Int. J. Cancer: **47**, 576–581 (1991)

Publication of the International Union Against Cancer
Publication de l'Union Internationale Contre le Cancer

RECOMBINANT INTERFERON ALPHA AND GAMMA MODULATE THE INVASIVE POTENTIAL OF HUMAN MELANOMA *IN VITRO*

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We have studied the effects of interferons (IFNs) on the attachment, collagenase IV activity, chemotactic migration and *in vitro* invasion of human melanoma (A2058) cells treated for various time periods with human recombinant interferon alpha (hrIFN-α) or gamma (hrIFN-γ). The cells treated with hrIFN alpha for a short time period attached more readily to purified basement membrane components, type IV collagen and laminin, than control cells. The stimulating effect of hrIFN gamma on the attachment was seen, however, when the cells were treated for a longer period of time (3 days) with this drug. The short-term treatment with hrIFN alpha also enhanced the *in vitro* invasion of cells through a reconstituted basement membrane compared to findings with untreated control cells. Pre-treatment of 3 days or more was, however, needed for hrIFN gamma to promote the invasion of A2058 cells. Both IFNs increased the secretion of basement membrane (type IV) collagen degrading metalloproteinase (collagenase IV) activity from human melanoma cells. Further, chemotaxis, *i.e.*, directed migration of A2058 cells to laminin, was enhanced by both IFNs. In contrast, the attachment, collagenase IV activity, chemotaxis, and *in vitro* invasion were markedly inhibited when the cells were treated for an extended time period (7 days) with the IFNs. Interferons also inhibited cell proliferation after 4 days of exposure. These results suggest that time of treatment with interferons modulates the invasive capacity of human melanoma cells *in vitro*, causing initially a transient enhancement of invasion followed by an inhibition of invasive propensity after extended exposure to these drugs, and that different biochemical steps required for successful invasion are regulated in parallel by interferons alpha and gamma.

Interferons (IFNs) possess several biological activities. In addition to their antiviral and antitumoral effects, interferons are able to affect such cellular processes as proliferation, differentiation and cell surface receptors (Pestka *et al.*, 1987). Several *in vitro* and *in vivo* studies show that interferons either enhance or suppress the invasive and metastatic potential of a number of different tumor cells (Siegal *et al.*, 1982; Brunda *et al.*, 1984; Nishimura *et al.*, 1985; Ramani and Balkwill, 1987, 1989; Ravine and Ledinko, 1986; Lollini *et al.*, 1987; Melchiori *et al.*, 1987). However, it is not known how these drugs affect the distinct steps of the metastatic cascade.

Metastasis is a multi-step process involving a variety of interactions between tumor cells and the host. Tumor cells must traverse basement membranes several times in order to metastasize. Tumor cell attachment, proteolysis and migration are the 3 essential events that occur during invasion (Liotta *et al.*, 1986). Various factors affecting tumor cell invasion have been reported. During hematogenous metastasis the blood-borne tumor cells can arrest in the capillary bed due to mechanical trapping or attach to basement membrane components of target organ, often showing a preference for a specific distant site (Nicolson, 1988). The attachment can be mediated by specific cell adhesion molecules such as fibronectin, laminin, type IV collagen, heparan sulfate proteoglycan and vitronectin (Nicolson, 1988). Once attached to and spread on the subendothelial basement membrane, tumor cells solubilize this matrix using a variety of degradative enzymes such as collagenase IV, stromelysin, plasminogen activator and various cathepsins (Liotta *et al.*, 1986; Tryggvason *et al.*, 1987). However, several findings strongly support a major role for a specific type IV collagen-degrading metalloproteinase, collagenase IV, in tumor cell invasion (Liotta *et al.*, 1980; Thorgeirsson *et al.*, 1985; Turpeenniemi-Hujanen *et al.*, 1985; Höyhtyä et al., 1990). Following degradation of the basement membrane, tumor cells invade the host tissue by active locomotion which may be guided by local tissue-specific chemoattractants (Hujanen and Terranova, 1985; Bresalier *et al.*, 1987). In this study we have tested the effects of human recombinant interferons alpha and gamma on human melanoma cell attachment, secretion of collagenase IV, directed migration and *in vitro* invasion. We also studied the effect of these drugs on cell growth and protein synthesis. Special emphasis was laid on the treatment time of the cells with these drugs.

MATERIAL AND METHODS

Cells and culture conditions

A2058 human melanoma cells were obtained from Meloy, Springfield, VA. The cells were routinely maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 50 μg/ml gentamycin at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Cells were subcultured weekly at a 1:4 split ratio. Routine tests showed the tumor cell line to be free from Mycoplasma.

Reagents

E. coli-derived human recombinant interferon alpha, hrIFN alpha2b (Introna®), was obtained from Schering (Plough, Union, NJ), and had a specific activity of 2×10^8 IU/mg protein on human cells. Lyophilized powder including phosphate buffer and 1 mg of human serum albumin was diluted in 1 ml of distilled water according to the manufacturer's recommendations. Human recombinant interferon gamma, hrIFN gamma, was a generous gift from Boehringer Ingelheim, Germany, and had a specific activity of 10×10^6 IU/mg protein on human cells. Lyophilized powder containing human serum albumin was diluted in phosphate buffered saline (PBS). Both IFNs were more than 98% pure, and were stored in aliquots at −70°C. Type IV collagen and laminin were prepared from EHS tumor (Kleinman *et al.*, 1982). Type IV collagen was extracted from the tumor with 2.0 M guanidine in 0.05 M Tris-HCl, pH 7.4, containing 0.005 M dithiothreitol. The collagen was further purified by DEAE-cellulose column chromatography (Kleinman *et al.*, 1982). Laminin was isolated from the 0.5 M NaCl extract (Timpl *et al.*, 1979).

