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Gamma Interferon Augments Fc_γ Receptor-Mediated Dengue Virus Infection of Human Monocytic Cells

UDO KONTNY,† ICHIRO KURANE, AND FRANCIS A. ENNIS*

Division of Infectious Diseases, Department of Medicine, University of Massachusetts Medical Center, Worcester, Massachusetts 01605

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It has been reported that anti-dengue antibodies at subneutralizing concentrations augment dengue virus infection of monocytic cells. This is due to the increased uptake of dengue virus in the form of virus-antibody complexes by cells via Fc_γ receptors. We analyzed the effects of recombinant human gamma interferon (rIFN-γ) on dengue virus infection of human monocytic cells. U937 cells, a human monocytic cell line, were infected with dengue virus in the form of virus-antibody complexes after rIFN-γ treatment. Pretreatment of U937 cells with rIFN-γ resulted in a significant increase in the number of dengue virus-infected cells and in the yield of infectious virus. rIFN-γ did not augment dengue virus infection when cells were infected with virus in the absence of anti-dengue antibodies. Gamma interferon (IFN-γ) produced by peripheral blood lymphocytes from dengue-immune donors after in vitro stimulation with dengue antigens also augmented dengue virus infection of U937 cells. IFN-γ did not augment dengue virus infections when cells were infected with virus in the presence of $F(ab')_2$ prepared from anti-dengue immunoglobulin G. Human immunoglobulin inhibited IFN-γ-induced augmentation. IFN-γ increased the number of Fc_γ receptors on U937 cells. The increase in the percentage of dengue antigen-positive cells correlated with the increase in the number of Fc_γ receptors after rIFN-γ treatment. These results indicate that IFN-γ-induced augmentation of dengue virus infection is Fc_γ receptor mediated. Based on these results we conclude that IFN-γ increases the number of Fc_γ receptors and that this leads to an augmented uptake of dengue virus in the form of dengue virus-antibody complexes, which results in augmented dengue virus infection.

Dengue is a tropical illness caused by dengue virus infection of humans and is transmitted by mosquitoes. The disease is endemic over a large part of Southeast Asia, the Pacific, Africa, and Central America. Clinically, infection with dengue virus presents in two major forms, dengue fever and dengue hemorrhagic fever-dengue shock syndrome (DHF-DSS). Dengue fever is a self-limited disease and represents most cases of dengue. In some cases, however, patients develop severe complications, DHF-DSS, which are life threatening. From 1980 through 1985, 500,000 cases of DHF-DSS were reported worldwide (16).

Human monocytes support active replication of dengue virus in vivo (11). Studies done by Halstead et al. have shown that monocytes are infected to a much greater extent in vitro in the presence of anti-dengue virus antibody (7, 10). This is due to the increased uptake by monocytes of dengue virus in the form of dengue virus-antibody complexes via Fc_γ receptors (4, 10). This phenomenon is referred as immune enhancement. Interestingly, most cases of DHF-DSS occur during secondary dengue infections when there are dengue virus antibodies present (2, 8, 9), and immune enhancement may play an important role in the pathogenesis of DHF-DSS (18). We have reported that T lymphocytes of dengue antibody-positive donors produce high titers of gamma interferon (IFN-γ) after stimulation with dengue antigens in vitro (I. Kurane, B. L. Innis, A. Nisalak, C. Hoke, S. Nimmannitya, A. Meager, and F. A. Ennis, submitted for publication). Therefore, it can be expected that IFN-γ is produced in vivo during secondary dengue infections. It has been reported that IFN-γ increases the number of Fc_γ receptors on human monocytes and monocytic cell lines (6, 17). In this study we investigated the effect of IFN-γ on dengue virus infection of U937 cells, a human monocytic cell line. IFN-γ augmented dengue virus infection of U937 cells when cells were infected in the presence of anit-dengue antibody. The augmenting effect of IFN-γ was Fc_γ receptor dependent. We conclude that IFN-γ increases the number of Fc_γ receptors and that this results in the augmented infection of the cells by dengue virus-antibody complexes.

