Cancer Chemother Pharmacol (1982) 9:75–80

Cancer Chemotherapy and Pharmacology

Characteristics of in vitro Antiproliferation Activity of Human Interferon-β

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Summary. *We compared the in vitro antiproliferative activity of highly purified interferon (IFN)-β ($> 10^7$ U protein/mg in antiviral activity) with that of IFNs-α and lymphoblastoid, using human cells of malignant and non-malignant origin. IFN-β was the least active of three IFNs in suppressing Daudi cell proliferation. Three hematological cells other than Daudi cells cultivated in suspension were insensitive to each of three IFNs. IFN-β was more active than IFNs-α and lymphoblastoid in suppressing all eight epithelioid cells tested and, particularly with respect to five epithelioid cells sensitive to IFN, IFN-β was seven to 49 times as active as IFN-α. These results indicate that suppression of cell proliferation by IFN depends not only on the target cell species but also on the IFN species, and emphasize the need for careful selection of the most appropriate IFN species in therapy.*

We found that the antiproliferative characteristics of the present IFN-β preparation were consistent with those reported previously, supporting the idea that IFN-β molecules in the present preparation were responsible for suppressing cell proliferation. The antiproliferation activity of our preparation was species-specific but not selective for cells of malignant origin; it was absorbable by IFN-sensitive but not by IFN-insensitive cells; and it was achieved by a cytostatic effect.

Introduction

Clinical trials using human interferon (IFN)-α derived from leukocytes and lymphoblastoid cells are currently under way to assess its antitumor activity. However, because of a limited supply and problems related to impurity, the antitumor activity of human IFN-β has been studied much less. A fairly well purified IFN-β preparation ($> 1 \times 10^7$ U/mg protein) is now available for clinical trial [5]. In the present study we characterized the activity of this IFN-β preparation in suppressing the in vitro proliferation of cells derived from patients with malignant and non-malignant diseases.

The mechanism of the antiviral activity of IFN has been studied extensively since its discovery and, although this is still controversial, the production of 2′–5′ oligonucleotide and the activation of protein kinase by IFN are considered to be responsible for its antiviral activity [8]. In contrast, the mechanism of the antiproliferation activity of IFN has been poorly understood, and as far as the action mechanism is concerned, the association of the antiproliferation activity of IFN with the antiviral activity is not established.

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Apart from its mechanism of action, it is essential to characterize the antiproliferation activity of IFN from the point of view of cancer therapy. The fact that IFN seems to be clinically effective in a limited number of tumors [1] shows the importance of careful selection of target tumors on the basis of characteristics of IFN in suppressing the tumor cell proliferation. In this regard, it has been proposed that the antiproliferation activity of IFNs is tissue-specific [3]. Therefore, we compared the potency and the specificity of the antiproliferative activity of the present IFN-β preparation with those of IFNs-α and lymphoblastoid, using different human cells. We found that the proliferation of IFN-sensitive human cells was suppressed to a different extent by IFNs-α, β, and lymphoblastoid, and that epithelioid cells were more, and lymphoblastoid Daudi cells less, sensitive to IFN-β than IFNs-α and lymphoblastoid. Our findings indicate that suppression of cell proliferation depends on both the target cell and the IFN species.

Materials and Methods

Interferons. Human IFN-α (4×10^6 U/mg protein) was kindly supplied by Dr K. Cantell. Human IFN-β, induced in human fibroblast cells by poly I · poly C, was a product of Toray Industries, Inc., Kamakura, Japan ($> 10^7$ U/mg protein) [5]. Human IFN-lymphoblastoid of Namalwa cells, a product of Wellcome Research Laboratories, Kent, England, was kindly supplied by Sumitomo Chemical Ind., Ltd , Osaka, Japan (Lot No. CIN/6, $> 5 \times 10^7$ U/mg protein). Mouse IFN was purified from mouse L-MS cells induced by Newcastle disease virus (10^6 U/mg protein). The antiviral activity was assayed by the cytopathic effect of vesicular stomatitis virus on FL cells, and expressed with reference to the National Institutes of Health (USA) standard.