Invasion assay

In vitro invasion of A2058 cells was measured by the method of Terranova *et al.* (1986). Briefly, reconstituted basement membrane barriers for cell invasion were placed into a modified Boyden chamber onto a type IV collagen-coated Nuclepore filter (5 μg/filter, pore size 1.0 μm) which separated

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Received: August 9, 1990 and in revised form October 26, 1990.

the upper and lower compartments. A suspension of tumor cells (1×10^6) with or without hrIFN alpha or gamma in DMEM containing 2% heat-inactivated FBS and 0.5% bovine serum albumin (BSA) was placed into the upper compartment and DMEM containing 0.5% BSA was introduced into the lower compartment. After a 48-hr incubation at 37°C in 95% air and 5% CO_2, the barrier was separated from the underlying Nuclepore filter. The cells that had penetrated the matrix and attached to the upper surface of the Nuclepore filter were detached with trypsin/EDTA solution (0.05%/0.1%) and counted. Results are expressed as the mean percentage of invading cells ± SD.

Attachment assay

Bacteriological Petri dishes (35 mm) were coated with type IV collagen and laminin. One milliliter of each protein (10 μg/ml) in 0.1 M acetic acid was added to the dishes and allowed to dry for 24 hr under UV light.

Tumor cells cultured for indicated time periods with or without hrIFN alpha or gamma were detached with 0.1% EDTA in PBS, pH 7.4. A 1-ml suspension of tumor cells (5×10^5/ml) in DMEM containing 0.5% BSA to inhibit non-specific binding was added to the coated dishes. After 60, 120 and 180 min time periods, incubation media with unattached cells were removed and the attached cells were washed gently twice with 1 ml of PBS, detached with trypsin/EDTA (0.05%/0.1%) and counted. Data are expressed as the mean percentage of attachment of triplicate assay plus and minus standard deviation (SD).

Chemotaxis assay

Chemotactic migration of tumor cells was determined by using a modified Boyden chamber assay. Briefly, type IV collagen-coated (5 μg/filter) polycarbonate filters (8 μm pores) were used to separate the upper and lower compartments of the chemotactic chamber. Cells ($2–3 \times 10^5$/ml) with or without hrIFN alpha or gamma in DMEM containing 0.5% BSA were placed into the upper compartment and DMEM with or without attractant containing 0.5% BSA into the lower compartment. After a 5-hr incubation at 37°C in a humidified atmosphere, filters were removed and stained. The cells that had not migrated were removed with cotton swabs. Migration of the cells was quantitated by counting 10 randomly selected high-power fields (×400) per filter. Data are expressed as the average number of migrating cells per high-power field of triplicate assay ± SD.

Assay of collagenase IV

The type IV collagen-degrading metalloproteinase activity was measured as described previously (Tryggvason *et al.*, 1980; Liotta *et al.*, 1981; Turpeenniemi-Hujanen *et al.*, 1986). Subconfluent tumor cell monolayers grown with or without hrIFN alpha or gamma for indicated time periods (0, 3 and 7 days) were washed with DMEM and subsequently incubated in serum-free medium (DMEM). Media were collected daily for 2 days and cell debris was removed by centrifugation at 600 *g* for 5 min. Media were concentrated 100-fold with ammonium sulfate precipitation (0–60%) followed by dialysis against 0.05 M Tris-HCl, pH 7.6, 0.2 M NaCl, at 4°C. The trypsin-activated type IV collagen degradation was assayed using soluble ^{3}H-proline-labelled type IV procollagen as a substrate.

Assay of leucine incorporation

Incorporation of ^{14}C-leucine to the cell proteins or secreted proteins was assayed from the A2058 cells grown in the presence of hrIFN alpha (2000 IU/ml) or hrIFN gamma (100 IU/ml) for various periods of time (0, 3 and 7 days). Cells were plated on 35-mm bacteriological dishes (5×10^5 cells/dish) and grown for 16 hr to allow them to attach in medium with 10% FBS which was then replaced by serum-free medium or medium with 2% FBS containing 0.5 μCi/ml ^{14}C-leucine (Amersham, Little Chalfont, UK, 54 mCi/mmol). At indicated time intervals, the supernatants were collected and the cells were washed 5 times with PBS and scraped off the dishes. Precipitates were formed from both supernatants and the cells with 10% TCA on ice for 30 minutes and washed 3 times with 5% TCA. The radioactivity of the precipitates was determined by using a scintillation counter. The results are expressed as cpm/1,000 cells (mean ± SD).

Assay of cell proliferation

The effect of interferons alpha and gamma on cell growth was determined by plating the A2058 cells (0.3×10^5) on 35-mm tissue culture dishes. After attachment of cells (16 hr) the dishes were washed 3 times with PBS and the cells grown in DMEM with or without 2% FBS and interferons alpha (2000 IU/ml) or gamma (100 IU/ml) for 10 days. The cells were detached with trypsin/EDTA (0.05%/0.1%) after a varying incubation period and counted. Data are expressed as the average number of cells of triplicate assays plus and minus standard deviation (SD).

Statistical analysis

The significance of differences in the assays was determined by Student's *t*-test.

