INFECTION AND IMMUNITY, Apr. 1983, p. 172–176
0019-9567/83/040172-05$02.00/0

Vol. 40, No. 1

Human Interferon-γ Enhances the Expression of Class I and Class II Major Histocompatibility Complex Products in Neoplastic Cells More Effectively than Interferon-α and Interferon-β

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Received 2 November 1982/Accepted 18 January 1983

Human interferon-γ was more effective than interferon-β or -α in stimulating production of immunoassociated antigens; HLA-A, -B, and -C; and β_2-microglobulin in human M14 and Namalva cells. The comparison was made on the basis of antiviral units, and the stimulation could be abolished by treatment of the interferon-γ preparation with pH 2 or anti-interferon-γ serum.

The interferon (IFN) system has a number of important functions in the host, among which antiviral, cell-modulating, and immunoregulatory activities are the most documented (12, 13, 22, 23). At least three antigenically distinct types of IFN have been identified so far: α, produced by B or natural killer cells; β, produced by fibroblast and epithelial cells; and γ, produced by sensitized or mitogen-activated T lymphocytes. There are indications that the antiviral effects, as well as the effects on other cellular functions, may be activated by the three types of IFN through different mechanisms of action (6, 10, 24). Moreover, IFN-γ is a more potent mediator of antitumor and anticellular activities than IFN-α and -β (10, 24–26).

It has been previously shown that IFN-α and -β enhance the expression of class I products (HLA-A, -B, and -C) of the major histocompatibility complex (MHC; 7, 9, 14, 15, 18). We have also found that class II products (immunoassociated antigens [Ia]) can be affected by IFN-β in human cells (3, 7).

This study shows that MHC expression is also enhanced by IFN-γ and that the action of IFN-γ, as determined on the basis of antiviral units, is up to 200 times more potent than the actions of other types of IFN.

MATERIALS AND METHODS

Cells. We used human melanoma cells (M14), human diploid cell strains derived from embryonic skin and muscle (E_1SM), and human B lymphoblastoid cells (Namalva). E_1SM and Namalva cells were maintained in Eagle minimum essential medium (Flow Laboratories, Inc., Irvine, Scotland) supplemented with 10 and 15% fetal calf serum, respectively. M14 cells were maintained in RPMI 1640 medium (Flow Laboratories) supplemented with 10% fetal calf serum.

IFNs. Partially purified IFN-α (specific activity, 10^6 IU/mg of protein) was obtained from G. B. Rossi, Istituto Superiore di Sanità, Rome. Partially purified IFN-β (specific activity, 10^6 IU/mg of protein) was produced in E_1SM cells and processed as previously described (7). We also used samples of human IFN-β purified to homogeneity (preparation no. 34370) and an anti-human IFN-β goat serum (preparation no. 925) kindly provided by A. Billiau, Rega Institute, Leuven, Belgium. The serum was raised against pure human IFN-β; its titer against 10 IU of human IFN-β per ml was 10^{-7}. Crude IFN-γ was prepared from peripheral blood leukocytes obtained from healthy donors and subjected to Ficoll-Hypaque gradient sedimentation. Activation was performed as previously described (11) with 0.2 μg of staphylococcal enterotoxin B per ml per 10^6 cells or by treatment for 30 min at 24°C with 10 IU of galactose oxidase (Worthington Diagnostics, Freehold, N.J.), per ml per 10^7 cells (5). We also used samples of partially purified IFN-γ (specific activity, 10^5 IU/mg of protein), a gift of J. Vilcek, New York, which was induced by tetradecanoil phorbol-acetate-phytohemagglutinin treatment (31). Rabbit antiserum against partially purified human IFN-γ was a gift of M. De Ley, Rega Institute, Leuven, Belgium. Its titer against 10 U of IFN-γ per ml was 10^{-3} (4). Throughout this paper, the amounts of IFN-α, -β, and -γ are referred to in terms of antiviral activity. IFNs were titrated on E_1SM cells with vesicular stomatitis virus (VSV) as the challenge virus as previously described (7). In our study, 1 U of IFN-α corresponds to 0.5 IU and 1 U of IFN-β corresponds to 2 IU; no standard is available so far for IFN-γ.

