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DIFFERENTIAL EFFECT OF INTERFERON EXPRESSION OF IgG- AND IgM-Fc RECEPTORS ON HUMAN LYMPHOCYTES[1]

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Mononuclear cells from the blood of healthy normal humans were kept in culture under nonstimulating conditions for 48 hr in the presence or absence of human leukocyte and fibroblast interferons. The number of cells bearing IgM-Fc and IgG-Fc receptors on the cultured cells was determined by rosette formation with ox red blood cells coated with IgM and IgG antibodies. Both interferons suppressed the expression of IgM-Fc receptors on the cells in culture and, conversely, augmented that of IgG-Fc receptors. The suppressed expression of IgM-Fc receptors and enhanced expression of IgG-Fc receptors occurred in parallel within 1 hr after incubation and reached a maximum at 6 hr. Expression of both receptors returned to normal levels after overnight cultivation, although the enhanced expression of IgG-Fc receptors persisted for a longer time than did the suppressed expression of IgM-Fc receptors. Treatment of leukocyte interferon with heat (at 56°C for 1 hr) and acid (pH 2.0) did not abolish these activities. The effects were found to be dose-dependent and blocked by 10^{-9} M ouabain. Pretreatment of lymphocytes with interferon for 3 hr was found to be as effective as having interferon present during the entire culture period. The enhanced expression of IgG-Fc receptors was observed on T- and non-T-enriched lymphocyte populations, and the suppressed expression of IgM-Fc receptors was also observed on T-enriched populations. The dose of interferon needed to induce these modulations of Fc receptors could also augment the natural cytotoxicity to human lymphoid (BJAB) cells and ADCC to chicken red blood cells by peripheral blood lymphocytes.

Increasing evidence indicates that interferon (IF)[2] exerts effects on host defense mechanisms in addition to its inhibition of virus growth. IF can act on lymphocytes to suppress their *in vitro* proliferative responses to mitogens and alloantigens (1, 2), inhibit *in vivo* and *in vitro* antibody production to T cell-dependent and -independent antigens (3–5), and enhance specific cytotoxicity of sensitized lymphocytes against tumor cells (6). IF can also activate macrophages to increase their phagocytic and cytotoxic functions (7, 8). Recently, IF has also been shown to have potent *in vivo* and *in vitro* effects on mouse and human natural killer (NK) and killer (K) cell activities (9–14). Normal lymphocytes after exposure to IF show selective increase of cell surface antigens such as H-2, HLA, or β_2-microglobulin (15, 16). Therefore, IF not only alters the function of lymphoid cells, but modulates cell surface antigens or receptors on the cell.

Receptors for the Fc portion of immunoglobulin are found on the surface of lymphocytes of many species and appear to be associated with functional subpopulations of lymphocytes. Receptors for IgG or IgM are detected on different human T cells and define two distinct subpopulations of T cells (17–20). T cells with IgM receptors (Tμ) provide help in T-dependent polyclonal response of B lymphocytes to pokeweed mitogen (PWM). T cells with IgG receptors (Tγ) after interaction with IgG immune complexes act as suppressor cells (18, 21). In addition some B lymphocytes express receptors for IgG- and IgM-Fc (22, 23). K cells involved in antibody-dependent cell-mediated cytotoxicity reactions also express IgG-Fc receptors (24, 25). NK cells of human peripheral blood involved in natural cell-mediated cytotoxicity have also been reported to carry the IgG-Fc receptors (26–28).

Although the involvement of IgG-Fc receptors in antibody-dependent cell-mediated cytotoxicity has been clearly recognized (25), the full significance of these receptors on lymphocytes is, in most cases, not yet understood. However, there may also be in other subpopulations some relationships between their specific functions and the expression of Fc receptors on the cell. Therefore, we examined in the present experiments whether or not IF, which can alter functions of various lymphoid cells, can also modulate the IgG- and IgM-Fc receptors. The results show that human IF acts directly on human lymphocytes to augment the IgG-Fc receptor expression and inversely suppress the IgM-Fc receptor expression.