RESULTS

We have studied the effects of interferons alpha and gamma on the *in vitro* invasion of human melanoma cells with special reference to the time of treatment. The most important events in the process of metastasis, namely the attachment of tumor cells to basement membrane components, expression of collagenase IV activity and directed migration of melanoma cells, were all studied separately in order to determine the specific step of invasion susceptible to the effects of interferons.

Effects of interferons alpha and gamma on the in vitro *invasion of A2058 cells*

An *in vitro* invasion assay (Terranova *et al.*, 1986), using a reconstituted basement membrane, was utilized to study the effects of IFN treatment on the invasive capacity of human melanoma cells. A short-term treatment with hrIFN alpha (0–3 days) caused a significant enhancement of invasion of A2058 cells ($p < 0.05$) (Fig. 1*a*). With hrIFN gamma, a pretreatment of 3 days was required to affect the invasion of the cells, after which a significant enhancement of invasion was seen with this drug ($p < 0.05$) compared to untreated control cells (Fig. 1*b*). However, when the cells were treated for a longer time (7 days) with the interferons, the penetration was markedly decreased (Figs. 1*a,b*). Interferons had no effect on

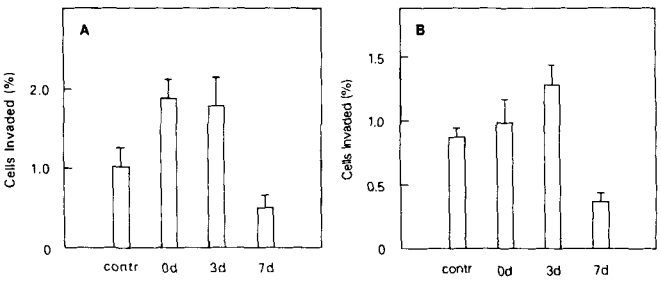

FIGURE 1 – Effect of interferons alpha and gamma on the *in vitro* invasion of A2058 human melanoma cells through a reconstituted basement membrane. Interferon was added to the cells in the beginning of the assay (= 0 days) or cells were pre-treated for various time periods (3 days, 7 days) with *(a)* interferon alpha (5000 IU/chamber) or *(b)* interferon gamma (100 IU/chamber). Bars, SD.

cell viability at the concentrations used during the same time period as in the assay indicated by the Trypan blue exclusion test.

Effects of interferons alpha and gamma on the attachment of A2058 cells

Cells attached more avidly to type IV collagen- and laminin-coated polystyrene dishes compared to uncoated dishes (not shown) as has been reported with other malignant cells (Terranova *et al.*, 1980). The attachment was markedly increased when hrIFN alpha was added directly to the assay solution (no pre-treatment) compared to control cells (Fig. 2*a*). However, a significant decrease in the attachment was observed when the cells were exposed to hrIFN alpha for a longer period of time (a 7-day treatment), whereas a shorter exposure (3 days) caused only a slight reduction in the attachment (Fig. 2*a*). Further, 100 IU/ml of hrIFN gamma also stimulated the attachment of human melanoma cells to purified basement membrane components but the stimulating effect was not seen until on the 3rd day of exposure. When the cells were treated for a longer time (7-day treatment) with hrIFN gamma, a significant inhibition of attachment was noted (Fig. 2*b*).

Effects of interferons alpha and gamma on the collagenase IV activity of A2058 cells

It has been shown (Turpeenniemi-Hujanen *et al.*, 1986) that collagenase IV represents the major type IV collagen-degrading enzyme activity in A2058 human melanoma cells upon examination of the cleavage products of the substrate by SDS-PAGE. Concentrating the medium before the assay of enzyme activity does not produce any inhibiting factors which would affect the enzyme activity, and the state of confluence does not affect the production of the enzyme in the medium of this cell line (Turpeenniemi-Hujanen *et al.*, 1986).

Figure 3*a* shows the effect of various concentrations of hrIFN alpha on collagenase IV activity. The drug was added to the cells when starting the collections of serum-free culture media for the enzyme assay. Interferon alpha caused a significant increase in the enzyme activity, the effect being concentration-dependent up to 5×10^4 IU/ml. We selected, however, the concentration of 2,000 IU/ml of hrIFN alpha for use in this study because concentrations of 5×10^3 IU/ml or more would hardly correspond to any pharmacological concentration present *in vivo* during the treatment of melanoma with hrIFN alpha.

An increase in collagenase IV production was found when the A2058 cells were pretreated with hrIFN alpha for 3 days (3 d) prior to the assay or when the drug was added to the cells at the beginning (0 d) of the 2-day collections of serum-free media for the enzyme assay (Fig. 3*b*). The differences in enzyme activity were statistically significant (Student's *t*-test) when compared to enzyme production for untreated control cells ($p < 0.05$). However, when the cells were pretreated for a longer time (7 days) with hrIFN alpha, the collagenase IV activity was markedly lower than that of cells treated for a short time or of untreated cells ($p < 0.01$) (Fig. 3*b*). The exposure of A2058 cells to hrIFN gamma also stimulated the collagenase IV activity. However, the activating effect with hrIFN gamma was not seen until on the 3rd day of the treatment when compared to untreated control cells ($p < 0.05$). In contrast, a significant inhibition of enzyme activity was noted when the cells were exposed to this drug for extended time periods (7–14 days) when compared to cells with a 3-day pre-treatment ($p < 0.01$). Here again, the inhibiting effect of hrIFN gamma appeared later (after a 7-day treatment) than with hrIFN alpha (Fig. 3*b*,*c*).