Cells. Human cells of malignant origin included Daudi cells from Burkitt's lymphoma (kindly supplied by Dr M. Shimoyama) and HeLa cells of uterine origin (supplied by Dr Y. Kojima). From Dainippon Pharmaceutical Co., Osaka, Japan, we purchased H.Ep#2 cells from a laryngeal tumor, J-111 cells from monocytic leukemia, and CCRF-SB cells from acute lymphoblastic leukemia. Human cells of non-malignant origin were amnionic FL cells (supplied by Dr T. Kishida); from Dainippon Pharmaceutical we purchased Detroit-98 cells from the sternal marrow of an adult Caucasian male with no history of malignancy, Bri 7 cells from normal peripheral B lymphocytes, and RPMI-1788 cells from normal peripheral leuko-

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cytes. The human cells were passaged in vitro in the culture medium indicated every 3–8 days.

Mouse leukemia L1210 and P388 cells were harvested from the peritoneal cavity of male DBA/2 Cr mice 5 days after IP inoculation of 10^6 cells. YAC-1 cells (supplied by Dr T. Kunimoto) were passaged in vitro.

Culture Medium. RPMI-1640 medium (Grand Island Biological Co., Grand Island, NY) containing 10% fetal bovine serum (FBS, GIBCO) and 100 μg kanamycin/ml (Banyu Pharmaceutical Co., Ltd, Tokyo, Japan) was used with Daudi, CCRF-SB, Bri 7, and RPMI 1788 cells. Eagle's minimum essential medium (MEM, Nissui Seiyaku Co., Ltd, Tokyo, Japan) containing 10% FBS and 100 μg kanamycin/ml was used with the remaining human cells. RPMI-1640 medium supplemented with 5% FBS, 100 μg kanamycin/ml and 20 μ*M* mercaptoethanol (Wako Pure Chemical Industries, Ltd, Tokyo, Japan) was used with the mouse L1210, P388, and YAC-1 cells.

Antiproliferation Activity of IFNs. The cells were cultivated in the presence or absence of IFNs for the periods indicated at 37° C in a CO_2 incubator (WJ-22-C, Hirasawa Works, Tokyo, Japan) containing 5%–10% CO_2. The cells in suspension culture were cultivated in glass test tubes. Cells forming a sheet in plastic vessels were cultivated in 3013F flasks or 3008 multiwell tissue culture plates (Falcon, Oxnard, CA, USA). Cells cultivated in vitro were counted directly by one of two methods: Those forming a cell sheet were first incubated with 0.05% trypsin-0.02% EDTA (GIBCO) and then mixed with an equivalent volume of physiological saline containing 10% newborn calf serum or FBS (GIBCO). Single-cell suspensions were prepared by pipetting. The cell number was counted with a hemocytometer. Alternatively, portions of, or whole, cell suspensions were mixed with Isoton (Coulter Electronics Inc., Hialeah, FA, USA) and counted on a Coulter counter (Model Z_B, Coulter Electronics, Inc.) at a setting where more than 95% of the viable cells were counted. Cell counting by these two methods gave essentially the same results unless the cell concentration of the test mixture was less than 10% of the control, in which case the dead cell population was increased significantly.

Cell concentrations were expressed as relative percentages of the control cell concentration. The IC_{50} was defined as the IFN concentration required to inhibit cell proliferation by 50%.

Trypan Blue Exclusion Test. The test cells were centrifuged at 200 *g* for 5 min (Model H-103RS centrifuge, Kokusan Centrifuge Co., Tokyo, Japan) at 4° C. The sediment was suspended in 5 m*M* phosphate-buffered saline (PBS, pH 7.4) and mixed with trypan blue solution (0.12% in PBS, Chroma-Gesellschaft, Stuttgart-Untertürkheim, FRG). Stained and unstained cells were counted using a hemocytometer.

Results

Differential Antiproliferation Activity of IFNs

As shown in Fig. 1 and Tables 1 and 2, the antiproliferation activity of IFNs-α, β and lymphoblastoid varied markedly, depending on the human cell species. Daudi cells were highly sensitive, although to different extents, to all of the tested

