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Research Article

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 125I- 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.

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 (FcRγ) 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 γ-interferon causes a dramatic (nearly 10-fold) increase in the number of FcRγ on normal human monocytes and on the human cell lines HL-60 and U-937. Alpha and β-interferons cause a modest but significant increase in these receptors. This report demonstrates that γ-interferon acts directly on human mononuclear phagocytes to increase FcRγ 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 γ-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 FcRγ)[1] are important for normal mononuclear phagocyte functions including clearance of immune complexes (1), phagocytosis (2), antibody-dependent cellular cytotoxicity (3), and tumoricidal actions in vivo of monoclonal antibodies (4). FcRγ probably also play

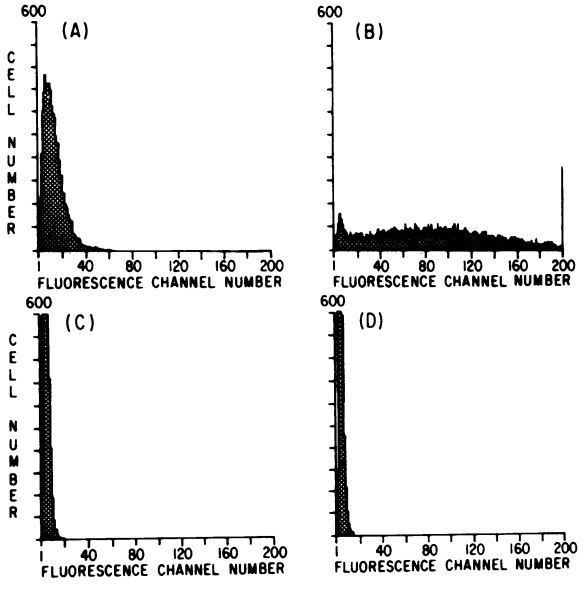

FIGURE 1 γ-Interferon augmentation of human monocyte FcRγ. 1×10^7 monocytes were cultured for 48 h in 120-ml teflon vessels (Scientific Specialties, Randallstown, MD) in 10 ml of RPMI 1640 plus 5×10^{-5} M 2-mercaptoethanol plus 10% autologous serum, with (*B* and *D*) or without (*A* and *C*) 10 U/ml recombinant γ-interferon. FITC-IgG binding was assayed by flow cytometry as described. FcRγ 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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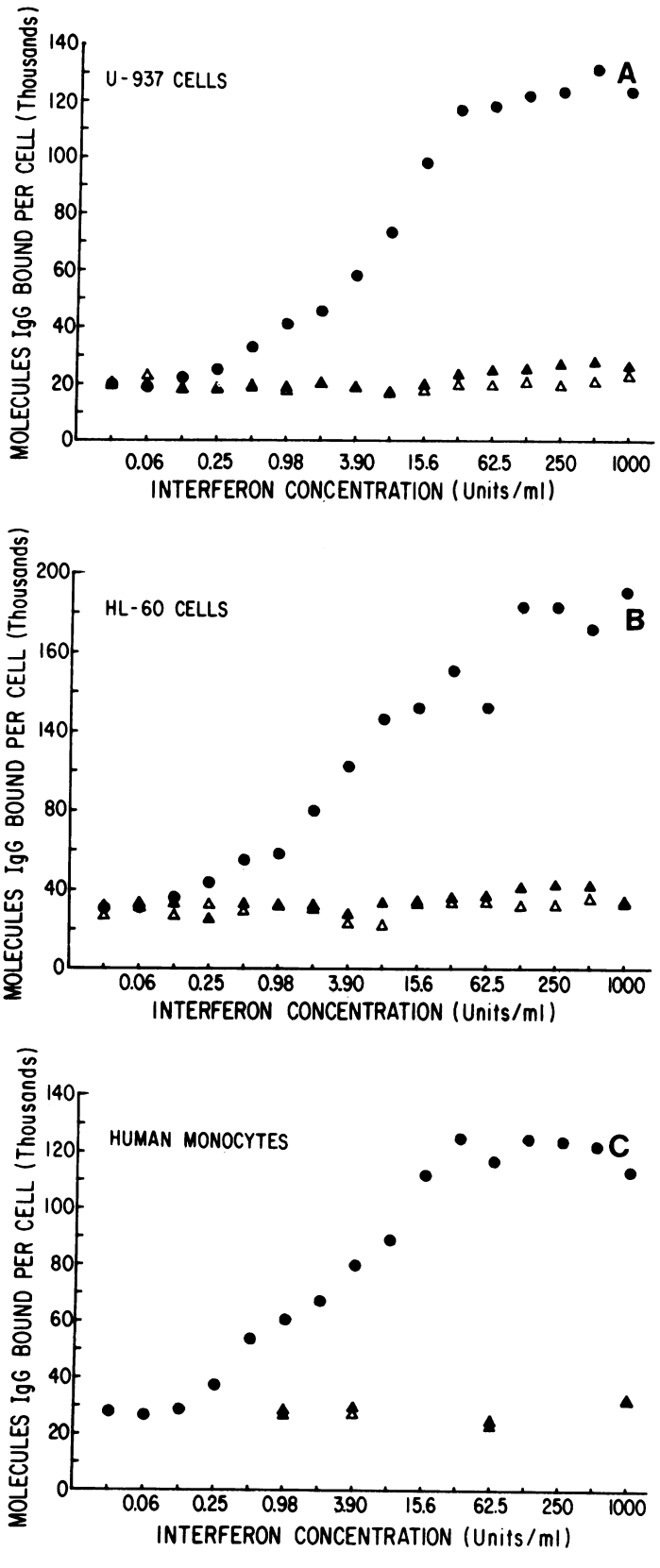
[1] *Abbreviations used in this paper:* FcRγ, receptors for the Fc portion of IgG; FITC, fluorescein isothiocyanate.


