by  $\alpha$ -NAE staining) accounted for 25.4% of the mononuclear preparation, 0.4% of the nonadherent cells and 75.0% of the adherent cells.

<sup>§</sup> Percent EA7S rosette-forming cells (HL-60) after an 18-h treatment with 10% supernatant fluid.

<sup>†</sup> Not done.

the supernatant fluids of lymphocytes infected with a variety of viruses (with a titer up to 1:500), and in the supernatant fluids from mixed cultures of lymphocytes and cell lines (Table I). Control supernatant fluids from lymphocytes or inducer cells cultured alone, or the direct addition of viruses to the indicator cell line never induced enhancement of FcR expression (data not shown). Cell separation experiments indicated that nonadherent peripheral blood mononuclear cells were responsible for the production of FcR-inducing factor; neither separated adherent monocytes (Table I), nor PMN (not shown) were able to produce the factor when infected with viruses or cocultured with tumor-derived cells. All the supernatant fluids, except for those obtained from the coculture with mouse L cells (not shown), contained high titers of  $IFN\alpha$ . The production of FcR-inducing factor by lymphocytes upon coculture with cell lines was inhibited by the presence of adherent cells. A similar inhibition has been described for IFN production (36).

*Induction of FcR by  $IFN\gamma$ .* Different IFN types were tested for their ability to induce FcR on HL-60 cells. A dose-dependent increase in the percentage of HL-60 cells able to form EA7S rosettes was observed with recombinant or purified  $IFN\gamma$  (Fig. 2). The titer of r $IFN\gamma$  was calculated on the basis of a specific activity of  $10^7$  antiviral U/mg protein. As noted in Materials and Methods, the comparisons between different types of IFN based on antiviral titers should be considered only approximate. Recombinant  $IFN\alpha$  (both A and D) and partially purified  $IFN\alpha$  did not induce expression of FcR even when used at concentrations as

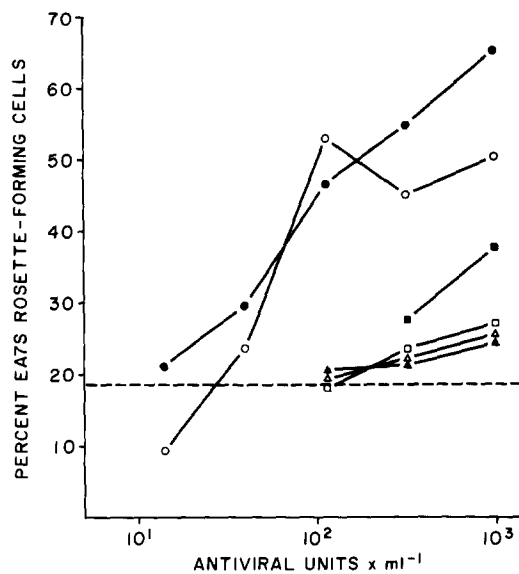


FIGURE 2. Enhancement of FcR expression on HL-60 cells treated with different types of IFN. HL-60 cells ( $3 \times 10^5$ /ml) were cultured for 18 h at 37°C in the presence of different concentrations of IFN preparations. After culture, the cells were washed and EA7S rosette formation was evaluated as described in Materials and Methods. The broken horizontal line indicates the percent EA7S rosette-forming cells in the untreated HL-60 cells. Cells were treated with: ●, purified  $IFN\gamma$ ; ○, r $IFN\gamma$ ; Δ, r $IFN\alpha$ ; ▲, r $IFN\beta$ ; □, purified  $IFN\alpha$ ; ■, purified  $IFN\beta$ .

high as  $10^3$  U/ml. The percentage of EA7S rosette-forming cells after incubation with partially purified IFN $\beta$  was slightly increased (up to 38% cells bound EA7S), but never reached the values observed after incubation with rIFN $\gamma$ . Kinetic studies with ML3 and HL-60 as indicator cells, indicated that EA7S rosette formation was induced as early as 4 and 6 h of incubation with both rIFN $\gamma$  and HK-SPN, the percentage of EA-rosette-forming cells reaching a plateau after 12 h of incubation (Fig. 3).

*HK-SPN and rIFN $\gamma$  Induce an Increase in the Number of the Binding Sites for Monomeric Human IgG1 on Promyelocytic Cell Lines.* Binding studies with  $^{125}\text{I}$ -labeled human myeloma IgG1 were performed to measure the variation of the number of binding sites and of receptor affinity on HL-60 and ML3 cells treated with HK-SPN or with rIFN $\gamma$  (Fig. 4). Scatchard plot analysis (Fig. 4, inset) revealed an increase in the number of binding sites for monomeric human IgG1 from  $10^4$  on the untreated cells to  $4.7 \times 10^4$  and  $7 \times 10^4$  on HL-60 cells treated with rIFN $\gamma$  or HK-SPN, respectively. The binding affinity, however, was the same in all the cell preparations tested. (HL-60,  $K = 1.5 \times 10^8 \text{ M}^{-1}$ , HL-60 + HK-SPN,  $K = 0.9 \times 10^8 \text{ M}^{-1}$ ; HL-60 + rIFN $\gamma$ ,  $K = 1.1 \times 10^8 \text{ M}^{-1}$ ). The 5- to 10-fold increase in the binding sites was reproducibly obtained in several experiments performed on different days with either cell line. >90% of the cells from the erythromyeloid cell line K562 form stable EA7S rosettes. Scatchard analysis showed that these cells bind with low affinity ( $K = 1.8 \times 10^6 \text{ M}^{-1}$ ) an extremely

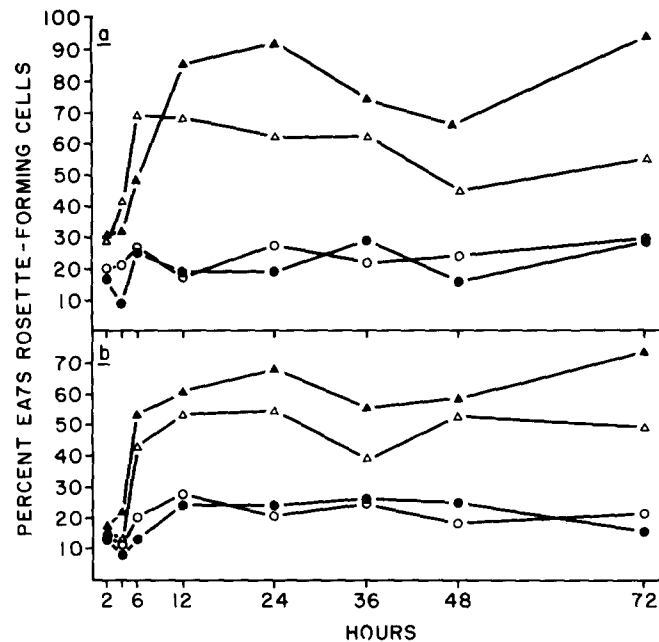


FIGURE 3. Kinetics of induction of FcR on ML3 (A) and HL-60 (B) cells. ML3 and HL-60 cells were cultured ( $2.5 \times 10^5$  cells/ml) at  $37^\circ\text{C}$  in culture medium containing different inducers. An aliquot of the cells was taken at the time points indicated, washed, and tested for FcR expression by EA7S rosette formation. O, control cells incubated in culture medium; ●, cells incubated in the presence of rIFN $\gamma$  ( $10^3$  U/ml); ▲, cells incubated in medium containing 10% (vol/vol) HK-SPN; △, cells incubated in medium containing 50 ng/ml of rIFN $\gamma$ .