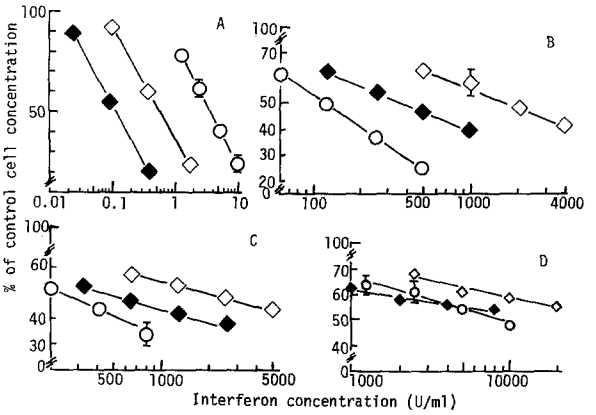

Fig. 1 A–D. Antiproliferation activity of IFN-α, -β and lymphoblastoid on human cells. Daudi (2×10^4/ml, **A**), H.Ep#2 (5×10^3/ml, **B**), Detroit-98 (5×10^3/ml, **C**), and Bri 7 (2×10^4/ml, **D**) cells were mixed with the indicated concentration of IFN-α (◇), β (○) or lymphoblastoid (◆) and cultivated for 4 or 5 days at 37° C in a CO_2 incubator. Cell concentrations were determined on a Coulter counter; the results are expressed as the relative percentage of test and control cell concentrations. Standard deviations are included wherever possible. The control cell concentrations ($\times 10^5$/ml, mean ± SD of triplicate) were 2.8 ± 0.10 (Daudi cells), 1.3 ± 0.026 (H.Ep#2 cells), 1.7 ± 0.061 (Detroid-98 cells), and 3.0 ± 0.10 (Bri 7 cells)

Table 1. Antiproliferation activity of IFN-α, -β and -lymphoblastoid on Daudi cells

IFNs	IC_{50} of IFN (U/ml)			
	Expt 1	Expt 2	Expt 3	Mean ± SD
Lymphoblastoid	0.11	0.10	0.11	0.11 ± 0.01*
α	0.44	0.47	0.57	0.49 ± 0.06**
β	2.85	3.40	3.60	3.28 ± 0.32**

* Significantly different ($P < 0.05$ by the *t*-test)
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IFNs; their proliferation was suppressed most markedly by IFN-lymphoblastoid and least by IFN-β (Fig. 1A). This finding was confirmed by a series of experiments in which the antiproliferation activity of the IFNs was expressed in terms of IC_{50} (Table 1).

In contrast, Bri 7 cells were resistant to all three IFNs; the IC_{50} of IFN was approximately 10,000 U/ml (Fig. 1D). CCRF-SB and RPMI-1788 cells were resistant to IFNs-α and β (Table 2).

Daudi, Bri 7, CCRF-SB, and RPMI-1788 cells proliferate in suspension. We also examined the antiproliferative activity of IFNs on human cells forming cell sheets. The relative antiproliferation activity of the three IFNs on H.Ep#2 and Detroit-98 cells was different from that on Daudi cells (Fig. 1B and C). In contrast to Daudi cells, in H.Ep#2 and Detroit-98 cells IFN-β suppressed proliferation most effectively. As both these are epithelioid cell lines, we examined whether IFN-β manifests greater antiproliferation activity than IFN-α on other epithelioid cells as well (Table 3). As indicated by the IC_{50} ratio, all five epithelioid cell lines tested were more sensitive to IFN-β than IFN-α by factors of 7–49, although IC_{50} of IFN-β ranged from 97 U/ml (H.Ep#2 cells) to 760 U/ml (FL cells).

Table 2. Antiproliferation activity of IFN-α and -β on suspension-cultured cells

Cells	Percent of control cell concentration in the presence of 10,000 U (per ml) of	
	IFN-α	IFN-β
Bri 7	58.3 ± 1.7[a, *]	47.7 ± 1.4[*]
CCRF-SB	59.5 ± 2.8[**]	49.2 ± 2.3[*, **]
RPMI-1788	101 ± 2.5[***]	89.5 ± 3.9[***]

The control cell concentrations ($\times 10^5$/ml, mean ± SD of triplicate) were 3.0 ± 0.06 (Bri 7 cells), 5.1 ± 0.31 (CCRF-SB cells), and 4.4 ± 0.09 (RPMI-1788 cells)