MATERIALS AND METHODS

Preparation of lymphocytes. Human peripheral blood mononuclear cells from healthy volunteers were prepared by the techniques of Ficoll-Isopaque gradients, as described previously (29). For assays of Fc-receptors and culture experiments, lymphocyte preparations depleted of monocytes by adherent techniques (partially purified lymphocytes) were usually used. One or 2×10^7 mononuclear cells suspended in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) (RPMI/10%

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[1] This work was supported in part by a grant-in-aid for Scientific Research and Cancer Research from the Ministry of Education, Science, and Culture, and a grant-in-aid from the Public Health Bureau, Ministry of Health and Welfare, Japan.
[2] Abbreviations used in this paper: IF, interferon; IFL, human leukocyte interferon; IFF, human fibroblast interferon; IFM, mouse interferon; EAμ, ox red blood cells coated with IgM antibody; EAγ, ox red blood cells coated with IgG antibody; RFC, rosette-forming cell; PWM, pokeweed mitogen; CRBC, chicken red blood cells; ADCC, antibody-dependent cell-mediated cytotoxicity; NK, natural killer cells; HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

FCS) were placed on a plastic Petri dish (6 x 6 cm in diameter, Falcon) and incubated for 1 hr at 37°C to attach the monocytes (30). After incubation, nonadherent cells were collected, washed by centrifugation, and suspended in RPMI/10% FCS. These methods generally yielded lymphocyte preparations containing less than 5.0% monocytes, as judged by the acid α-naphthyl acetate esterase activity and phagocytosis of yeast particles (30). In order to obtain highly purified lymphocytes, monocytes were depleted by carbonyl-iron treatment (30, 31). This method generally yielded lymphocyte preparations containing less than 0.1% monocytes, as judged by nonspecific esterase staining or yeast-phagocytosis (30, 31).

Cell cultures. Suspensions of lymphocytes were adjusted to a concentration of 1×10^6 cells/ml in plastic tubes and incubated in 5% CO_2/95% air at 37°C. RPMI/10% FCS was used in all the experiments with the exception of the natural cell-mediated cytotoxicity assay in which RPMI 1640/10% FCS plus 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) was used.

Detection of Fc-receptors. Ox red blood cells (ORBC) coated with IgM (EAμ) or IgG (EAγ) were prepared essentially as described by Moretta *et al.* (17). Anti-ORBC serum was prepared in rabbits by i.v. immunization with ORBC. An IgM-rich immune serum was obtained from the rabbits 1 or 2 weeks after a single administration of ORBC. IgG-rich hyperimmune sera were prepared from the rabbits that received a successive administration of ORBC once a week for 5 weeks. The IgG and IgM fractions were obtained by precipitation with 33% ammonium sulfate followed by Sephadex G-200 gel (Pharmacia, Uppsala, Sweden) filtration. These were further purified by a rabbit IgM or IgG immunoadsorbent column. The purity of these fractions was tested by immunoelectrophoresis. The EAγ was prepared with ORBC coated with 1:20 diluted anti-ORBC IgG fraction (protein concentration, 10 mg/ml; hemolysis titer, 1:20,000) at 37°C for 30 min. The EAμ was prepared by incubation of ORBC with anti-ORBC IgM fraction (protein concentration, 0.8 mg/ml; hemolysis titer, 1:20,000) at 37°C for 90 min. These EAγ and EAμ thus obtained were washed three times with phosphate-buffered saline, pH 7.2 (PBS), and resuspended in RPMI 1640 medium and stored at 4°C until use.