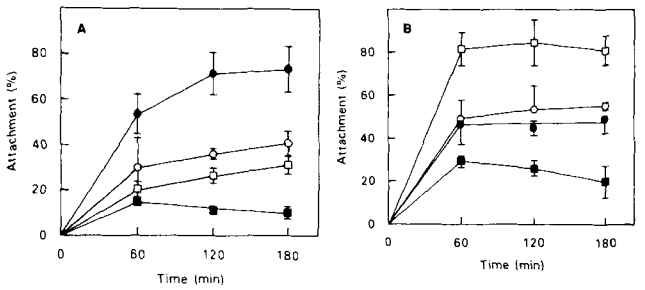

FIGURE 2 – Time-course for the attachment of interferon-treated A2058 human melanoma cells to purified basement membrane components. Cells were grown for indicated time periods with *(a)* interferon alpha (2000 IU/ml) or *(b)* interferon gamma (100 IU/ml). (○—○) control; (●—●) 0d; (□—□) 3d; (■—■) 7d. Bars, SD.

Effects of interferons alpha and gamma on the chemotactic migration of A2058 cells

Laminin and its receptor promote the migration of A2058 human melanoma cells (Wewer *et al.*, 1987). Therefore, we tested the effect of interferons alpha and gamma on the chemotactic migration of human melanoma cells to laminin. Interferons themselves as attractants had no effect on the directed movement (not shown). Interestingly, chemokinesis, *i.e.*, random migration of A2058 cells, was greatly increased when the same concentrations of interferons were added to both compartments of the chemotactic chamber (not shown). Laminin alone stimulated the chemotaxis of A2058 cells about 3-fold at the concentration of 100 μg/ml compared to control cells (Fig. 4*a*). Interferons alpha and gamma appeared to stimulate the chemotaxis of these cells to laminin when these drugs were added directly to the upper compartment of the chamber containing the assay solution with the cells (Fig. 4*a*). The difference, however, was not statistically significant ($p = 0.165$ for IFN alpha, and $p = 0.228$ for IFN gamma). In contrast, extended exposure (7 days) of the cells to hrIFN alpha significantly inhibited the laminin-induced chemotaxis when compared to the untreated cells ($p = 0.034$) or to the cells to which hrIFN alpha was added in the beginning of the assay ($p = 0.004$) (Fig. 4*a*). A longer pre-treatment time (7 days) with hrIFN gamma also suppressed the chemotactic migration of A2058 cells ($p = 0.004$) when compared to the cells without pre-treatment (0 days), but again the inhibiting effect of hrIFN gamma seemed to appear somewhat later than with hrIFN alpha, so that a 7-day pre-treatment with hrIFN gamma did not totally abolish the laminin-induced chemotaxis as did the corresponding treatment with hrIFN alpha (Fig. 4*b*).

Effects of interferons alpha and gamma on the protein synthesis of A2058 cells

Protein synthesis and the amount of secreted newly synthesized proteins in the media were studied using a ^{14}C-leucine incorporation test (see "Material and Methods"). The aim of the experiment was to assess protein synthesis under the assay conditions used (with or without 2% FBS) as well as to study the influence of the extended treatment time with IFNs on the cells. Interferon alpha increased leucine incorporation only into the medium proteins after short-term treatments (no pre-treatment, $p < 0.05$, and a 3-day pre-treatment, $p < 0.01$). In the presence of 2% serum, an increase in leucine incorporation in the cells without pre-treatment with hrIFN alpha was not statistically significant ($p = 0.078$) (Table IA). Interferon gamma decreased the leucine incorporation in the cells and in the medium after a 7-day pre-treatment. However, the difference between the values in the serum-free medium and in the untreated control cells was not statistically significant ($p = 0.128$) (Table IB). Neither was the decrease in the leucine incorporation in the cells with 2% serum after a 3-day pre-treatment with hrIFN gamma statistically significant

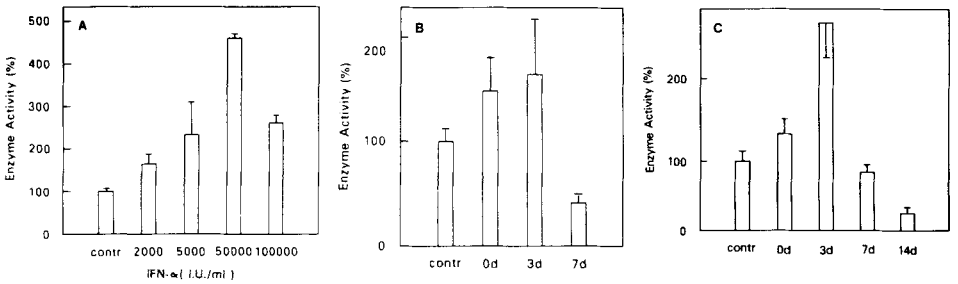

FIGURE 3 – Effect of interferons alpha and gamma on collagenase IV activity. *(a)* Effect of varying concentrations of interferon alpha. Collagenase IV activity of A2058 cells pretreated for various time periods (0 days, 3 days, 7 days) with *(b)*, interferon alpha (2000 IU/ml) and *(c)*, interferon gamma (100 IU/ml). Bars, SD.

