

Induction of Mouse IgG2a- and IgG3-dependent Cellular Cytotoxicity in Human Monocytic Cells (U937) by Immune Interferon¹

Yukio Akiyama, Michael D. Lubeck, Zenon Steplewski,² and Hilary Koprowski

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

ABSTRACT

The effects of natural and recombinant human γ -interferon (IFN- γ) on mouse monoclonal antibody-dependent cellular cytotoxicity (ADCC) mediated by U937 human monocytic-like cells were examined. The efficiency of mouse monoclonal antibody of different isotypes in inducing ADCC was also compared. The number of receptors for the Fc portion of immunoglobulin G (IgG) (FcR) for mouse IgG2a and IgG3 on U937 cells, as detected by IgG antibody-sensitized erythrocyte rosette formation, was significantly enhanced by IFN- γ . In contrast, FcR for mouse IgG1 and IgG2b were not detected even after IFN- γ stimulation. U937 cell-mediated ADCC against sheep or ox red blood cell targets was minimal. However, after incubation with human purified IFN- γ , U937 cells exhibited increased activity in IgG2a- and IgG3-dependent lysis, whereas their activity in IgG1- and IgG2b-dependent lysis was low. ADCC stimulated by IFN- γ was inhibited by Protein A. When mouse peritoneal exudate cells were used, FcR for all IgG isotypes were easily detected, and all IgG isotypes mediated ADCC. Taken together, these results indicate that IFN- γ induces U937 cell ADCC with mouse IgG2a and IgG3 partly through augmentation of FcR expression. Recombinant IFN- γ showed the same effect as natural IFN- γ . These effects of IFN- γ were completely abrogated by anti-IFN- γ serum but not by anti-IFN- α or normal rabbit serum. Addition of polymyxin B or lipopolysaccharide did not affect the activity of IFN- γ .

INTRODUCTION

Murine MoAbs³ have been used for the detection of tumor-associated antigens in the blood of cancer patients or on the surface of tumor cells (2, 11, 12, 14, 15, 23). In addition, several murine MoAbs have been shown to be effective in inhibiting tumor growth (3, 9, 10, 13, 28), e.g., murine MoAb against human CRC cells specifically inhibited tumor growth in nude mice (10) and were shown to depend on host macrophages for tumor cell destruction (9). Furthermore, human macrophages as well as cultured human monocytes were found to express FcR that cross-react strongly with murine IgG2a; such macrophages kill human CRC cells in the presence of anti-CRC MoAb *in vitro* (24, 25). Those results suggested the importance of FcR for ADCC of mononuclear phagocytes and for the tumoricidal action of MoAb.

IFN- γ is a secretory product of activated lymphocytes and has

¹ Supported by Research Grants CA 10815, CA 21124, CA 25874, and CA 37864, and Grant AI-19607 from the National Institute of Allergies and Infectious Diseases, and a grant from the Mrs. Smith Lead Foundation.

² To whom requests for reprints should be addressed.

³ The abbreviations used are: MoAb, monoclonal antibody(ies); ADCC, antibody-dependent cellular cytotoxicity; CRC, colorectal carcinoma; EA7S, immunoglobulin G antibody-sensitized erythrocytes; FcR, receptors for the Fc portion of immunoglobulin G; IFN- γ , immune interferon; LPS, bacterial lipopolysaccharide; ORBC, ox red blood cells; PEC, murine peritoneal exudate cells; PMN, polymorphonuclear leukocytes; SRBC, sheep red blood cells.

Received May 15, 1984; accepted July 19, 1984.

various biological activities. In addition to its antiviral and antiproliferative activities, IFN- γ reportedly stimulates tumoricidal (17) and microbicidal activity in human monocytes (19). Furthermore, recent studies have shown that IFN- γ causes a dramatic increase in the expression of FcR for human monomeric IgG1 on normal human monocytes and on myelomonocytic human cells (8, 20).

In the present study, we examined whether monocyte-like U937 cells are able to mediate MoAb-dependent lysis after treatment with IFN- γ . The efficiency of mouse MoAb of different isotypes in inducing ADCC was also compared.

MATERIALS AND METHODS

Cells. U937 monocyte-like cells, derived from a patient with histiocytic lymphoma (26), were maintained in suspension culture in RPMI 1640 medium supplemented with 10% fetal bovine serum (Dutchland Laboratories, Inc., Denver, PA), 2 mM glutamine, and gentamicin (50 μ g/ml; hereafter referred to as complete culture medium). Murine PEC were obtained from CBA mice inoculated with thioglycolate as described previously (9) and used as a positive control in the ADCC and FcR expression assays.