Rosette-formation of test lymphocytes with EAγ and EAμ was performed as described previously (32). The lymphocytes were washed twice and resuspended in RPMI 1640/10% FCS. One-tenth milliliter of the cell suspension (1×10^6/ml) and an equal volume of 1×10^8/ml sensitized ORBC were mixed, spun down at 1000 rpm for 10 min at 4°C, and then kept at 4°C for 1 to 3 hr. After incubation, mixtures were resuspended very gently by pipetting. A drop of resuspended cells was placed on a hemocytometer and at least 250 cells were counted. Adherence of three or more erythrocytes was considered positive. Uncoated ORBC served as controls. All data were determined in duplicate. The specificity of the receptors for IgM and IgG was assessed by blocking experiments of EAμ and EAγ rosettes by using purified human IgM and IgG or Fc-piece purified from human IgG and myeloma protein of IgM type.

By using these methods and IgM and IgG antibodies, either IgM or IgG receptors at the levels near the maximum values could be detected even before culture (32).

Purification of T and non-T cells. T cells were separated from non-T cells according to their capacity to form rosettes with neuraminidase-treated SRBC (En-RFC), as previously described (32). Briefly, highly purified lymphocytes were mixed with En, spun at 1000 rpm for 10 min, and incubated at 4°C for 1 hr. The En-RFC (T) cells were separated from the nonrosette-forming (non-T) cells by Ficoll-Isopaque centrifugation at 2000 rpm for 30 min. If the enrichment of the En-RFC was not satisfactory, the same procedures were repeated. The En-RFC was dissociated by hypotonic shock. These fractionation procedures gave T cell-enriched preparations (96 to 98%) and non-T cell-enriched preparations from which En-RFC was depleted to less than 5%.

Interferons. Human leukocyte IF (IFL), which was obtained from normal buffy coats stimulated by Newcastle disease virus and partially purified by acidic methanol precipitation and subsequent precipitation by pH change, was kindly provided by Dr. N. Ishida of Sendai. The specific activity of this preparation was 1×10^6 international units (U)/ml. The partially purified preparation of human fibroblast IF (IFF) produced in human diploid fibroblasts by a superinduction procedure (33) with polyinosinic:polycytidylic acid was provided by Dr. H. Kobayashi of Tokyo. The specific activity was 2×10^7 U/ml. Mouse fibroblast IF (IFM) with a titer of 200,000 U/ml was a gift from Dr. N. Ishida. Titration of IF was carried out, as previously described (34).

Treatment of cells with IF. Unfractionated or fractionated mononuclear cells were suspended at 1×10^6 cells/ml in RPMI/10% FCS, and 1 ml of cell suspensions were placed in tubes to which were added 0.1 ml of IF at varying concentrations. Cells were incubated for various periods of time at 37°C in 5% CO_2/95% air. The cells were then washed twice with RPMI/10% FCS and the values of EAμ- and EAγ-RFC were then examined.

Heat- and pH-stability assays of interferon effect on Fc receptors. To examine the heat stability of human IF effect on Fc receptors, the IFL samples diluted in RPMI/10% FCS were incubated at 56°C for 60 min. For pH-stability assay, the samples were dialyzed against over 50 volumes of 0.1 M glycine-HCl buffer (pH 2.0) at 4°C for 24 hr. The pH was then brought back to 7.2 by dialysis against RPMI 1640 medium (34). The effect of heat- or pH-treated IF on Fc receptors was then examined immediately after the treatment (34).

Assay for natural cell-mediated cytotoxicity. The human lymphoblastoid B cell line BJAB, which is derived from an African Burkitt's lymphoma patient but is Epstein-Barr virus-negative, was used as target cells. The cells were grown in RPMI/10% FCS. Approximately 2×10^7 BJAB cells in 0.5 ml were incubated with 100 μCi ^{51}Cr ($Na_2\,^{51}CrO_4$, Dai-ichi Laboratory, Tokyo) for 1 hr at 37°C. The labeled target cells were then washed three times with warm RPMI 1640 medium and resuspended in RPMI/10% FCS with 10 mM HEPES. The cell suspensions were incubated further for 1 hr at 37°C. This incubation of cells at 37°C before use was effective in minimizing the spontaneous ^{51}Cr release from BJAB cells during assay. After incubation, the cells were washed twice and diluted to 1×10^7 cells/ml in the medium.

