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Comparison of Effects of Leukocyte and Fibroblast Interferon on Immunological Parameters in Cancer Patients*

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Abstract—*Patients with metastatic cancer were given single intramuscular injections of 10^7 units of partially purified preparations of either leukocyte or fibroblast IFN. Serum levels of interferon, of β_2-microglobulin and of carcino-embryonic antigen (CEA), as well as NK activity of circulating lymphocytes, were followed over a period of* 96 hr *post injection. In confirmation of previous studies, levels of circulating IFN were lower after injection of fibroblast IFN than after injection of leukocyte IFN. Despite this difference in pharmacokinetics, the natural killer activity of circulating lymphocytes was enhanced with both IFNs. Levels of CEA were not influenced by the IFN injections. Leukocyte but not fibroblast IFN caused an increase in serum levels of β_2-microglobulin in the circulation. A similar difference between leukocyte and fibroblast IFN in their ability to influence the β_2-microglubulin system was observed in experiments on cell cultures. Only leukocyte IFN was able to cause release of β_2-microglobulin by either leukocytes or fibroblasts.*

INTRODUCTION

THE USE of interferon (IFN) as a therapeutic agent for tumour-bearing patients has gained considerable interest after the reports demonstrating potent enhancing effects of IFN on the immune system. Thus, besides its direct effect on tumor cell multiplication [1], IFN may influence the host–tumor relationship by activating cells with potential antitumor activity such as Natural Killer (NK) cells [2, 3], Killer (K) cells [3] or macrophages [4]. IFN also plays a role by its capacity to modify the metabolism of certain molecules involved in immune functions, such as antigen receptors [5], histocompatibility antigens [6–8] or receptors for the Fc fragment of immunoglobulins [9, 10].

Human IFN in sufficient amounts to perform clinical trials can be prepared in various ways. Fresh leukocytes [11] or cultured lymphoblastoid cells [12], upon infection with certain viruses, produce IFN consisting mainly of α-type molecules (HuIFN-α) as defined by the International Committee on Interferon Nomenclature [13]. Cultured human fibroblast, after induction with double-stranded RNA, produce β-type IFN (HuIFN-β) [14]. These two types of IFN differ in their chemical structure [15, 16] and pharmacological properties [17, 18]. They also differ in at least some biological properties, e.g., in their relative effects on the growth of cultured human cells [19] and in their dose–response curves in certain assay systems [20].

The purpose of the present study was to compare these two IFNs as to their effects on certain immunological parameters which could be used as biological markers to follow potential therapeutic effects.

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In particular, the following parameters were chosen: (a) serum levels of carcinoembryonic antigen (CEA), a non-specific marker of tumor proliferation; (b) serum levels or release by cultured cells of β_2-microglobulin, a molecule associated with the major histocompatibility complex (MHC) products; and (c) NK activity of circulating lymphocytes, i.e. spontaneous cytotoxic effect of lymphocytes exerted on certain tumor cell lines.

The effects of IFNs were studied both *in vitro*, by addition of IFN to cultured cells, or *in vivo*, by measuring the parameters in patients given intramuscular injections.

We show that both leukocyte and fibroblast IFNs enhance NK cell activity *in vivo*, but have no effect on the level of serum CEA. Leukocyte but not fibroblast IFN causes an increase in the β_2-microglobulin levels both *in vivo* and *in vitro*.

MATERIALS AND METHODS

Patients

The study was done on 10 patients treated at the Institut Curie for advanced metastatic cancer. Age, sex and diagnosis are given in Table 1.

Interferons

Leukocyte [11] and fibroblast [14] IFNs were prepared as described earlier. Both preparations had a specific activity of *ca.* 10^6 units/mg protein.

Analytical procedures

Serum was obtained from 10-ml blood samples collected in glass tubes, allowed to clot at room temperature and centrifuged for 10 min at 1000 *g*. The samples were stored at −20°C until analyzed. Interferon concentrations in serum were determined using a cytopathic effect inhibition assay on trisomic cells (GM 2504, obtained from Dr. A. Greene, Institute of Medical Research, Amden, NY) challenged with vesicular stomatitis virus. Interferon titrations were calibrated with laboratory standards of leukocyte and fibroblast IFNs and all concentrations were expressed in international units.

β_2-Microglobulin levels were assayed using a solid phase radio-immunoassay (Phadebas, Pharmacia, Uppsala, Sweden).

The level before treatment $[(\beta_2m)_0]$ was taken as a reference and the results were expressed as the percent increase of this basic level to $(\beta_2m)_t$, according to the following formula: $(\%\ \text{increase} = 100 \times [(\beta_2m)_t - (\beta_2m)_0]/(\beta_2m)_0$.

Since in serum the β_2-microglobulin levels can be influenced by renal function, creatinin levels were determined in each sample. Creatininaemia was found to be constant throughout the follow-up period in each patient.

