INFECTION AND IMMUNITY, Oct. 1983, p. 122–125
0019-9567/83/100122-04$02.00/0

Vol. 42, No. 1

Differential Effects of Gamma Interferon on Expression of HLA Class II Molecules Controlled by the DR and DC Loci

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Received 12 April 1983/Accepted 22 July 1983

Interferon-γ affected the expression of the products of the immunoassociated antigen complex by a differential modulation of DR- and DC-locus-controlled molecules. In melanoma M14 cells treated with interferon-γ, levels of DR molecules were increased two- to threefold, whereas levels of DC molecules were increased six- to sevenfold. Similar effects were induced on the two allelic products of each locus.

A wide variety of cell regulatory effects have been attributed to the interferons (IFNs), besides the well-known antiviral properties (10). General agreement indicates immune IFN-γ as the most potent modulator of cell functions (1, 9, 16), especially with respect to those related to the immune system (17). It is conceivable that at least some of these activities may be connected to modulation of major histocompatibility complex-controlled molecular components.

Several studies have shown an IFN-induced enhanced expression of class I molecules (HLA-A, -B, and -C) both in humans and in mice (11, 18). Such an effect has been observed with all IFN types; α, β, and γ (3, 6, 7, 11, 18, 29). As far as the effect on class II molecules (immunoassociated antigens [Ia]) is concerned, IFN-α has been repeatedly shown to be ineffective (8), whereas in previous studies we have demonstrated a sizable increase of Ia expression in human cell lines treated with IFN-β (3, 6, 7).

The present work shows the effects of IFN-γ on class II expression in melanoma M14 cells. Previous quantitation by radioimmunoassay (RIA) of Ia molecules revealed a significant increase, after treatment of cells with IFN-γ, that was up to 200-fold more effective than was IFN-β (7). A detailed analysis of IFN-γ effects was carried out in this work, taking into account the recognized genetic heterogeneity of class II molecules. In fact, recent evidence has been provided for the existence in humans of the two classes of Ia molecules which have been well characterized in the mouse: I-A and I-E (2). The corresponding human homologs, DR and DC, have been shown to differ from each other for both alpha and beta subunits (19, 22).

The data obtained show that treatment with IFN-γ induces an increase in the level of both molecular subsets. However, the magnitude of the IFN-γ effect on DC molecules is much more pronounced than the effect on DR molecules.

MATERIALS AND METHODS

Cells. M14 human melanoma cells were grown in RPMI 1640 (Flow Laboratories, Irvine, Scotland), supplemented with 2 mM L-glutamine and 10% fetal calf serum.

Interferon. Crude IFN-γ was prepared from peripheral blood leukocytes, as previously described, by activation with 0.2 μg of staphylococcal enterotoxin B per ml per 10^6 cells (7) or by treatment for 30 min at 24°C with 10 U of galactose oxidase (Worthington Biochemicals Corp., Freehold, N.J.), per ml per 10^7 cells (7). Partially purified IFN-γ, induced by PHA-TPA (specific activity, 10^5 U per mg of protein, gift of J. Vilcek, New York) was also used. A rabbit antiserum against partially purified human IFN-γ was a gift of M. De Ley, Leuven, Belgium. The neutralization titer of the antiserum was 10^{-3} against 10 U of IFN-γ per ml. The characterization of the antiserum has been previously reported (5). This antiserum was used against both crude and partially purified IFN-γ (ammonium sulfate-precipitated, specific activity, $10^{4.6}$ U per mg of protein). IFN was titrated on human E_1SM diploid fibroblasts with vesicular stomatitis virus as the challenge virus, as previously described (6). In a typical experiment, cells were seeded at one-half the saturation density in the presence or absence of IFN. At 48 h, cells were washed twice with phosphate-buffered saline and lysed with 75 mM Tris-hydrochloride (pH 7.8), containing 2% Renex-30 detergent. Parallel cultures were trypsinized and counted; cell viability was always higher than 90%. The antiviral state of the cultures was determined by vesicular stomatitis virus yield reduction as described in reference 6.

RIA. Quantitation of antigens was performed by inhibition reactions (RIA) in which cell lysates (treated or not treated with IFN-γ) were used as competitors of the binding reaction between specific antisera and ^{125}I-labeled purified Ia molecules (6, 7). The amount of inhibitor corresponding in RIA to 50% inhibition was

taken as 1 50% inhibition unit (IU_{50}). Throughout this paper results are expressed as IU_{50} per 10^6 cells. It must be pointed out that the IU_{50}s of each product tested are arbitrary units and therefore not comparable to each other. Comparison of the expression of different products was made as the ratio between values of treated over control cells. A lysate from M14 cells was used as the standard sample in each RIA.