For assay of cytotoxicity, 0.2 ml of lymphocyte suspensions at varying concentrations was mixed with 2 μl of 1×10^7/ml ^{51}Cr-labeled BJAB cells in Falcon round-bottomed microtest plates in triplicate (35). After rocking, the plates were cultured in 5% CO_2/95% air at 37°C for 4 hr. At the end of the incubation period, 0.1 ml of the supernatant was removed from the microtest wells and counted in an autogamma counter. In all the experiments percentages of natural cytotoxicity were calculated from the means of triplicate assay by the following formula.

$$\frac{\text{Experimental release} - \text{spontaneous release}}{\text{Total release} - \text{spontaneous release}} \times 100$$

where the spontaneous release is cpm released from target cells alone, and total release is from repeated freezing and thawing

of target cells. Spontaneous release rarely exceeded 10%.

Assay for antibody-dependent cell-mediated cytotoxicity (ADCC). ADCC assays were carried out by microcytotoxicity method as described previously (30). Briefly, 2×10^5 effector cells, 2×10^4 ^{51}Cr-labeled target CRBC and 2×10^{-5} diluted rabbit anti-CRBC serum were put into a microtest well in triplicate and then incubated for 4 hr at 37°C. Percentages of ADCC were calculated from the means of triplicate assay by the same formula as described in natural cytotoxicity. The spontaneous release is cpm released from target cells in the presence of effector cells and nonimmune serum and rarely exceeded 10%.

RESULTS

Suppression of IgM receptor expression and enhancement of IgG receptor expression by IF. Partially purified lymphocytes were cultivated in the presence or absence of 20 U/ml of IFL at 37°C for 24 hr and the values of EAμ- and EAγ-rosette-forming cells (RFC) were assayed at intervals during culture. Figure 1 shows representative results obtained with two different healthy donors. The results show that with either donor the number of EAμ-RFC in IFL-treated cells decreased 1 hr after cultivation, reached a minimum at 6 hr and, after overnight culture, returned to the control values. Conversely, the numbers of EAγ-FRC in IFL-treated cells increased 1 hr after cultivation and reached a maximum at 6 hr. After overnight culture, these decreased but still remained at levels somewhat higher than that of control. The total number of cells in culture, when incubated either with or without IFL, did not change at least for 24 hr. Figure 2 summarizes the overall results with ten normal donors in which the values of EAμ- and EAγ-FRC on IFL-treated cells were represented as percent of control. The results clearly show that IFL at 20 U/ml induced the suppressed expression of IgM receptors and enhanced expression of IgG receptors on cells, which appeared within 1 hr after culture, reached a maximum at 6 hr and returned almost to normal levels after overnight cultivation, although the enhanced expression of IgG receptors persisted for longer periods of time (18 or 24 hr) than did the suppressed expression of IgM receptors.

The amounts of IFL added to partially purified lymphocytes varied from 0.2 to 200 U/ml, and cells were incubated for 6 hr at 37°C (Table I). The results show that the effect of IFL on either IgM-Fc or IgG-Fc receptors was dose-dependent. IFL as low as 2 U/ml was effective.

Characterization of IF-action modulating Fc receptors. IFL at 20 U/ml was exposed to heat (56°C 30 min) or acid (pH 2.0) and then its effects on the EAμ- and EAγ-RFC on lymphocytes were examined (Table II). The lymphocytes after exposure to either heat- or acid-treated IFL showed suppressed expression of IgM receptors and enhanced expression of IgG receptors, as did those after exposure to untreated IFL.