MATERIALS AND METHODS

Virus and antibody. Dengue virus type 2, New Guinea C strain, was used for infection. The virus was supplied by Walter E. Brandt of the Walter Reed Army Institute of Research, Washington, D.C. The virus was passed in mouse brain and then propagated in mosquito cells (C6/36) as previously described (14). The titer of the virus pool used in these experiments was 10^7 PFU/ml in Vero cells as determined by previously described methods (12). Ascitic fluid from mice hyperimmunized with dengue virus type 2 was used as a source of anti-dengue virus type 2 antibody. This antibody was also supplied by Walter E. Brandt. The titer of this antibody was 1:1,024 as determined by a plaque neutralization test (14). Hyperimmune ascitic fluid was heated at 56°C for 30 min to destroy complement activity before use.

Cells cultures. U937, a monocytic cell line that was derived from a patient with histiocytic lymphoma (19), was used. Cells were cultured in RPMI 1640 medium (Flow Laboratories, McLean, Va.) supplemented with 10% fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.).

IFNs and anti-IFN antibodies. Human recombinant IFN-γ (rIFN-γ; Hoffman-LaRoche, Inc., Nutley, N.J.) and human lymphoblastoid IFN-α (Lee-Biomolecular Research, San

* Corresponding author.
† Present address: University of Tübingen Medical School, 7400 Tübingen, Federal Republic of Germany.

Diego, Calif.) were used. For neutralization experiments, a monoclonal antibody to human IFN-γ (Interferon Sciences, New Brunswick, N.J.) and a rabbit polyclonal antibody to IFN-α (Interferon Sciences) were used.

Treatment of cells with human γ-globulin. U937 cells were incubated with 10 mg of of human immunoglobulin (γ-globulin) (Sigma Chemical Co., St. Louis, Mo.) per ml or bovine albumin fraction V powder (GIBCO) in RPMI for 20 min at 4°C. The cells were washed three times and then infected by dengue virus-antibody complexes. The human γ-globulin used in the experiments had no neutralizing activity (<1:10) for dengue virus type 2 when it was tested in a plaque-neutralization assay at a concentration of 10 mg/ml.

Culture fluid of PBMC stimulated with dengue antigen. Peripheral blood mononuclear cells (PBMC) from a donor who previously had been infected with dengue virus type 3 and subsequently developed antibodies to dengue virus type 3 were stimulated with a dengue virus type 3 antigen preparation or control antigen for 7 days. This antigen was prepared from dengue virus-infected Vero cells that were treated with 0.025% glutaraldehyde and sonicated. Control antigen was prepared from uninfected Vero cells, which were also glutaraldehyde treated and sonicated. Culture fluids were tested for IFN-γ and IFN-α by Anthony Meager, London, by using a radioimmunoassay with specific monoclonal antibodies for human IFN-γ and IFN-α (15).

Infection of cells with dengue virus. U937 cells were washed once in RPMI 1640 containing 1% fetal calf serum and suspended to a concentration of 10^6 cells per 0.1 ml. They were then incubated with dengue virus type 2 or with dengue virus type 2-antibody complexes for 2 h. These virus-antibody complexes were made before infection by adding 10 μl of anti-dengue virus type 2 mouse ascites fluid at a dilution of 1:200 to 5×10^6 PFU of dengue virus type 2 in 0.5 ml and were incubated for 1 h at 4°C. The multiplicity of infection (MOI) of the virus inoculum was 5 PFU per cell. Cells were washed twice after 2 h of incubation with virus or virus-antibody complexes and then cultured at a concentration of 2×10^5 cells per ml in RPMI 1640 containing 10% fetal calf serum for 24 h. Cells were then stained for the presence of dengue viral antigen by immunofluorescence as previously described (14). The mouse anti-dengue virus type 2 serum described above and a fluorescein isothiocyanate-conjugated sheep anti-mouse immunoglobulin G (IgG) antibody (Cappel Laboratories, Malvern, Pa.) were used.

