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Potentiation of growth suppression and modulation of the antigenic phenotype in human melanoma cells by the combination of recombinant human fibroblast and immune interferons

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Summary. Administration of interferon as a single therapeutic regimen in cancer patients with various neoplasias has had only limited efficacy in ameliorating the negative clinical course of their disease. In the present study, we have evaluated the effect of recombinant human fibroblast (IFNβ) and immune (IFNγ) interferon, alone and in combination, on growth, differentiation and the expression of class I and II histocompatibility locus antigens (HLA) and melanoma-associated antigens on the human melanoma cell line H0-1. The effect of combinations of interferons on the antigenic profile of human melanoma cells displaying different organ colonization and spontaneous metastatic potential in athymic nude mice was also determined. H0-1 cells were more sensitive to the antiproliferative activity of IFNβ than to IFNγ and the combination of interferons resulted in a potentiation of growth suppression. The antiproliferative effect of both interferons was greater in later-passage than in earlier-passage H0-1 cells, possibly reflecting alterations in the evolving tumor cell population as a result of long-term in vitro propagation and/or the selective outgrowth of cells with an increased growth rate. The enhanced growth suppression observed in H0-1 cells treated with the combination of IFNβ plus IFNγ was not associated with a significant increase in the level of melanin, a marker of melanoma differentiation, above that observed with either interferon used alone. IFNβ and IFNγ differentially modulated the expression of class I and II HLA and melanoma-associated antigens in H0-1 cells and a series of melanoma cells with different organ colonization and metastatic potential, including MeWo, MeM 50-10, MeM 50-17, 3S5 and 70W. No consistent potentiation or antagonism in the expression of any specific antigen was observed in any of the melanoma cell lines exposed to the combination of interferons. The present study demonstrates that the combination of IFNβ plus IFNγ can potentiate growth suppression in H0-1 human melanoma cells and that this effect is not associated with an increase in differentiation or a potentiation in antigenic modulation. In addition, no direct correlation between the expression of any specific antigen or its modulation by IFNβ or IFNγ, alone or in combination, and organ colonization and metastatic potential in nude mice was observed in the different melanoma cell lines.

Key words: Human melanoma cells – Recombinant interferons – Growth suppression – Differentiation – Antigenic phenotype

Introduction

Interferons induce a wide variety of effects on target cells (for review see [2, 8, 17, 42, 43]. Included among the diversity of interferon-induced modifications in cells are induction of an antiviral state [43]; a direct antiproliferative effect toward both normal and tumor cells [2, 8, 18, 43]; induction of altered gene expression, including new gene transcription and an enhanced expression of previously expressed genes [2, 12, 14, 28, 32]; modulation of differentiation, either an induction or an inhibition in differentiation depending on the target cell [2, 4, 8, 46]; and immunological modifications of tumor cells, as indicated by the enhanced expression of class I HLA, the induction of class II HLA and increased expression of specific tumor-associated antigens [14, 17, 19].

Of potential clinical interest has been the observation that specific interferons can induce an antitumor effect in vivo in humans (reviewed in [16, 31, 43]). Unfortunately, the ability of interferon to serve as a single modality therapy for most cancers has proven unsuccessful [7, 16, 31, 43]. The mechanism by which interferons induce a clinical response in specific patients is not known. It is possible that these effects are caused by a direct antiproliferative activity (perhaps by the induction of differentiation) and/or an effect on the patient's immune system

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(either a direct effect on immunological cells or by inducing immunological modification of the tumor cells) [2, 4, 8, 14, 17–19, 42, 43, 46]. The observation that the combination of different classes of interferons, such as leukocyte (IFNα) and immune (IFNγ) or fibroblast (IFNβ) and IFNγ, or of interferon with other differentiation-modulating agents, such as the antileukemic compound mezerein, can result in an enhanced antiproliferative effect may provide a basis for the better application of interferons for cancer therapy (reviewed in [2, 43]).

In the present study, we have determined the effect of recombinant human IFNβ and IFNγ, alone and in combination, on the growth and differentiation of the human melanoma cell line, H0-1. Studies have also been conducted to determine the effect of the two types of interferons, alone and in combination, on the antigenic phenotype of H0-1 cells and a series of human melanoma cells that exhibit stable alterations in their organ colonization and metastatic potential in nude mice [5, 26, 29].

Materials and methods

Cell lines and interferons. The H0-1 human melanoma cell line has been described previously [9, 15]. The MeWo cell line and its wheat-germ-agglutinin-resistant variants, MeM 50-10, MeM 50-17, 3S5 and 70W, have been described previously [5, 26, 29]. MeM 50-10, MeM 50-17 and 70W are "high-colonization" variants of MeWo cells, which give greater numbers of pulmonary and extra-pulmonary metastases after intravenous inoculation. The MeWo cell line is more metastatic ("spontaneous metastases") than MeM 50-10, MeM 50-17 and 70W after subcutaneous or subdermal inoculation into nude mice. The 3S5 cell line is deficient in both colonization and spontaneous metastatic potential. All of the cell lines were grown in Dulbecco's modified Eagle's medium (Gibco, N.Y.) supplemented with 5% fetal bovine serum (Hyclone, Utah), referred to as DMEM-5, at 37° C in a 5% CO_2/95% air humidified incubator. Cultures were fed two times per week and subcultured at a 1 : 3 to 1 : 10 ratio, depending on the cell line, prior to reaching confluency.

