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# Recombinant Immune Interferon Increases Immunoglobulin G Fc Receptors on Cultured Human Mononuclear Phagocytes

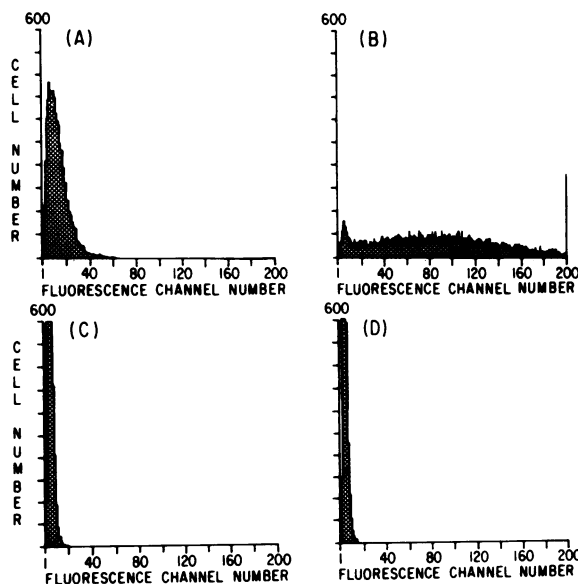
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**ABSTRACT** Although recent studies suggest that interferons can increase the number of IgG Fc receptor (FcR $\gamma$ ) sites on mouse macrophages, direct assessment of similar effects on human mononuclear phagocytes is lacking. We therefore measured the specific binding of  $^{125}$ I- and fluorescein-labeled IgG1 to human monocytes and leukemic cell lines after culture in vitro with highly purified human interferons. We report that natural and recombinant human  $\gamma$ -interferon causes a dramatic (nearly 10-fold) increase in the number of FcR $\gamma$  on normal human monocytes and on the human cell lines HL-60 and U-937. Alpha and  $\beta$ -interferons cause a modest but significant increase in these receptors. This report demonstrates that  $\gamma$ -interferon acts directly on human mononuclear phagocytes to increase FcR $\gamma$  sites, it identifies a qualitative difference in the physiologic actions of human type I and type II interferons, and it suggests that HL-60 and U-937 cells will be important models for further study of the molecular mechanisms of interferon action. The results reported here could also be the basis for a bioassay to assess the pharmacokinetics and variability of  $\gamma$ -interferon action on monocytes of individual patients during treatment in vitro and in vivo.

## INTRODUCTION

Receptors for the Fc portion of IgG (Fc receptors or FcR $\gamma$ )<sup>1</sup> are important for normal mononuclear phagocyte functions including clearance of immune com-