Preparation of $F(ab')_2$ from anti-dengue virus type 2 IgG. $F(ab')_2$ was prepared from anti-dengue virus type 2 mouse ascites fluid with an Immuno Pure $F(ab')_2$ preparation kit (Pierce Chemical Co., Rockland, Ill.). Mouse ascites fluid was diluted 1:2 with the binding buffer (pH 8.2), and 1 ml of the diluted fluid was applied to a 1-ml column of immobilized protein A. The column was washed with 15 column volumes of the binding buffer. IgG was eluted with 5 column volumes of the elution buffer (pH 2.8). Eluted IgG was dialyzed against 20 mM sodium acetate at pH 4.2 and concentrated to an IgG concentration of 20 mg/ml with a minicon-A25 (Amicon Corp., Danvers, Mass.). Then 10 mg of IgG was treated with 12.5% immobilized pepsin (Pierce) in 1 ml of 20 mM sodium acetate (pH 4.2) at 37°C for 4 h. The crude digest (10 mg in 3 ml) was applied to a 3-ml column of immobilized protein A, and $F(ab')_2$ was eluted with 6 ml of the binding buffer. IgG and $F(ab')_2$ were dialized against phosphate-buffered saline (pH 7.4) at 4°C for 72 h. The purity of $F(ab')_2$ and IgG was examined by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis. $F(ab')_2$ did not contain any detectable IgG. $F(ab')_2$ and purified IgG were concentrated to a concentration of 1 mg/ml with a minicon-A25. The neutralizing antibody titers of purified IgG and $F(ab')_2$ at 1 mg/ml were 1:320 and 1:160, respectively, determined by a plaque neutralization test for dengue virus type 2 (14).

Detection of Fc_γ receptors. Fc receptors were analyzed by quantitative flow cytometry with a monoclonal antibody, MAb 32, kindly provided by Paul Guyre of Dartmouth Medical School, Hanover, N.H. (1). MAb32 is an IgG1 antibody which reacts with the high-affinity Fc receptor on U937 cells and human monocytes (1). Cells (2.5×10^6) were incubated with MAb32 at a final dilution of 1:3 in 150 μl of RPMI 1640 containing 40 mg of human γ-globulin per ml at 4°C for 2 h. Cells were washed twice in phosphate-buffered saline containing 2% bovine serum albumin and incubated with 100 μl of FITC-conjugated goat $F(ab')_2$ anti-mouse IgG antibody (Caltag Laboratories, South San Francisco, Calif.) at a final dilution of 1:30 at 4°C for 2 h. Cells were then washed two times in phosphate-buffered saline containing 2% bovine serum albumin and fixed with 2% paraformaldehyde. Stained cells were analyzed with a fluorescence-activated cell sorter (Coulter 753; Coulter, Hialeah, Fla.). Quantitative fluorescein microbeads (Flow Cytometry Standards Corp., Research Triangle Park, N.C.) were used for calibration to convert the fluorescence intensity values obtained from the flow cytometer into the number of molecules of second antibody bound per cell. Since the number of molecules of second antibody bound per cell is expected to be proportional to the number of molecules of the first antibody, which is the MAb32 to the high-affinity Fc receptor, the number of second antibody molecules bound per cell can serve as a relative measurement of the number of Fc receptors per cell.

RESULTS

IFN-γ augments dengue virus infection of U937 cells in the presence of anti-dengue antibody. U937 cells were incubated with 100 U of rIFN-γ per ml for 24 h and then infected with dengue virus at an MOI of 5 PFU per cell in the presence of anti-dengue mouse serum. The percentage of dengue antigen-positive cells was determined by indirect immunofluorescence staining 24 h after infection. Anti-dengue serum at final dilutions of $1:10^3$, $1:10^4$, and $1:10^5$ augmented infection of nontreated U937 cells and further augmented dengue virus infection when U937 cells were pretreated with rIFN-γ (Fig. 1). Normal mouse serum, which did not contain detectable levels of anti-dengue antibody, did not augment dengue virus infection of the nontreated or the IFN-γ-treated U937 cells (data not shown). Based on these results, we decided to use anti-dengue serum at a final dilution of $1:10^4$ in the following experiments.

U937 cells were incubated with variable amounts of rIFN-γ for 24 h and infected with dengue virus at an MOI of 5 PFU per cell in the presence of the anti-dengue mouse serum at a final dilution of $1:10^4$. Pretreatment of U937 cells with rIFN-γ at concentrations from 1 to 10,000 U/ml increased the percentage of dengue antigen-positive cells. The percentage of antigen-positive cells reached a maximum level by pretreatment with IFN-γ at 100 U/ml (Fig. 2). When U937 cells were infected with dengue virus in the absence of anti-dengue antibody, pretreatment with rIFN-γ did not increase the percentage of antigen-positive cells (1 to 3% without rIFN-γ treatment and 1 to 4% with rIFN-γ treatment). Pretreatment of U937 cells with 100 U of rIFN-γ per ml also increased dengue virus titers in the culture fluids when cells were infected with virus in the presence of