Interferon. Purified human IFN- γ (10⁸ antiviral units/mg protein on Hep-2 cells) was obtained from Interferon Science, Inc., New Brunswick, NJ. Human recombinant IFN- γ (specific activity, $\sim 10^7$ units/mg protein on HeLa cells) from *Escherichia coli* was kindly supplied by Genentech, Inc., San Francisco, CA. Rabbit antiserum against human IFN- γ and sheep antiserum against human IFN- α were obtained from Interferon Science, Inc.

Monoclonal Antibodies. IgG2a, IgG2b, or IgG3 MoAbs reacting with SRBC were prepared by Dr. W. C. Raschke, La Jolla Cancer Research Foundation, La Jolla, CA, and obtained through American Type Culture Collection, Rockville, MD. A MoAb (IgG1), reacting with ORBC and cross-reactive with SRBC, was established in our laboratory. MoAbs were used as unconcentrated hybridoma culture supernatant.

EA7S Rosette Formation. SRBC (or ORBC) were coated with equivalent amounts of antibodies to saturate binding sites and FcR detected as described previously (25).

Stimulation of U937 Cells. U937 cells were cultured in 24-well No. 3524 tissue culture plates (Costar, Cambridge, MA) at an initial concentration of 5×10^5 /ml in a total volume of 2 ml with or without IFN- γ at various concentrations in complete culture medium. After several hr of culture, cells were harvested and washed 3 times. Cells were then incubated for 2 hr at 37° in RPMI 1640 without fetal bovine serum to allow detachment of IgG possibly absorbed from the serum. Cell counts and viability were determined visually with the trypan blue exclusion test.

Cytotoxicity Assay. ADCC against SRBC or ORBC was quantified as described previously (16). Briefly, effector cells (a 100- μ l suspension in complete culture medium) at various concentrations were mixed with 100 μ l of 2×10^5 ⁵¹Cr-labeled SRBC or ORBC target cells and 50 μ l of MoAb at different dilutions in round-bottomed microtiter wells. Plates (Costar; No. 3799) containing the cell mixture were incubated for 18 hr and centrifuged. Supernatants (125 μ l) were harvested for γ -counting. Percentage of cytotoxicity was calculated as follows:

$$\frac{(A - B) - (C - B)}{(T - B) - (C - B)} \times 100$$

where *A* is the mean cpm from cultures of effector cells plus MoAb, *B* is the mean background cpm, *C* is the mean cpm from ⁵¹Cr-labeled effector cells incubated alone, and *T* is the mean cpm in the supernatants of detergent-lysed target cells. The spontaneous release from RBC targets incubated with medium alone was between 7 and 10% of *T*.

To determine the role of FcR of U937 cells in ADCC, *Staphylococcus aureus* Protein A (Sigma Chemical Co., St. Louis, MO) was used. SRBC (2×10^6) in 100 μ l were mixed with 50 μ l of MoAb and incubated alone or with various concentrations of Protein A in 50 μ l for 30 min before addition of 50 μ l of effector cells. Effects of polymyxin B and LPS (*E. coli* 0128:B12) on anti-SRBC ADCC or FcR expression were also examined. U937 cells (5×10^5 /ml) were incubated for 36 hr in the presence or absence of IFN- γ with or without the addition of polymyxin B (25 μ g/ml) or various concentrations of LPS. Cells were then washed and assayed for ADCC and for FcR expression (24, 25). In ADCC assay, IgG2a was used at a 1:5 dilution. Polymyxin B and LPS were purchased from Burroughs Wellcome Co., Research Triangle Park, NC, and Sigma Chemical Co., respectively.

RESULTS

Effect of IFN- γ on Expression of FcR for Mouse IgG on U937 Cells. FcR expression was detected by EA7S rosette formation; 7.2 and 0.5% of the population of unstimulated U937 cells expressed FcR for mouse IgG2a and IgG3, respectively, whereas FcR cross-reactive with mouse IgG1 and IgG2b were not detected (Table 1). After stimulation with IFN- γ , there was a steady increase in the number of EA7S rosette-forming cells for mouse IgG2a. IFN- γ also induced a significant increase in FcR expression for mouse IgG3 ($p < 0.02$ at 100 units per ml of IFN- γ); however, binding of IgG1 and IgG2b to U937 cells was not detected by this assay even after IFN- γ stimulation. The apparent increase of IgG2a- and IgG3-specific FcR induced by IFN- γ was observed already at an IFN- γ concentration of 10 units/ml. Kinetics studies indicated that the percentage of EA7S-rosette-forming cells reached a plateau after 12 hr of incubation. While IFN- γ induced a dramatic increase in FcR expression for mouse IgG2a on U937 cells, α - and β -interferon had only minor effects (data not shown).