Recombinant fibroblast interferon ([Ser^{17}]IFNβ) (Betaseron), with a serine substituted for a cysteine at position 17 of the molecule [36], was supplied by Triton Biosciences, Alameda, Calif. Lyophilized [Ser^{17}]IFNβ was dissolved in 1.0 ml sterile distilled water, divided into aliquots at 1×10^6 units/ml and frozen at –70° C. Recombinant immune interferon (IFNγ) was produced and purified to homogeneity as described previously [30]. Aliquots of 5×10^5 units/ml, prepared in DMEM-5, were stored at –70° C and thawed immediately prior to use. IFNγ was kindly provided by Dr. Sidney Pestka, Robert Wood Johnson Medical School, Piscataway, N.J., USA.

The interferon titers were determined by a cytopathic-effect-inhibition assay with vesicular stomatitis virus on a bovine kidney cell line (MDBK) or human fibroblast Ag-1732 cells [45].

Growth and melanin assays. The effect of IFNβ and IFNγ, alone and in combination, on the growth of H0-1 cells was determined as previously described [9]. Briefly, 2.5×10^4 cells were seeded per 35-mm tissue-culture plate; 24 h later the medium was changed for medium with and without interferons; 4 days later the medium was changed again for medium with and without interferons and on day 7 the cells were resuspended by brief treatment with trypsin/versene and counted using a Z_M Coulter Counter (Hialeah, Fla.). Results are the average of triplicate samples that varied by <10%.

The effect of IFNβ and IFNγ, alone and in combination, on the synthesis of melanin by H0-1 melanoma cells was determined as described previously [9, 10]. Since previous studies [9] have indicated that the synthesis of melanin is significantly increased in H0-1 cells treated with the combination of mezerein plus IFNβ, these agents were incorporated as controls for the induction of melanin synthesis in H0-1 cells.

Monoclonal antibodies and conventional antisera. The anti-melanoma-associated monoclonal antibodies (mAb) included the anti-(high-M_r antigen) mAb 225.28, the anti-(115-kDa antigen) mAb 345.134, the anti-(100-kDa antigen) mAb 376.96, and the anti-(96-kDa antigen) mAb CL203.4 [13, 24, 25, 37, 38, 53, 54]. Immunochemical studies have shown that the mAb CL203.4 recognizes intercellular adhesion molecule 1 (ICAM-1). The anti-HLA mAbs included the W6/32 to a framework determinant of HLA class I antigens and the anti-(HLA-DR) mAb CL413 [3, 41, 44, 54]. Fluorescein-isothiocyanate-conjugated $F(ab')_2$ fragments of goat anti-(mouse Ig) antibodies (FITC-GaM) were purchased from Jackson Immuno Research Laboratories, Avondale, Pa.

Serological assays. Serological assays and fluorescence-activated cell sorter (FACS) analysis were performed as described previously [20, 21, 33]. Indirect immunofluorescence was performed by mixing 50 μl single-cell suspension (5×10^6/ml) with 50 μl spent medium from a hybridoma secreting the mAb to be tested. This amount was found to be saturating for all of the antibodies used. At the end of a 30-min incubation at 4° C, cells were washed twice with cold phosphate-buffered saline (PBS) containing 0.001 M sodium azide and incubated with 50 μl FITC-GaM preparation for 30 min at 4° C. After two washings with PBS/azide, cells were analyzed by flow cytometry using a FACStar (Becton Dickinson, Mountain View, Calif.). The Consort 30 software program that runs this instrument was used for the analysis of the data obtained in the list mode. For each sample, 10 000 cells were analyzed by gating of the data obtained in the list mode. All of the fluorescence data were obtained and expressed in a logarithm base 10 scale. The background fluorescence values were obtained by analyzing cells sequentially incubated with nonreactive mAbs and FITC-GaM and were subtracted from the fluorescence values obtained from each experimental point. Results are expressed as mean fluorescence intensity = (mean channel fluorescence in fluorescence-positive antibody-binding cells × percentage of fluorescence-positive antibody-binding cells) – (mean channel fluorescence in unstained cells × percentage of fluorescence-positive cells in the unstained population). The negative peak for the control cell population was set so that no more than 10% of the cell population would fall into the positive peak region. The mean fluorescence of the control peak was determined by the intrinsic autofluorescence of the cell population in addition to any non-specific antibody binding that may have occurred. Any variation of the MFI values ⩾30% in either direction from control value was considered as a change in expression. All studies were performed a minimum of three to four times with duplicate samples in each experiment. Replicate samples within individual experiments varied by ⩽10% and variation between experiments was generally ⩽20%.

Results

Effect of IFNβ and IFNγ, alone and in combination, on the Growth and differentiation of H0-1 human melanoma cells

The growth of H0-1 melanoma cells was suppressed in a dose-dependent manner by IFNβ and IFNγ (Fig. 1). IFNβ displayed a greater antiproliferative effect toward H0-1 cells than did IFNγ. When used in combination, a potentiation of growth suppression was observed with both low (200 units/ml) and high (4000 units/ml) doses of the combination of interferons. The antiproliferative effect of IFNβ and IFNγ, alone or in combination, was greater in H0-1 cells that had been grown in culture for extended periods of time (unpublished data). In early-passage (<50) H0-1 cells, the average population doubling time was approximately 36 h, whereas in later-passage (>150) H0-1 cells the average population doubling time was approximately 23 h.