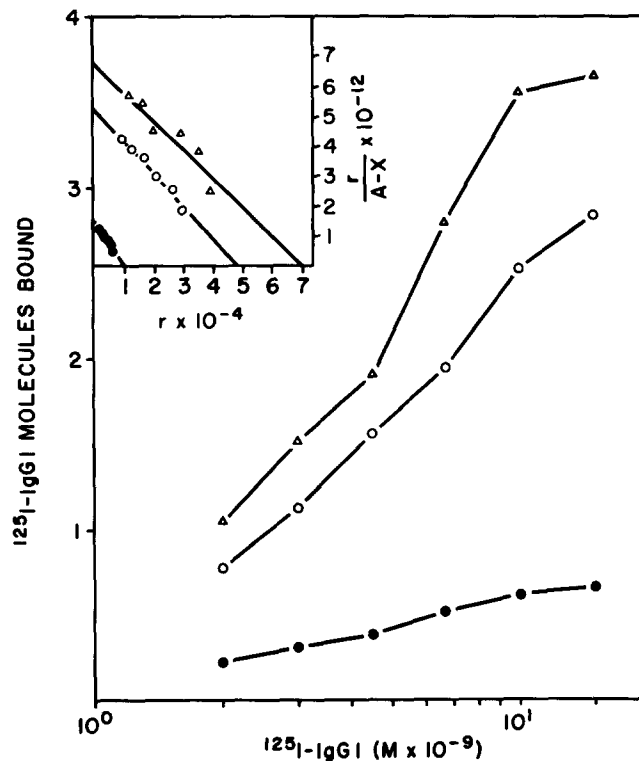


FIGURE 4. Binding of human monomeric  $^{125}\text{I}$ -labeled IgG1 to HL-60 cells. HL-60 cells were incubated ( $3 \times 10^5$  cells/ml) for 18 h at  $37^\circ\text{C}$  with different inducers, washed and tested for binding of  $^{125}\text{I}$ -IgG1 monomeric human Ig as described in Materials and Methods. ●, cells incubated in culture medium; Δ, cells incubated in culture medium containing 10% HK-SPN; ○, cells incubated in the presence of rIFN $\gamma$  (50 ng/ml). Scatchard plot of results from the same experiment is shown in the inset.  $r$ , number of binding sites;  $A$ , molar concentration of  $^{125}\text{I}$ -labeled IgG1 added;  $x$ , molar concentration of  $^{125}\text{I}$ -labeled IgG1 molecules bound; mol wt for human IgG1 = 150,000.

high number of IgG1 molecules ( $r_{\text{max}} = 1.9 \times 10^6$ ) and that this property is not modified by treatment with HK-SPN (data not shown).

*Characterization of the FcR-Inducing Factor in HK-SPN as IFN $\gamma$ .* To ascertain whether the increase in the percentage of EA7S rosette-forming cells after incubation with HK-SPN could be attributed to the presence of IFN, the effect of anti-IFN $\gamma$  and anti-IFN $\alpha$  sera on FcR induction was tested. Anti-IFN antibodies (anti-IFN $\alpha$ ,  $10^3$  neutralizing U/ml; anti-IFN $\gamma$ , 250 neutralizing U/ml) or NRS were added to cultures of HL-60 cells together with increasing concentrations of rIFN $\gamma$  or HK-SPN. Expression of FcR after culture was measured by EA7S rosette formation, as described. If rabbit anti-IFN $\gamma$  serum was present, no rosette formation was observed at any of the inducing concentrations of rIFN $\gamma$  (Fig. 5, left panel) or HK-SPN (Fig. 5, right panel) used. Neither NRS at the same concentration, nor sheep anti-IFN $\alpha$  serum inhibited the induction of FcR on HL-60 cells.

The molecular weight of the factor present in HK-SPN was determined by gel filtration. Each fraction eluted was tested for: (a) antiviral activity on CB2 and



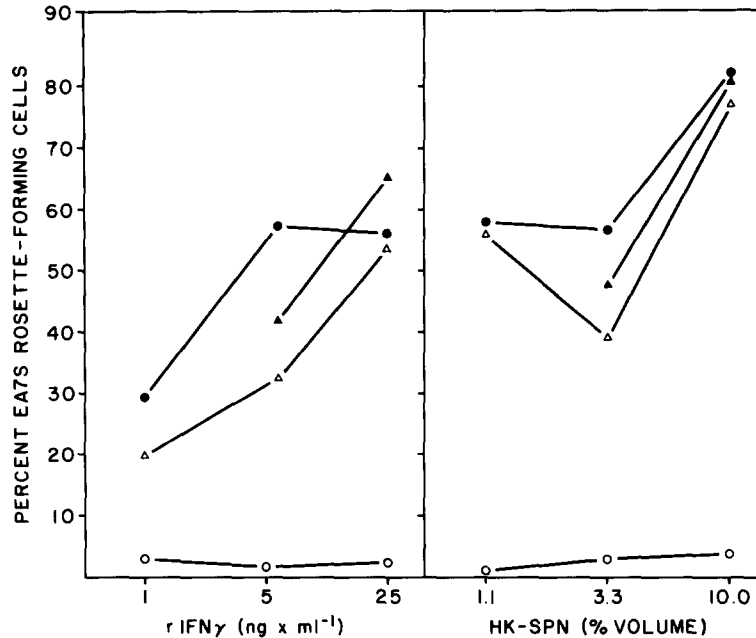


FIGURE 5. Effect of anti-IFN sera on FcR induction. HL-60 cells ( $3 \times 10^5$  cells/ml) were cultured in the presence of increasing concentrations of rIFN $\gamma$  (left panel) or HK-SPN (right panel) and a constant concentration of anti-IFN $\gamma$ , anti-IFN $\alpha$  antiserum or NRS. After an 18-h incubation, the percentage of EA7S-rosette-forming cells was scored. ●, control cells incubated in culture medium; ○, cells cultured in the presence of rabbit anti-IFN $\gamma$  serum (1:40 dilution, 250 neutralization units/ml); Δ, cells cultured in the presence of sheep anti-IFN $\alpha$  serum ( $10^3$  neutralization U/ml; 1:80 dilution); ▲, cells cultured in the presence of NRS (1:40 dilution).

HF-28 fibroblasts, as measured by the inhibition of cytopathic effect induced on these cells by VSV (Fig. 6,A); (b) induction of HLA-DR antigens on a human melanoma line, a specific property of IFN $\gamma$  (37) (Fig. 6,B), and (c) induction of FcR on HL-60 cells, by  $^{125}$ I-labeled human monomeric IgG1 binding assay (Fig. 6,C). The antiviral activity, when tested on CB2 bovine fibroblasts, corresponded to a single peak with a molecular weight of 20,000. The activity eluted as two peaks of  $\sim 40$ –50,000 and 20,000 molecular weight, respectively, when tested on HF-28 human fibroblasts. The antiviral activity present in the 20,000-dalton peak, but not that present in the 40–50,000-dalton peak, was completely inhibited by sheep anti-IFN $\alpha$  serum. As previously reported (36), the activity of the 40–50,000 peak, but not that of the 20,000 peak, was destroyed by 56°C or pH 2.0 treatment (not shown). These data indicate that IFN $\gamma$  comprises the high molecular weight peak, whereas IFN $\alpha$  is present in the low molecular weight one.