CEA levels in serum were assayed without extraction using the double antibody radioimmunoassay kit provided by the Commissariat à l'Energie Atomique (Saclay, France). With this assay, levels below 10 μg/l are considered normal.

Preparation of lymphocyte and fibroblast suspensions

Peripheral blood was collected in a syringe containing heparin (Calciparine, Laboratories Choay, Paris, France) and lymphocytes were prepared on a Ficoll gradient (Ficoll Radio Selectan, Sigma, St. Louis, Missouri, USA, and Schering, Bonn, W. Germany) as previously described [21]. The lymphocytes were resuspended at a final concentration of 10^7 cells/ml in RPMI 1640 medium (Eurobio, Paris, France) supplemented with 20% foetal calf serum (FCS, Gibco, Glasgow, Scotland).

Human diploid fibroblasts (strain MRC5) used for experiments on β_2-microglobulin release were cultivated as monolayers in Eagle's Minimum Essential Medium with 10% FCS. For experiments on β_2-microglobulin release, 2.5×10^5 cells were seeded on cover slips.

Lymphocyte cytotoxicity assay

Cells of the K 562 line (kindly provided by Dr. M. Fellous, Hospital St. Louis, Paris, France) were used as target cells. They were grown in RPMI 1640 medium supplemented with 10% FCS and 40 mg/l Gentalline (Unilabo, Paris, France).

The cytotoxicity assay was performed according to Cerottini and Brunner [22]. Briefly, K 562-cells were labelled by adding 200 μCi of $Na_2[^{51}Cr]O_4$ (CEA, Saclay, France) to 10^6 cells in 0.2 ml of growth medium. After incubation for 1 hr at 37°C, the cells were extensively washed and resuspended at 10^5 cells/ml in growth medium. Aliquots of 100 μl of this suspension were mixed with equal volumes of lymphocyte suspensions at effector/target ratios varying from 25/1 to 100/1. The cell mixures were incubated for 4 hr at 37°C. At the end of incubation period, 100 μl aliquots of the supernatants were transferred into vials and counted in a gamma counter (C.G. 4000 Intertechnique, Paris, France).

The percentage of specific $[^{51}Cr]$ release was determined according to the formula: $(E - S)/(T - S)$, where E is the amount of $[^{51}Cr]$ released from the target cells in the presence of lymphocytes, S is the spontaneous release of

Table 1. Concentrations of interferon and β_2-microglobulin, and NK cell activity in patients given single intramuscular injections of leukocyte or fibroblast IFN

Experimental group No.	Patient Code	Age	Sex	Diagnosis*	IFN treatment Preparation type†	Dose (units × 10^6)	Peak IFN concentration in serum (units/ml) Leukocyte IFN	Fibroblast IFN	NK cell activity‡ (% [^{51}Cr] release) 0 h	48 h	β_2-Microglobulin level in serum mg/ml at: 0 h	24 h	% increase Leukocyte IFN	Fibroblast IFN
1	YAO	57	F	Breast cancer	Leukocyte	10	112		4	46	2.7	4.5	67	
					Fibroblast	10		< 32	< 1	50	2.7	2.2		− 19 ·
	LEG	68	F	Kidney cancer	Fibroblast	10		32	15	31	3.1	4.1		32
					Leukocyte	10	82		16	38	3.2	6.1	91	
	EUV	50	M	Osteosarcoma	Leukocyte	10	500		6	61	2.4	4.7	96	
					Fibroblast	10		32	19	46	2.4	2.5		4
	VAL	59	M	Epithelioma of	Fibroblast	10		32	57	60	2.1	1.9		−10
				rectum	Leukocyte	10	225		21	70	2.3	3.3	43	
	PET	67	F	Epithelioma of	Leukocyte	10	96		24	65	3.8	5.4	42	
				rectum	Fibroblast	10		< 32	15	65	3.4	3.2		−6
2	FAB	28	F	Ovarian cancer	Leukocyte	10	132		34	89	1.9	3.8	100	
					Leukocyte	10	316		35	91	2.0	3.7	95	
	PIC	52	M	Melanosarcoma	Fibroblast	10		52	66	79	1.6	2.0		25
					Fibroblast	10		114	71	72	1.5	1.8		12
3	PIA	57	F	Fibrosarcoma	Fibroblast	6		64	34	58	2.0	2.0		0
					Leukocyte	6	128		33	62	2.8	4.0	43	
	TCH	70	F	Breast cancer	Fibroblast	3		< 32	26	53	3.7	3.7		0
					Leukocyte	3	< 32		49	59	2.5	3.9	56	
	BER	73	M	Squamous cell	Fibroblast	1		ND	43	42	ND	ND		—
				carcinoma (upper respiratory and digestive tract)	Leukocyte	1	ND		52	51	ND	ND	—	

*All patients were in a metastatic stage of the disease.
†Intramuscular injection given in indicated sequence with a 1 week interval.
‡Effector/target cell ratio: 100/1.