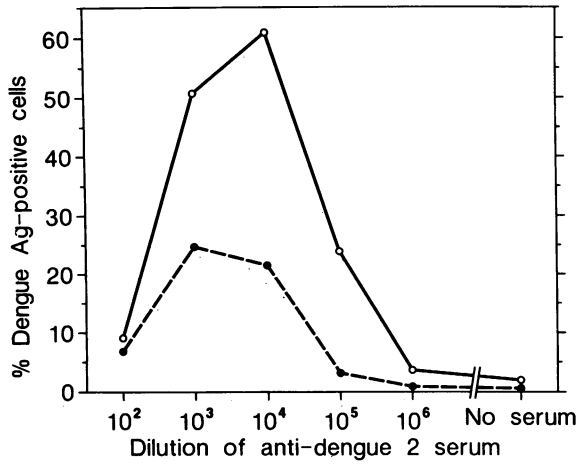

FIG. 1. Effect of dilution of anti-dengue serum on dengue virus infection of U937 cells. U937 cells were incubated with or without 100 U of rIFN-γ per ml for 24 h and infected with dengue virus at an MOI of 5 PFU/ml in the presence of variable dilutions of anti-dengue mouse serum. The percentage of dengue antigen-positive cells was determined by indirect immunofluorescence staining 24 h after infection. Symbols: ○, U937 cells pretreated with rIFN-γ; ●, nontreated U937 cells. The percentage of antigen-positive cells was compared between IFN-γ-pretreated cells and nontreated cells at same dilutions of anti-dengue virus type 2 serum. $P < 0.001$ at serum dilutions of 10^{-3}, 10^{-4}, and 10^{-5}; $P < 0.05$ at serum dilution of 10^{-6}. $P > 0.05$ (not significant) at a serum dilution of 10^{-2} and with no serum.

antibody but not when cells were infected in the absence of antibody (Table 1).

IFN-γ produced by dengue-immune lymphocytes after stimulation with dengue antigen augments dengue virus infection of U937 cells. IFN-γ produced by human lymphocytes in vitro was used in the following experiments. PBMC from a dengue antibody-positive donor were cultured with dengue antigen for 7 days, and the culture fluid was collected. The culture fluid contained 650 U of IFN-γ per ml and no detectable IFN-α as determined by radioimmunoassays specific for human IFN-γ and IFN-α. U937 cells were incubated for 24 h with various dilutions of this culture fluid and infected with dengue virus-antibody complexes. The diluted culture fluids from lymphocytes of the dengue-immune donor that had been stimulated with dengue antigens and contained 10 or 100 U of IFN-γ per ml augmented dengue virus infection of U937 cells as well as recombinant IFN-γ (Table 2).

TABLE 1. Virus titers in the culture fluids of dengue virus-infected U937 cells pretreated with IFN-γ[a]

IFN-γ (U/ml)	Virus titer (PFU/ml)	
	With anti-dengue antibody	Without antibody
0	5.0×10^3	3.5×10^2
1	4.5×10^4	4.0×10^2
10	6.0×10^4	6.5×10^2
100	9.0×10^4	7.0×10^2
1,000	1.0×10^5	6.0×10^2

[a] U937 cells were incubated with various concentrations of rIFN-γ for 24 h and infected with dengue virus at an MOI of 5 PFU per cell in the presence of anti-dengue mouse serum at final dilution of 10^{-4} or in the absence of antiserum. Cells were cultured at 3×10^5 cells per ml in RPMI 1640 containing 10% fetal calf serum for 24 h. Titers of dengue virus contained in the culture fluids were determined by using a plaque titration assay.

Anti-IFN-γ antibody inhibits IFN-γ-induced augmentation of dengue virus infections. To confirm that the IFN-γ contained in the culture fluid is responsible for the augmented dengue virus infection shown in Table 2, culture fluid which contained 10 U of IFN-γ per ml was incubated with a monoclonal anti-IFN-γ antibody and then used to treat U937 cells. Culture fluid pretreated with an anti-IFN-γ antibody did not augment dengue virus infection, but the culture fluid pretreated with an anti-IFN-α antibody did augment infection (Table 3). We also tried to block the effect of rIFN-γ by using a monoclonal anti-IFN-γ antibody to confirm that the rIFN-γ-induced augmentation of infection was due to IFN-γ and not to other substances derived from the production of this rIFN-γ in *Escherichia coli* (3). U937 cells were pretrea-