Unseparated HK-SPN enhanced the expression of HLA-DR antigens on the SK-Mel 23 melanoma cell line. The factor inducing HLA-DR expression was eluted in the fractions corresponding to an approximate molecular weight of 40–50,000 (Fig. 6,B). Induction of HLA-DR antigens was never observed in the fractions in which IFN $\alpha$  activity was eluted. Likewise, only the fractions corre-

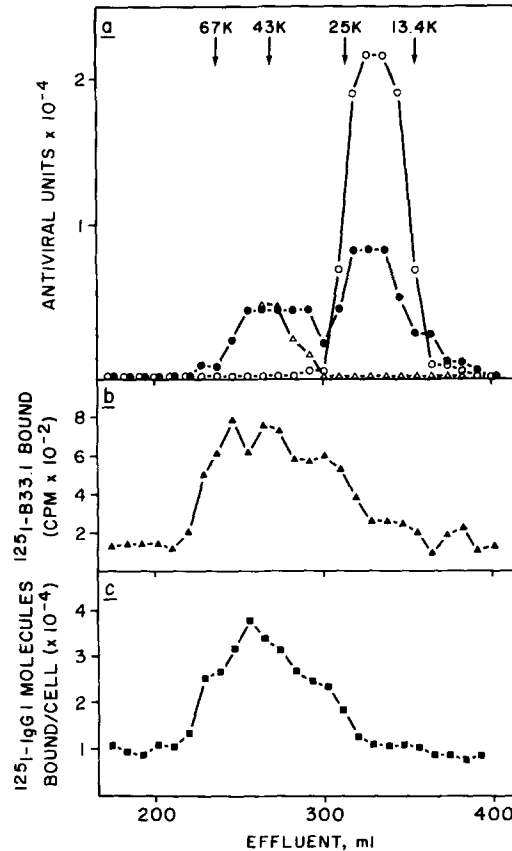


FIGURE 6. Gel filtration of HK-SPN. 2 ml of 200-fold concentrated HK-SPN was applied to a Sephadex G100 column. Each 9-ml fraction was dialyzed against RPMI, filter-sterilized and tested as described in Materials and Methods for: (a) antiviral activity on bovine CB2 fibroblasts (○), human HF-28 fibroblasts (●), and human HF-28 fibroblasts in the presence of sheep anti-IFN $\alpha$  antiserum ( $\Delta$ ); (b) induction of HLA-DR antigens on SK-Mel 23 human melanoma cell line ( $\blacktriangle$ ); (c) induction of binding of human monomeric <sup>125</sup>I-IgG1 to HL-60 cells ( $\blacksquare$ ). Molecular weight markers: bovine serum albumin (67K); ovalbumin (43K); chymotrypsin (25K); and ribonuclease A (13.4K).

sponding to a molecular weight of 35–50,000 were able to increase significantly the <sup>125</sup>I-labeled IgG1 binding on HL-60 cells (Fig. 6, C).

*Analysis of the Cell Types on which IFN $\gamma$  Induces Expression of FcR.* The target specificity of the FcR enhancing activity of IFN $\gamma$  was tested on cell lines and on cells freshly obtained from human peripheral blood. Myeloid, B- and T-lymphoid cell lines were incubated for 18 h at 37°C in the presence of rIFN $\gamma$  (25 ng/ml) or rIFNA (10<sup>3</sup> U/ml). The presence of FcR was tested by the EA7S rosette assay. Incubation with rIFN $\gamma$ , but not with rIFNA, resulted in a significant increase in the proportion of EA7S rosette-forming cells with all the myeloid cell lines tested (Table II). No increase, upon treatment with either of the two IFN types was observed on erythromyeloid K562 cells, which are spontaneously 98% positive for EA7S rosetting, nor on any of the B and T lymphoblastoid lines tested. The same specificity for myelomonocytic cells was also observed when

TABLE II  
Effect of rIFN $\gamma$  and rIFNA on FcR Expression on Human Cell Lines

Cell lines	Treatment*		
	None	rIFN $\gamma$	rIFNA
Myeloid			
HL-60	15.1 <sup>‡</sup>	84.4	16.3
ML1	78.8	87.9	ND <sup>§</sup>
ML2	71.2	88.3	ND
ML3	15.5	76.3	14.8
KG1	3.7	44.6	11.0
U937	42.5	83.3	50.9
K562	98.0	99.0	ND
Lymphoid			
B origin			
Raji	0.0	0.0	0.0
Daudi	6.6	11.2	6.1
8866	0.9	0.9	0.0
BC10R	12.0	12.0	14.6
T origin			
HSB-2	0.0	0.0	0.0
Molt 4	0.0	0.0	0.0
Jurkat	3.6	0.9	0.8

\* Cells ( $3 \times 10^5$ /ml) were incubated (18 h at 37°C) in medium with or without 25 ng/ml rIFN $\gamma$  or  $10^3$  U/ml rIFNA.

<sup>‡</sup> Percent EA7S rosette-forming cells.

<sup>§</sup> Not done.

TABLE III  
Effect of IFN $\gamma$  and HK-SPN on the Expression of FcR on Normal and Leukemic Cells

Responder cells	Treatment*		
	None	HK-SPN	rIFN $\gamma$
Normal bone marrow (2) <sup>‡</sup>	14.6, 5.6 <sup>§</sup>	56.5, 31.2	56.1, 45.6
Chronic myelogenous leukemia (7)	22.9 $\pm$ 18.3	68.9 $\pm$ 12.8	58.2 $\pm$ 16.1
Myelofibromatosis	6.5	37.2	45.6
AML (M1) (3)	1.8 $\pm$ 3.2	3.8 $\pm$ 6.5	6.7 $\pm$ 7.2
AML (M2) (4)	6.4 $\pm$ 5.5	3.0 $\pm$ 7.3	15.0 $\pm$ 9.1
AML (M3)	1.9	6.7	20.0
AML (M4) (2)	16.8, 39.2	20.8, 56.7	52.6, 81.2
ALL (pre-B) (2)	2.0, 0.0	0.0, 3.4	0.0, 0.0
ALL (T) (2)	0.0, 0.0	0.0, 0.0	0.0, 3.4

\* Mononuclear cells ( $2 \times 10^6$ /ml) were incubated for 18 h at 37°C in culture medium with or without 10% HK-SPN or 50 ng/ml rIFN $\gamma$ .

<sup>‡</sup> Number of donors tested.

<sup>§</sup> Percent EA7S rosette forming cells. Individual values are given when less than three donors were tested, and mean  $\pm$  SD when three or more donors were tested.

normal bone marrow or leukemic peripheral blood cells were tested (Table III). Both HK-SPN and rIFN $\gamma$  were able to induce FcR expression on nonadherent mononuclear cells obtained from two normal marrow specimens at levels significantly higher than on untreated cells. A variable proportion of cells ( $22.9 \pm 18.3$ ) in nonadherent mononuclear cells from the peripheral blood of seven CML patients formed EA7S rosettes. In all cases, the proportion of EA7S-rosette-forming cells increased after incubation with either inducer. The same results were obtained with nonadherent mononuclear cells from a patient affected with myelofibromatosis. Cells from 10 AML patients were tested: upon treatment with rIFN $\gamma$ , FcR was induced in a small proportion of cells obtained from the patients with M1, M2, and M3 leukemias; significant FcR increase was observed with the cells of the two patients with myelomonocytic leukemia (M4). rIFN $\gamma$  was always more efficient than HK-SPN in inducing FcR on AML cells, as well as on myelofibromatosis and bone marrow cells. In none of the four ALL tested (two of B- and two of T-cell origin) was FcR expression increased upon treatment with either HK-SPN or rIFN $\gamma$ .