Antigens and antisera. Class II antigens were obtained from human lymphoblastoid cell lines, as previously described (20). They were devoid of HLA-A, -B, and -C and β_2-microglobulin and on electrophoretic analysis showed the two-band pattern typical of Ia molecules. The antigens were labeled with ^{125}I as described previously (28). The antigen-antiserum combinations varied according to the Ia molecular species to be determined: (i) DC1 molecules (corresponding to MB1 or MT1 supertypic specificities defined by cytotoxicity, [21, 24, 27]) were obtained from Ia antigens prepared from Daudi cells (DRw6, DC1) and enriched by papain digestion (23). Antiserum was Fe 131/6 (21, 26, 27). (ii) DC4 molecules (corresponding to MB3 or MT4 specificities, [24, 25]) were prepared from LG38 cells (DR5 and -5 and DC4 and -4 after adsorption with a rabbit antiserum specifically directed against the α subunit of DR molecules (19). Antiserum was Douville (anti-DR4+MB3), kindly provided by R. Duquesnoy, Milwaukee, Wis. (iii) DR4 molecules were obtained from U698M cells (DR2, and -4 and DC1, and -4). Antiserum was Fe 59/15 (28). (iv) DRw6 molecules were prepared from Daudi cells. Antiserum was Fe 88/37 (28). (v) Total Ia molecules were prepared from U698M cells. Antiserum was rabbit 7147 serum raised against Daudi cell membranes (6). This antiserum binds DR and DC molecules; its reaction with U698M ^{125}I-labeled purified extract is inhibited by cell lysates, irrespective of their DR phenotype (6, 28).

RESULTS

Effect of IFN-γ on the overall expression of class II molecules. Table 1 shows the effect of IFN-γ on class II molecules, as quantitated by RIA employing a rabbit anti-human Ia serum. This antiserum recognizes human class II molecules of all loci. Therefore, the test reveals the overall quantitative modification of Ia molecules. The enhancing effect of IFN-γ is dose dependent, and it appears to reach a plateau at about 200 U/ml. Repeated experiments with IFN-γ doses giving nearly maximum effect gave similar results, irrespective of the methods of preparation or purification. Comparison with control values shows a highly significant difference ($P < 0.001$).

Effect of IFN-γ on the expression of the products of DR and DC loci. By preliminary tests the M14 cell line was defined as: DR4 and -w6 and DC1 and -4. The molecules carrying each of these alloantigenic determinants were quantitated separately by measuring the inhibiting activity of cell lysates in different RIAs.

Figure 1 shows the data obtained by using cells treated for 48 h with increasing concentrations of IFN-γ. The two allelic forms of the DR locus, carrying the DR4 and DRw6 determinants, show a two to threefold increase, as compared to controls. The two DC allelic forms carrying DC1 and DC4 determinants show approximately a six to sevenfold increase.

TABLE 1. Ia levels in M14 treated with IFN-γ

Expt.	IFN-γ treatment (U/ml)	Ia levels[a] (IU_{50} per 10^6 cells)
1	0	38
	25	47
	50	49
	75	60
	100	65
	150	78
	200	80
	400	79
2	0	38
	200	104
3	0	48
	200	96
4	0	38
	200	93
5	0	46
	200	84
6	0	38
	200	85
	200, pH 2 treated[b]	41

[a] Values of controls and cultures treated with 200 U of IFN-γ per ml were used for Student's t analysis: differences were highly significant ($P < 0.001$). For details see text.

[b] Treatment at 0°C for 2 h.

Specificity of IFN-γ action. The described effects appear to be due to IFN-γ molecules, since they were observed with IFN preparations obtained from different sources, induced with different protocols, had different degrees of purity, and were abolished by exposure of IFN-γ preparations to pH 2 (see Table 1). Furthermore, specific antiserum completely abolished both the antiviral activity (Fig. 2B) and the modulating effects on the two Ia molecular subsets (Fig. 2A).

DISCUSSION

It was previously shown in this laboratory that IFN-γ enhances the overall expression of class II HLA antigens and that this type of IFN is significantly more effective than IFN-β (7). This report shows the effect of IFN-γ on two of the three different loci controlling class II molecules that are known so far. The DR locus, in both allelic forms, shows a two- to threefold increase of expression after exposure to IFN-γ; the two allelic forms of DC are much more markedly increased, up to six- to sevenfold. IFN-γ seems to act on those important cell membrane compo-

nents by a fine tuning modulation and not by an indiscriminate variation of the expression. This is the first report of differential expression of Ia products controlled by separate loci, after treatment with IFN.