Effects of IFN- γ on Mouse MoAb-dependent ADCC Mediated by U937 Cells. The possibility that IFN- γ enhances not only FcR expression in U937 cells, but also the activity of these

cells in ADCC against RBC targets was tested. The ADCC activity mediated by U937 cells cultured in the absence of IFN- γ was usually low or barely detectable (Table 2). IFN- γ induced a dose-dependent IgG2a- and IgG3-dependent cytotoxicity in U937 cells reaching a plateau after incubation with IFN- γ for 36 hr. In contrast, IgG1- and IgG2b-dependent ADCC were increased only slightly under these conditions (Table 2). No spontaneous cytotoxicity by untreated or IFN- γ -treated U937 cells was observed against RBC (not shown). Since the IgG1 MoAb against ORBC also reacts with SRBC, IgG1-dependent ADCC to SRBC targets was also determined. Under these conditions, IFN- γ -stimulated U937 cells mediated the same level of ADCC activity against ORBC targets (data not shown). Treatment with IFN- γ resulted in slower growth in U937 cells; after 48 hr of culture, cell proliferation was inhibited 10, 17, and 22% by 100, 500, and 1000 units per ml of IFN- γ , respectively.

Table 3 shows a titration of the hybridoma supernatants in the cytotoxicity assays mediated by U937 cells and murine PEC. When U937 cells were used, IgG2a- and IgG3-dependent lysis was maximal generally at a 1:10 dilution of MoAb. ADCC activities were lower at higher effector:target cell ratios (2:1). Only low levels of target cell lysis mediated by U937 cells were observed in the presence of IgG1 and IgG2b at all MoAb dilutions used. However, when murine PEC were tested for ADCC to RBC targets under the same conditions as used for U937 effector cells, IgG2a, IgG1, and IgG2b MoAbs exhibited similarly efficient RBC lysis by PEC. IgG3-dependent ADCC levels using PEC were undetectable at a MoAb dilution of 1:20, and at a 1:40 dilution, all MoAb-mediated cytolysis decreased (data not shown).

To determine the role of FcR on U937 cells in ADCC, the effect of Protein A on mouse IgG2a- and IgG3-dependent ADCC was examined. As shown in Table 4, IFN- γ -induced ADCC was inhibited by Protein A in a dose-dependent manner. Protein A at a final concentration of 20 μ g/ml caused a 68 and 82% inhibition of IgG2a- and IgG3-dependent ADCC, respectively.

To evaluate the possibility that contaminating endotoxin accounted for the stimulation of U937 cells by IFN- γ , U937 cells were incubated with IFN- γ in the presence or absence of polymyxin B, an agent that blocks LPS activity (18). After 36 hr of

Table 1
Differential enhancement of FcR expression for mouse IgG on U937 cells treated with IFN- γ
U937 cells (5×10^5 /ml) were cultured in the presence of different concentrations of IFN- γ . An aliquot of the cells was taken at the time points indicated, washed, and tested for FcR expression by rosette formation. Thioglycolate-induced murine PEC were used as controls.

Cells	Concentration of IFN- γ (units/ml)	Period of stimulation (hr)	% of EA7S rosette-forming cells				
			IgG1	IgG2a	IgG2b	IgG3	
U937	0	None	— ^a	7.2 \pm 2.0 ^b	—	0.5 \pm 0.2	
		10	12	—	18.7 \pm 4.8	—	1.3 \pm 0.3
			24	—	23.4 \pm 7.2	—	1.8 \pm 0.4
	100	36	—	20.7 \pm 1.7	—	1.6 \pm 0.4	
		12	—	21.6 \pm 4.5	—	4.5 \pm 0.2	
			24	—	22.2 \pm 5.3	—	4.6 \pm 1.1
	1000	36	—	21.9 \pm 2.9	—	4.2 \pm 0.9	
		12	—	24.6 \pm 6.2	—	4.1 \pm 0.3	
			24	—	25.1 \pm 6.8	—	3.8 \pm 0.7
		36	—	25.6 \pm 5.8	—	3.9 \pm 0.7	
Murine PEC	None	None	52.5	42.8	63.0	54.3	

^a —, not detectable.