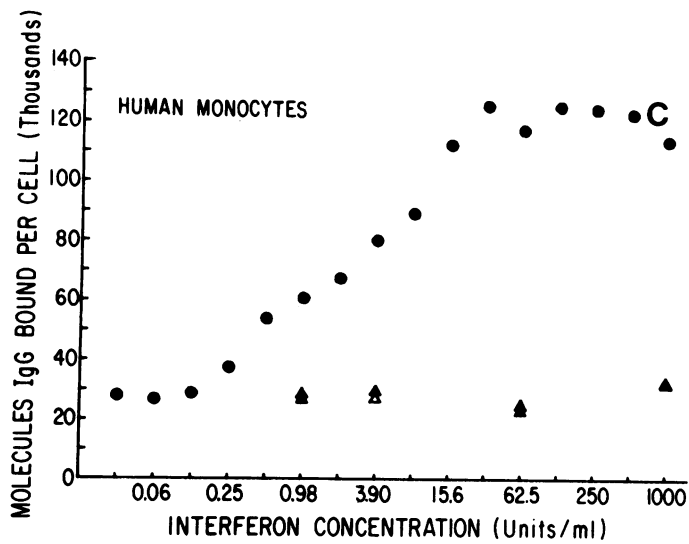
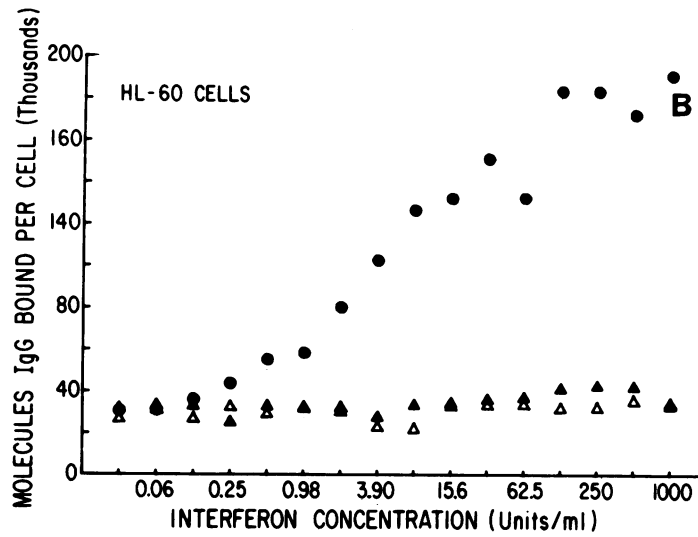
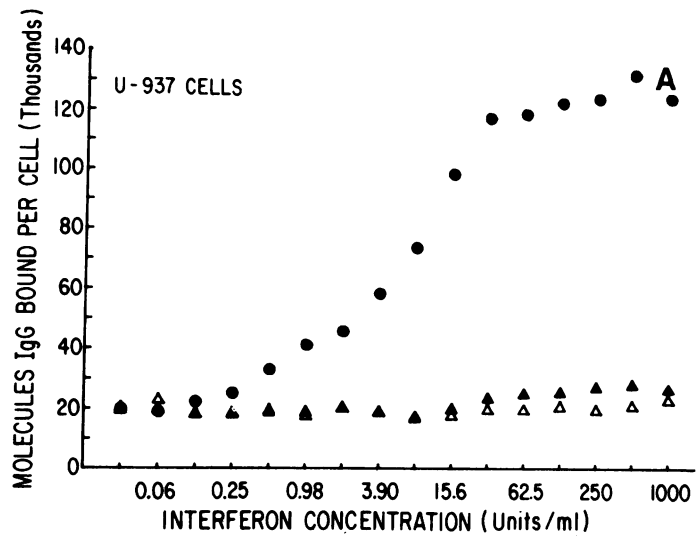
plexes (1), phagocytosis (2), antibody-dependent cellular cytotoxicity (3), and tumoricidal actions in vivo of monoclonal antibodies (4). FcR $\gamma$  probably also play



**FIGURE 1**  $\gamma$ -Interferon augmentation of human monocyte FcR $\gamma$ .  $1 \times 10^7$  monocytes were cultured for 48 h in 120-ml teflon vessels (Scientific Specialties, Randallstown, MD) in 10 ml of RPMI 1640 plus  $5 \times 10^{-5}$  M 2-mercaptoethanol plus 10% autologous serum, with (B and D) or without (A and C) 10 U/ml recombinant  $\gamma$ -interferon. FITC-IgG binding was assayed by flow cytometry as described. FcR $\gamma$  increased from a mean of 18,000 sites/cell for control cultures (A) to 162,000 sites/cell following interferon treatment (B). Nonsaturable binding of the fluorescent IgG was identical for both treatments (C and D).

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<sup>1</sup> Abbreviations used in this paper: FcR $\gamma$ , receptors for the Fc portion of IgG; FITC, fluorescein isothiocyanate.



a role in several autoimmune diseases (5), in B cell mitogenesis (6) and in prostaglandin and collagenase release (7) by macrophages. Although lymphokines stimulate some of these cellular functions, both the identification of the active moiety and the mechanisms underlying the effects of lymphokines are not well known. Defective expression of FcR $\gamma$  on macrophages from C3H/HeJ mice is corrected by murine  $\beta$ - and  $\gamma$ -interferons (8), and murine  $\alpha$ -interferon has similar activity (9). The augmentation of human monocyte FcR $\gamma$ , which we have described (10), is one of the largest lymphokine-induced macrophage activation effects reported to date (11). During purification of the "Fc receptor-augmenting factor" we noted many physicochemical similarities to immune ( $\gamma$ ) interferon, and we now show that human  $\gamma$ -interferon is a potent Fc receptor-augmenting factor, which probably accounts for the activity we identified in crude lymphokine preparations.