In previous studies, we have demonstrated that IFNβ (2000 units/ml) could induce a 1.7-fold increase in melanin

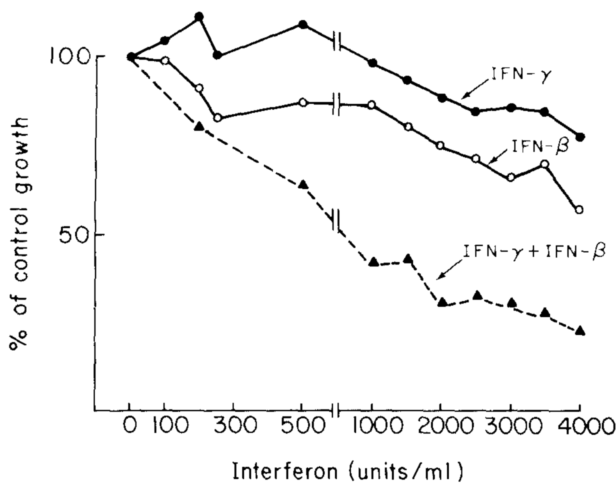

Fig. 1. Effect of interferon β and γ (IFNβ, and IFNγ), alone and in combination, on the growth of H0-1 human melanoma cells. Samples of 25000 cells were seeded per 35-mm tissue-culture plate; 24 h later the medium was changed for medium with and without interferons; 4 days later the medium was changed again for medium with and without interferons and on day 7 the cells were resuspended by brief treatment with trypsine/versene and counted using a Z_M Coulter Counter (Hialeah, Fla.). Results are the average of triplicate samples and varied by ≤10%

Table 1. Melanin synthesis in early-passage (<50) H0-1 human melanoma cells grown in the presence of IFNβ, IFNγ, IFNβ + IFNγ, mezerein and IFNβ + mezerein

Experimental conditions[a]	Concentration	total melanin (μg/10⁶ cells)	Change[b] (-fold)
Control	–	4.54	1.00
IFNβ	1000 U/ml	7.20	1.59
IFNγ	1000 U/ml	5.59	1.23
IFNβ+IFNγ	500+500 U/ml	6.19	1.36
Mezerein	50 ng/ml	8.33	1.84
Mezerein+IFNβ	50 ng/ml+1000 U/ml	22.64	4.99

[a] H0-1 cells were seeded at 1×10^6 cells/10-cm plate, the medium was changed with DMEM-5 or DMEM-5 with the indicated concentration of test compound 24 h later and plates were refed with the appropriate additions 4 days later. After 7 days incubation at 37°C, cell numbers were determined and the melanin was extracted and quantificed by absorbance at 400 nm as described previously [9, 10]

[b] The difference in melanin content between treated and control cultures

synthesis, a marker of melanoma cell differentiation, in H0-1 cells [9]. However, when 2000 units/ml IFNβ was combined with 10 ng/ml of the compound mezerein, a synergistic suppression in growth and a 4.9-fold increase in melanin synthesis were induced. We have therefore determined whether the ability of the combination of IFNβ plus IFNγ to potentiate growth suppression in H0-1 cells was associated with an increased level of melanin synthesis (Tables 1 and 2). Employing both early (<50) and later-passage (>150) H0-1 cells, we have found that combinations of IFNβ plus IFNγ that potentiate growth suppression did not induce an increase in melanin synthesis above that observed with either interferon used alone. However, the combination of IFNβ plus mezerein did induce an increase in melanin synthesis in both early- and late-passage H0-1 cells. Lower doses of interferons and mezerein, employed alone or in combination, exerted a greater antiproliferative

Table 2. Melanin synthesis in late-passage (>150) H0-1 human melanoma cells grown in the presence of IFNβ, IFNγ, IFNβ+IFNγ, mezerein and IFNβ+mezerein

Experimental conditions[a]	Concentration	Total melanin (μg/10⁶ cells)	Change[b] (-fold)
Control	–	6.86	1.00
IFNβ	100 U/ml	10.00	1.46
IFNβ	250 U/ml	9.45	1.38
IFNγ	100 U/ml	4.29	–0.62
IFNγ	250 U/ml	9.35	1.36
IFNβ+IFNγ	50+ 50 U/ml	8.07	1.18
IFNβ+IFNγ	125+125 U/ml	9.13	1.33
Mezerein	10 ng/ml	8.36	1.22
Mezerein+IFNβ	10 ng/ml+250 U/ml	13.55	1.98

[a] H0-1 cells were seeded at various densitites because of the increased senstivity of late-passage H0-1 cells to interferon-induced growth suppression. Control cells were seeded at 1×10^5 cells/10-cm plate; IFNβ-treated cultures were seeded at 4×10^5 or 5×10^5 cells/10-cm plate for 125 U/ml and 250 U/ml plates, respectively; IFNγ-treated cultures were seeded at 4×10^5 cells/10-cm plate; (IFNβ+IFNγ)-treated cultures were seeded at 5×10^5 cells/10-cm plate; mezerein-treated cultures were seeded at 5×10^5 cells/10-cm plate; and (mezerein+IFNβ)-treated cultures were seeded at 6×10^5 cells/10-cm plate. The medium was changed with DMEM-5 or DMEM-5 with the indicated concentration of test compound 24 h after seeding and cultures were refed with the appropriate additions 4 days later. After 7 days incubation at 37°C, cell numbers were determined and the melanin was extracted and quantified by absorbance at 400 nm as described previously [9, 10]

[b] The difference in melanin content between treated and control cultures

and a differentiation-induction effect in late- versus early-passage H0-1 cells. In the experiments reported in Tables 1 and 2, different levels of IFNβ and mezerein were used to account for the different response of early- versus late-passage H0-1 cells to these agents, alone and in combination. The doses chosen induce comparable inhibitory effects on cell proliferation (IFNβ and IFNγ, alone and in combination) without inducing cell toxicity, as indicated by trypan blue dye exclusion (data not shown).