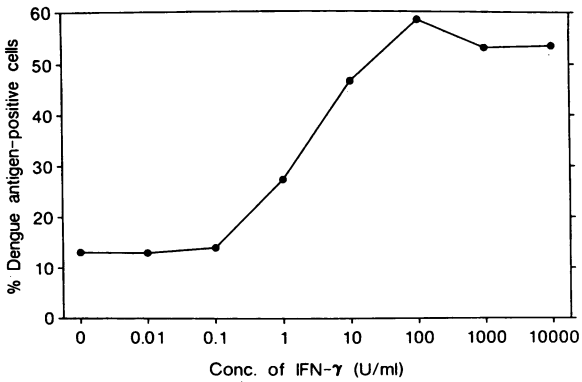

FIG. 2. Dengue virus infection of U937 cells pretreated with IFN-γ. U937 cells were incubated with variable concentrations of IFN-γ for 24 h and then infected with dengue virus-antibody complexes at an MOI of 5 PFU per cell. The percentage of dengue antigen-positive cells was determined by indirect immunofluorescent staining 24 h after infection. The percentage of antigen-positive cells was compared between IFN-γ-pretreated cells and nontreated cells. $P < 0.001$ at IFN-γ concentration of 1, 10, 100, 1,000, and 10,000 U/ml. $P > 0.05$ (not significant) at 0.01 and 0.1 U/ml.

TABLE 2. Culture fluid of dengue-immune donor PBMC stimulated with dengue antigen augments dengue virus infection of U937 cells

Source of IFN-γ	Titer (U/ml)	% of dengue antigen-positive cells[a]
None	0	11.5
Dengue culture fluid[b]	1	13.2[c]
	10	21.6[d]
	100	32.6[e]
Recombinant IFN-γ	100	42.6[e]

[a] U937 cells were incubated with rIFN-γ or with dengue virus-stimulated culture fluid for 24 h and then infected with dengue virus-antibody complexes at an MOI of 5 PFU per cell. The percentage of dengue antigen-positive cells was determined by indirect immunofluorescence 24 h after infection. The percentage of antigen-positive cells was compared between IFN-γ-pretreated cells and nontreated cells.

[b] The PBMC from a dengue antibody-positive donor were cultured with dengue antigen for 7 days, and culture fluid was collected. This culture fluid, which contained IFN-γ at a titer of 650 U/ml and no detectable IFN-α as determined by radioimmunoassay, was diluted to contain 1, 10, or 100 U of IFN per ml for pretreatment of U937 cells.

[c] $P > 0.05$ (not significant).

[d] $P < 0.01$.

[e] $P < 0.001$.

TABLE 3. IFN-γ contained in the culture fluid of PBMC is responsible for augmenting dengue virus infection of U937 cells

Source of IFN-γ	Antibodies[a]	% of dengue antigen-positive cells[b]
Dengue culture fluid[c]	None	66.0
	Anti-IFN-γ	20.5
	Anti-IFN-α	59.6
Control culture fluid[d]	None	20.9
None	None	15.0
	Anti-IFN-γ	16.4
	Anti-IFN-α	12.1

[a] Dengue culture fluid which contained IFN-γ was duluted to 10 U/ml and then incubated with 1,000 U of monoclonal anti-IFN-γ per ml and 2,000 U of anti-IFN-α at 4°C for 2 h.

[b] U937 cells were incubated with culture fluids for 24 h and infected with dengue virus-antibody complexes at an MOI of 5 PFU per cell. The percentage of dengue antigen-positive cells was determined by indirect immunofluorescent staining 24 h after infection.

[c] Dengue culture fluid was obtained as described in footnote *b* of Table 2 and was diluted to contain 10 U of IFN-γ per ml.

[d] PBMC of the same donor were cultured with a control antigen for 7 days, and a culture fluid was collected. This culture fluid, which contained no detectable IFN-γ or IFN-α, was diluted similarly.

ted with 10 U of rIFN-γ which had been incubated with a monoclonal IFN-γ antibody or a polyclonal anti-IFN-α antibody per ml. An anti-IFN-γ antibody inhibited the augmenting effect of rIFN-γ, but anti-IFN-α antibody had no effect (data not shown). These results confirm that IFN-γ is responsible for the augmentation of dengue virus infection shown in Table 2 and Fig. 1 and 2.