^b Mean \pm S.D. of 3 separate experiments. Data for murine PEC are means of 2 separate experiments.

Table 2

Differential stimulation of mouse IgG-dependent lysis by IFN- γ -treated U937 cells

U937 cells were incubated at 5×10^6 /ml for various periods of time with IFN- γ at 0, 10, 100, 500, or 1000 units/ml. Cells were then washed and assayed for ADCC against ^{51}Cr -labeled SRBC or ORBC targets at an effector:target cell ratio of 0.5:1. MoAb was used as hybridoma culture supernatant at a 1:5 dilution. Spontaneous cytotoxicity by untreated or IFN- γ -treated U937 cells in the absence of MoAb was not detected.

Concentration of IFN- γ (units/ml)	Period of stimulation (hr)	% of ADCC with MoAb			
		IgG1	IgG2a	IgG2b	IgG3
0	0	0.6 \pm 0.2 ^a	0.7 \pm 0.5	-0.4 \pm 0.5	0.8 \pm 0.5
10	18	0.6 \pm 0.2	2.9 \pm 0.7	0.9 \pm 0.2	2.2 \pm 0.7
	36	1.1 \pm 0.4	4.1 \pm 1.3	0.9 \pm 0.4	3.2 \pm 1.1
100	54	1.2 \pm 0.5	4.7 \pm 1.1	0.8 \pm 0.3	3.5 \pm 1.0
	18	1.1 \pm 0.5	11.1 \pm 2.5	1.3 \pm 0.3	12.9 \pm 4.5
	36	3.3 \pm 0.7	16.7 \pm 5.5	1.6 \pm 0.5	17.8 \pm 4.8
500	54	2.5 \pm 1.3	15.8 \pm 3.5	3.2 \pm 0.4	18.4 \pm 5.4
	18	3.1 \pm 0.9	15.7 \pm 3.5	1.2 \pm 0.4	14.7 \pm 5.1
	36	4.2 \pm 0.9	22.0 \pm 5.3	2.1 \pm 1.3	21.8 \pm 4.4
1000	54	2.9 \pm 1.0	22.5 \pm 5.1	2.6 \pm 0.5	19.2 \pm 4.2
	18	1.6 \pm 0.7	17.4 \pm 3.4	2.0 \pm 0.6	16.5 \pm 2.6
	36	3.3 \pm 0.6	23.8 \pm 4.2	3.4 \pm 1.3	23.7 \pm 5.0
	54	4.1 \pm 1.0	25.0 \pm 4.8	3.8 \pm 1.2	22.4 \pm 3.1

^a Mean \pm S.D. of 3 separate experiments. Values represent percentage of specific lysis at the effector:target ratio of 0.5:1.

Table 3

Titration of mouse IgG in ADCC mediated by IFN- γ -treated U937 cells

U937 cells were incubated at 5×10^6 /ml for 40 hr with IFN- γ (0 or 500 units/ml). Cells were then washed and assayed for ADCC against ^{51}Cr -labeled SRBC or ORBC targets in the presence of MoAb as hybridoma culture supernatant at the MoAb dilutions shown. Thioglycolate-induced murine PEC were also used as effector cells.

Effector cells	Effector:target cell ratio	% of ADCC at antibody dilution											
		IgG1			IgG2a			IgG2b			IgG3		
		1:5	1:10	1:20	1:5	1:10	1:20	1:5	1:10	1:20	1:5	1:10	1:20
Untreated U937	0.5:1	0.6 ^a	0.1	-0.4	0.9	0.9	0.4	0.2	-0.3	-0.5	0.3	-0.2	-0.2
	1:1	0.8	0.4	-0.1	0.4	0.4	0.2	-0.2	0.1	-0.4	0.5	0.1	-0.2
IFN- γ -treated U937	0.5:1	3.2	2.8	1.8	21.8	20.7	13.6	2.4	1.9	1.1	23.2	23.2	14.7
	1:1	2.9	3.1	1.7	22.1	22.4	12.9	2.3	2.2	1.2	23.8	25.3	12.8
	2:1	2.5	2.2	1.3	15.2	14.8	10.2	1.8	1.7	0.9	18.3	18.6	11.5
Murine PEC	0.5:1	35.7	36.4	28.4	44.4	41.7	37.1	48.7	51.4	49.3	18.9	12.3	1.0
	1:1	37.0	36.1	35.4	41.6	38.7	34.5	47.6	44.6	48.0	17.3	7.4	1.0

^a Representative results (means) of 3 separate experiments. Values represent percentage of specific lysis.