Effect of IFNβ and IFNγ, alone and in combination, on the antigenic phenotype of H0-1 melanoma cells and human melanoma cells displaying different organ colonization and spontaneous metastatic potential in nude mice

By employing both a single-step selection following mutagenesis and spontaneous selection for resistance to wheat-germ agglutinin, Kerbel and colleagues [5, 26, 29] have isolated a series of human MeWo melanoma cell lines that stably express altered organ colonization and spontaneous metastatic phenotypes in nude mice. A partial list of some of the WGA-resistant MeWo cell lines and their reported properties is shown in Table 3. The cell lines used for analyzing the effects of IFNβ and IFNγ, alone and in combination, on their antigenic phenotype included H0-1, MeWo, 3S5, 70W, MeM 50-10 and MeM 50-17. These cell lines were analyzed for changes in the surface expression of class I and II HLA, and a series of melanoma-associated antigens, including a 96-kDa antigen (identical to ICAM-1), a 100-kDa antigen, a 115-kDa antigen and a high-M_r melanoma-associated antigen.

Table 3. Properties of mutagenized-single-step- and spontaneous-selected wheat-germ-agglutinin(WGA)-resistant human MeWo melanoma cells with differing metastatic potential in nude mice

Cell line[a]	WGA sensitivity (D_{50}[b], μg/ml)	No. of lung nodules[c] and other sites[d]	Incidence and location of metastases at other sites	Spontaneous metastatic potential after	
				Subdermal injection[e]	Subcutaneous injection[e]
MeWo parent (p38)	6	9,37,45,66,>200 >200	0/6	Lung and lymph node metastases	Mainly lung metastases
3S5 (p44)	>125	0,0,0,0,1,2	0/6	No metastases	No metastases
70W (p25)	18	2,>200,>200,>200	5/6 skin, brain, mesentery, rib cage, muscle, abdominal wall	No metastases	No metastases
MeM 50-10 (p48F)	Similar to 70W	Similar to 70W	Skin metastases	Not tested	Not tested
MeM 50-17 (p54)	23	0,2,2,7,80,87	1/6, rib cage	Not tested	Not tested

[a] The properties and various designations of the cell lines have been described previously in [5, 26, 29]. 3S5 and 70W cells are spontaneous MeWo variants, which were obtained by continued gradual incremental growth in increasing concentrations of WGA [19]. MeM 50-10 and MeM 50-17 cells were derived from MeWo cells following a single exposure to 100 μg/ml methylmethanesulfonate and growth in 50 μg/ml WGA [29]

[b] D_{50}, WGA concentration that reduced the incorporation of [^{3}H]thymidine to 50% of control. (Data for 3S5 and 70W from [26])

[c] NIH Swiss nude mice were given an i. v. injection of 5×10^6 tumor cells and the number of surface tumor nodules in the lung were counted 40 days later. The organ colonization data for MeWo, 3S5, 70W and MeM 50-17 have been presented in [26]

[d] Organ colonization data for MeWo, 3S5, 70W and Mem 50-17 from [26]

[e] Spontaneous metastasis profiles observed after subcutaneous or subdermal inoculation of MeWo, 70W and 3S5 from [5]