[a] Mean ± SD of triplicate

[*, **, ***] Significantly different ($P < 0.05$ by the t-test) respectively

Table 3. Antiproliferation activity of IFN-α and -β on epithelioid cells

Cells	IC_{50} of IFNs[a]		Relative activity of IFN-β/IFN-α
	IFN-α	IFN-β	
HeLa	6,800 ± 5,200	202 ± 70[*]	29 ± 13
H.Ep # 2	4,900 ± 1,800	97 ± 29[*]	49 ± 4.4
J-111	1,260 ± 340	189 ± 64[*]	7.1 ± 2.0
FL	9,600 ± 3,800	760 ± 48[*]	12 ± 5.8
Detroit-98	2,200 ± 570	111 ± 8[*]	20 ± 4.2

Summarized results obtained with three or more experiments are presented. The ratio of IC_{50} of IFN-α and -β is shown as the relative activity of IFN-β over IFN-α

[a] U/ml, mean ± SD

[*] Significant at $P < 0.05$ (t-test) compared with the activity of IFN-α

Table 4. Stability of the antiproliferation activity of IFN-β in Daudi cells

IFN-β preparation	Percent of controll cell concentration[a] after 4-day cultivation
Preincubated at 4° C	25.2 ± 1.5[b, *]
Preincubated at 37° C	43.5 ± 1.0
Preincubated at 37° C with Daudi cell culture fluid	43.0 ± 0.71
Daudi cell culture fluid in the abscence of IFN-β	101 ± 4.7

In the presence of RPMI-1640 medium containing fetal bovine serum (10%) and kanamycin (100 µg/ml), Daudi cells (2×10^4/ml) were cultivated for 2 days at 37° C in a CO_2 incubator. Then the culture fluid was collected and mixed with an equivalent volume of medium containing or not containing 20 U/ml IFN-β. This mixture was re-incubated for 1 day at 37° C. Alternatively, IFN-β (10 U/ml) was incubated under the above conditions in the absence of culture fluid. These IFN-β preparations were incubated with Daudi cells for examination of their antiproliferation activity

[a] Control cell concentration was 4.5×10^5/ml

[b] Mean ± SD of triplicate

[*] Significantly different ($P < 0.05$ by the t-test) from any other group

Table 5. Antiproliferation activity of IFN-α and -β on precultured H.Ep # 2 cells

IFN species	IC_{50} of IFN (U/ml)			
	Expt 1	Expt 2	Expt 3	Mean ± SD
α	2,400	4,500	7,500	4,800 ± 209[*]
β	630	147	115	297 ± 235[*]

H.Ep # 2 cells were precultured at 37° C in IFN-free medium in a CO_2 incubator. One day later, IFN-α or -β was added and after further 4-day cultivation, its antiproliferative activity was assayed

[*] Significantly different from the control ($P < 0.05$ by the t-test)

However, not all epithelioid cells are sensitive to IFN-β. We found that G-361 cells from a human malignant melanoma, KB cells from a nasopharyngeal tumor, and L-132 cells from human fetal lung were IFN-β-insensitive, as indicated by an IC_{50} greater than 3,500 U/ml (data not shown). On the other hand, in these experiments also, IFN-β suppressed cell proliferation more effectively than IFN-α by factors of 1.5 (G-361 cells) and 5.4 (L-132 cells) at IC_{50}, and a factor of 10 (KB cells) at IC_{20}.

Although these results do not permit the firm conclusion that IFN-β is superior to IFN-α in suppressing epithelioid cell proliferation, they lead us to posit that, based on its antiviral activity, IFN-β suppresses the proliferation of IFN-sensitive epithelioid cells more effectively than IFN-α. Therefore, we next characterized the antiproliferation activity of IFN-β.

Stability of the Antiproliferation Activity of IFN-β

The preceding study had shown that, in contrast to all other cells tested, in Daudi cells IFN-β was less effective in suppressing cell proliferation. Therefore, we examined whether the effect of IFN-β is negatively affected by the substrates, including metabolites and enzymes, released from Daudi cells in the course of cultivation. For this purpose, we assayed the stability of the IFN-β antiproliferation activity in Daudi cell culture fluid (Table 4). Compared with the results obtained upon incubation of IFN-β at 4° C in the presence of RPMI-1640 medium, incubation at 37° C resulted in a marked loss of antiproliferation activity. On the other hand, when IFN-β was incubated at 37° C in the presence of Daudi cell fluid, its antiproliferation activity was not further decreased. These results indicate that the activity of IFN-β was stable in Daudi cell culture fluid and that its observed poor activity on Daudi cells, compared with that of IFNs-α and lymphoblastoid (Table 1), was not ascribable to its inactivation during cell culture.