*Increased Numbers of FcR for Human Monomeric IgG1 are Induced by rIFN $\gamma$  on Peripheral Blood Monocytes and PMN.* Normal peripheral blood monocytes and PMN were incubated (18 h at 37°C) in RPMI-10% FBS containing 50 ng/ml of rIFN $\gamma$ . After washing, the binding assay of  $^{125}\text{I}$ -labeled human IgG1 was performed as described. Scatchard plot of the results obtained with monocytes in one typical experiment out of six performed with similar results is shown in Fig. 7. The number of FcR binding sites on monocytes after incubation with rIFN $\gamma$  was increased 2.1- to 5.2-fold (from  $10,461 \pm 3,585$  to  $32,059 \pm 13,662$  per cell). HK-SPN was unable to induce an increase of FcR expression on monocytes. rIFN $\alpha$  did not increase FcR by itself and it partially prevented the induction by IFN $\gamma$  (not shown). This inhibitory effect of rIFN $\alpha$  was not observed on the myeloid cell lines, on which the HK-SPN is effective. The Scatchard plot of the binding of IgG1 to uninduced monocytes was not perfectly linear and did not provide an accurate determination of the affinity of the receptor (Fig. 7). The FcR on rIFN $\gamma$ -induced monocytes has an affinity ( $K = 1.58 \pm 0.81 \times 10^8 \text{ M}^{-1}$ ), comparable to that observed on both induced and uninduced HL-60 cells. On PMN from three different donors tested in the same experiment (Fig. 8), rIFN $\gamma$  induced the appearance of  $13,641 \pm 2,374$  specific binding sites per cell with an average affinity of  $1.32 \pm 0.28 \times 10^8 \text{ M}^{-1}$ . Untreated PMN did not show specific high affinity binding for monomeric IgG1. The reactivity of antibody B73.1 on the PMN preparations, as analyzed by indirect immunofluorescence (flow cytometry), was not affected by treatment with rIFN $\gamma$  (data not shown). Table IV shows the results obtained in one experiment of binding of human monomeric IgG1 to myeloid cells at different stages of maturation obtained from the peripheral blood of a CML patient. The myeloid cells have been separated into cells with  $d > 1.077 \text{ g/ml}$  (mostly mature PMN); nonadherent cells with  $d < 1.077 \text{ g/ml}$ , reacting with antibody B13.4 (32) (metamyelocytes and bands); nonadherent cells, not reacting with B13.4 (mostly myelocytes); and adherent cells with  $d < 1.077 \text{ g/ml}$  (mostly monocytes). Only monocytes and, among the myeloid cell preparations, the B13.4(+) cells showed significant binding on day 0. A marked increase in the number of IgG1 molecules bound per cell was

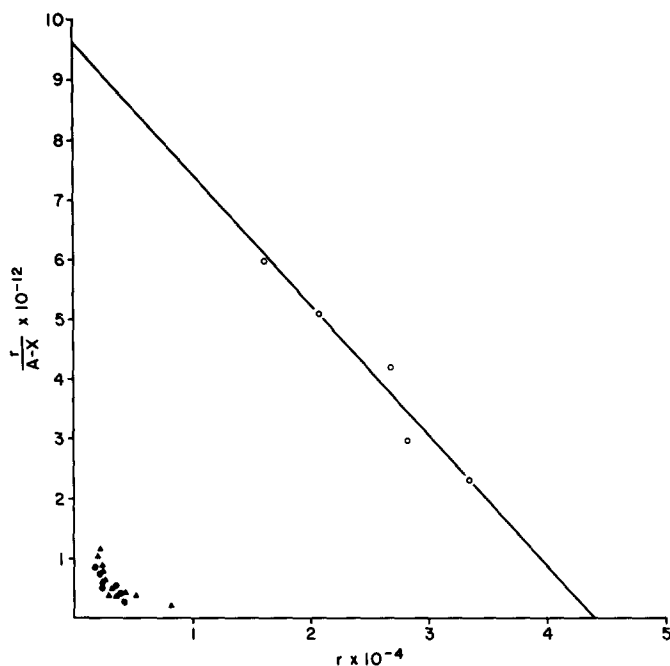


FIGURE 7. Enhancement of expression of FcR for human monomeric IgG1 on normal peripheral blood monocytes. Binding assay of human monomeric  $^{125}\text{I}$ -labeled IgG1 was performed as described in Materials and Methods, on adherent peripheral blood monocytes from one donor. Data were plotted according to Scatchard. Monocytes were incubated with:  $\Delta$ , culture medium;  $\blacktriangle$ , HK-SPN (10%, vol/vol);  $\bullet$ , rIFN $\alpha$  ( $10^3$  U/ml);  $\circ$ , rIFN $\gamma$  (50 ng/ml).

observed in all cell preparations after an 18-h incubation in the presence of rIFN $\gamma$ ; this increase was particularly evident in the B13.4 (+) cells. In this experiment, incubation of monocytes at  $37^\circ\text{C}$  for 18 h determined a decrease in binding: the presence of rIFN $\gamma$  during the incubation simply maintained the original binding ability. A similar but more modest decrease of binding ability, upon an 18-h incubation, was sometimes observed with cultures of normal monocytes, in agreement with previous reports (26). However, monocytes from normal donors, cultured in the presence of rIFN $\gamma$ , always showed a several-fold increase in binding ability over that observed at the beginning of the culture (not shown).

*Immunoglobulin Subclass Specificity of the FcR Induced by rIFN $\gamma$  on Myeloid Cells.* The specificity of the FcR induced, on cells of myeloid origin, by rIFN $\gamma$  was tested in competition experiments in which monoclonal mouse immunoglobulins were used as cold competitors for  $^{125}\text{I}$ -labeled monomeric human IgG1. Cold human IgG1 completely blocks the binding of the  $^{125}\text{I}$ -labeled IgG1. Only IgG2a and IgG3 mouse immunoglobulins were able to inhibit human IgG1 binding on monocytes, both untreated (Fig. 9, a) and treated with rIFN $\gamma$  (Fig. 9, b). The same results were obtained when untreated (Fig. 9, c) and rIFN $\gamma$ -treated HL-60 cells (Fig. 9, d), or rIFN $\gamma$ -induced peripheral blood PMN (not shown) were tested.

*rIFN $\gamma$ -Dependent FcR Induction on Myeloid Cell Lines: Sensitivity to RNA and*

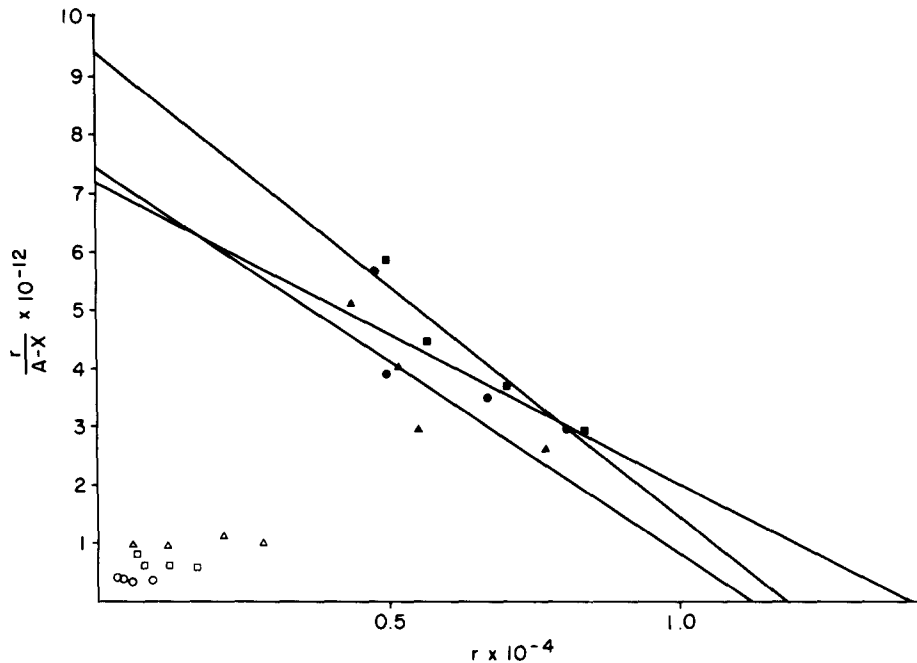


FIGURE 8. Induction of FcR for human monomeric IgG1 on normal peripheral mature PMN. Binding of human monomeric  $^{125}\text{I}$ -labeled IgG1 was performed, as described, on PMN preparations ( $20 \times 10^6$  cells/ml) from three different normal donors incubated for 18 h at  $37^\circ\text{C}$  in culture medium (O, □, Δ) or in medium containing 50 ng/ml rIFN $\gamma$  (●, ■, ▲). Data were plotted according to Scatchard.