In a typical experiment, cells were seeded at half the saturation density in the presence or absence of IFN. After 48 h, cells were washed twice with phosphate-buffered saline and lysed with 75 mM Tris-hydrochloride, pH 7.8, containing 2% Renex-30 (30). Parallel cultures were trypsinized and counted; cell viability was always higher than 90%. The antiviral state of each culture was determined by VSV titer reduction, as described previously (7).

Radioimmunoassay. (i) ^{125}I-labeled antigens. Purified β_2-microglobulin (β_2-m) was provided by N. Tanigaki, Buffalo, N.Y. It showed a single 12,000-dalton band on electrophoretic analysis. Papain-solubilized HLA-A, -B, and -C antigens were prepared from lymphoid RPMI 1788 cells (19). On electrophoretic analysis, the antigens showed only two bands of 34,000 and 12,000 daltons. Separation of the heavy and light polypeptides was done by acid dissociation of the material (1). Detergent-purified human Ia molecules were obtained from Renex-30-solubilized membranes of U698M cells as previously described (29, 30). Ia preparations, devoid of HLA-A, -B, and -C and β_2-m molecules (28), showed the two-band pattern (27,000 and 34,000 daltons) typical of Ia molecules on electrophoretic analysis. Labeling of the antigens was performed as previously described (19, 30).

(ii) Inhibitors. Cell lysates were used as inhibitors in the radioimmunoassay. Cells were washed twice with phosphate-buffered saline and resuspended in 75 mM Tris-hydrochloride, pH 7.8, containing 2% Renex-30.

(iii) Antisera. Rabbit anti-human Ia serum, raised against glycoproteins of Daudi cells, was used. It was directed against both α and β Ia subunits (27). Rabbit serum against human HLA-A, -B, and -C was raised against purified antigens of RPMI 1788 cells as previously described (21). Both antisera were provided by N. Tanigaki. Rabbit anti-human β_2-m serum was raised against a purified preparation of human β_2-m as previously described (20).

Radioimmunoassay procedure. The direct binding test was used in the radioimmunoassay (29). Antiserum dilutions were chosen so as to obtain about 60% of maximum binding in the absence of the inhibitor. Each inhibitor was tested in different amounts as described previously (29). Controls without inhibitor and with normal serum instead of antiserum were included in each assay. Percent inhibition was expressed by the following formula:

$$100 - \frac{\text{cpm bound with inhibitor} - \text{cpm bound by normal rabbit serum}}{\text{cpm bound without inhibitor} - \text{cpm bound by normal rabbit serum}} \times 100.$$

The amount of inhibitor causing 50% inhibition in the radioimmunoassay was considered 1 50% inhibition unit (IU_{50}). It must be pointed out that the IU_{50}s of the three products tested are arbitrary units and therefore not comparable to one another. For β_2-m, 1 IU_{50} corresponds to 0.6 ng. An M14 cell lysate expressing all three antigens was used as a standard sample in each radioimmunoassay. In four experiments, the coefficient of variation (standard deviation/mean) never exceeded 15% for each antigen tested. No correction of the experimental values obtained in different experiments was therefore carried out.

RESULTS

Differential effects of IFN-α, -β, and -γ on MHC expression. M14 cells were treated for 48 h with graduated amounts of IFN-α, -β, or -γ; the cellular contents of MHC products were determined as described above. Results of a representative experiment are reported in Fig. 1. IFN-α and -β caused a dose-dependent increase of HLA-A, -B, and -C and β_2-m expression that reached a plateau two to three times base levels after treatment with 1,000 to 5,000 U/ml, as previously reported (3, 7). Only IFN-β was effective in enhancing Ia expression in M14 cells; the enhancement was slightly evident at up to 1,000 U/ml and reached a plateau that was two times base levels at 5,000 U/ml. With IFN-γ, like IFN-α and -β, the expression of all three antigens increased in a dose-dependent manner to two to three times base levels. However, the effects of IFN-γ were evident at doses significantly lower than those of IFN-α and -β.

We compared the efficacies of the three types of IFN in enhancement of MHC product expression. To do this, we calculated the IFN concentration that caused an enhancement corresponding to 50% of the maximal effect obtainable with any IFN (P/2 value). Furthermore, we extended the studies to Namalva lymphoblastoid cells. Data obtained for M14 and Namalva cells are reported in Table 1. With M14 cells, IFN-α and -β had similar potencies in enhancing HLA-A, -B, and -C and β_2-m expression (P/2, 650 and 500 U/ml, respectively, for HLA-A, -B, and -C and 830 U/ml [both IFNs] for β_2-m). For Ia expression, IFN-α was ineffective in M14 cells. IFN-γ appeared to be 32 times more potent than IFN-β in this respect, and 14 to 16 times more effective than IFN-β and -α in enhancing expression of HLA-A, -B, and -C. In the case of β_2-m expression in M14 cells, only a fivefold difference in P/2 values was found between IFN-γ and IFN-α or -β.