Human γ-globulin blocks IFN-γ-induced augmentation of dengue virus infection. It has been reported that IFN-γ increases Fc_γ receptors on U937 cells (6, 17). We tried to determine whether the IFN-γ-induced augmentation of dengue virus infection was Fc_γ receptor mediated. U937 cells that had been treated with 100 U of rIFN-γ per ml for 24 h were incubated with γ-globulin at 4°C for 20 min. Cells were then infected with dengue virus-antibody complexes. γ-Globulin inhibited infection by dengue virus-antibody complexes of U937 cells that were pretreated with IFN-γ, whereas bovine serum albumin at the same concentration had no effect (Table 4). These results suggest that the IFN-γ-induced augmentation of dengue virus infection is mediated by Fc_γ receptors.

IFN-γ does not augment dengue virus infection of U937 cells in the presence of the $F(ab')_2$ fraction of anti-dengue IgG antibody. We then used the $F(ab')_2$ fraction of anti-dengue IgG to confirm that IFN-γ-induced augmentation of dengue virus infection is Fc_γ receptor mediated. Pretreatment of U937 cells with IFN-γ did not augment infection when cells were infected with dengue virus in the presence of $F(ab')_2$ prepared from anti-dengue IgG, but IFN-γ pretreatment augmented infection when cells were infected with virus in the presence of purified anti-dengue IgG at 0.1 to 10 μg/ml (Table 5). This result confirms that the IFN-γ-induced augmentation of dengue virus infection is mediated by Fc_γ receptors on U937 cells.

TABLE 4. Inhibition of IFN-γ-induced augmentation of dengue virus infection by human γ-globulin[a]

Blocking reagent	% of dengue antigen-positive cells	
	IFN-γ pretreatment	No pretreatment
None	40.8	11.5
γ-Globulin	1.8	<1.0
Bovine serum albumin	41.6	11.6

[a] U937 cells that had been treated with 100 U of rIFN-γ per ml for 24 h were incubated with human γ-globulin (10 mg/ml) or bovine serum albumin (10 mg/ml) at 4°C for 20 min. Cells were infected with dengue virus-antibody complexes at an MOI of 5 PFU per cell. The percentage of dengue antigen-positive cells was determined by indirect immunofluorescence 24 h after infection.

TABLE 5. $F(ab')_2$ prepared from anti-dengue IgG does not augment dengue virus infection of U937 cells pretreated with IFN-γ[a]

Final concn of IgG and $F(ab')_2$ (μg/ml)	% of dengue antigen-positive cells			
	IFN-γ pretreatment		No treatment	
	IgG	$F(ab')_2$	IgG	$F(ab')_2$
0	1.0	1.0	1.4	1.4
0.001	1.3	1.3	1.0	0.5
0.01	6.2	0.9	3.5	1.5
0.1	36.5[b]	2.1	9.8[b]	1.2
1	73.7[b]	0.4	34.2[b]	1.2
10	36.5[b]	<0.3	16.8[b]	<0.3
100	11.6[c]	0.4	7.0[c]	<0.3

[a] U937 cells were incubated with or without 100 U/ml of rIFN-γ for 24 h and infected with dengue virus at an MOI of 5 PFU/ml in the presence of various concentrations of purified anti-dengue IgG or $F(ab')_2$ prepared from IgG. The percentage of dengue antigen-positive cells was determined 24 h after infection. The percentage of antigen-positive cells was compared between IFN-γ-pretreated cells and nontreated cells at same concentrations of IgG and $F(ab')_2$.

[b] $P < 0.001$.

[c] $P < 0.005$. Results were not significant ($P > 0.05$) except where indicated.

Augmentation of dengue virus infection correlates with increase in the number of Fc_γ receptors. We tried to determine whether there is a correlation between the number of Fc_γ receptors and the percentage of dengue antigen-positive cells. U937 cells were incubated with variable concentrations of IFN-γ for 24 h and examined for Fc_γ receptor expression by quantitative fluorescence-activated cell sorter analysis after exposure to MAb32, which is specific for the human $Fc_\gamma R1$. The percentage of antigen positive cells was determined 24 h after infection. There was a good correlation between the percentage of dengue antigen-positive cells and the number of Fc_γ receptors (Fig. 3). This result is consistent with those shown in Tables 4 and 5 and indicates that augmentation of dengue virus infection induced by IFN-γ is mediated by Fc_γ receptors.