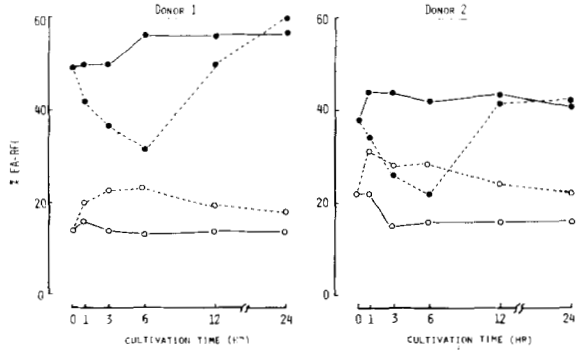

Figure 1. Effect of human leukocyte interferon on the expression of IgM and IgG receptors. The values represent the percentages of EAμ-RFC in the culture with (●-----●) and without (●——●) IFL at 20 units/ml and those of EAγ-RFC in the culture with (○-----○) and without (○——○) IFL at 20 units/ml.

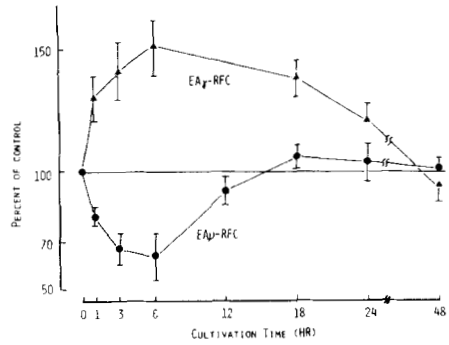

Figure 2. Effect of human leukocyte interferon on the expression of IgM and IgG receptors: summary of the results from 10 normal donors. To minimize some consistent subject-to-subject variations in such variables as EAμ- and EAγ-RFC percentages among the total lymphocytes, the percentages of both RFC in each subject at each time were expressed as a percentage of control. The values represent the mean ± S.D. in 10 normal donors.

TABLE I

Interferon action on Fc receptors: dose-response

IFL	% EAμ-RFC				% EAγ-RFC			
	Expt. 1	Expt. 2	Expt. 3	Mean % of control	Expt. 1	Expt. 2	Expt. 3	Mean % of control
units/ml								
None	33.1	28.5	59.9		19.3	9.6	13.8	
0.2	38.2	29.4	53.7	103	20.7	10.7	ND[a]	109
2	24.5	25.9	45.1	80.1	27.0	13.6	ND	141
20	21.5	11.2	43.9	59.2	26.1	20.1	24.4	174
200	ND	10.3	33.2	45.8	26.8	27.3	ND	211

[a] Not determined.

Human fibroblast IF (IFF) at 2 to 200 U/ml also suppressed the IgM receptor expression and enhanced the IgG receptor expression. In contrast, no changes in expression of IgM and IgG receptors were produced by mouse IF (IFM) even at 2000 U/ml.

Lymphocytes were cultivated for 6 hr with 20 U/ml of IFL in the presence or absence of 10^{-9} M ouabain, an inhibitor of cell membrane ATPase responsible for the uptake of potassium and sodium, which is also known to inhibit the antiviral effect of IF (36), and the effect of ouabain on the IF-induced modulation of Fc receptors was examined (Table III). The results clearly show that both the suppressed expression of IgM receptors and enhanced expression of IgG receptors by IFL were blocked completely by the presence of ouabain.

To examine whether the exposure of lymphocytes with IF for a limited time results in the modulation of Fc receptors, the experiments were performed by incubating lymphocytes with 20 U/ml of IFL for 1, 2, and 3 hr, after which the cells were washed free of IFL and re-cultivated in the IFL-free medium for 5, 4, and 3 hr, respectively (Table IV). The results show that exposure of cells to IFL for 3 hr was as effective as exposure to IFL during the entire culture period (6 hr).