Data for Namalva cells were consistent with those obtained for M14 cells, and differences in potency were even more pronounced. In all three instances, the P/2 values of IFN-γ were 100 to 200 times lower than those of IFN-α and -β. It is noteworthy that in Namalva cells, Ia expression was also stimulated by treatment with IFN-α.

IFN-γ: effects on MHC expression and virus replication, as compared with effects of IFN-β. We compared the extent of MHC expression enhancement by IFN-γ and -β preparations of various degrees of purity. IFN-β enhanced Ia expression regardless of the presence of contaminants, because pure IFN-β exerted the same effect as crude (data not shown) and partially purified IFN (Table 2). The results of seven experiments with 5,000 U of partially purified IFN-β per ml were analyzed by Student's t test, and the result was $0.01 > P > 0.001$. In addition, the effect was abolished by monospecific anti-IFN-β serum. All of these findings paralleled the

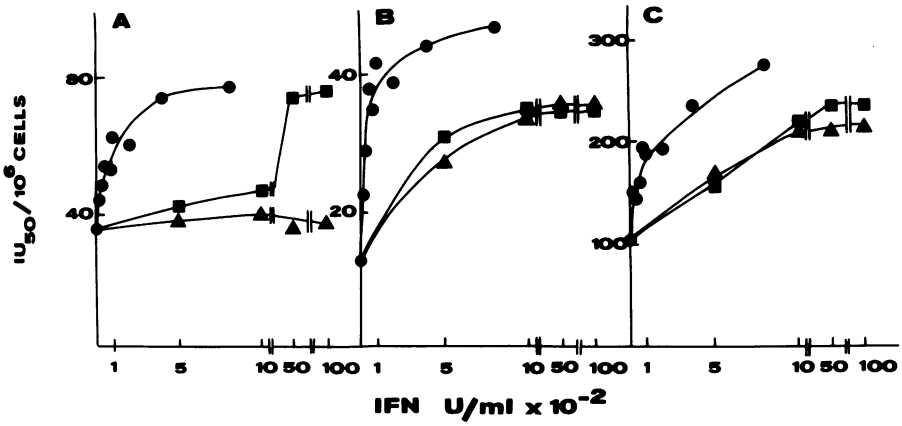

FIG. 1. MHC expression in M14 cells treated with IFN-α, -β, and -γ. Cells were treated for 48 h with graduated amounts of IFN and then assayed by radioimmunoassay for Ia (A), HLA-A, -B, and -C (B), and β_2-m (C). Symbols: ▲, IFN-α; ■, IFN-β; ●, IFN-γ.

establishment of the antiviral state (Table 2). Similar results were obtained for crude and partially purified IFN-γ preparations. The results of six experiments with 200 U of crude IFN-γ per ml were analyzed by Student's *t* test, and the result was $P < 0.001$. Furthermore, treatment with pH 2 or with anti-IFN-γ serum completely abolished the enhancement of Ia expression. The antiviral state followed the addition of active IFN-γ under various experimental conditions (Table 2). HLA-A, -B, and -C and β_2-m expression paralleled Ia expression (data not shown). We can exclude the possibility that the enhancing effect was due to interleukin 2 (IL2), a frequent contaminant of IFN-γ preparations. In fact, treatment with partially purified IL2 did not enhance MHC expression (data not shown), at least at the IL2 level generally present in IFN-γ preparations.