DISCUSSION

In this report we demonstrate that IFN-γ augments dengue virus infection of U937 cells in the presence of anti-dengue antibodies. This effect is Fc_γ receptor mediated, because (i) IFN-γ had no augmenting effect on the infection of U937 cells when cells were infected with dengue virus in the absence of anti-dengue antibody, (ii) IFN-γ did not augment dengue virus infection when cells were infected with virus complexed to the $F(ab')_2$ fraction prepared from anti-dengue IgG, (iii) IFN-γ had no augmenting effect when Fc_γ receptors on U937 cells were blocked by γ-globulin, and (iv) there was a good correlation between the percentage of dengue antigen-positive cells and the number of Fc_γ receptors on U937 cells. We observed that IFN-γ increased the number of Fc_γ receptors on U937 cells, as previously reported by other investigators (6, 17). Based on these

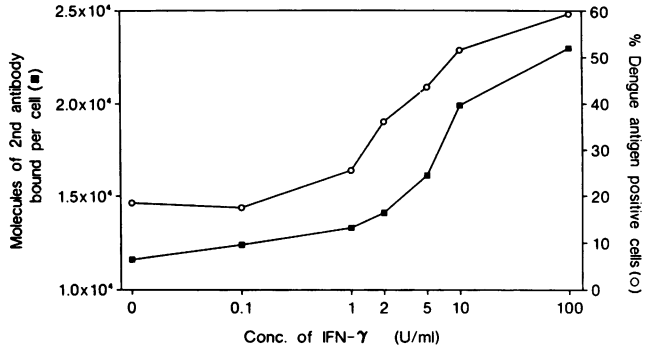

FIG. 3. Correlation between IFN-γ increased Fc_γ receptors and dengue virus infection. U937 cells were incubated with variable titers of IFN-γ for 24 h. Cells were infected with dengue virus-antibody complexes at an MOI of 5 PFU/cell. The percentage of dengue antigen-positive cells was determined by indirect immunofluorescent staining 24 h after infection. The relative numbers of Fc_γ receptors was measured by quantitative fluorescence-activated cell sorter analysis as described in Materials and Methods.

observations, we conclude that the increase in the number of Fc_γ receptors on U937 cells induced by IFN-γ leads to an augmented uptake of dengue virus in the form of dengue virus-antibody complexes, which results in a higher percentage of dengue virus-infected cells and in higher yields of infectious dengue virus. IFN-γ also augmented dengue virus infection of human monocytes enriched from peripheral blood mononuclear cells, when monocytes were infected with virus in the presence of anti-dengue antibodies. IFN-γ did not augment dengue virus infection of human monocytes when cells were infected in the absence of antibody (data not shown).

It has been reported that IFN-α has no or little effect on the number of Fc_γ receptors on monocytic cells (6, 17). We found that IFN-α suppressed dengue virus infection of U937 cells at doses higher than 1 U/ml even when cells were infected in the presence of anti-dengue antibody (data not shown). We attribute this suppressive effect to the antiviral activity of IFN-α. We recently reported that high levels of IFN-α were produced by dengue virus-infected monocytes (13) and by HLA DR antigen-positive, non-T lymphocytes cultured with dengue virus-infected monocytes (12); furthermore the IFN-α produced was active in limiting infection of human monocytes by dengue virus (12, 13).

It has been hypothesized that increased infection of monocytes with dengue virus in the form of dengue virus-antibody complexes may occur in vivo and play an important role in the pathogenesis of DHF-DSS (8). This is supported by epidemiological studies, which reported that most cases of DHF-DSS occur during secondary dengue infections when anti-dengue antibodies are present (2, 9, 18). We have reported that IFN-γ is produced by previously sensitized human T lymphocytes after secondary antigenic stimulation (5, 20). We have also found that the T lymphocytes of individuals who have antibodies to dengue viruses proliferate and produce high titers of IFN-γ after stimulation with dengue antigens in vitro (Kurane et al., submitted). The IFN-γ produced by dengue virus stimulation of immune T cells was active in augmenting dengue virus infection of U937 cells and human monocytes. Therefore, we hypothesize that IFN-γ is produced by dengue-specific T lymphocytes during secondary dengue infections after stimulation with conserved dengue antigens and that the IFN-γ produced might contribute to the pathogenesis of DHF-DSS by enhancing Fc_γ receptor expression on human monocytes, thereby increasing the number of infected monocytes and the yields of infectious dengue virus in the presence of anti-dengue virus antibodies.