## METHODS

**Cells.** HL-60 and U-937 cells (gifts of Dr. R. Gallo, National Cancer Institute, and Dr. P. Ralph, Sloan Kettering Institute, respectively) were cultured in RPMI 1640 (KC Biological, Inc., Lenexa, KS) plus 10% fetal bovine serum (Sterile Systems, Inc., Logan, UT). Human monocytes were obtained by adherence to plastic (7) and subsequent release by incubation for 2 h at 4°C. These cultures contained 84–94% monocytes as determined by morphology and specific staining with monoclonal antibody 63D3 (12).

**Analysis of Fc receptors.** We (10) and others (13) have characterized Fc receptors on human monocytes, HL-60 cells (14), and U-937 cells (15) using a standard radiolabeled IgG1-binding assay. In this report we also use a flow cytometric method for measurement of Fc receptors (16). Human IgG1 was purified from the plasma of a patient with myeloma (kindly provided by Dr. G. Cornwell, Dartmouth Medical School) radioiodinated, and  $^{125}\text{I}$ -IgG1-binding to HL-60 and U-937 cells was quantified by slight modification (see Figs. 1 and 2 legends) of methods previously reported (10, 14). Fluorescein isothiocyanate-labeled IgG1 (FITC-IgG1; fluorescein/protein ratio 3.8) was prepared according to Goding (17). For flow cytometric analysis, cells cultured in human serum were washed five times with 1 ml RPMI 1640, and stained for 2 h at 37°C with  $4 \times 10^{-8}$  M FITC-IgG1, in the absence or presence of  $4 \times 10^{-6}$  M unlabeled IgG (to assess nonsaturable binding). Cells were then washed twice with ice-cold Dulbecco's phosphate-buffered saline containing 2 mg/ml bovine serum albumin (PBS-BSA), resuspended in PBS-BSA, and analyzed on a Cytofluorograf System 50H (Ortho Diagnostic Systems, Inc., Westwood, MA) using 488 nm laser excitation. To convert the fluorescence values obtained from the flow cytometer to molecules

of IgG bound per cell, U-937 cells which received identical treatment, were labeled with FITC-IgG1 for assay by fluorescence as above and with  $^{125}\text{I}$ -IgG1 for assay of IgG1 binding by our standard assay (14). Fluorescence was converted to IgG per cell according to the method of Titus et al. (16).

**Interferons.** Human  $\alpha$ -,  $\beta$ -, and  $\gamma$ -interferons (kindly provided by Dr. P. Weck, Genentech, Inc.) were produced in bacterial cultures following cloning of the appropriate genes into *Escherichia coli* (18–20). The interferons used in this study were >95% pure as determined by gel electrophoresis and silver staining (21). For treatment of cells, interferons were diluted in Iscove's-modified Dulbecco's-modified Eagle's medium (KC Biological, Inc., Lenexa, KS).

## RESULTS

Fig. 1 shows the immunofluorescence due to binding of FITC-labeled IgG1 to Fc receptors on normal human monocytes following 48-h culture with or without 10 U/ml  $\gamma$ -interferon. Similar results were obtained with monocytes from each of 14 normal donors. Fc receptor sites increased dramatically on the  $\gamma$ -interferon-treated monocytes, and ranged on individual cells from the control level (15,000–35,000 sites) to >200,000 sites/cell. This marked heterogeneity will permit cell sorting for cells with high or low FcR $\gamma$ , to evaluate the relationship between the number of FcR $\gamma$  sites, and FcR $\gamma$ -dependent functions. The maximal effect is at 48–72 h using 10–100 antiviral U/ml  $\gamma$ -interferon ( $\alpha$ -interferon as standard), with a half-maximal effect between 1 and 5 U/ml. We have found flow cytometry to be superior to radioligand binding for analyses of monocytes, since it permits computer regions to be set for independent evaluation of lymphocytes and monocytes without their prior physical separation. Since our monocyte preparations contained 6–16% lymphocytes in 14 experiments, we have not ruled out the possibility that the lymphocyte is important for the FcR $\gamma$  augmentation reported here. However, since we found identical effects at all levels of lymphocyte contamination, and direct effects on HL-60 and U-937 cells (Fig. 2), this possibility seems unlikely. Fig. 2 shows the specific binding of labeled-IgG1 to human monocytes, HL-60, and U-937 cells after treatment for 16 h with various concentrations of  $\alpha$ -,  $\beta$ -, or  $\gamma$ -interferons. While  $\gamma$ -interferon dramatically increased IgG1-binding to Fc receptors.  $\alpha$ - and  $\beta$ -interferons had minor but significant ( $P < 0.01$ ) effects. In four experiments, the dose that gave a half-maximal increase in IgG binding ranged from 1 to 5