As for the antiviral state observed under the various experimental conditions (Table 2), it appears that (i) both crude and purified preparations of either IFN-β or -γ were able to inhibit VSV replication to the same extent, (ii) inhibition was reversed by preincubating either IFN with a specific antiserum or treating IFN-γ with acid, and (iii) the extent of virus inhibition was lower in IFN-γ-treated cultures than in IFN-β-treated cultures. In fact, treatment with IFN-β inhibited VSV replication almost 4 logs, whereas

TABLE 1. Effects of IFN-α, -β, and -γ preparations on MHC expression in M14 and Namalva cells

Cell line	IFN type	IFN P/2 value (U/ml)		
		Ia	HLA-A, -B, and -C	β_2-m
M14	α	>10,000	650	750
	β	2,450	500	750
	γ	75	40	140
Namalva	α	2,000	4,000	2,000
	β	1,800	4,000	2,000
	γ	20	20	20

TABLE 2. Ia expression and VSV titer in M14 cells treated with IFN-β or -γ

Treatment	Ia expression ($IU_{50}/10^6$ cells)[a]	VSV titer (log PFU/ml)[b]
None	38	7.9
IFN-β		
200 U/ml, partially purified	37	4.1
5,000 U/ml, partially purified	85	3.5
5,000 U/ml, pure	79	3.3
5,000 U/ml, pure, + anti-IFN-β serum[c]	36	8.0
IFN-γ		
200 U/ml, crude	73	6.5
1,000 U/ml, crude	78	6.5
200 U/ml, partially purified	85	6.6
200 U/ml, crude, + anti-IFN-γ serum[d]	39	7.8
200 U/ml, crude; acid (pH 2)[e]	41	7.9

[a] Cells were harvested 48 h after seeding and assayed by radioimmunoassay.

[b] Cells were washed three times with phosphate-buffered saline and infected with VSV (5 PFU per cell) 24 h after seeding. Virus was collected after 20 h and titrated by plaque assay on mouse L929 cells.

[c] IFN-β was preincubated with a 10-fold excess of anti-human IFN-β serum at 4°C for 90 min.

[d] IFN-γ was preincubated with a fourfold excess of anti-human IFN-γ serum at 4°C for 90 min.

[e] IFN-γ was exposed to acid at 4°C for 120 min.

IFN-γ was able to reduce VSV titers only up to 1.5 to 2 logs. This reduced protection against VSV infection was also observed in other cell lines (data not shown) and is consistent with data in literature (24).

DISCUSSION

Previous studies have shown that IFN-α and -β enhance the expression of class I (HLA-A, -B, and -C) (7, 14, 15, 18) and class II (Ia) (3, 7) antigens, as well as that of β_2-m (3, 7, 9, 14). The present study shows that MHC expression can also be stimulated in a dose-dependent manner by IFN-γ. Using antiviral units as a measure, we determined that IFN-γ was significantly more effective than IFN-α and -β, as indicated by the findings that the dose-response curves for IFN-γ were steeper than those obtained for IFN-α and -β (Fig. 1) and the IFN-γ P/2 values were up to 200 times lower, in terms of conventional antiviral units, than those of the other IFN types.

The possibility that the MHC expression-enhancing effect and higher potency of IFN-γ preparations are due to the presence of contaminants is minimal, since the same effect has been observed with preparations of IFN-γ obtained from different sources, induced by different mitogens, and having different degrees of purity. Moreover, the effect is completely abolished by treatment with acid (pH 2) or antiserum to IFN-γ (Table 2).

It has been shown that Ia, HLA-A, -B, and -C, and β_2-m play an important role in triggering and controlling immune responses (8, 17). For instance, Ia molecules are likely to be products of immune response (Ir) genes (16), and HLA-A, -B, and -C are involved in T-lymphocyte killing of virus-infected cells (32). IFNs are among the soluble mediators synthesized during immune responses and, in turn, have immunoregulatory properties (reviewed in reference 25). In this respect, IFN-γ has been demonstrated to be the most potent modulator (25, 26). The present study shows that IFN-γ is also the most effective in enhancing MHC expression, whereas its antiviral properties are lower than those of IFN-α and -β, at least with respect to VSV replication.

All of these findings support the argument that IFN-γ plays a major role in the modulation of various components involved in the immune system. Moreover, our findings suggest that the immunoregulatory activities of IFNs are mediated, at least in part, by the regulation of MHC expression.

ACKNOWLEDGMENTS

This work was supported in part by grants 82.00297.96 and 82.00397.96 from Consiglio Nazionale delle Ricerche, Progetto Finalizzato Controllo della Crescita Neoplastica, and by Istituto Pasteur, Fondazione Cenci Bolognetti.

We are indebted to R. Tosi, F. Dianzani and G. B. Rossi for reading the manuscript and to O. Acuto for providing IL2.

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