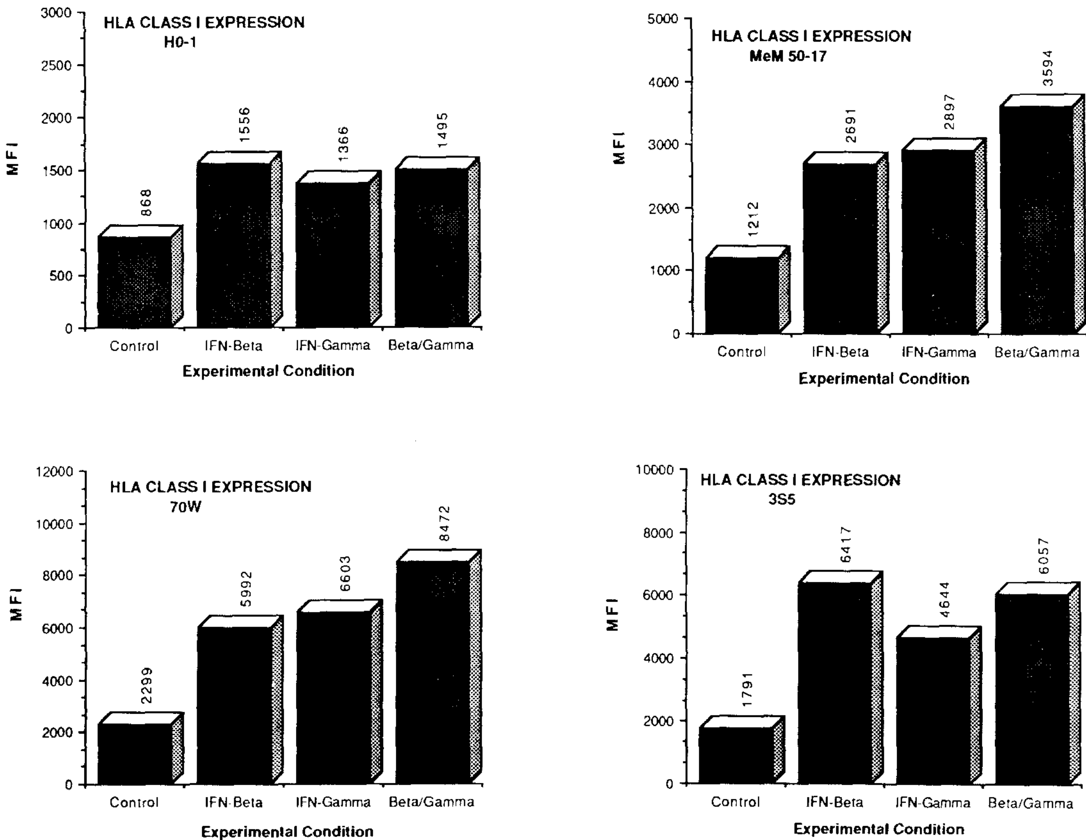

Fig. 2. Effect of IFNβ and IFNγ, alone and in combination, on the expression of HLA class I by H0-1, MeM 50-17, 70W and 3S5 cells. Cultures were exposed to 2000 units/ml IFNβ of IFNγ, or 1000 units/ml IFNβ plus 1000 units/ml IFNγ, for 72 h prior to analysis by fluorescence-activated cell sorting (FACS) using an anti-(HLA class I) mAb. Experimental details can be found in Materials and methods. Similar results have been obtained in two additional experiments using duplicate samples and when cells were exposed to the interferons for 96 h as opposed to 72 h

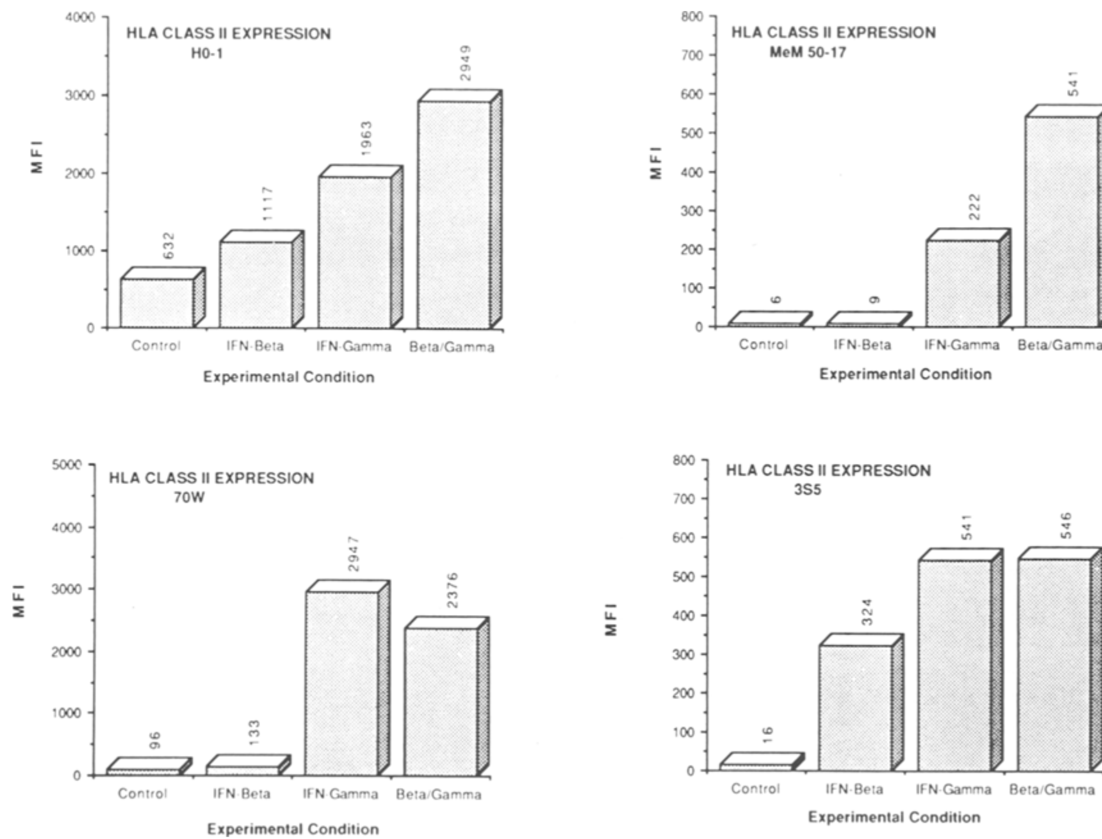

Fig. 3. Effect of IFNβ and IFNγ, alone and in combination, on the expression of HLA class II by H0-1, MeM 50-17, 70W and 3S5 cells. Experimental details are similar to those described in Fig. 2. Quantitatively similar results have been obtained in two additional experiments using duplicate samples and when cells were exposed to the interferons for 96 h as opposed to 72 h

Preliminary studies indicated that optimal alterations in the expression of the various antigens analyzed had occurred by 72–96 h when the various melanoma cell lines were exposed to 1000–2000 units/ml IFNβ or 500–2000 units/ml IFNγ. Experiments were therefore conducted using 2000 units/ml IFNβ or IFNγ and 1000 units/ml of each interferon for combination studies (a total of 2000 units/ml) and a treatment period of 72 h.