The evidence for distinct loci controlling class II molecules in humans has been the subject of several previous publications (19; R. Sorrentino, G. Corte, F. Calabi, N. Tanigaki, and R. Tosi, Mol. Immunol., in press) and will not be discussed here. There is evidence that human DR is homologous to mouse I-E and that human DC is homologous to mouse I-A (2).

The differences between these molecular species controlled by the Ia region should correspond to differentiated functions. However, very little is known on this particular point.

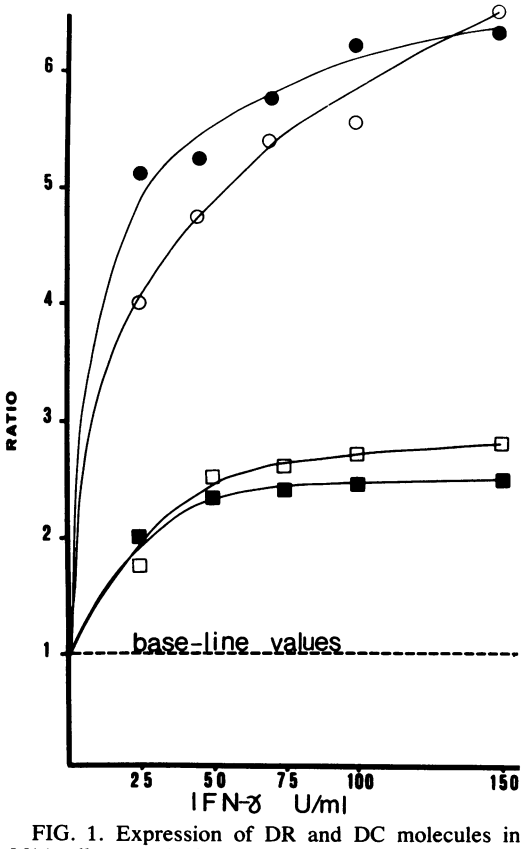

FIG. 1. Expression of DR and DC molecules in M14 cells treated with IFN-γ. Lysates of cells treated for 48 h with graded amounts of partially purified IFN-γ were used in RIA for quantitation of DR4 (□), DRw6 (■), DC1 (●) and DC4 (○). Data are expressed as the ratio of IFN-treated values to control values. For each antigen, base-line values expressed as $IU_{50}/10^6$ cells were: DR4, 84; DRw6, 75; DC1, 26; DC4, 10. Student's t test was made on results of four experiments carried out with 250 U of IFN-γ per ml. For all antigens tested differences were highly significant ($0.01 < P < 0.001$).