TABLE IV  
*Binding of Monomeric Human IgG1 to Peripheral Blood Cells from a CML Patient*

Cell preparation*	Time 0 $^\ddagger$	18 h culture	
		Medium $^\ddagger$	rIFN $\gamma$ $^\ddagger$
d < 1.077, nonadherent, B13.4 (-)	1,741 $^\S$	2,564	9,394
d < 1.077, nonadherent, B13.4 (+)	4,067	6,559	23,403
d > 1.077, PMN	2,544	2,813	5,654
d < 1.077, adherent	12,222	4,162	9,652

\* Cells at different stages of maturation were obtained as described in Material and Methods.

$^\ddagger$  Cells were tested immediately after separation or after an 18-h incubation in culture medium or in the presence of 50 ng/ml of rIFN $\gamma$ .

$^\S$  Number of  $^{125}\text{I}$ -labeled IgG1 molecules bound per  $10^6$  cells after a 2-h incubation at  $4^\circ\text{C}$  with  $15 \text{ nM}^{125}\text{I}$ -labeled IgG1.

*Protein Synthesis Inhibitors.* To ascertain whether de novo RNA and protein synthesis are necessary in the myeloid cells in order to express FcR upon induction with rIFN $\gamma$ , cycloheximide (in concentrations ranging from 50–200  $\mu\text{g}/\text{ml}$ ) and actinomycin D (0.5–2  $\mu\text{g}/\text{ml}$ ) were added to the cells (HL-60 and ML3) during an 8-h incubation in the presence of 50 ng/ml of rIFN $\gamma$ . The background percentage of EA7S rosette-forming cells in the HL-60 and ML3

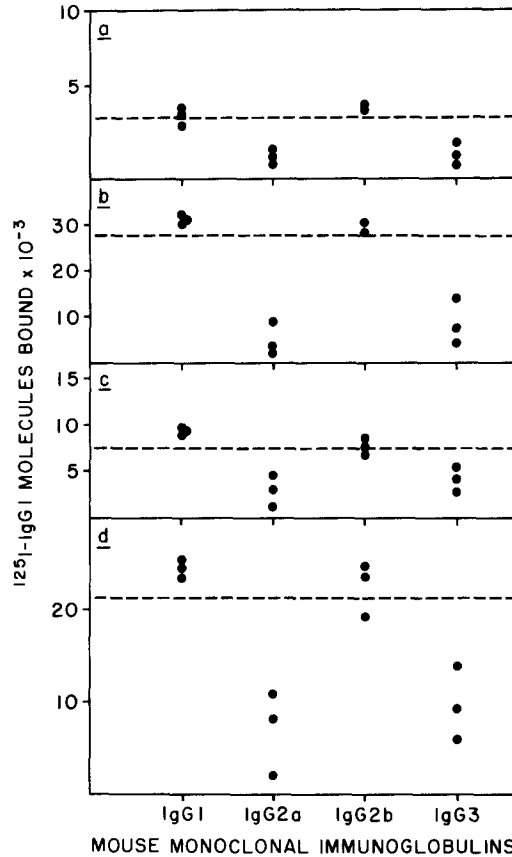


FIGURE 9. Mouse IgG isotype specificity of the FcR for human monomeric IgG1 induced by rIFN $\gamma$  on myeloid cells. Target cells were: *a*, normal peripheral blood monocytes; *b*, peripheral blood monocytes induced by rIFN $\gamma$  (50 ng/ml); *c*, HL-60 cells; *d*, rIFN $\gamma$  treated HL-60 cells (50 ng/ml). Induction and inhibition experiments were as described in Materials and Methods. Points represent individual monoclonal antibodies tested of the indicated Ig subclass. Broken lines indicate the number of <sup>125</sup>I-labeled human monomeric IgG1 molecules bound/10<sup>6</sup> cells in the absence of cold competitor Ig.

cell lines was 16.7% and 10%, increasing upon 8-h incubation with rIFN $\gamma$  to 65.7% and 71.2%, respectively. Both cycloheximide and actinomycin D, at the concentrations used, reduced the number of rosette-forming cells to values close to background values.

### Discussion

A lymphokine able to rapidly enhance the expression of the FcR for IgG1 present on human cell lines is contained in medium conditioned by mixed leukocyte cultures (23, 25). In this report, we identify the lymphokine able to induce FcR as immune IFN. We demonstrate that IFN $\gamma$  selectively induces the expression of the high-affinity FcR for monomeric human IgG1 on both human monocytic and myeloid cells, including mature peripheral blood PMN, which do not spontaneously express this type of FcR.

As a source of the FcR-enhancing lymphokine, we used supernatant fluids from human leukocytes infected with a variety of viruses or cultured with cell lines. Infection of fibroblasts with HSV has been reported to induce FcR expression (39); infection of myelomonocytic cells with the other virus types that we used for supernatant fluid production did not directly induce FcR, showing that a lymphokine and not the virus itself was responsible for the FcR induction. Treatment of the supernatants at pH 2.0, a procedure performed to inactivate the infectious viruses, also inactivated the lymphokine. The identification of the factor present in the leukocyte supernatant fluids, specifically in HK-SPN, as immune interferon is supported by both indirect and direct evidence.

The production of IFN $\gamma$  in lymphocyte cultures incubated with influenza viruses for 24 h has been considered dependent on the sensitization of the lymphocyte donors to the virus (40, 41). However, we have shown previously that lymphocytes cultured with cell lines or with viruses, such as NDV, against which donors are rarely sensitized, consistently produce IFN $\gamma$  together with IFN $\alpha$  in less than 24 h (36). Induction of IFN $\gamma$  does not seem to depend on alloantigenic stimulation under these experimental conditions, because a rapid production of IFN $\gamma$  in response to alloantigens is observed only when primed lymphocytes are used (34), and autologous derived cell lines are able to induce IFN $\gamma$  production (36). The addition of an anti-IFN $\gamma$  antiserum to the cultures of HL-60 cells containing HK-SPN completely abrogated FcR induction, as tested by EA7S rosette formation. In those experiments, the induced cells were washed free of serum and preincubated for 2 h at 37°C in serum-free medium to avoid competition of the IgG contained in the serum for the binding to the FcR. Under those conditions, normal rabbit IgG or IgG aggregates present in the serum do not prevent detection of the FcR. The FcR of monocytes is modulated after interaction with immune complexes (42, 43). However, the inability of an anti-IFN $\alpha$  antiserum, reacting with and inactivating the IFN $\alpha$  present at high titer in the HK-SPN, to prevent FcR enhancement excludes the possibility that modulation of the FcR is induced by IFN-anti IFN immune complexes.