Effect of IF on Fc receptors of fractionated lymphocytes. The effect of IFL on Fc receptor expression was examined with

TABLE II

Interferon action on Fc receptors: stability to heat and acid treatment, and species-specificity of interferon

IF			% EAμ-RFC[a]				% EAγ-RFC[a]			
Dose		Treatment	Expt. 1	Expt. 2	Expt. 3	Mean % of control	Expt. 1	Expt. 2	Expt. 3	Mean % of control
units/ml										
None			59.9	56.6	29.4		19.3	13.9	9.6	
IFL	20		43.9	40.6	15.2	65.5	26.1	24.4	20.1	173
IFL	20	Heat	43.9	ND[b]	12.2	57.4	25.8	23.1	13.8	148
IFL	20	Acid	43.7	ND	18.6	68.2	26.7	25.6	13.3	154
IFF	2		48.0	40.3	ND	75.7	ND	17.5	12.7	130
IFF	20		40.5	35.6	ND	65.3	ND	20.1	13.2	142
IFF	200		38.5	29.8	ND	58.5	ND	21.0	15.0	154
IFM	20		51.7	57.2	ND	93.7	ND	15.5	ND	112
IFM	2000		55.0	55.6	ND	95.0	ND	13.0	ND	94.2

[a] All assays were performed with partially purified lymphocytes cultivated with or without the indicated interferons for 6 hr.
[b] Not determined.

TABLE III

Blocking by ouabain of interferon action on Fc receptors[a]

	Expt. No.	Substances			
			Ouabain	IFL	Ouabain + IF
% EAμ-RFC	1	24.2	26.3	15.5	25.4
	2	43.7	46.3	35.6	44.7
% EAγ-RFC	1	16.6	16.7	25.8	14.7
	2	12.0	11.4	24.1	15.1

[a] Partially purified lymphocytes were cultivated at 37°C for 6 hr in the presence of 10^{-9} M ouabain alone, 20 units/ml of human leukocyte interferon (IFL) alone, or 10^{-9} M ouabain plus 20 units/ml of IFL, or in the absence of either substance.

TABLE IV

Incubation times required for suppression of Fcμ receptor and enhancement of Fcγ receptor by interferon

IFL	Incubation Time with IF,	% EAμ-RFC[a] (±S.D.)	% EAγ-RFC[a] (±S.D.)
	hr		
None	0	45 (4)	23 (2)
+	1	44 (3)	22 (3)
+	2	41 (5)	22 (2)
+	3	32 (1)	31 (2)
+	For entire periods (6 hr)	25 (3)	30 (2)

[a] The values represent the means and S.D. of two experiments in the same subject.

highly purified lymphocytes and their T- and non-T-enriched populations (Table V). With highly purified lymphocytes, suppressed expression of IgM-Fc receptors and enhanced expression of IgG-Fc receptors were observed, indicating that the IF-induced modulation of IgM- and IgG-Fc receptors may not be dependent on the presence of monocytes. IgM-Fc receptors could be detected on T- but not non-T-enriched cells and the receptor expression on this T-enriched population was also suppressed by IFL. IgG-Fc receptors could be detected on both T- and non-T enriched populations and the enhanced expression of the receptors by IF was observed with both populations.

In vitro augmentation of NK activity and ADCC by interferon. Partially purified lymphocytes were cultured in the presence of IFL or IFF ranging from 0.2 to 200 U/ml at 37°C for 6 hr, washed, and then tested for their cytotoxicity against ^{51}Cr-labeled BJAB cells. As clearly shown in Table VI, either IFL or IFF at 2 U/ml or more was able to augment the cytotoxicity to BJAB cells, and their effects were dose-dependent. The results also showed that the minimal amount of IF required for the augmentation of NK activity is similar to that needed to produce the modulations of IgM and IgG receptors.

TABLE V

Effect of IFL on expression of IgM-Fc and IgG-Fc receptors on purified lymphocytes and T- and non-T cell-enriched populations[a]

Fractions of Lymphocytes	Donors	% EAμ-RFC			% EAγ-RFC		
		Control	IFL	Mean % of control	Control	IFL	Mean % of control
Unfractionated	A	39.4	26.1	58.0	13.5	25.7	174
	B	37.2	18.5		8.8	13.8	
T	A	57.7	36.0	61.9	6.9	13.2	165
	B	44.6	27.4		13.9	19.2	
Non-T	A	3.0	2.0	ND[b]	25.5	39.5	142
	B	ND	ND		26.0	33.2	

[a] Unfractionated, T-, and non-T lymphocytes obtained from the donors A and B were incubated with IFL at 20 units/ml at 37°C for 6 hr and the values of EAμ- and EAγ-RFC were examined.
[b] Not determined.