U-937 CELLS
A
MOLECULES IgG BOUND PER CELL (Thousands)
140
120
100
80
60
40
20
0
0.06
0.25
0.98
3.90
15.6
62.5
250
1000
INTERFERON CONCENTRATION (Units/ml)
HL-60 CELLS
B
MOLECULES IgG BOUND PER CELL (Thousands)
200
160
140
80
40
0
0.06
0.25
0.98
3.90
15.6
62.5
250
1000
INTERFERON CONCENTRATION (Units/ml)
HUMAN MONOCYTES
C
MOLECULES IgG BOUND PER CELL (Thousands)
140
120
100
80
60
40
20
0
0.06
0.25
0.98
3.90
15.6
62.5
250
1000
INTERFERON CONCENTRATION (Units/ml)

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 FcRγ on macrophages from C3H/HeJ mice is corrected by murine β- and γ-interferons (8), and murine α-interferon has similar activity (9). The augmentation of human monocyte FcRγ, 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 (γ) interferon, and we now show that human γ-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}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 4×10^{-8} M FITC-IgG1, in the absence or presence of 4×10^{-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}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 α-, β-, and γ-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 γ-interferon. Similar results were obtained with monocytes from each of 14 normal donors. Fc receptor sites increased dramatically on the γ-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 FcRγ, to evaluate the relationship between the number of FcRγ sites, and FcRγ-dependent functions. The maximal effect is at 48–72 h using 10–100 antiviral U/ml γ-interferon (α-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 FcRγ 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 α-, β-, or γ-interferons. While γ-interferon dramatically increased IgG1-binding to Fc receptors. α- and β-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. 2.5×10^5 HL-60 or U-937 cells/well were cultured for 16 h in 200 μl complete medium in 96-well microtiter trays (Costar, Data Packaging, Cambridge, MA). Human monocytes were cultured as described for Fig. 1 using 2×10^6 cells/ml per 30 ml teflon vessel. After incubation with interferons, U-937 and HL-60 cells were labeled with ^{125}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 α-(▲), β-(△), and γ-(●) interferon for U-937 cells (2 *A*), HL-60 cells (2 *B*), and normal monocytes (2 *C*).

U γ-interferon/ml. The maximal increase after 16-h treatment was obtained with ~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, cytocrits, and flow cytometric analysis of cell size.

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

We have found in other experiments that treatment of U-937 cells with recombinant γ-interferon results in enhanced antibody-dependent cell-mediated cytotoxicity of chick erythrocytes, which is roughly proportional to the increase in FcRγ sites. Thus, treatment with γ-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 γ-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 α- and β-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 γ-interferon increases Fc receptor-dependent phagocytosis by increasing FcRγ sites. 10 U/ml γ-interferon, a concentration well below circulating levels of cloned α-interferon achieved following parenteral administration to humans (23), caused a five- to 10-fold increase in FcRγ 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 γ-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).