FIGURE 2 Concentration dependence of Fc receptor augmentation.  $2.5 \times 10^5$  HL-60 or U-937 cells/well were cultured for 16 h in 200  $\mu\text{l}$  complete medium in 96-well microtiter trays (Costar, Data Packaging, Cambridge, MA). Human monocytes were cultured as described for Fig. 1 using  $2 \times 10^6$  cells/ml per 30 ml teflon vessel. After incubation with interferons, U-937 and HL-60 cells were labeled with  $^{125}\text{I}$ -IgG1 and the average number of IgG molecules bound per cell was determined as previously described (14). Human monocytes were stained and analyzed by flow cytometry as described. Fig. 2 shows the response to  $\alpha$ -( $\blacktriangle$ ),  $\beta$ -( $\triangle$ ), and  $\gamma$ -( $\bullet$ ) interferon for U-937 cells (2 A), HL-60 cells (2 B), and normal monocytes (2 C).

U  $\gamma$ -interferon/ml. The maximal increase after 16-h treatment was obtained with  $\sim 100$  U/ml and ranged from 4.8- to 7.4-fold. Alpha and beta interferons caused only a 20–50% increase, even at concentrations between 100 and 10,000 U/ml. The increased number of Fc receptors was not accompanied by alterations in cell size or proliferative rate, as determined by Coulter counts, cytocris, and flow cytometric analysis of cell size.

Scatchard analysis of IgG1 binding by U-937 cells following treatment for 40 h with  $\alpha$ -,  $\beta$ -, or  $\gamma$ -interferons showed that while 10 U/ml  $\gamma$ -interferon caused an 8.5-fold increase in the number of FcR $\gamma$ , the binding affinity for IgG did not change significantly (ranging in three experiments from  $5.5 \times 10^8$  M $^{-1}$  to  $2.7 \times 10^8$  M $^{-1}$ , independent of treatment).

We have found in other experiments that treatment of U-937 cells with recombinant  $\gamma$ -interferon results in enhanced antibody-dependent cell-mediated cytotoxicity of chick erythrocytes, which is roughly proportional to the increase in FcR $\gamma$  sites. Thus, treatment with  $\gamma$ -interferon alone is a sufficient stimulus for this type of macrophage activation. However, a second type of Fc receptor present on human neutrophils has been described by Fleit et al. (26), who reported that it is absent from HL-60 cells, U-937 cells, and human monocytes. It will be important for interpretation of functional assays to determine whether this second Fc receptor is expressed on interferon-treated cells.

## DISCUSSION

The major finding in this report is that recombinant human  $\gamma$ -interferon, which is clearly not contaminated with other human lymphokines, causes a dramatic increase in the number of human monocyte Fc receptors. Since an effect of similar magnitude is not obtained with recombinant or natural  $\alpha$ - and  $\beta$ -interferons, even at concentrations as high as 10,000 U/ml, these findings demonstrate an important qualitative difference between type I and type II interferons. They also extend to human mononuclear phagocytes the finding by Vogel et al. (8) that murine  $\gamma$ -interferon increases Fc receptor-dependent phagocytosis by increasing FcR $\gamma$  sites. 10 U/ml  $\gamma$ -interferon, a concentration well below circulating levels of cloned  $\alpha$ -interferon achieved following parenteral administration to humans (23), caused a five- to 10-fold increase in FcR $\gamma$  on monocytes from each of 14 donors tested in vitro. The increasing evidence that antibody-mediated tumor regression in vivo requires functional macrophage Fc receptors (4) suggests to us that cloned  $\gamma$ -interferon could have important effects at concentrations that will be tolerated in vivo, and may prove to be an important adjunct to monoclonal antibody therapy. These results

introduce a new measure of human mononuclear phagocyte function that may provide important information in diseases such as systemic lupus erythematosus, where there is an as yet poorly understood defect in FcR function (5).

## ACKNOWLEDGMENTS

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