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LITERATURE CITED

1. **Anderson, C. L., P. M. Guyre, J. C. Whitin, D. H. Ryan, R. J. Looney, and M. W. Fanger.** 1986. Monoclonal antibodies to Fc receptors for IgG on human mononuclear phagocytes: antibody characterization and induction of superoxide production in a monocyte cell line. J. Biol. Chem. **261**:12856–12864.
2. **Burke, D. S., A. Nisalak, D. E. Johnson, and R. M. Scott.** 1988. A prospective study of dengue infections in Bangkok. Am. J. Trop. Med. Hyg. **38**:172–180.
3. **Caray, P. W., D. W. Geungi, D. Pennica, R. Yelverton, R. Narajan, 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 (London) **295**:503–508.
4. **Daughaday, C. C., W. E. Brandt, J. M. McCowan, and P. K. Russell.** 1981. Evidence for two mechanisms of dengue virus infection of adherent human monocytes: trypsin-sensitive virus receptors and trypsin-resistent immune complex receptors. Infect. Immun. **32**:469–473.
5. **Ennis, F. A., and A. Meager.** 1981. Immune interferon produced to high levels by antigenic stimulation of human lymphocytes with influenza virus. J. Exp. Med. **154**:1279–1289.
6. **Guyre, P. M., P. Morganelli, and R. Miller.** 1983. Recombinant immune interferon increases immunoglobulin G Fc receptors on cultured human mononuclear phagocytes. J. Clin. Invest. **72**: 393–397.
7. **Halstead, S. B.** 1979. In vivo enhancement of dengue virus infection in rhesus monkeys by passively transferred antibody. J. Infect. Dis. **140**:527–533.
8. **Halstead, S. B.** 1981. The pathogenesis of dengue: molecular epidemiology in infectious disease. Am. J. Epidemiol. **114**:632–647.
9. **Halstead, S. B., S. Nimmannitya, and S. N. Cohen.** 1970. Observations related to pathogenesis of dengue hemorrhagic fever. IV. Relation of disease severity to antibody response and virus recovered. Yale J. Biol. Med. **42**:311–328.
10. **Halstead, S. B., E. J. O'Rourke, and A. C. Allison.** 1977. Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. J. Exp. Med. **146**:201–217.
11. **Halstead, S. B., E. J. O'Rourke, and A. C. Allison.** 1977. Dengue viruses and mononuclear phagocytes. II. Identity of blood and tissue leukocytes supporting in vitro infection. J. Exp. Med. **146**:218–229.
12. **Kurane, I., and F. A. Ennis.** 1987. Induction of interferon-α from human lymphocytes by autologous, dengue virus-infected monocytes. J. Exp. Med. **166**:999–1010.
13. **Kurane, I., and F. A. Ennis.** 1988. Production of interferon alpha by dengue virus-infected human monocytes. J. Gen. Virol. **69**: 445–449.
14. **Kurane, I., D. Hebblewaite, W. E. Brandt, and F. A. Ennis.** 1984. Lysis of dengue virus-infected cells by natural cell-mediated cytotoxicity and antibody-dependent cell-mediated cytotoxicity. J. Virol. **52**:223–230.
15. **Kurane, I., A. Meager, and F. A. Ennis.** 1986. Induction of interferon alpha and gamma from human lymphocytes by dengue virus-infected cells. J. Gen. Virol. **67**:1653–1661.

16. **Monath, T. B.** 1985. Flaviviruses, p. 955–1004. *In* B. W. Fields (ed.), Virology. Raven Press, Inc., New York.
17. **Perussia, B., E. T. Dayton, R. Lazarus, V. Fanning, and G. Trinchieri.** 1983. Immune interferon induces the receptor for monomeric IgG1 on human monocytic and myeloid cells. J. Exp. Med. **158**:1092–1113.
18. **Sangkawibha, N., S. Rojanasuphot, S. Ahandrik, S. Viriyapongse, J. Jatanasen, V. Salitul, B. Phanthumachinda, and S. B. Halstead.** 1984. Risk factors in dengue shock syndrome: a prospective epidemiologic study in Rayond, Thailand. Am. J. Epidemiol. **120**:653–669.
19. **Sundstroem, C., and K. Nilsson.** 1976. Establishment and characterization of a human histiocytic lymphoma cell line (U937). Int. J. Cancer **17**:565–577.
20. **Yamada, Y. K., A. Meager, A. Yamada, and F. A. Ennis.** 1986. Human interferon alpha and gamma production by lymphocytes during the generation of influenza virus specific cytotoxic T lymphocytes. J. Gen. Virol. **67**:2325–2334.