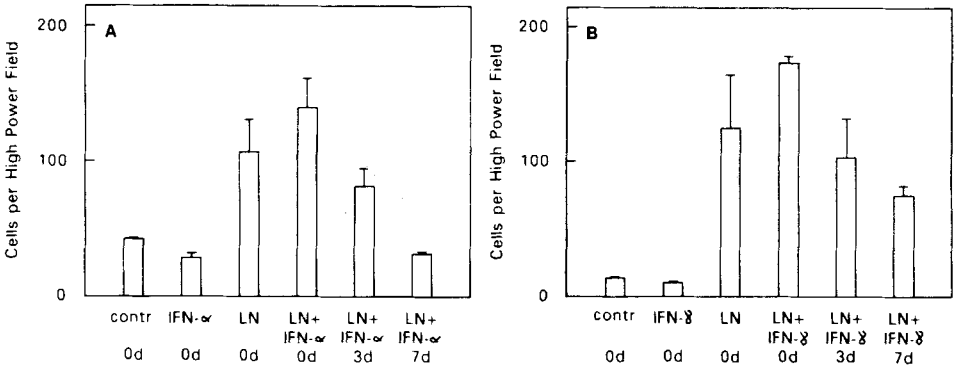

FIGURE 4 – Effect of interferon treatment on the chemotaxis of A2058 human melanoma cells to laminin. Interferon was added to the cells at the beginning of the assay (=0 days) or cells were pre-treated for extended time periods (3 days, 7 days) with *(a)*, interferon alpha (2000 IU/ml) or *(b)* interferon gamma (100 IU/ml). The data are expressed as a mean number of migrating cells per high-power field (×400) of triplicate assays. Bars, SD.

TABLE IA,B – EFFECTS OF INTERFERONS ALPHA AND GAMMA ON ^{14}C-LEUCINE INCORPORATION OF A2058 CELLS. A, IFN ALPHA; B, IFN GAMMA

A

Pre-treatment time (days)	− Serum (10^2)		+ 2% Serum (10^2)	
	Medium	Cells	Medium	Cells
Control	1.42 ± 0.15	10.77 ± 3.97	2.08 ± 0.23	16.41 ± 5.90
0	1.98 ± 0.23[1]	12.81 ± 0.45	2.66 ± 0.33	30.35 ± 3.67
3	3.66 ± 0.19[2]	14.89 ± 0.57	1.77 ± 0.59	20.14 ± 10.06
7	1.78 ± 0.31	6.01 ± 0.50	2.32 ± 0.47	24.82 ± 3.32

B

Pre-treatment time (days)	− Serum (10^2)		+ 2% Serum (10^2)	
	Medium	Cells	Medium	Cells
Control	2.57 ± 0.81	18.31 ± 3.71	2.05 ± 0.12	24.92 ± 6.91
0	2.44 ± 0.32	19.50 ± 0.13	3.09 ± 0.35[1]	20.35 ± 3.74
3	2.88 ± 0.70	12.20 ± 0.07	3.80 ± 0.61[1]	13.83 ± 2.59
7	1.28 ± 0.24	4.42 ± 0.71[1]	1.31 ± 0.20[1]	3.77 ± 0.21[1]

Cells were grown in the presence of IFN alpha (2000 IU/ml) or gamma (100 IU/ml) for various time periods. ^{14}C-leucine incorporation was determined after 24 hr of incubation from the cells and culture media with or without 2% FBS. The results are expressed as cpm/1,000 cells–[1]$p < 0.05$. –[2]$p < 0.01$ relative to control.

($p = 0.074$). In contrast, when medium was supplemented with 2% serum, the secretion of newly synthesized proteins in the medium was markedly increased after short-term treatments (no pre-treatment, $p < 0.05$; and 3-day pre-treatment, $p < 0.05$) (Table IB).

Effects of interferons alpha and gamma on the growth of A2058 cells

The growth of A2058 cells was tested in the presence or absence of 2% FBS. Control cells or cells treated with hrIFN alpha or gamma proliferated little during the first 4 days in 2% serum (Fig. 5). However, both interferons significantly inhibited the growth of these cells after the 4th day of incubation. It must be emphasized that these cells did not grow very well in serum-free medium over long periods of time.

DISCUSSION

Tumor cell attachment, secretion of collagenase IV and directed migration are thought to play an important role in the spread of cancer. The purpose of this study was to determine the effect of interferons on these distinct steps of invasion by tumor cells. Here, we show that human recombinant interferons alpha and gamma possess a dual effect on the invasive capacity of human melanoma cells *in vitro*. Our results show that a short-term pre-treatment with these drugs enhanced the

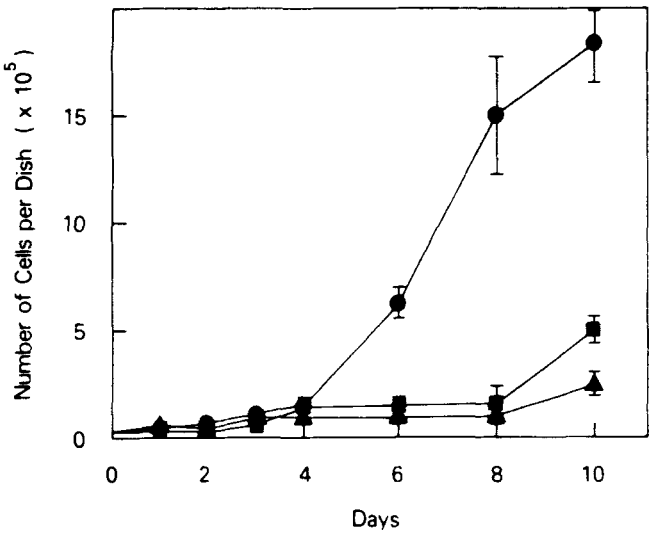

FIGURE 5 – Effect of interferons alpha and gamma on the growth of A2058 human melanoma cells. Cells were first plated as described in "Material and Methods". After incubation in 2% serum for indicated time periods (0–10 days) with or without hrIFN alpha or gamma, the cells were trypsinized and counted. The data are expressed as the average number of cells of triplicate assays plus and minus SD. (●—●) control; (■—■) hrIFN alpha; (▲—▲) hrIFN gamma.