The FcR enhancing activity contained in HK-SPN was eluted from a Sephadex G100 column in the fractions corresponding to a molecular weight of 40–50,000. These fractions contained antiviral activity not inhibitable by the anti-IFN $\alpha$  antiserum and detectable only on homologous human fibroblasts. A second peak, corresponding to a molecular weight of 20,000, contained antiviral activity that was identifiable as IFN $\alpha$  by its activity on both human and bovine cells (34) and by its complete inhibition with anti-IFN $\alpha$  antiserum. The fractions containing this second peak of antiviral activity had no effect on FcR expression. The same fractions from the G100 column that induced FcR expression were also able to induce expression of HLA-DR antigens (as detected by antibody B33.1) on human melanoma cells. The presence of anti-IFN $\gamma$  serum during the induction completely abolished the ability of these fractions, of HK-SPN and of other leukocyte-conditioned media, to induce expression of HLA-DR on both melanoma and on the ML3 promyelocytic cell line.

Crude or partially purified preparations of human IFN $\alpha$  have been reported to induce a modest and transient enhancement of FcR expression on lymphocytes



and lymphoid and nonlymphoid cell lines (27, 28, 45). However, the results of our tests of several IFN preparations for their ability to induce FcR expression provide a direct demonstration that IFN $\gamma$  is responsible for the induced expression of FcR for monomeric IgG1 on human cells of myeloid origin. Only purified IFN $\gamma$  and rIFN $\gamma$  produced in *E. coli* were able to enhance or induce FcR expression; using different preparations of IFN $\alpha$  or IFN $\beta$ , we observed no effect on FcR expression in either myelomonocytic or lymphoid human cells. Similar to our findings with human cells, IFN $\gamma$  has been shown to enhance FcR expression on mouse macrophages (46).

The effect of IFN on FcR expression is prevented in the presence of cycloheximide or actinomycin D, as are most of the antiviral and anticellular effects of IFN, which require active RNA and protein synthesis: this fact is generally interpreted as a requirement for the synthesis of a second intracellular mediator of proteinaceous nature (47). The molecular mechanism by which IFN induces or increases the expression of FcR or other surface proteins, such as HLA-A,B,C and HLA-DR antigens (48) is not known. The rapid induction of FcR on the IFN-treated cells might be due to changes in cellular membrane properties (49, 50) that allow the expression of previously undetectable preformed receptors, and not to de novo synthesis.

The expression of FcR during myeloid differentiation induced by chemical inducers (32) or by leukocyte-conditioned medium (26), represents only one of many different morphological, enzymatic, and phenotypic parameters of induced maturation (26, 51). In the presence of IFN $\gamma$ , FcR expression in myelomonocytic cells is at a maximum by 8 h after the beginning of the culture and is maintained for at least 72 h; at least during the initial 24 h, FcR expression is not accompanied by other morphological or cytochemical changes or functional activities (e.g. Ab-CMC) characteristic of differentiated myeloid cells. Although these data might suggest a separate regulation of differentiation and induction of FcR on myeloid cells, recent evidence indicates that immune IFN is the major factor in conditioned medium from lectin-stimulated leukocytes responsible for the induction of differentiation of myeloid cells along the monocytic pathway (Dayton, Perussia, and Trinchieri, manuscript in preparation).

Our analysis of the effect of IFN $\gamma$  on cell lines and on fresh cells from patients or healthy donors shows that FcR expression is enhanced or induced only on cells of myelomonocytic origin. K562 cells, which have a dual differentiation potential toward erythroid or myeloid cells (52), never showed an increase in the number of FcR receptor binding sites for human IgG1. These cells bind with very low affinity monomeric human IgG1, and the 72,000-dalton IgG binding protein, characteristic of human monocytes, has not been precipitated from these cells (12). Instead, IgG binding ability of K562 cells has been associated with a 43,000-dalton protein, possibly identified as actin (12). Though HK-SPN and IFN $\gamma$  had a similar FcR enhancing ability on cell lines, only IFN $\gamma$  was effective on fresh monocytes. It is possible that the inability of HK-SPN to induce FcR on fresh monocytes is due to the inhibitory effect of the IFN $\alpha$  contained in the preparation, as preliminary experiments have shown that IFN $\alpha$  is able to block the IFN $\gamma$ -mediated enhancement of FcR and the HK-SPN contains predominantly IFN $\alpha$ . The ability of different cell types to respond to HK-SPN would be

inversely correlated with their susceptibility to the effect of IFN $\alpha$ : this might explain the observation that IFN $\gamma$  was more effective than HK-SPN in inducing FcR on AML cells. A several-fold increase in the number of IgG1 molecules bound per cell, similar to observations on monocytes and on HL-60 and ML3 promyelocytic cell lines, was also observed after IFN $\gamma$  treatment on both mature (B13.4[+]), and immature (B13.4[-]) myeloid cells obtained from the peripheral blood of CML patients. IFN $\alpha$  and  $\beta$  preparations did not significantly increase the number of binding sites even when used at concentrations of  $10^3$  U/ml. These data show that myeloid cells at any stage of maturation, from myelocytes or more immature cells, to metamyelocytes and mature neutrophils, can be induced by IFN $\gamma$  to express FcR. The significant enhancement of FcR on the more differentiated M3 and M4 AML, compared with the modest effect observed in most cases of M1 and M2, suggests that the ability to respond to IFN $\gamma$  with FcR induction might be acquired at the promyelocytic stage. Data showing that the promyelocytic cell lines HL-60 or ML3 are more sensitive responders to IFN $\gamma$  than the myeloblastic line KG1 support this hypothesis.

The binding equilibrium constant of the FcR induced on promyelocytic cell lines and on peripheral blood monocytes ( $1.44 \pm 0.51 \times 10^8$  M $^{-1}$  and  $3.01 \pm 0.81 \times 10^8$  M $^{-1}$ , respectively) was not appreciably affected by IFN $\gamma$  treatment. The equilibrium constant could not be accurately determined on fresh monocytes, because the Scatchard plot was not perfectly linear, as reported by other authors (13, 38).

The ability of IFN $\gamma$  to induce on mature PMN a number of binding sites ( $13,641 \pm 2,374$ ) similar to that of resting monocytes, with an affinity ( $1.32 \pm 0.30 \times 10^8$  M $^{-1}$ ) of the same order of magnitude as that of monocytes or of HL-60 cells was an unexpected finding. The FcR induced on PMN is inhibited by the same mouse IgG isotypes as the FcR of monocytes and HL-60 cells. Mature PMN, in agreement with previous reports (9), do not express high affinity receptors for monomeric IgG. Morphological observation of EA7S rosette-forming PMN preparations shows both increased numbers of rosettes and of E bound per cell, confirming that IFN $\gamma$  affects PMN and not contaminant monocytes. However, IFN $\gamma$  does not induce any increase in the expression of the B73.1 antigen, present at variable density on the PMN of different donors, and representing the receptor for aggregated immunoglobulins or a molecule functionally associated with it (14). This is in agreement with our previous report that IFN does not affect the expression of the B73.1 antigen on human NK cells (15). Our results recall the recent findings that activation of murine macrophages selectively enhances expression of the FcR1 for monomeric IgG2a, whereas the expression of the FcR2 for aggregated IgG2b is decreased (22). Unlike mature PMN, immature myeloid cells from CML patients show a low but significant binding of monomeric IgG1 even in the absence of IFN $\gamma$  stimulation. The presence of the receptor for monomeric IgG on immature myeloid cells might be transient and restricted to specific stages of normal neutrophil maturation. Alternatively, a population of cells with intermediate myelomonocytic characteristics might exist in the peripheral blood of CML patients.