Table 4

Blocking by Protein A of ADCC induced by IFN- γ

U937 cells were incubated at 5×10^6 /ml for 36 hr with or without IFN- γ . Cells were then washed and assayed for ADCC against ^{51}Cr -labeled SRBC targets. Targets were mixed with MoAb and incubated alone or with Protein A for 30 min before addition of effector cells. The dilution of MoAb was 1:5.

Pretreatment	Protein A concentration ($\mu\text{g}/\text{ml}$)	% of ADCC with MoAb			
		IgG2a		IgG3	
		0.25:1 ^a	0.5:1	0.25:1	0.5:1
Medium	0	0.6 ^b	1.2	0.8	0.7
IFN- γ (500 units/ml)	0	15.2	20.6	18.2	21.2
	1	7.6	10.4	14.0	21.6
	5	7.2	9.4	5.2	5.2
	20	4.1	6.6	3.3	3.6
	80	2.7	4.1	2.5	2.7

^a Effector:target cell ratio.

^b Means of triplicate cultures.

incubation, IgG2a-dependent ADCC and FcR expression of IgG2a was determined. As shown in Table 5, polymyxin B at a concentration of 25 $\mu\text{g}/\text{ml}$ did not alter the stimulation of U937 cells by IFN- γ . The possibility of a synergistic effect of LPS and IFN- γ was also analyzed, since LPS is known to be an activator and costimulator of macrophage-mediated cytotoxicity. LPS did not augment the effect of a suboptimal dose of IFN- γ . In addition, LPS alone had no effect on U937 cell ADCC or on FcR expression.

Table 5

Effect of polymyxin B and LPS on the activation of ADCC by IFN- γ

	% of ADCC with IgG2a MoAb at effector:target ratios		% of IgG2a EA7S rosette-forming cells
	0.5:1	1:1	
None	0.4 ^a	0.6	8.3
IFN- γ (500 units/ml)	21.1	18.6	29.3
IFN- γ + polymyxin B	22.6	19.2	28.6
Polymyxin B	0.8	0.8	10.1
IFN- γ (50 units/ml)	8.8	11.0	25.3
IFN- γ + LPS	0.1 $\mu\text{g}/\text{ml}$	10.1	9.8
	1 $\mu\text{g}/\text{ml}$	8.3	10.3
	10 $\mu\text{g}/\text{ml}$	7.8	12.5
	LPS		
0.1 $\mu\text{g}/\text{ml}$	0.4	0.5	11.4
1 $\mu\text{g}/\text{ml}$	0.4	0.5	8.7
10 $\mu\text{g}/\text{ml}$	0.6	0.7	9.3

^a Means of duplicate cultures and representative results of 3 separate experiments.

Inhibition of Interferon-induced ADCC and FcR Expression by Anti-IFN Serum. To conclusively identify IFN- γ as an enhancer of mouse MoAb-dependent lysis and FcR expression for mouse MoAb, the effect of anti-IFN- γ serum on IFN- γ -induced enhancement of ADCC and FcR expression was tested. As shown in Table 6, anti-IFN- γ serum treatment abolished the capacity of IFN- γ to induce IgG2a- and IgG3-dependent ADCC.

Furthermore, no rosette formation for IgG2a and IgG3 FcR was observed at the inducing concentration of IFN- γ used. Neither sheep anti-IFN- α serum nor normal rabbit serum at the same concentrations inhibited the induction of ADCC and FcR expression by IFN- γ .

We also examined the effect of recombinant IFN- γ , which is clearly not contaminated with other human lymphokines, on mouse MoAb-dependent lysis and FcR expression for mouse MoAb. The effects of recombinant IFN- γ were similar to those of natural IFN- γ ; recombinant IFN- γ induced a preferential enhancement of IgG2a- and IgG3-dependent U937 cell ADCC (Table 7); the dose-response curve in ADCC was the same as for natural IFN- γ on the basis of antiviral activity (data not shown); FcR expression for IgG2a and IgG3 but not for IgG1 and IgG2b on U937 cells was increased; and treatment of recombinant IFN- γ with anti-IFN- γ serum before addition to the cultures blocked any augmentation of ADCC and FcR expression by recombinant IFN- γ . Pretreatment with antiserum to human IFN- α or normal rabbit serum and the addition of polymyxin B did not alter the activity of recombinant IFN- γ (data not shown).