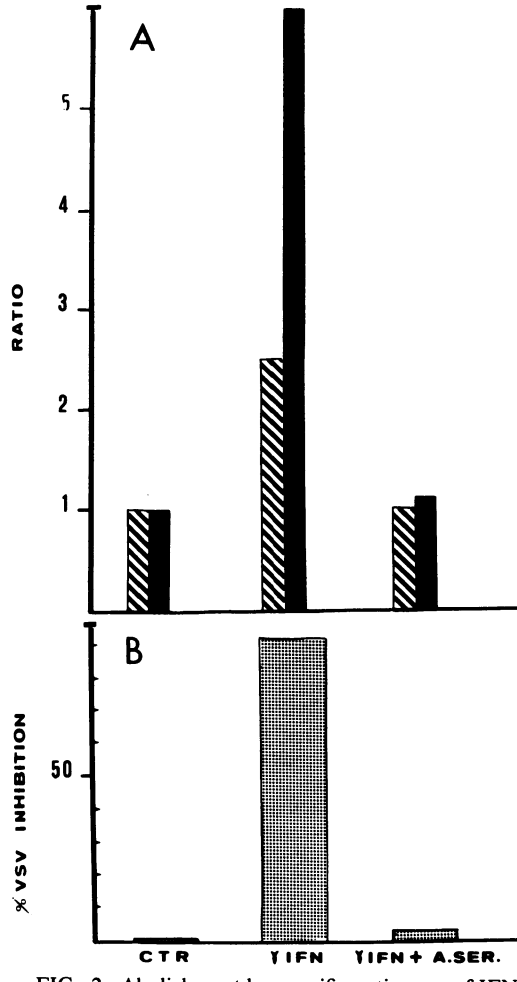

FIG. 2. Abolishment by specific antiserum of IFN-γ effects on DR and DC expression and on VSV replication in M14 cells. Three conditions were carried out: untreated cells (CTR), cells treated with 150 U of IFN-γ per ml, preincubated for 90 min at 4°C with or without a fourfold excess of a rabbit anti-human IFN-γ serum (γ-IFN+A.SER.). (A) DR4 (▨) and DC1 (■) levels. Data are expressed as in Fig. 1. Base-line values are as in Fig. 1. (B) Vesicular stomatitis virus yield: 24 h after seeding, cells of the same three culture conditions of (A) were washed three times and infected with vesicular stomatitis virus (5 PFU per cell). Virus collected after 20 h was titrated by plaque assay on mouse L929 cells. Data are expressed as percent inhibition with respect to control cultures. Control values were $10^{7.5}$ PFU/ml.

There is only one report suggesting a specific involvement of DC molecules in the generation of effector T cells mediating specific cytolytic activity (4); DR molecules, instead, play a role in the activation of mixed lymphocyte reaction and in antigen presentation (13). In addition, they are strongly related to a variety of diseases, some of them of possible autoimmune etiology. More-

over, it has been reported that Ia-positive nonlymphoid cells (12) could be involved, in addition to macrophages, in antigen presentation.

The choice of the M14 cell line as a model system was determined by the fact that this line was used in previous studies on IFN effects on HLA antigens (3, 6, 7) and possesses a suitable HLA phenotype, i.e., two recognizable alleles at both DR and DC loci.

It is well known that IFNs are modulators of functions related to cell differentiation (14, 15), and Ia antigens may be considered, in some respect, as differentiation-related antigens (3; Sorrentino et al., in press). Work is in progress to establish whether this phenomenon occurs also in normally circulating immune-responsive cells. If this is true, these findings may be connected with the immune-regulating activities of IFNs, especially the γ type and may have important implications for immunomodulation, cell-mediated responses to foreign and transformed cells, and autoimmune disorders.

ACKNOWLEDGMENTS

We are indebted to F. Dianzani for helpful suggestions and critical revision of this manuscript and to G. B. Ferrara for providing human alloantisera. The M14 cell line was kindly provided by P. Natali.

This work was supported in part by grants from CNR, Progetti Finalizzati Controllo della Crescita Neoplastica Contratto 82.00297.96, Ingegneria Genetica e Basi Molecolari delle Malattie Ereditarie Contratto 83, and Malattie da Infezione Contratto 82.02393.52.