The effect of leukocyte IF on the ADCC activity of peripheral blood lymphocytes was examined. Highly purified lymphocytes from five different donors were cultured in the presence or absence of 20 U/ml of IFL at 37°C for 6 hr, washed, and then tested for their cytotoxicity against ^{51}Cr-labeled CRBC in the presence of heat-inactivated rabbit anti-CRBC serum. As shown in Table VII, 20 U/ml of IFL were able to augment the ADCC activity of lymphocytes from four out of five donors.

DISCUSSION

The present study indicates that human leukocyte and fibroblast IF as low as 2 U/ml can suppress the expression of IgM-Fc receptors on human lymphocytes and, conversely, augment that of IgG-Fc receptors. This was studied by the detection of EAμ- and EAγ-RFC on IF-treated lymphocytes in culture. The results indicate that the suppressed expression of IgM receptors and the contiguous enhanced expression of IgG receptors occurred within 1 hr after culture, continued until 6 hr, and returned to normal levels after longer incubation, although the enhanced expression of IgG receptors was still observed after 24 hr.

TABLE VI

Effect of human interferon on natural killer cell-mediated cytotoxicity by human peripheral blood lymphocytes

IF	Concentration	% Natural cytotoxicity[a]				Mean % Augmentation
	Expt. no.	1	2	3	4	
	units/ml					
IFL	0	36.4	36.2	22.9	41.8	
	0.2	39.7	36.4	23.1	40.0	1.8
	2	43.3	40.6	37.9	42.5	24.8
	20	42.8	47.7	49.5	43.7	42.8
	200	57.0	53.3	51.4	53.9	64.3
IFF	0	22.7	20.2	23.5		
	2	37.9	23.7	23.0		27.3
	20	49.5	27.2	29.6		59.7
	200	51.4	30.7	41.2		84.3

[a] The cells incubated at 37°C for 6 hr in the presence or absence of IFL or IFF were washed twice with RPMI 1640 medium and incubated with ^{51}Cr-labeled BJAB cells for 4 hr.

TABLE VII

Effect of human interferon on antibody-dependent cell-mediated cytotoxicity by human peripheral blood lymphocytes

Donor	% ADCC Activity[a]		% Augmentation
	IF (−)	IF (+)	
1	13.1	25.6	95.4
2	25.8	28.5	10.5
3	17.6	24.7	40.3
4	40.1	50.6	26.2
5	37.8	61.3	62.2

[a] The highly purified lymphocytes incubated at 37°C for 6 hr in the presence or absence of 20 units/ml of human leukocyte interferon were washed twice with RPMI 1640 medium and incubated for 4 hr with ^{51}Cr-labeled CRBC coated with 2×10^{-5} diluted antibody. Effector/Target cell ratio was 10:1.

The effects of human leukocyte IF were heat- and acid-stable, as are their antiviral effects (37, 38). Mouse fibroblast IF induced neither suppressed expression of IgM receptors nor enhanced expression of IgG receptors. Exposure of lymphocytes to 20 U/ml IF for 3 hr was necessary for significant decrease of IgM receptor expression. Similarly, at least several hours of treatment with IF at 37°C is necessary before antiviral activity develops (39). Both changes of IgM- and IgG-receptors by IF were blocked by 10^{-9} M ouabain, an ATPase inhibitor that is known to inhibit the antiviral action of IF (36). Thus the substance(s) in the interferon preparations that induced the changes of Fc receptors have several characteristics of IF (40).

IF suppressed the expression of IgM receptors on the monocyte-depleted lymphocytes and purified T cells. The expression of IgG receptors, which were found on non-T as well as T cells, was also augmented by IF in the absence of monocytes. Therefore, IF may act directly on the lymphocytes to induce these changes. Previous investigations have shown that IF requires no macrophages for its *in vitro* or *in vivo* augmentation of NK activity (13).