metastatic propensity of the cells including cell attachment, secretion of collagenase IV, cell migration and *in vitro* invasion through a reconstituted basement membrane, whereas long-term exposure (7 days) of the cells to the IFNs markedly inhibited these cellular activities implicated in invasion *in vivo*.

Tumor cells adhere preferentially to type IV collagen via laminin as they penetrate the basement membranes (Terranova *et al.*, 1980). Several laminin binding proteins (integrins) have been identified on a variety of cell types (Rao *et al.*, 1983; Gehlsen *et al.*, 1988), and also on human melanoma cells (Kramer *et al.*, 1989). Since interferons can alter the expression of cell surface antigens (Pestka *et al.*, 1987), it is possible that the increased attachment to basement membrane components of the short-term interferon-treated melanoma cells was due to enhanced expression of laminin binding proteins on the cell surface.

The expression of collagenase IV activity in culture and the *in vivo* metastatic potential of tumor cells have been correlated in several studies (Liotta *et al.*, 1980; Turpeenniemi-Hujanen *et al.*, 1985; Thorgeirsson *et al.*, 1985). Duncan and Bergman (1989) showed that a 48-hr treatment of human dermal fibroblasts with human recombinant interferon alpha, beta or gamma markedly increased collagenase type I production by these cells. Similarly, the ability of IFN alpha and beta to increase the production of trypsin-activatable collagenase for the type IV collagen by Ewing sarcoma cells reported by Siegal *et al.* (1982) supports our finding that interferon alpha and gamma can enhance collagenase IV secretion from cancer cells in short-term treatments. These data suggest that enhanced collagenase activity in interferon-treated cells may be due to elevated interferon-induced expression of collagenase IV gene rather than increased autoactivation of latent collagenase produced by interferon-treated cells. The data from the biphasic effect of interferons on all the steps required for the invasion of melanoma cells support the previous suggestion that the genetic control of the expression of collagenase IV might be linked to the control of other biochemical properties in cancer cells required for metastasis (Turpeenniemi-Hujanen *et al.*, 1985; Thorgeirsson *et al.*, 1985).

Tumor cell migration is one of the important cellular activities implicated in invasion. Transformed cells are able to move towards the source of a chemo-attractant, and tumor cells possess specific surface receptors for chemo-attractants. Changes in cell movement and in the cytoskeleton can be induced by a variety of agents such as polypeptide growth factors (response modifiers), tumor promoters, transforming viruses, and electrical fields. Laminin, a basement-membrane-specific glycoprotein, exhibits various biological activities including promotion of cell attachment, growth and differentiation. It also appears to be involved in the directional movement of a variety of normal and transformed cells, including melanoma cells (McCarthy *et al.*, 1984; Situ *et al.*, 1984), probably through an interaction with its receptor (McCarthy *et al.*, 1984). Interferons inhibit chemotaxis of non-transformed (Tamm *et al.*, 1981; Brouty-Boyé and Zetter, 1980) and of several transformed cells (Melchiori *et al.*, 1987) *in vitro*. Our results show that interferon alpha and gamma are able to modulate the directed migration of these cells to laminin, depending on the treatment time. In addition, these data raise the possibility that interferons alpha and gamma may regulate the number of laminin receptors (integrins) on the cell surface.

In our studies interferons alpha or gamma themselves were not chemotactic for human melanoma cells. However, chemokinesis, *i.e.*, random movement of the cells, was markedly increased. This observation may be of clinical importance in terms of tumor cell detachment from the primary tumor and subsequent formation of distant metastases, since the detachment of cells from the tumor bulk is one of the prerequisites for metastasis.

A critical property of malignant cells is their capacity to invade basement membranes, an essential step in metastasis. Several studies report either stimulatory or inhibitory effects of interferons on tumor cell invasion and metastasis (Siegal *et al.*, 1982; Brunda *et al.*, 1984; Nishimura *et al.*, 1985; Lollini *et al.*, 1987; Ramani and Balkwill, 1987, 1989). In other studies, a pre-treatment of malignant or transformed cells with interferons has inhibited invasion *in vitro* (Ravine and Ledinko, 1986; Melchiori *et al.*, 1987). Our results, however, show that interferons alpha and gamma enhanced the invasive capacity of human melanoma cells *in vitro* after a short pre-treatment. Also in agreement with our findings is an investigation by Siegal *et al.* (1982) who showed a considerable enhancement of the invasiveness *in vitro* of Ewing sarcoma cells treated with IFN alpha and beta. The metastatic potential of cancer cells was increased also *in vivo* by using pre-treatments with gamma IFN (Ramani and Balkwill, 1987; Lollini *et al.*, 1987). The enhanced metastasis also correlated to an increase in major histocompatibility complex (MHC) expression (Lollini *et al.*, 1987). Taken together, these data suggest that the enhanced metastatic potential of interferon-treated cells may be related to antigen-inducing effects of interferon *in vivo* and may also be due to a short exposure of the cancer cells to the drugs.