Antiproliferation Activity of IFNs on Epithelioid Cells

The above experiments had shown that, judging by antiviral activity, IFN-β was more potent than IFN-α in suppressing epithelioid cell proliferation. During proliferation, the epithelioid cells attached to the culture flasks and IFN had been present from the outset of cultivation. Therefore, it was necessary to rule out the possibility that the greater antiproliferative effect of IFN-β was due to its greater interference with cell attachment to the flasks.

Comparison of the results shown in Tables 3 and 5 shows that when H.Ep#2 cells were precultured in the absence of

IFN and then grown in IFN-containing medium, their proliferation was not different from that observed in experiments in which IFN was present throughout the culture period. Furthermore, in the latter, as in the former, experiments, IFN-β suppressed cell proliferation more potently than IFN-α. These results ruled out the possibility that the greater effectiveness of IFN-β was due to its greater interference with cell attachment to the flasks.

Additive Antiproliferation Activity of IFN-α and -β

Next we examined whether in combination, IFN-α and -β, which individually exerted differential antiproliferation activity on various human cells, were effective in suppressing the proliferation of H.Ep#2 cells and whether suppression was additive or synergistic. As shown in Table 6, individually, IFN-α and -β inhibited cell proliferation by 21% and 13%, respectively; when they were given in combination suppression was 33%, indicating that the antiproliferatin activity was additive.

Species Specificity of the Antiproliferation Activity of IFN-β

As the antiproliferation activity of IFN-β was reported to be species-specific, we examined whether this was also true for the present IFN-β. Human and mouse cells were incubated in the presence of IFN-β or mouse IFN for 6 and 4 days, respectively, and the cell concentration was determined. As shown in Table 7, human IFN suppressed human cell proliferation, while mouse IFN preferentially suppressed the murine cells, indicating that the IFN-β-induced suppression was species-specific.

Cytostatic Suppression of Cell Proliferation by IFN-β

To examine whether the IFN-β-induced suppression of human cell proliferation was due to cytotoxic or cytostatic activity, we preincubated Daudi cells with IFN-β and then cultivated these cells in IFN-β-free medium. The cell concentration and viability were determined at different intervals (Fig. 2). Cells preincubated with an excess of IFN-β (50 IC_{50}) were perfectly viable before cultivation; however, their proliferation was

Table 6. Additive antiproliferation activity of IFN-α and -β on H.Ep # 2 cells

IFNs	Cell concentrations after 5-day culture ($\times 10^5$/ml, mean ± SD)	Percent proliferation inhibition (mean ± SD)
None (control)	2.3 ± 0.08	
α, 1,000 U/ml	1.8 ± 0.01*	21.3 ± 0.7
β, 100 U/ml	2.0 ± 0.04*	13.0 ± 1.8
α, 1,000 U/ml plus β, 100 U/ml	1.5 ± 0.02*	33.5 ± 1.0

The results are presented as percent proliferation inhibition, which is defined as

$$\left(1 - \frac{\text{Cell concentration of the test mixture}}{\text{Cell concentration of the control mixture}}\right) \times 100$$

* Significantly different from the control ($P < 0.05$) by the t-test

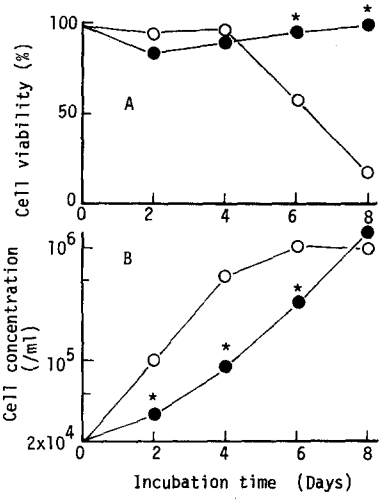

Fig. 2. A and **B** Cytostatic antiproliferation activity of IFN-β. Daudi cells (2×10^5/ml) were preincubated in the presence (●) or absence (○) of 500 U IFN-β/ml for 1 day at 37° C in a CO_2 incubator. After washing with RPMI medium by centrifugation at 200 *g* for 5 min, they were re-incubated in IFN-β-free medium. At the indicated times, cell viability was determined by trypan blue exclusion, using a hemocytometer (**A**) and total cell concentrations were established using a Coulter counter (**B**). *, statistically different ($P < 0.05$) from the corresponsing controls (t-test)