To define the specificity of the induced human FcR and to compare it with the specificity of the better characterized murine FcR, we also tested the ability

of mouse monoclonal IgG of different isotypes to inhibit human IgG1 binding. This approach seemed valid based on the biochemical similarities between human monocyte FcR and murine FcR1 and between the human PMN FcR and murine FcR2. Mouse IgG isotypes are easily obtained from supernatant fluids of murine somatic B-cell hybrid cultures, enabling a better comparison of the human and the mouse FcR. Moreover, in the light of current therapeutic attempts in humans using murine monoclonal antibodies, information on the ability of the various isotypes to bind to different human FcR, characteristics of cells with different effector functions, might help in interpreting the possible *in vivo* effects of these murine Ig isotypes. Our results show that culture supernatants containing IgG2a and IgG3, but not those containing IgG1 or IgG2b, efficiently compete with human IgG1 for binding to monocytes, IFN $\gamma$ -treated PMN and promyelocytic cell lines. These results confirm the similarities of the human FcR induced by IFN $\gamma$  with the mouse FcR1. However, in the mouse, binding of IgG3 to the FcR1 has not been observed and the receptor for IgG3 has been described as a separate, low affinity receptor. This finding might suggest a minor divergence of the human FcR during evolution. Our observation that IFN $\gamma$  enhances FcR expression on human myelomonocytic cells with specificity for some isotypes of mouse monoclonal antibodies, together with the ability of IFN $\gamma$  to induce immature myeloid cells and monocytes to exert antibody-dependent cytotoxicity, mediated through the FcR (unpublished results), suggest the possibility that IFN $\gamma$  and mouse monoclonal antibodies might have a synergistic therapeutic effect, e.g. in tumor cell destruction.

### Summary

We report here that FcR for human monomeric IgG1 can be induced on cells of myeloid origin cultured in the presence of IFN $\gamma$  for 8 h. Supernatant fluids from cultures of lymphocytes infected with a variety of viruses or cocultured with cell lines have the same FcR enhancing effect as IFN $\gamma$ . We identify the factor in the supernatant fluid responsible for the induction as immune interferon. Among the different types of IFN, only the  $\gamma$  type (both purified and recombinant) specifically induces the appearance of FcR for monomeric IgG1 on normal and leukemic myeloid cells but not on cells of lymphoid origin. This effect is also evident on mature PMN. We show that the specificity and the affinity of the receptor induced on HL-60 promyelocytic cells, peripheral blood monocytes, and PMN are identical to those of the receptor spontaneously present on the same cells, except for PMN, which do not spontaneously express this type of receptor. The results of inhibition experiments performed with mouse IgG of different isotypes indicate that the receptor can be inhibited by murine IgG2a and IgG3. These results suggest that the receptor present on human monocytes or immature myeloid cells, selectively inducible by IFN $\gamma$ , has a specificity similar to the FcR1 described on mouse macrophages.

We thank Drs. Perumal Thiagarajan, Jaime Caro, and James Hoxie for providing the samples of peripheral blood and bone marrow; Dr. Pier Luigi Meroni for the gift of IgG1 human myeloma sera; Marina Hoffman for editing; Peg Engle for art work, and Marion

Kaplan for preparing the manuscript.

Received for publication 6 June 1983.

### References

1. Unkeless, J. C., H. Fleit, and I. S. Mellman. 1981. Structural aspects and heterogeneity of immunoglobulin Fc receptors. *Adv. Immunol.* 31:247.
2. Dickler, H. B. 1976. Lymphocyte receptors for immunoglobulins. *Adv. Immunol.* 24:167.
3. Theofilopoulos, A. N., and F. J. Dixon. 1978. The biology and detection of immune complexes. *Adv. Immunol.* 28:89.
4. Kulczycki, A., Jr., L. Solanki, and L. Cohen. 1981. Isolation and partial characterization of Fc-binding proteins of human leukocytes. *J. Clin. Invest.* 68:1558.
5. Silverstein, S. C., R. M. Steinman, and Z. A. Cohn. 1977. Endocytosis. *Annu. Rev. Biochem.* 46:669.
6. Werb, Z., and S. Gordon. 1975. Elastase secretion by stimulated macrophages. Characterization and regulation. *J. Exp. Med.* 142:361.
7. Rabellino, E. M., G. D. Ross, H. T. K. Trang, N. Williams, and D. Metcalf. 1978. Membrane receptors of mouse leukocytes. II. Sequential expression of membrane receptors and phagocytic capacity during leukocyte differentiation. *J. Exp. Med.* 147:434.
8. Crabtree, G. R. 1980. Fc receptors of a human promyelocytic leukemia cell line: evidence for two types of receptors defined by binding of the staphylococcal protein A-IgG1 complex. *J. Immunol.* 125:448.
9. Kurlander, R. J., and J. Batker. 1982. The binding of human immunoglobulin G1 monomer and small, covalently cross-linked polymers of immunoglobulin G1 to human peripheral blood monocytes and polymorphonuclear leukocytes. *J. Clin. Invest.* 69:1.
10. Anderson, C. L., and G. N. Abraham. 1980. Characterization of the Fc receptor for IgG on a human macrophage cell line, U937. *J. Immunol.* 125:2735.
11. Lawrence, D. A., W. W. Weigle, and H. L. Spiegelberg. 1975. Immunoglobulins cytophilic for human lymphocytes, monocytes, and neutrophils. *J. Clin. Invest.* 55:368.
12. Anderson, C. L. 1982. Isolation of the receptor for IgG from a human monocyte cell line (U937) and from human peripheral blood monocytes. *J. Exp. Med.* 156:1794.
13. Fleit, H. B., S. D. Wright, and J. C. Unkeless. 1982. Human neutrophil Fc receptor distribution and structure. *Proc. Natl. Acad. Sci. USA* 79:3275.
14. Perussia, B., O. Acuto, C. Terhorst, J. Faust, R. Lazarus, V. Fanning, and G. Trinchieri. 1983. Human Natural Killer cells analyzed by B73.1, a monoclonal antibody blocking Fc receptor functions. II. Studies of B73.1 antibody-antigen interaction on the lymphocyte membrane. *J. Immunol.* 130:2142.
15. Perussia, B., S. Starr, S. Abraham, V. Fanning, and G. Trinchieri. 1983. Human Natural Killer cells analyzed by B73.1, a monoclonal antibody blocking Fc receptor functions. I. Characterization of the lymphocyte subset reactive with B73.1. *J. Immunol.* 130:2133.
16. Alexander, M. D., J. A. Andrews, R. G. Q. Leslie, and N. J. Wood. 1978. The binding of human and guinea-pig IgG subclasses to homologous macrophage and monocyte Fc receptors. *Immunology.* 35:115.
17. Walker, W. S. 1976. Separate Fc receptors for immunoglobulins IgG2a and IgG2b on an established cell line of mouse macrophages. *J. Immunol.* 116:911.
18. Unkeless, J. C. 1977. The presence of two Fc receptors on mouse macrophages: evidence from a variant cell line and differential trypsin sensitivity. *J. Exp. Med.*