Receptors for IgG-Fc have been demonstrated in T, B, K and NK subpopulations of lymphocytes in man and animals (23–27, 41). Since the receptors probably participate in some way in the specific functions of the subpopulations, modulation of the Fc receptor expression by IF may directly or indirectly influence the course of these immune responses.

K cells in ADCC systems and NK cells involved in the killing of tumor cell targets are known to carry IgG receptors (24, 25). The present results show that IF enhanced the expression of IgG receptors on non-T cells as well as T cells, suggesting the possibility that it can enhance the expression of IgG receptors on K and NK cells. If this is the case, the capacity of K and NK cells to lyse the targets may be influenced by the increased expression of IgG receptors by IF. As to the effect of IF on ADCC, the controversial results have been reported. Herberman *et al.* (9) but not Trinchieri *et al.* (11) have demonstrated that ADCC can be augmented by IF. The present results show that ADCC mediated by K cells against CRBC could be augmented by IF. The reason for this discrepancy remains unknown. However, it may depend on the titers of antibody coated with erythrocyte-targets; in our experiences, IF was less effective on ADCC against the targets when tested with highly sensitized CRBC.

Recent work has also shown that IF has potent *in vivo* and *in vitro* effects on natural cell-mediated cytotoxicity by mouse and human NK cells (9–14). The present results show that IF also augmented cytotoxicity at the same doses needed to enhance the expression of IgG receptors on cells. It has been shown that augmentation by IF of human natural cell-mediated cytotoxicity by NK cells occurs within 1 hr after exposure of cells with IF and persists for 1 day (14). Our results show that the increased expression of IgG receptors also occurs immediately after exposure with IF and also persists for 1 day. These results suggest the possibility that increased expression of IgG receptors by IF is associated with augmentation of NK activity. Pollack and Emmons (28) have recently shown that IgG receptors on NK cells are implicated either to be the "active site" for natural cell-mediated cytotoxicity or to be closely linked to the active site. Zarling and co-workers (14) have also demonstrated that removal of IgG receptors-bearing cells prevented interferon from augmenting cytotoxicity. Therefore, it is possible that there is a direct relationship between the increased expression of IgG receptors and the increased activity of NK cells.

Previous investigations have shown that IgG receptor-bearing T and non-T cells after binding with immune complexes have a suppressive influence on antibody responses to T-dependent antigens (41, 42). Thus, the capability of IgG receptor-bearing cells to suppress antibody responses may also be influenced by the expression of IgG receptors. The increased expression of IgG receptors by IF may, therefore, be reflected in the augmentation of suppressor activity of Fc receptor-bearing cells. It has been reported that IF can inhibit antibody production to T cell-dependent and -independent antigens *in vivo* and *in vitro* (3–5).

Receptors with a high avidity for IgM-Fc have been demonstrated in human T cells (17–20), although some B cells also carry the receptor (22, 23). T cells bearing IgM receptors (Tμ) appear to facilitate the PWM-induced differentiation of B cells into plasma cells in contrast to the suppressor role played by T cells bearing IgG receptors (Tγ) (18, 21). Although the significance of the receptors on these subpopulations is not yet understood, it is possible that the suppressive activity of Tγ cells and the enhancing activity of Tμ cells on PWM-induced B cell differentiation are influenced by the expression of IgM- and IgG-receptors. We have found that IFL at 20 U/ml or more can suppress the PWM-induced B cell differentiation, although it remains unknown whether or not the modulations of IgM and IgG receptors are directly related to this suppression (unpublished observation).

The changes of Fc receptors by IF might also play an important role in affecting other immune responses such as delayed-type hypersensitivity (43), the proliferative response of lymphocytes to T cell-mitogens (1, 2), transplantation or tumor im-

munity (44–46), and phagocytosis (7), each of which can be modulated by IF.

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