In previous studies, we have demonstrated that both IFNγ and IFNβ enhance the expression of class I HLA on MeWo and MeM 50-10 cells [34, 35]. Similarly, IFNγ and IFNβ, alone and in combination, also increase class I HLA expression in early-passage (passage 35) H0-1 cells [1, 11]. As shown in Fig. 2, both IFNγ and IFNβ also enhanced the expression of class I HLA on H0-1 (>150 passages), MeM 50-17, 70W and 3S5. The different cell lines varied both in their de novo expression of this antigen as well as in the level of modulation by interferon. When both interferons were used in combination, an approximately additive increase in class I HLA expression was observed in the MeWo, MeM 50-10, MeM 50-17 and 70W cell lines. In contrast, no significant further change in class I HLA expression was seen in early- (passage 35) [1] or late- passage (passage >150) H0-1 or 3S5 cells treated with IFNγ plus IFNβ. The percentage of class-I-HLA-positive cells prior to interferon treatment was generally >80%. Consequently, the changes in percentage positive cells we have observed following interferon treatment was generally minimal. The major effect of interferon in each cell system analyzed was a change in the shift in mean fluorescence, resulting in corresponding increases in mean fluorescence intensity values.

MeM 50-10 and MeWo cells have previously been shown to display a differential response to the induction of class II HLA-DR by treatment with either IFNγ or IFNβ [34, 35]. MeM 50-10 cells could be induced to express HLA-DR, whereas early-passage MeWo cells were noninducible for expression of this antigen by either interferon. As shown in Fig. 3, only late-passage H0-1 cells expressed HLA-DR de novo, and the level of expression was increased by both IFNβ and IFNγ. When employed together, an additive increase in HLA-DR expression was observed in both early- (passage 35) [1] and late-passage (passage >150) H0-1 cells. Both of the organ-colonization variants of MeWo cells, MeM 50-17 and 70W, were induced to express HLA-DR when exposed to IFNγ, but not to IFNβ. In the MeM 50-17 cell line, an increase in HLA-DR expression was observed when cells were exposed to IFNβ plus IFNγ. In contrast, the combination of IFNγ plus IFNβ reduced the expression of HLA-DR in 70W cells. In the non-metastatic MeWo variant, 3S5, both IFNγ and IFNβ induced the expression of HLA-DR and no significant further increase in HLA-DR expression was found when both interferons were used in combination.

When exposed to IFNγ, a variable induction or an increase in the expression of ICAM-1 was observed in all of the melanoma cell lines studied (Fig. 4). A small increase or induction in ICAM-1 expression was also ap-