Our *in vitro* studies demonstrate that, after a transient enhancement of invasive propensity, interferons alpha and gamma markedly suppress the cellular functions of human melanoma cells, including their ability to invade. This effect of interferons, however, needs a treatment time of 7 days or more. After this time, the interferons have also a strong inhibitory effect on the cell growth and the amount of newly synthesized proteins in the cells. This kind of biphasic effect of interferons on the invasive behavior of melanoma cells in our model may be due to the possibility that these drugs are able to modulate the metabolism of their own receptors.

Different species of interferons have divergent effects on cellular functions (Krown, 1986). Our study shows that the effect of interferons alpha and gamma on the distinct steps of invasion appear to be parallel except that the stimulatory effect

of interferon gamma on cell attachment and secretion of collagenase IV appears later than with interferon alpha, and that treatment time with interferons plays an important role in the modulation of cellular functions implicated in invasion. Our results support the clinical use of interferons alpha and gamma in the treatment of cancer in combination therapy in which another systemic drug should be used, at least at the beginning of the treatment, in order to avoid adverse stimulating effects of interferons.

ACKNOWLEDGEMENTS

We thank Ms. M. Syrjäkari, A. Korkeakoski, K. Järvenpää, E. Lukkarinen and V. Vähäsaari for excellent technical assistance, Dr. H. Sarelin (Boehringer Ingelheim) for generously providing the recombinant interferon gamma, and Ms. S. Annanperä for preparing the manuscript. This work was supported by grants from Finnish Cancer Foundation and from the Jusélius Foundation.

REFERENCES

BRESALIER, R.S., HUJANEN, E.S., RAPER, S.E., ROLL, F.J., ITZKOWITZ, S.H., MARTIN, G.R. and KIM, Y.S., An animal model for colon cancer metastasis: establishment and characterization of murine cell lines with enhanced liver-metastasizing ability. *Cancer Res.*, **47**, 1398–1406 (1987).

BROUTY-BOYÉ, D. and ZETTER, B., Inhibition of cell mobility by interferon. *Science*, **208**, 516–518 (1980).

BRUNDA, M.J., ROSENBAUM, D. and STERN, L., Inhibition of experimentally induced metastasis by interferon alpha: correlation between the modulatory effect of interferon treatment on natural killer cell activity and inhibition of metastasis. *Int. J. Cancer*, **34**, 421–426 (1984).

DUNCAN, M.R. and BERMAN, B., Differential regulation of glycosaminoglycan, fibronectin and collagenase production in cultured human dermal fibroblasts by interferon-alpha, -beta, and -gamma. *Arch. Dermatol. Res.*, **281**, 11–18 (1989).

GEHLSEN, K.R., DILLNER, L., ENGVALL, E. and RUOSLAHTI, E., The human laminin receptor is a member of the integrin family of cell adhesion receptors. *Science*, **241**, 1228–1229 (1988).

HÖYHTYÄ, M., HUJANEN, E., TURPEENNIEMI-HUJANEN, T., THORGEIRSSON, U., LIOTTA, L.A. and TRYGGVASON, K., Modulation of type IV collagenase activity and invasive behaviour of metastatic human (A2058) cells *in vitro* by monoclonal antibodies to type IV collagenase. *Int. J. Cancer*, **46**, 282–286 (1990).

HUJANEN, E.S. and TERRANOVA, V.P., Migration of tumor cells to organ-derived chemoattractants. *Cancer Res.*, **45**, 3517–3521 (1985).

KLEINMAN, H.K., MCGARVEY, M.L., LIOTTA, L.A., GEHRON-ROBEY, P., TRYGGVASON, K. and MARTIN, G.R., Isolation and characterization of type IV procollagen, laminin, and heparan sulfate proteoglycan from EHS sarcoma. *Biochemistry*, **21**, 6188–6193 (1982).

KRAMER, R.H., MCDONALD, K.A. and VU, M.P., Human melanoma cells express a novel integrin receptor for laminin. *J. biol. Chem.*, **264**, 15642–15649 (1989).

KROWN, S.E., Interferons: not just alpha. *Cancer Treat. Rep.*, **70**, 1353–1355 (1986).

LIOTTA, L.A., RAO, C.N. and WEWER, U.M., Biochemical interactions of tumor cells with the basement membrane. *Ann. Rev. Biochem.*, **55**, 1037–1057 (1986).

LIOTTA, L.A., TRYGGVASON, K., GARBISA, S., GEHRON-ROBEY, P. and ABE, S., Partial purification and characterization of a neutral protease which cleaves type IV collagen. *Biochemistry*, **20**, 100–104 (1981).

LIOTTA, L.A., TRYGGVASON, K., GARBISA, S., HART, I., FOLTZ, C.M. and SHAFIE, S., Metastatic potential correlates with enzymatic degradation of basement membrane collagen. *Nature (Lond.)*, **284**, 67–68 (1980).

LOLLINI, P-L., GIOVANNI, C., DEL RE, B., NICOLETTI, G., PRODI, G. and NANNI, P., Interferon-mediated enhancement of metastasis. Are MHC antigens involved? *Clin. exp. Metast.*, **5**, 277–287 (1987).