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REFERENCES

1. Leslie, R. G. Q. 1980. Macrophage handling of soluble immune complexes. *Immunol. Today. (Amst.).* 1:78–84.
2. Shaw, D. R., and F. M. Griffin, Jr. 1981. Phagocytosis requires repeated triggering of macrophage phagocytic receptors during particle ingestion. *Nature (Lond.).* 289:409–411.
3. Larrick, J. W., D. G. 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.* 125:6–12.
4. Herlyn, D., and H. Koprowski. 1982. IgG2a monoclonal antibodies inhibit human tumor growth through interaction with effector cells. *Proc. Natl. Acad. Sci. USA.* 79:4761–4765.
5. Frank, M. M. (moderator). 1983. Immunoglobulin Fc receptor functional activity in autoimmune diseases. *Ann. Intern. Med.* 98:206–218.
6. Morgan, E. L., and W. O. Weigle. 1981. Regulation of the immune response: III. The role of macrophages in the potentiation of the immune response by Fc fragments. *J. Immunol.* 126:1302–1306.
7. Passwell, J., F. S. Rosen, and E. Merler. 1980. The effect of Fc fragments of IgG on human mononuclear cell responses. *Cell. Immunol.* 52:395–403.
8. Vogel, S. N., D. S. Finbloom, K. E. English, D. L. Rosenstreich, and S. G. Langreth. 1983. Interferon-induced enhancement of macrophage Fc receptor expression: β-interferon treatment of C3H/HeJ macrophages results in increased numbers and density of Fc receptors. *J. Immunol.* 130:1210–1214.
9. Yoshie, O., I. S. Mellman, R. J. Broeze, M. Garcia-Blanco, and P. Lengyel. 1982. Interferon action: effects of mouse alpha and beta interferons on rosette formation, phagocytosis, and surface-antigen expression of cells of the macrophage-type line RAW309Csl. *Cell. Immunol.* 73:128–140.
10. 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.* 126:666–668.
11. Nakagawara, A., N. M. DeSantis, N. Nogueria, and C. F. Nathan. 1982. Lymphokines enhance the capacity of human monocytes to secrete reactive oxygen intermediates. *J. Clin. Invest.* 70:1042–1048.
12. Ugolini, V., G. Nuney, G. R. Smith, K. P. Stastney, and J. D. Capra. 1980. Initial characterization of monoclonal

antibodies against human monocytes. *Proc. Natl. Acad. Sci. USA.* 77:6764–6768.
13. 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.* 69:1–8.
14. Crabtree, G. R. 1980. Fc receptors of a human promyelocytic leukemic cell line: evidence for two types of receptors defined by binding of the staphylococcal protein A-IgG1 complex. *J. Immunol.* 125:448–453.
15. Anderson, C. L., and G. N. Abraham. 1980. Characterization of the Fc receptor for IgG on a human macrophage cell line, U-937. *J. Immunol.* 125:2735–2741.
16. Titus, J. A., B. F. Haynes, C. A. Thomas, A. S. Fauci, and D. M. Segal. 1982. Analysis of Fc-gamma receptors on peripheral blood leukocytes by flow microfluorometry I. Receptor distributions on monocytes, T-cells, and cells labeled with the 3A2 anti-T-cell monoclonal antibody. *Eur. J. Immunol.* 12:474–479.
17. Goding, J. W. 1976. Conjugation of antibodies with fluorochromes: modifications to the standard methods. *J. Immunol. Methods.* 13:215–226.
18. Goeddel, D. V., D. W. Leung, T. J. Dull, M. Gross, R. M. Lawn, R. McCandliss, P. H. Seeburg, A. Ullrich, E. Yelverton, and P. W. Gray. 1981. The structure of eight distinct cloned human leukocyte interferon cDNAs. *Nature (Lond.).* 290:20–27.
19. Derynck, R., J. Content, E. DeClercq, G. Volckaert, J. Tanernier, R. Devos, and W. Fiers. 1980. Isolation and structure of a human fibroblast interferon gene. *Nature (Lond.).* 285:542–547.
20. Gray, P. W., D. W. Leung, D. Pennica, E. Yelverton, R. Najarian, C. C. Simonsen, R. Derynck, P. J. Sherwood, D. M. Wallace, S. L. Berger, A. D. Levinson, and D. V. Goeddel. 1982. Expression of human immune interferon cDNA in *E. coli* and monkey cells. *Nature (Lond.).* 295:503–508.
21. Oakley, B. R., D. R. Kirsch, and N. R. Morris. 1980. A simplified ultrasensitive silver stain for detecting proteins in polyacrylamide gels. *Anal. Biochem.* 105:361–363.
22. Fleit, H. B., S. D. Wright, and J. C. Unkeless. 1982. Human neutrophil Fc receptor distribution and structure. *Proc. Natl. Acad. Sci. USA.* 79:3275–3279.
23. Gutterman, J. V., S. Fine, J. Quesada, S. J. Horning, J. F. Levine, R. Alexanian, J. Bernhardt, M. Kramer, H. Spiegel, K. Colburn, P. Trown, T. Merigan, and Z. Dziewanowski. 1982. Recombinant leukocyte A interferon: pharmacokinetics, single dose tolerance, and biologic effects in cancer patients. *Ann. Intern. Med.* 96:549–555.
24. Guyre, P. M., J. E. Bodwell, and A. Munck. 1981. Glucocorticoid actions on the immune system: inhibition of production of an Fc receptor-augmenting factor. *J. Steroid Biochem.* 15:35–39.