DISCUSSION

We have demonstrated here that natural and recombinant IFN- γ stimulates human monocytic cell line U937 to lyse SRBC or

ORBC *in vitro* in the presence of mouse MoAb. IFN- γ -stimulated U937 cells armed with IgG2a or IgG3 MoAb efficiently lysed target cells. The IFN- γ -induced enhancement of ADCC activity was accompanied by increased expression of FcR for mouse IgG2a and IgG3. FcR for mouse IgG1 and IgG2b on U937 cells were not detected after IFN- γ stimulation. The complete abrogation of natural and recombinant IFN- γ activity by anti-IFN- γ serum and the lack of effects of polymyxin B and LPS on IFN- γ activity confirmed IFN- γ as the agent which stimulates FcR expression and murine MoAb-dependent ADCC.

Murine macrophages have at least 3 different FcR for mouse IgG of different isotypes (4, 27). FcR1 is trypsin sensitive and specifically binds to monomeric IgG2a; FcR2 is trypsin resistant and preferentially binds complexed IgG1 and IgG2b; and FcR3 is trypsin sensitive and specifically binds aggregated IgG3. The independent regulation of mouse FcR specific for IgG1 and IgG2b immune complexes and for IgG2a has been reported (5). In the human system, 2 different FcR for human IgG have been identified on human leukocytes (6). A high-affinity FcR for human IgG1 is present on human monocytes but not on PMN, and a low-affinity FcR is present on PMN and NK/K cells but not on monocytes. It has recently been suggested that human monocytes cultured *in vitro* might bear both types of receptors (7). An FcR that binds human monomeric IgG1 has been purified by immunoabsorption to human IgG1 from both freshly isolated human monocytes and from U937 cells (1). This *M_r* 73,000 molecule is not detectable on normal peripheral blood PMN using the same technique. Recent studies have shown that the binding of IgG1 monomers to U937 cells is detected following activation by lymphokines; enhanced expression of the low-affinity FcR was not concomitantly observed (7), demonstrating the independent regulation of human leukocyte FcRs. Taken together, these results suggest that the human U937 cell and monocyte FcR is analogous to the mouse FcR1. In addition, IFN- γ -stimulated U937 cells exhibited significant FcR expression for IgG3. This finding is consistent with previous results (20) which demonstrate that IgG2a and IgG3, but not IgG1 or IgG2b, effectively compete with human IgG1 for binding to untreated and IFN- γ -treated human monocyte and promyelocytic cell lines. In the mouse, binding of IgG3 to FcR2 has not been observed, and the receptor for IgG3 has been described as a separate, low-affinity receptor (4). Although a corresponding FcR3 on human cells has not yet been demonstrated, it will be interesting to ascertain whether mouse IgG3 binds to a previously undescribed FcR or to the FcR reactive with IgG2a.

All mouse IgG classes mediated ADCC against SRBC or ORBC when mouse PEC were used as effector cells, consistent with a previous report (22). Ralph and Nakoinz (21) also report

Table 6

Neutralization of the IFN- γ -induced stimulation of ADCC and FcR expression by antiserum to IFN- γ

IFN- γ was neutralized with different dilutions of anti-IFN- γ antiserum for 45 min at 37° and added to the cultures based on the initial concentration of 500 units/ml. Anti-IFN- α serum and normal rabbit serum were used as control. U937 cells (5×10^6 /ml) were stimulated for 36 hr before assays. In ADCC assay, effector:target cell ratio was 0.5:1 and MoAb were used at a 1:5 dilution.

Pretreatment	% of ADCC		% of EA7S rosette-forming cells	
	IgG2a	IgG3	IgG2a	IgG3
Medium	1.1 ^a	0.6	5.1	0.4
IFN- γ (500 units/ml)	20.5	19.6	25.0	4.3
IFN- γ + anti-IFN- γ serum				
250 units/ml ^b	4.8	5.0	— ^c	—
500 units/ml	2.0	0.4	—	—
1000 units/ml	0.5	0.3	—	—
IFN- γ + anti-IFN- α serum				
250 units/ml	24.0	18.8	20.6	3.6
500 units/ml	23.8	23.0	21.6	3.9
1000 units/ml	23.5	17.9	22.7	3.8
IFN- γ + normal rabbit serum				
Final dilution, 1:40	19.8	19.2	25.0	4.2
Final dilution, 1:20	24.2	19.0	29.0	4.4
Final dilution, 1:10	18.7	18.8	27.7	4.1

^a Means of duplicate cultures from 3 separate experiments.