LITERATURE CITED

1. **Blalock, J. E., J. A. Georgiades, M. P. Langford, and H. M. Johnson.** 1980. Purified immune interferon has more potent anticellular activity than fibroblast or leukocyte interferon. Cell. Immunol. **49**:390–394.
2. **Bono, R., and J. L. Strominger.** 1982. Direct evidence of homology between human DC1 antigens and murine I-A molecules. Nature (London) **299**:836–848.
3. **Capobianchi, M. R., F. Ameglio, A. Dolei, and R. Tosi.** 1982. Expression of MHC products in human cell lines treated with interferons, p. 485–487. *In* R. F. Revoltella, G. M. Pontieri, C. Basilico, G. Rovera, R. M. Gallo, and J. H. Subak-Sharpe (ed.), Expression of differentiated functions in cancer cells. Raven Press, New York.
4. **Corte G., A. Moretta, E. Cosulich, D. Ramarli, and A. Bargellesi.** 1982. A monoclonal antibody selectively inhibits the generation of effector T cells mediating specific cytolytic activity. J. Exp. Med. **156**:1539–1544.
5. **De Ley, M., J. Van Damme, A. Billiau, and P. De Somer.** 1981. The preparation of antibodies directed against human immune interferon. J. Virol. Methods **3**:149–153.
6. **Dolei, A., F. Ameglio, M. R. Capobianchi, and R. Tosi.** 1981. Human β-type interferon enhances the expression and shedding of Ia-like antigens. Comparison to HLA-A, B, C, and β_2-microglobulin. Antiviral Res. **1**:367–381.
7. **Dolei, A., M. R. Capobianchi, and F. Ameglio.** 1983. 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. Infect. Immun. **40**:172–176.
8. **Fellous, M., M. Kamoun, I. Gresser, and R. Bono.** 1979. Enhanced expression of HLA antigens and β_2-microglobulin on interferon-treated human lymphoid cells. Eur. J. Immunol. **9**:446–452.
9. **Fleischmann, W. R.** 1982. Potentiation of the direct anticellular activity of mouse interferons: mutual synergism and interferon concentration dependence. Cancer Res. **42**:869–875.
10. **Gresser, I.** 1980. Interferon II. Academic Press, Inc., New York.
11. **Heron, I., M. Hokland, and K. Berg.** 1978. Enhanced expression of β_2-microglobulin and HLA antigens on human lymphoid cells by interferon. Proc. Natl. Acad. Sci. U.S.A. **75**:6215–6219.
12. **Hirschberg, H., L. R. Braathen, and E. Thorsby.** 1982. Antigen presentation by vascular endothelial cells and epidermal Langerhans cells: the role of HLA-DR. Immunol. Rev. **66**:57–77.
13. **Katz, D. H., and B. Benacerraf (ed.).** 1979. The role of products of histocompatibility gene complex in immune responses. Academic Press, Inc., New York.
14. **Rossi, G. B., A. Dolei, L. Cioè, C. Peschle, and E. Affabris.** 1980. Interactions of interferon with *in vitro* model systems involved in hematopoietic cell differentiation. 1980. Ann. N.Y. Acad. Sci. **350**:279–293.
15. **Rossi, G. B., A. Dolei, L. Cioè, and C. Peschle.** 1982. Effects of interferon on erythropoietic differentiation. Tex. Rep. Biol. Med. **41**:381–387.
16. **Rubin, B. Y., and S. L. Gupta.** 1980. Differential efficacies of human type I and type II interferons as antiviral and antiproliferative agents. Proc. Natl. Acad. Sci. U.S.A. **77**:5928–5932.
17. **Sonnenfeld, G.** 1980. Modulation of immunity by interferon, p. 113–131. *In* I. E. Pick (ed.), Lymphokine reports. Academic Press, Inc., New York.
18. **Sonnenfeld, G., D. Meruelo, H. O. McDevitt, and T. C. Merigan.** 1981. Effect of type I and type II interferons on murine thymocyte surface antigen expression: induction or selection? Cell Immunol. **57**:427–439.
19. **Tanigaki, N., and R. Tosi.** 1982. The genetic control of human Ia alloantigens: a three-loci model derived from the immunochemical analysis of "supertypic" specificities. Immunol. Rev. **66**:5–37.
20. **Tanigaki, N., and R. Tosi.** 1982. Assessment of the specificity of human alloantisera and alloantigens by the use of radio-iodinated human Ia antigens. Tissue Antigens **20**:1–21.
21. **Tanigaki, N., R. Tosi, D. Centis, G. B. Ferrara, and D. Pressman.** 1981. Further characterization of two "DR supertypic" specificities. Additional evidence that they reside on molecules different from those carrying HLA-DR locus specificities. Hum. Immunol. **3**:93–108.
22. **Tanigaki, N., R. Tosi, R. J. Duquesnoy, and G. B. Ferrara.** 1983. Three Ia species with different structures and alloantigenic determinants in an HLA homozygous cell line. J. Exp. Med. **157**:231–247.
23. **Tanigaki, N., R. Tosi, and D. Pressman.** 1980. Purification and separation of subsets of human Ia molecules by papain digestion. Immunology **39**:615–622.
24. **Terasaki, P. I. (ed.).** 1980. Histocompatibility testing, 1980. University of California Press, Berkeley.
25. **Tosi, R.** 1981. MT3, p. 568. *In* P. I. Teresaki (ed.), Histocompatibility testing 1980. University of California Press, Berkeley.
26. **Tosi, R., and N. Tanigaki.** 1980. MT1, p. 558. *In* P. I. Terasaki (ed.), Histocompatibility testing 1980. University of California Press, Berkeley.
27. **Tosi, R., N. Tanigaki, D. Centis, G. B. Ferrara, and D. Pressman.** 1978. Immunological dissection of human Ia molecules. J. Exp. Med. **148**:1592–1611.
28. **Tosi, R., N. Tanigaki, D. Centis, P. L. Rossi, G. Alfano, G. B. Ferrara, and D. Pressman.** 1980. HLA-DR typing by radioimmuno-assay. Transplantation **29**:302–305.
29. **Wallach, D., M. Fellous, and M. Revel.** 1982. Preferential effect of γ interferon on synthesis of HLA antigens and their mRNAs in human cells. Nature **29**:833–836.