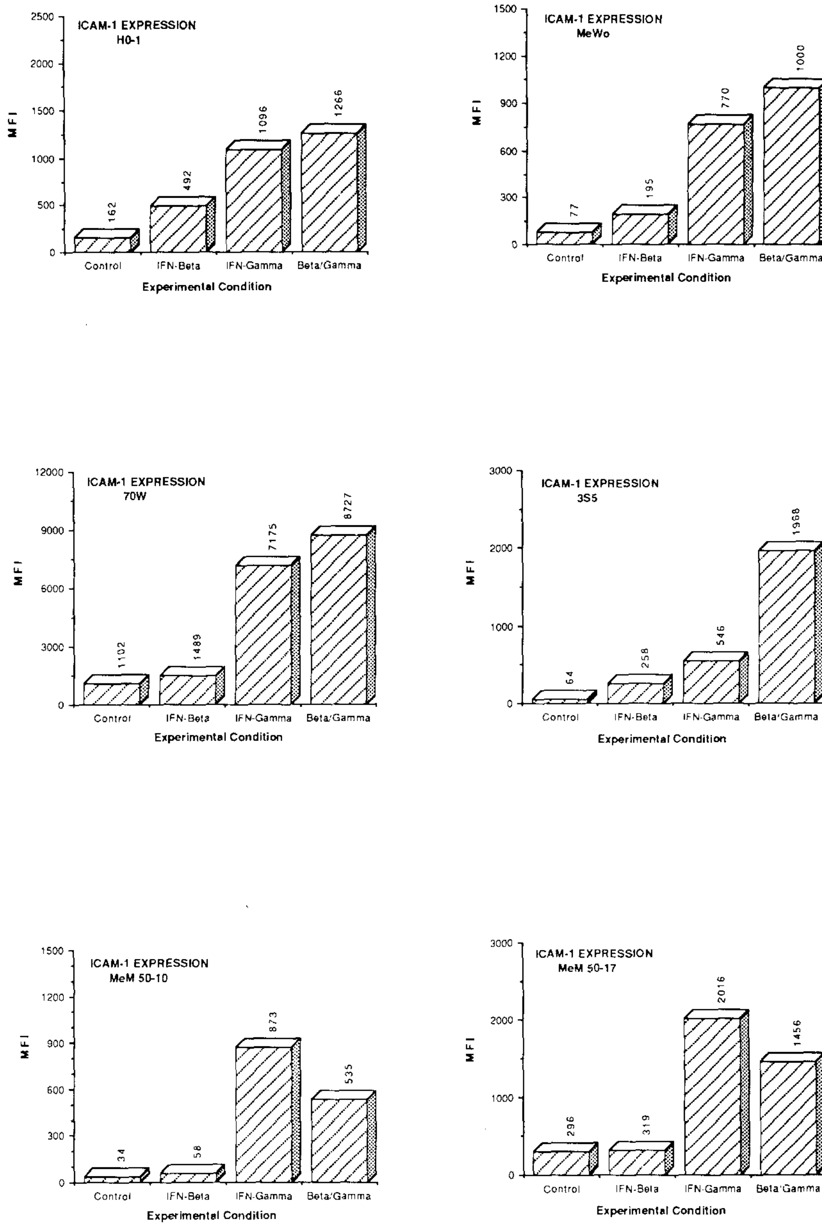

Fig. 4. Effect of IFNβ and IFNγ, alone and in combination, on the expression of ICAM-1 by H0-1, MeWo, 70W, 3S5, MeM 50-10 and MeM 50-17 cells. Experimental details are similar to those described in Fig. 2. Quantitatively similar results have been obtained in two additional experiments using duplicate samples and when cells were exposed to the interferons for 96 h as opposed to 72 h

Table 4. Effect of IFNβ and IFNγ, alone and in combination, on the expression of 100-kDa, 115-kDa and high-M_r melanoma-associated antigens by human melanoma cells

Cell type[a]	100-kDa antigen[b]			115-kDa antigen			High-M_r antigen		
	β	γ	β+γ	β	γ	β+γ	β	γ	β+γ
H0-1 (175)	↑	NC	↑↑	↑	↑	↑	↓	↓	↓
MeWo (49)	NC	↓	↓	↑	↓	↓	NC	NC	NC
MeM 50-10 (57)	NC	NC	NC	↓	↓	↓	NC	NC	NC
MeM 50-17 (63)	↑	NC	↑	↑	↓	↑↑	↓	↓	↓
70W (29)	↑	↓	↑	↑	↓	NC	↓	↓↓	↓↓
3S5 (50)	↑	↓	↑	↑	↑	↓	↓	↓↓	↓

[a] The various human melanoma cell lines were assayed after the passage number (in parentheses) indicated

[b] Arrows indicate the direction of antigenic change as determined by FACS analysis after exposure to the specific interferon for 72 h. Experimental details can be found in Materials and methods. ↑, Increased; ↓, decreased. Double arrows indicate antigens more sensitive to change than those represented by single arrows. NC, indicates no significant change in antigen expression

parent when H0-1 (passage >150), MeWo, 70W or 3S5 cells were grown for 72 h in 2000 units/ml IFNβ. When IFNγ and IFNβ were employed in combination, a further increase in ICAM-1 expression was observed in H0-1 (passage >150), MeWo, 70W and MeM 50-17 cells. In contrast, the level of ICAM-1 was decreased in both MeM 50-10 and MeM 50-17 cells exposed to the combination of interferons.

The effect of IFNβ and IFNγ, alone or in combination, on the expression of a 100-kDa, a 115-kDa and a high-M_r melanoma-associated antigen is shown in Table 4. When employed alone, IFNβ increased the expression of the 100-kDa antigen in all of the melanoma cell lines, with the exception of MeWo and MeM 50-10, which did not significantly change. In contrast, IFNγ reduced the expression of the 100-kDa antigen in MeWo, 70W and 3S5 cells, and did not alter its expression in H0-1, MeM 50-10 or MeM 50-17 cells. The effect of IFNβ plus IFNγ, varied from cell line to cell line and no consistent pattern of change was observed. Cell-line-specific alterations in the expression of the 115-kDa melanoma-associated antigen was also observed in cultures treated with IFNβ, IFNγ or IFNβ plus IFNγ (Table 4). A consistent change in expression of the high-M_r antigen was observed in the interferon-treated melanoma cell lines. With the exception of MeWo and MeM 50-10 cells, in which no change in the expression of the high-M_r antigen was observed following treatment with any of the interferon preparations, the remaining four melanoma cell lines displayed variable degrees of reduction in expression of the high-M_r antigen following growth in IFNβ, IFNγ or IFNβ plus IFNγ.

Discussion

Interferons are known to modulate the growth of tumor cells both in vitro and in vivo (reviewed in [4, 18, 43]). Although extensively studied, the mechanism involved in this growth suppression is not currently known. Possible mechanisms by which interferons could exert their antitumor effect include a direct inhibitory effect on biochemical processes in tumor cells that mediate cellular proliferation and/or an alteration in the antigenic phenotype of the tumor cells, resulting in a more effective immunological response by the host (reviewed in [2, 4, 8, 17–19, 42, 43, 46]). In the present study we have addressed these issues, as well as the potential utility of combinations of interferon in augmenting both the antiproliferative and antigenic modulatory effects of these cytokines. Recombinant IFNβ and IFNγ induced a dose-dependent suppression in the growth of the human melanoma cell line H0-1 in vitro. When both interferons were employed in combination, the growth suppression was greater than when either interferon preparation was used alone. This observation is similar to those found when employing other tumors, including patient-derived early-passage glioblastoma multiforme, bladder carcinoma, colon carcinoma, lung adenocarcinoma, transitional bladder carcinoma, renal carcinoma, bronchiogenic carcinoma and fibrosarcoma cell lines [6, 23, 40, 47, 48, 52]. The ability of the combination of interferons to suppress the growth of H0-1 cells was not associated with an increase in melanin synthesis above that observed with either interferon employed alone (Tables 1 and 2). This is in marked contrast to the effect of IFNβ plus mezerein on H0-1 cells, which results in a synergistic suppression in cell growth and a concomitant increase in melanin synthesis. The difference observed between the two agents may be related to the fact that growth suppression induced by a 7-day exposure to IFNβ plus mezerein is irreversible, whereas removal of IFNβ plus IFNγ after 7 days results in a gradual revovery of cell growth [1, 9].