Table 7. Species specificity of the antiproliferation activity of IFN-β

IFN	Percentage of control cell concentration in the presence of IFN (mean ± SD) in				
	Human cells		Mouse cells		
	H.Ep # 2	Daudi	L1210	P388	YAC-1
Human IFN-β	33.2 ± 1.3[a]	3.7 ± 0.7[b]	78.8 ± 9.3[c]	100 ± 2.5[d]	79.5 ± 3.8[e]
Mouse IFN	102 ± 3.5[a]	81.9 ± 3.9[b]	7.3 ± 2.8[c]	75.7 ± 1.2[d]	50.7 ± 3.9[e]

Human cells (2×10^4/ml, Daudi; 5×10^3/ml, H.Ep # 2) were cultivated for 6 days at 37° C in a CO_2 incubator in the presence or absence of 1,000 U human IFN-β or mouse IFN/ml. Mouse cells (10^3/ml, L1210; 5×10^3/ml, P388; 5×10^4/ml, YAC-1) were cultivated in the presence or absence of either type of IFN (4,000 U/ml, L1210; 1,000 U/ml, P388 and YAC-1) for 4 days. The control cell concentrations were as follows ($\times 10^5$/ml, mean ± SD of triplicate or more): 2.2 ± 0.20 (Daudi), 8.1 ± 0.26 (H.Ep # 2), 2.2 ± 0.39 (L1210), 4.4 ± 0.16 (P388), and 11.1 ± 0.70 (YAC-1)

[a, b, c, d, e] Significant ($P < 0.05$ by the t-test) respectively

Table 8. Antiproliferation activity of IFN-β after absorption with IFN-β-sensitive and IFN-β-insensitive cells

Absorption with		Antiproliferation activity of unabsorbed fractions of IFN-β (IC_{50} determined on Daudi cells)			
		Expt 1	Expt 2	Expt 3	Mean ± SD of the relative antiproliferation activity
None		11.7[a] (1.00)[b]	4.4 (1.00)	2.7 (1.00)	1.00
Bri 7 cells,	3×10^6	9.7 (0.83)	4.9 (1.11)	2.8 (1.04)	0.99 ± 0.11*
Daudi cells,	3×10^3	12.6 (1.08)	5.5 (1.25)	3.3 (1.22)	1.18 ± 0.07
Daudi cells,	3×10^6	20.0 (1.71)	7.8 (1.77)	4.4 (1.63)	1.70 ± 0.05*

IFN-β (50 U/ml) was incubated for 1 h on ice with or without the indicated number of Daudi and Bri 7 cells, then the mixtures were centrifuged at 200 *g* for 5 min. The unabsorbed IFN fraction was assayed on Daudi cells for its antiproliferation activity

[a] U/ml determined on the assumption that IFN-β was recovered quantitatively

[b] Antiproliferation activity expressed as the IC_{50} ratio relative to the activity of unabsorbed IFN

* Significant ($P < 0.05$ by the *t*-test)

significantly suppressed. Nevertheless, the viability of IFN-pretreated cells was high throughout the experimental period, indicating that the observed suppression was not ascribable to the delayed proliferation of a cell population surviving the cytotoxic effect of IFN-β, but to the IFN-β-induced cytostatic suppression of the entire cell population.

Cellular Absorption of the IFN-β Antiproliferation Activity

Next we examined whether the suppression of Daudi cell proliferation was associated with IFN-β binding to the cell surface (Table 8). When IFN-β was incubated with Daudi cells the antiproliferation activity became significantly lower than that of unabsorbed IFN-β. Furthermore, the apparent IC_{50} determined in Daudi cells was increased by a factor of 1.70, indicating that about one-third of IFN-β present in the incubation mixture was bound. In contrast, Bri 7 cells, which are much less sensitive to IFN-β than Daudi cells (Fig. 1) did not absorb a detectable amount of IFN-β. These results are consistent with the hypothesis of other investigators that IFN binding to the cell surface was related to the suppression of cell proliferation.