MCCARTHY, J.B., PALM, S.L. and FURCHT, L.T., Laminin and fibronectin promote the haptotactic migration of B16 mouse melanoma cells *in vitro*. *J. Cell Biol.*, **98**, 1474–1480 (1984).

MELCHIORI, A., ALLAVENA, G., BÖHM, J., REMY, W., SCHMIDT, J., PARODI, S., SANTI, L. and ALBINI, A., Interferons inhibit chemotaxis of transformed cells and their invasion of a reconstituted basement membrane. *Anticancer Res.*, **7**, 475–480 (1987).

NICOLSON, G.L., Organ specificity of tumor metastasis: role of preferential adhesion, invasion and growth of malignant cells at specific secondary sites. *Cancer Metast. Rev.*, **7**, 143–188 (1988).

NISHIMURA, J., MITSUI, K., ISHIKAWA, T., TANAKA, Y., YAMAMOTO, R., SUHARA, Y. and ISHITSUKA, H., Antitumour and antimetastatic activities of human recombinant interferon alpha A/D. *Clin. exp. Metast.*, **3**, 295–304 (1985).

PESTKA, S., LANGER, J.A., ZOON, K.C. and SAMUEL, C.E., Interferons and their actions. *Ann. Rev. Biochem.*, **56**, 727–778 (1987).

RAMANI, P. and BALKWILL, F.R., Enhanced metastases of a mouse carcinoma after *in vitro* treatment with murine interferon gamma. *Int. J. Cancer*, **40**, 830–834 (1987).

RAMANI, P. and BALKWILL, F.R., Action of recombinant alpha interferon against experimental and spontaneous metastases in a murine model. *Int. J. Cancer*, **43**, 140–146 (1989).

RAO, C.N., BARSKY, S.H., TERRANOVA, V.P. and LIOTTA, L.A., Isolation of a tumor cell laminin receptor. *Biochem. biophys. Res. Comm.*, **111**, 804–808 (1983).

RAVINE, T.J. and LEDINKO, N., Treatment with human recombinant leukocyte interferons inhibits *in vitro* invasive ability of human lung carcinoma cells. *Clin. exp. Metast.*, **4**, 191–203 (1986).

SIEGAL, G.P., THORGEIRSSON, U.P., RUSSO, R.G., WALLACE, D.M., LIOTTA, L.A. and BERGER, S.L., Interferon enhancement of the invasive capacity of Ewing sarcoma cells *in vitro*. *Proc. nat. Acad. Sci (Wash.)*, **79**, 4064–4068 (1982).

SITU, R., LEE, E.C., MCCOY, J.P. and VARANI, J., Stimulation of tumor cell motility by laminin. *J. Cell Sci.*, **70**, 167–176 (1984).

TAMM, I., PFEFFER, L.M. and MURPHY, J.S., Assay of the inhibitory activities of human interferons on the proliferation and locomotion of fibroblasts. *In: Methods in enzymology*, Vol. **79**, pp. 404–413, Academic Press, New York (1981).

TERRANOVA, V.P., HUJANEN, E.S., LOEB, D.M., MARTIN, G.R., THORNBURG, L. and GLUSHKO, V., Use of a reconstituted basement membrane to measure cell invasiveness and select for highly invasive tumor cells. *Proc. nat. Acad. Sci. (Wash.)*, **83**, 465–469 (1986).

TERRANOVA, V.P., ROHRBACH, D.H. and MARTIN, G.R., Role of laminin in the attachment of PAM 212 (epithelial) cells to basement membrane collagen. *Cell*, **22**, 719–726 (1980).

THORGEIRSSON, U.P., TURPEENNIEMI-HUJANEN, T., WILLIAMS, J.E., WESTIN, E.H., HEILMAN, C.A., TALMADGE, J.E. and LIOTTA, L.A., NIH/3T3 cells transfected with human tumor DNA containing activated *ras* oncogenes express the metastatic phenotype in nude mice. *Mol. cell. Biol.*, **5**, 259–262 (1985).

TIMPL, R., ROHDE, H., ROBEY, P.G., RENNARD, S.I., FOIDART, J.-M. and MARTIN, G.R., Laminin-A glycoprotein from basement membranes. *J. biol. Chem.*, **254**, 9933–9937 (1979).

TRYGGVASON, K., GEHRON-ROBEY, P. and MARTIN, G.R., Biosynthesis of type IV collagen. *Biochemistry*, **19**, 1284–1289 (1980).

TRYGGVASON, K., HÖYHTYÄ, M. and SALO, T., Proteolytic degradation of extracellular matrix in tumor invasion. *Biochim. biophys. Acta*, **907**, 191–217 (1987).

TURPEENNIEMI-HUJANEN, T., THORGEIRSSON, U.P., HART, I.R., GRANT, S.S. and LIOTTA, L.A., Expression of collagenase IV (basement membrane collagenase) activity in murine tumor cell hybrids which differ in metastatic potential. *J. nat. Cancer Inst.*, **75**, 99–103 (1985).

TURPEENNIEMI-HUJANEN, T., THORGEIRSSON, U.P., RAO, C.N. and LIOTTA, L.A., Laminin increases the release of type IV collagenase from malignant cells. *J. biol. Chem.*, **261**, 1883–1889 (1986).

WEWER, U.M., TARABOLETTI, E.G., SOBEL, M.E., ALBRECHTSEN, R. and LIOTTA, L.A., Role of laminin receptor in tumor cell migration. *Cancer Res.*, **47**, 5691–5698 (1987).