^b Neutralization units.

^c —, not detectable.

Table 7

Effect of recombinant IFN- γ on ADCC and FcR expression by U937 cells

U937 cells (5×10^6 /ml) were stimulated with IFN- γ for 36 hr. Cells were then washed and assayed for ADCC and for FcR expression (rosette formation). Recombinant IFN- γ was neutralized with anti-IFN- γ antiserum for 45 min at 37° and added to the cultures. In the ADCC assay, effector:target cell ratio was 0.5:1, and MoAb were used at a 1:5 dilution.

Pretreatment	% of ADCC				% of EA7S rosette-forming cells			
	IgG1	IgG2a	IgG2b	IgG3	IgG1	IgG2a	IgG2b	IgG3
Medium	0.8 ^a	1.0	-1.0	1.0	— ^b	7.5	—	1.0
Recombinant IFN- γ (500 units/ml)	2.6	22.5	1.2	22.1	—	22.5	—	5.6
Recombinant γ IFN + anti-IFN- γ serum (500 neutralization units/ml)	0.3	0.8	-0.2	0.9	—	ND	—	ND

^a Means of triplicate cultures.

^b —, not detectable; ND, not determined.

the apparent lack of restriction among mouse IgG isotypes in inducing ADCC against SRBC targets or mouse T-lymphoma targets mediated by human PMN or nonadherent mononuclear leukocytes, respectively (21, 22).

Our findings that IFN- γ induces FcR expression and ADCC with specificity for mouse IgG2a and IgG3 MoAb suggest the possible use of antitumor MoAbs of these isotypes in combination with IFN- γ in the immunotherapy of human cancer.

ACKNOWLEDGMENTS

We wish to thank Joan MacDonald Smith for excellent technical assistance and Marina Hoffman for editorial assistance.