Discussion

In previous studies on the antiproliferation activity of human IFN-β, a crude IFN-β preparation, as determined by its antiviral activity (2×10^6 U/mg protein or less) [2, 6], was used. Our earlier investigation, in which we used purified IFN-β ($> 10^7$ U/mg protein), showed that IFN-β molecules were responsible for the antiviral activity [5]. As reported in experiments using a crude IFN-β preparation [8], the antiproliferative effect of IFN-β was due to its cytostatic activity and it was human cell-specific but not tumor-specific.

The present study is an extension of earlier findings on the antiproliferation activity of IFN-β. Bri 7, CCRF-SB, and RPMI-1788 cells cultured in suspension were insensitive to IFNs-α and -β (Table 2). Our comparative absorption test (Table 8) suggested that the lack of cell surface binding sites was responsible for their IFN-insensitivity.

Another group of epithelioid human cells, HeLa, H.Ep#2, J-111, FL, and Detroit-98 cells and lymphoblastoid Daudi cells were sensitive to IFNs. However, their relative sensitivity to the three tested IFNs varied. IFN-β was the least effective of the three IFNs in suppressing Daudi cell proliferation. Our finding that the activity of IFN-β was stable upon incubation with culture medium and with Daudi cell culture fluid (Table 4) indicated that the inferior antiproliferative potency of IFN-β in Daudi cells was not due to its loss of activity during incubation, but was a reflection of its poor intrinsic potency with respect to these cells.

In contrast, while their IFN-sensitivity varied, the above five epithelioid cell lines (Table 3) and the tested G-361, KB, and L-132 cells (data not shown) were more sensitive to IFN-β than to IFN-α by a factor of 1.5–49, indicating the relative specificity of IFN-β for suppressing epithelioid cells. Since these results were consistent irrespective of whether IFN was added at the time of, or 1 day after, culture initiation (Table 5), the greater epithelioid cell sensitivity to IFN-β was not due to its interference with initial cell attachment to the culture flasks, but to interference with cell proliferation per se.

These results lead us to posit that suppression of cell proliferation depends on both the target cell species and the IFN species. However, suppression was not tissue-specific, as has been proposed [3], because IFN-β, at lower concentrations than were used in epithelioid cell experiments, suppressed the proliferation of the Daudi cells, although it was less effective in Daudi and more effective in epithelioid cells than was IFN-α.

Our finding that suppression of cell proliferation is IFN species-dependent suggested that the suppression mechanisms differ from one IFN to the other. Therefore, we tested whether, in combination, IFN-α and -β would produce a synergistic antiproliferation effect. We found that suppression of Daudi cell proliferation was additive rather than synergistic (Table 6). Fleischmann et al. [4] have reported that in combination, mouse IFN-α and -γ produced a synergistic therapeutic effect in tumor-bearing animals. Although IFN-activated host antitumor immunity may play a role in the eventual therapeutic effect, we hypothesized that a direct synergistic antiproliferation effect on the inoculated tumor cells was involved, since they administered the IFNs at the tumor site and determined the therapeutic effect by the tumor size. Taking this into account, our results lead us to conclude that there is not so much difference between the suppression

mechanisms of IFN-α and -β, although IFN exerted species-dependent antiproliferation activity.

Our observation that the antiproliferative activity is IFN species-dependent stresses the necessity for careful selection of IFN species for therapy. Therefore, it is advisable to screen the IFN species by in vitro stem cell assay [7] in which stem cells of primary tumors are tested for their drug sensitivity. The potency of IFN-β, lymphoid, and myeloid in inhibiting the colony formation of primary tumor cells has been compared [2]; it was found that in most of the tumors tested IFN-β was either as potent as, or more potent than, the other IFN species. These, as well as the present results, suggest that in some tumors, IFN-β may induce a more beneficial effect than other IFN species and that it would be worthwhile to conduct clinical trials of IFN-β independent of IFN-α.

Acknowledgements. We thank Drs S. Tsukagoshi and S. Kobayashi for their encouragement in the course of this study, and Dr K. Cantell of the State Serum Institute in Helsinki, Finland and Sumitomo Chemical Ind. Ltd for supplying IFN-α and lymphoblastoid, respectively. We also thank Y. Hamazaki for preparing this manuscript.

This research was supported in part by Grants-in-Aid for a group study on the clinical application of interferon from the Japanese Ministry of Health and Welfare, and for Cancer Research from the Japanese Ministry of Education, Science, and Culture.

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Received November 5, 1981/Accepted July 21, 1982