Human leukocyte interferon and IFNβ bind to the same high-affinity surface receptor (type I receptor), whereas IFNγ binds to a different cell membrane receptor (type II receptor) (reviewed in [42, 43]). In a recent study, Schiller et al. [48] have analyzed the effect of IFNβ and IFNγ on the expression and internalization of cell-surface receptors for the respective interferons in the human colon carcinoma cell line, SKCO1. In this cell line, a synergistic suppression in cell growth was evident in cultures grown in the combination of [Ser^{17}]IFNβ plus IFNγ. Exposure of SKCO1 cells to IFNγ was shown to reduce the binding of ^{125}I[Ser^{17}]IFNβ to its receptor by decreasing the affinity of the receptor without altering the number of binding sites. Similarly, IFNγ has been shown to reduce the binding of leukocyte interferon (IFN-α2) in the T986 human neuroblastoma cell line by reducing the binding affinity for the type I receptor [22]. In contrast, pretreatment of SKCO1 cells with [Ser^{17}]IFNβ resulted in an increase (10%– to 35%) in ^{125}I-IFNγ-specific binding. This altered binding was shown by Scatchard analysis to involve an increase in the number of IFNγ receptor sites per cell with a decrease in binding affinity to the receptors. Similarly, in previous studies we have demonstrated that a 96-h treatment of H0-1 melanoma cells with IFNβ or mezerein results in either no change or an increase in the binding of labeled IFNγ to surface receptors [11]. In contrast, the combination of IFNβ plus mezerein increased IFNγ binding to H0-1 cells two- to four-fold in H0-1 cells. The significance of this altered binding of respective interferons to target cells, as it relates to the synergistic suppression of growth by interferons in human melanoma cells, remains to be determined. It is possible that the binding of both IFNβ and IFNγ to their respective cell-surface receptors potentiates the expression of gene(s) that mediate an inhibition of cellular proliferation in target cells. It is equally possible that the combination of interferons synergistically inhibits cellular proliferation by reducing the expression of gene(s) that function as positive regulators of cellular proliferation. Further studies are required to distinguish between these possibilities as well as other potential mechanism(s) by which the combination of IFNβ and IFNγ potentiate growth suppression in diverse cell types.

Interferons have been shown to enhance the expression of both histocompatibility and tumor-associated antigens in a wide spectrum of tumor cell types in vitro (reviewed in [14, 17, 19]). With respect to class I HLA antigens, both types I (α/β) and II (γ) interferon differentially increase surface expression of these antigens in both normal and tumor cells. In contrast, a preferential induction of class II HLA antigens by IFNγ has been observed. However, in the

present study and in several previous studies IFNβ has also been found to induce to variable degrees HLA-DR expression in specific human melanoma and breast carcinoma cultures [1, 14, 33, 35]. An apparent induction in HLA-DR expression de novo by IFNβ was observed in the 3S5 cell line, whereas an enhancement in HLA-DR expression by IFNβ was observed in HLA-DR-positive H0-1 cells (Fig. 3). The 3S5 cell line did not display any constitutive expression of HLA class II antigens. When exposed to IFNβ, a small induction in HLA-DR expression (between 150 and 425 mean fluorescence intensity units in different experiments) was observed in several independent experiments. In contrast, H0-1 cells express both baseline levels of HLA-DR in the absence of IFNβ and inducible levels of HLA-DR expression when exposed to IFNβ. These results indicate that different human melanoma cell cultures will vary in both their baseline (constitutive) and inducible levels of HLA-DR when exposed to IFNβ. Although the mechanism underlying this differential response to IFNβ between different human melanoma cell lines is not known, melanoma cultures displaying inducible and non-inducible expression of HLA-DR following exposure to IFNβ should prove valuable in elucidating the molecular basis of this response.

An important molecule involved in cell-to-cell interactions, which is paramount in immunological recognition processes, is ICAM-1 (reviewed in [49]). In previous studies, it has been shown that ICAM-1 is induced in a similar manner as HLA-DR by IFNγ on melanocytes and melanoma cells [35, 44, 50, 51]. In addition, immunochemical staining indicated a markedly higher reactivity of anti-ICAM-1 (mAb CL203.4) with surgically removed metastatic lesions than with primary lesions [27, 39]. In the present study, we demonstrate that two high-colonization variant melanoma cell sublines, 70W and MeM 50-17 (Fig. 4), are more susceptible to up-regulation of ICAM-1 expression than is the non-metastatic variant melanoma cell subline, 3S5. These results are consistent with previous observations indicating a greater responsiveness to ICAM-I up-regulation by IFNγ of the MeM 50-10 subline versus its parental cell line MeWo [35]. The mechanism underlying this differential sensitivity of high-colonization variants versus non-metastatic human melanoma cell lines to up-regulation of ICAM-1 by IFNγ is not presently known. It may reflect differences in the amount of transcriptional regulatory molecules in the different cell types and/or the ability to induce these transcriptional regulatory molecules following treatment with IFNγ. It is clear, however, from our present studies that this unique series of MeWo human melanoma cell lines, which display stable differences in organ colonization or spontaneous metastatic potential [5, 26, 29], will prove useful in analyzing the role of ICAM-1, HLA class I and II antigens and melanoma-associated antigens in the metastatic process.

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