REFERENCES

- Anderson, C. L. Isolation of the receptor for IgG from a human monocyte cell line (U937) and from human peripheral blood monocytes. *J. Exp. Med.*, **156**: 1794-1806, 1982.
- Atkinson, B. F., Ernst, C. S., Herlyn, M., Steplewski, Z., Sears, H. F., and Koprowski, H. Gastrointestinal cancer-associated antigen in immunoperoxidase assay. *Cancer Res.*, **42**: 4820-4823, 1982.
- Badger, C. C., and Bernstein, I. D. Therapy of murine leukemia with monoclonal antibody against a normal differentiation antigen. *J. Exp. Med.*, **157**: 828-842, 1983.
- Diamond, B., and Yelton, D. E. A new Fc receptor on mouse macrophages binding IgG3. *J. Exp. Med.*, **153**: 514-519, 1981.
- Ezekowitz, R. A. B., Bampton, M., and Gordon, S. Macrophage activation selectively enhances expression of Fc receptors for IgG2a. *J. Exp. Med.*, **157**: 807-812, 1983.
- Fleit, H. B., Wright, S. D., Durie, C. J., Valinsky, J. E., and Unkeless, J. C. Ontogeny of Fc receptors and complement receptor (CR3) during human myeloid differentiation. *J. Clin. Invest.*, **73**: 516-525, 1984.
- Fleit, H. B., Wright, S. D., and Unkeless, J. C. Human neutrophil Fc γ receptor distribution and structure. *Proc. Natl. Acad. Sci. USA*, **79**: 3275-3279, 1982.
- Guyre, P. M., Morganeli, P. M., and Miller, R. Recombinant immune interferon increases immunoglobulin G Fc receptors on cultured human mononuclear phagocytes. *J. Clin. Invest.*, **72**: 393-397, 1983.
- Herlyn, D., and Koprowski, H. IgG2a monoclonal antibodies inhibit human tumor growth through interaction with effector cells. *Proc. Natl. Acad. Sci. USA*, **79**: 4761-4765, 1982.
- Herlyn, D., Steplewski, Z., Herlyn, M., and Koprowski, H. Inhibition of growth of colorectal carcinoma in nude mice by monoclonal antibody. *Cancer Res.*, **40**: 717-721, 1980.
- Herlyn, M., Sears, H. F., Steplewski, Z., and Koprowski, H. Monoclonal antibody detection of a circulating tumor-associated antigen. I. Presence of antigen in sera of patients with colorectal, gastric, and pancreatic carcinoma. *J. Clin. Immunol.*, **2**: 135-140, 1982.
- Herlyn, M., Steplewski, Z., Herlyn, D., and Koprowski, H. Colorectal carcinoma-specific antigen: detection by means of monoclonal antibodies. *Proc. Natl. Acad. Sci. USA*, **76**: 1438-1442, 1979.
- Kirsh, M. E., and Hammerling, U. Immunotherapy of murine leukemia by monoclonal antibodies. I. Effect of passively administered antibody on growth of transplanted tumor cells. *J. Immunol.*, **127**: 805-810, 1981.
- Koprowski, H., Herlyn, M., Sears, H., and Steplewski, Z. Specific antigen in serum of patients with colon carcinoma. *Science (Wash. DC)*, **212**: 53-55, 1981.
- Koprowski, H., Steplewski, Z., Herlyn, D., and Herlyn, M. Study of antibodies against human melanoma produced by somatic cell hybrids. *Proc. Natl. Acad. Sci. USA*, **75**: 3405-3409, 1978.
- Larrick, J. W., Fisher, D. G., Anderson, S. J., and Koren, S. Characterization of a human macrophage-like cell line stimulated *in vitro*: a model of macrophage functions. *J. Immunol.*, **125**: 6-12, 1980.
- Le, J., Prensny, W., Yip, Y. K., Chang, Z. L., Hoffman, T., Stevenson, H. C., Balazs, I., Sadiq, J. R., and Vilcek, J. Activation of human monocyte cytotoxicity by natural and recombinant immune interferon. *J. Immunol.*, **131**: 2821-2826, 1983.
- Morrison, D. C., and Jacobs, D. M. Binding of polymyxin B to the lipid A portion of bacterial lipopolysaccharides. *Immunochemistry*, **13**: 813-818, 1976.
- Murray, M. W., Berish, Y. R., and Rothermel, C. D. Killing of intracellular *Leishmania donovani* by lymphokine-stimulated human mononuclear phagocytes. Evidence that interferon- γ is the activating lymphokine. *J. Clin. Invest.*, **72**: 1506-1510, 1983.
- Perussia, B., Dayton, E. T., Lazarus, R., Fanning, V., and Trinchieri, G. Immune interferon induces the receptor for monomeric IgG1 on human monocytic and myeloid cells. *J. Exp. Med.*, **158**: 1092-1113, 1983.
- Ralph, P., and Nakoinz, I. Cell-mediated lysis of tumor targets directed by murine monoclonal antibodies of IgM and all IgG isotypes. *J. Immunol.*, **131**: 1028-1031, 1983.
- Ralph, P., Nakoinz, I., Diamond, B., and Yelton, D. All classes of murine IgG antibody mediate macrophage phagocytosis and lysis of erythrocytes. *J. Immunol.*, **125**: 1885-1888, 1980.
- Sears, H. F., Herlyn, M., Del Vilano, Z., Steplewski, Z., and Koprowski, H. Monoclonal antibody detection of circulating tumor-associated antigen. II. A longitudinal evaluation of patients with colorectal cancer. *J. Clin. Immunol.*, **2**: 141-149, 1982.
- Steplewski, Z., Herlyn, D., Maul, G., and Koprowski, H. Hypothesis: macrophages as effector cells for human tumor destruction mediated by monoclonal antibody. *Hybridoma*, **2**: 1-5, 1983a.
- Steplewski, Z., Lubeck, M. D., and Koprowski, H. Human macrophages armed with murine immunoglobulin G2a antibodies to tumors destroy human cancer cells. *Science (Wash. DC)*, **221**: 865-867, 1983b.
- Sundstrom, C., and Nilsson, K. Establishment and characterization of a human histiocytic lymphoma cell line (U937). *Int. J. Cancer*, **17**: 565-577, 1976.
- Unkeless, J. C. The presence of two Fc receptors on mouse macrophages: evidence from variant cell lines and differential trypsin sensitivity. *J. Exp. Med.*, **145**: 931-947, 1977.
- Young, W. W., Jr., and Hakomori, S. Therapy of mouse lymphoma with monoclonal antibodies to glycolipid: secretion of low antigenic variants *in vivo*. *Science (Wash. DC)*, **211**: 487-489, 1981.