- 145:931.
19. Diamond, B., and Yelton, D. E. 1981. A new Fc receptor on mouse macrophages binding IgG3. *J. Exp. Med.* 153:514.
  20. Vogel, S. N., and D. L. Rosenstreich. 1979. Defective Fc receptor-mediated phagocytosis in C3H/HeJ macrophages. I. Correction by lymphokine-induced stimulation. *J. Immunol.* 123:2842.
  21. Yudoj, J., T. Masuda, M. Mujama, M. Maeda, and Y. Ichikawa. 1978. Interaction of lymphocytes and macrophage cell line cells (M1 cells). I. Functional maturation and appearance of Fc receptors in M1 cells. *Cell Immunol.* 39:5.
  22. Ezekowitz, R. A. B., M. Bampton, and S. Gordon. 1983. Macrophage activation selectively enhances expression of Fc receptors for IgG2a. *J. Exp. Med.* 157:807.
  23. Koren, H. S., S. J. Anderson, and J. W. Larrick. 1979. In vitro activation of a human macrophage-like cell line. *Nature (Lond.)*. 279:328.
  24. Larrick, J. W., D. S. Fischer, S. J. Anderson, and H. S. Koren. 1980. Characterization of a human macrophage-like cell line stimulated in vitro: a model of macrophage functions. *J. Immunol.* 125:6.
  25. Guyre, P. M., G. R. Crabtree, J. E. Bodwell, and A. Munck. 1981. MLC-conditioned media-stimulate an increase in Fc receptors on human macrophages. *J. Immunol.* 126:666.
  26. Talieh-Dayton, E., B. Perussia, and G. Trinchieri. 1983. Correlation between differentiation, expression of monocyte-specific antigens, and cytotoxic functions in human promyelocytic cell lines treated with leukocyte-conditioned medium. *J. Immunol.* 130:1120.
  27. Fridman, W. H., I. Gresser, M. T. Bandu, M. Aguet, and C. Neauport-Sautes. 1980. Interferon enhances the expression of Fc(gamma) receptors. *J. Immunol.* 124:2436.
  28. Itoh, K., M. Inoue, S. Kataoka, and K. Kumaga. 1980. Differential effect of interferon expression of IgG- and IgM-Fc receptors on human lymphocytes. *J. Immunol.* 124:2589.
  29. Gallagher, R. E., S. J. Collins, J. Trujillo, K. McCredia, M. Ahearn, S. Tsai, R. Metzgar, G. Aulakh, R. Ting, F. Ruscetti, and R. Gallo. 1979. Characterization of the continuous differentiating myeloid cell line (HL60) from a patient with acute promyelocytic leukemia. *Blood.* 54:713.
  30. Minoru, F., H. P. Koefler, and J. Minowada. 1981. Membrane differentiation in human myeloid cells: expression of unique profiles of cell surface glycoproteins in myeloid leukemia cell lines blocked at different stages of differentiation. *Proc. Natl. Acad. Sci. USA* 78:6299.
  31. Bennett, J. M., D. Catovsky, G. Flandrin, D. A. G. Galton, H. R. Gralnick, and C. Sultan. 1976. Proposals for the classification of the acute leukaemias. *Br. J. Haematol.* 33:451.
  32. Perussia, B., D. Lebman, S. H. Ip, G. Rovera, and G. Trinchieri. 1981. Terminal differentiation surface antigens of myelomonocytic cells are expressed in human promyelocytic leukemia cells (HL60) treated with chemical inducers. *Blood.* 58:836.
  33. Hunter, W. M., and F. C. Greenwood. 1962. Preparation of iodine-131-labeled human growth hormone of high specific activity. *Nature (Lond.)*. 194:495.
  34. Perussia, B., L. Mangoni, H. D. Engers, and G. Trinchieri. 1980. Interferon production by human and murine lymphocytes in response to alloantigens. *J. Immunol.* 125:1589.
  35. Scatchard, G. 1949. The attraction of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.* 51:660.
  36. Trinchieri, G., D. Santoli, R. R. Dee, and B. B. Knowles. 1978. Anti-viral activity induced by culturing lymphocytes with tumor-derived or virus-transformed cells.

- Identification of the anti-viral activity as interferon and characterization of the human effector lymphocyte subpopulation. *J. Exp. Med.* 147:1299.
37. Basham, T. Y., and T. C. Merigan. 1983. Recombinant interferon- $\gamma$  increases HLA-DR synthesis and expression. *J. Immunol.* 130:1492.
  38. Kaplan, G., and G. Gaudernack. 1982. In vitro differentiation of human monocytes. Differences in monocyte phenotypes induced by cultivation on glass or on collagen. *J. Exp. Med.* 156:1101.
  39. Bauke, R., and P. G. Spear. 1979. Membrane proteins specified by Herpes Simplex Viruses. V. Identification of an Fc-binding glycoprotein. *J. Virol.* 32:779.
  40. Ennis, F. A., and A. Meager. 1982. Immune interferon produced to high levels by antigenic stimulation of human lymphocytes with influenza virus. *J. Exp. Med.* 154:1279.
  41. Rasmussen, L. E., G. W. Jordan, D. A. Stevens, and T. C. Merigan. 1974. Lymphocyte interferon production and transformation after herpes simplex infections in humans. *J. Immunol.* 112:728.
  42. Ragsdale, C. G., and W. P. Arend. 1980. Loss of Fc receptor activity after culture of human monocytes on surface-bound immune complexes. *J. Exp. Med.* 151:32.
  43. Griffin, F. M. Jr. 1980. Effects of soluble immune complexes on Fc receptor- and C3b receptor-mediated phagocytosis by macrophages. *J. Exp. Med.* 152:905.
  44. Pober, J. S., M. A. Gimbrone, R. S. Cotran, C. S. Reiss, S. J. Burakoff, W. Fiers, and K. A. Ault. 1983. Ia expression by vascular endothelium is inducible by activated T cells and human  $\gamma$ -interferon. *J. Exp. Med.* 157:1339.
  45. Yoshie, O., I. S. Mellman, R. G. Broeze, M. Garcia-Blanco, and P. Lengyel. 1982. Interferon action: effects of mouse  $\alpha$  and  $\beta$  interferons on rosette formation, phagocytosis, and surface-antigens expression of cells of the macrophage-type line RAW 309Cl.1. *Cell. Immunol.* 73:128.
  46. Vogel, S. N., L. L. Weedon, R. N. Moore, and D. L. Rosenstreich. 1982. Correction of defective macrophage differentiation in C3H/HeJ mice by an interferon-like molecule. *J. Immunol.* 128:1981.
  47. Samuel, C. E. 1982. Molecular mechanisms of interferon action: interferon-mediated phosphorylation of ribosome-associated protein P1 and protein synthesis initiation factor eIF-2. In *The Interferon System: A Review to 1982. Tex. Rep. Biol. Med.* 41:463.
  48. Dolei, A., M. R. Capobianchi, and F. Ameglio. 1983. Human interferon- $\gamma$  enhances the expressions of class I and class II major histocompatibility complex products in neoplastic cells more effectively than interferon  $\alpha$  and interferon  $\beta$ . *Infect. Immun.* 40:172.
  49. Pfeffer, L. M., E. Wang, and I. Tamm. 1980. Interferon effects on microfilament organization, cellular fibronectin distribution, and cell motility in human fibroblasts. *J. Cell Biol.* 85:9.
  50. Pfeffer, L. M., E. Wang, and I. Tamm. 1980. Interferon inhibits the redistribution of cell surface components. *J. Exp. Med.* 152:469.
  51. Perussia, B., D. Leberman, G. Pegoraro, B. Lange, C. Damsky, D. Aden, J. Vartikar, G. Trinchieri, and G. Rovera. 1982. Induction of differentiation of human myeloid leukemia cells by phorbol diesters. In *Maturation Factors and Cancer*. M.A.S. Moore, editor, Raven Press, New York. 273.
  52. Anderson, L. C., M. Jokinen, and C. G. Gahmberg. 1979. Induction of erythroid differentiation in the human leukemia cell line K562. *Nature (Lond.)* 278:364.