[^{51}Cr] measured in control cultures (target cells mixed with culture medium) and T is the maximum release when control cultures are treated with 2 N HCl. Spontaneous release did not exceed 15%. All assays were done in triplicate cultures. The variability for the triplicate determination did not exceed 10%.

RESULTS

Modification of immune parameters following i.m. injection of IFN in patients

In a first experimental group, single i.m. injections of 10^7 units of either leukocyte or fibroblast IFN were given to 5 patients with metastatic cancer (Table 1). Each patient received two injections, with a time interval of 1 week, the first injection consisting of one type of IFN, the second injection consisting of the other type. Blood samples were taken a few minutes before injection and 1, 4, 9, 24, 35, 48, 72 and 96 hr post-injection. A second experimental group was treated similarly, except that each patient received consecutive injections of the same type of IFN. In the third group, the effect of different doses of IFN was studied.

Table 1 gives an overview of the results obtained. It is clear that leukocyte IFN was easily detectable in the serum of injected patients; with fibroblast IFN serum, titers of antiviral activity were much lower and sometimes undetecable. Injections of leukocyte IFN were followed by an increase in β_2-microglobulin levels. This was not the case with fibroblast IFN. In contrast, fibroblast IFN, as well as leukocyte IFN, caused an increase in NK cell activity. It can be seen that NK cell activity fluctuated independently of interferon administration, as evident from comparison of the zero time points within each patient. Statistical analysis (Student's t-test) of the NK cell activity values of all patients, except BER, who received only 1×10^6 units of IFN, revealed that the average spontaneous change in specific chrome release (X = difference between first and second zero time point in each patient) was 0.3 ± 11.0 (95% two-sided confidence limits; $n = 10$), against 23.3 ± 13.2 ($n = 9$) for the change induced by fibroblast IFN injection and 39.9 ± 12.3 ($n = 9$) for that induced by leukocyte IFN. Hence, both increases are statistically significant at the $P < 0.01$ level. The analysis also shows that the increase induced by fibroblast IFN was smaller than that induced by leukocyte IFN. However, with the present, relatively small number of observations, this difference between the two IFN treatments was only significant at the $P < 0.10$ level. From the data of group 3 (Table 1), it can be seen that the threshold dose required to obtain this effect was probably similar (3×10^6 units) for the two IFN preparations. However, in view of the fluctuations of NK cell activity within patients, ascertainment of this point needs additional determinations in a larger group of patients.

Figure 1 shows two representative cases in which patients YAD and EUV received first an i.m. injection of 10^7 units of leukocyte IFN, followed by the same dose of fibroblast IFN. It apears that both IFNs enhanced NK cell activity with similar kinetics: a small peak immediately after IFN administration, the maximum increase being at 48 hr after the injection. After this maximum, NK cell activity decreased at 96 hr to the starting level. By contrast, the two IFNs differed with respect to the levels of serum antiviral activity and β_2-microglobulin. Leukocyte IFN administration was followed by a peak of antiviral acitivity in the first hours after the injection (maximum at 4 hr) and by an increase in serum β_2-microglobulin (over 100% of the starting level), peaking at 24 hr, both parameters returning to starting level thereafter. Administration of fibroblast IFN was followed by low or undetectable antiviral activity in the serum.

As another parameter of possible effects of interferon on the tumor–host relationship, the serum levels of CEA were studied. The results of these determinations are summarized in Table 2. No modification under the influence of interferon therapy was observed, regardless of whether pretreatment levels were high, low or undetectable.

Enhancement of β_2-microglobulin production by IFN in vitro

Suspensions of peripheral blood lymphocytes (5×10^6 cells/ml) or monolayers of cultured fibroblasts (*ca.* 2.5×10^5 cells/ml) were incubated at 37°C with 10^4 units/ml of either leukocyte or fibroblast IFN. Supernatant fluid samples were collected at 4 and 24 hr, and β_2-microglobulin contents were measured. Figure 2 shows the results obtained in these experiments. All lymphocyte cultures (Fig. 2), including untreated controls, released measurable quantities of β_2-microglobulin. Fibroblast IFN had no effect on this spontaneous release. In contrast, cultures treated with leukocyte IFN released about 3 times more than untreated controls both at 4 and 24 hr. A rather similar situation was encourted with fibroblast cultures (Fig. 2B), with the difference that in this case, β_2-microglobulin release was low in the supernatants of

Table 2. Serum levels of CEA (μg/l) in interferon-treated patients

Patients*	Hours after 1st injection of IFN									Hours after 2nd injection of IFN								
	0	1	4	9	24	35	48	72	96	0	1	4	9	24	35	48	72	96
YAD	24	35	24	36	19	ND	30	ND	29	31	43	31	34	99	39	24	24	23
LEG	0†	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
EUV	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VAL	5	8	5	5	10	5	6	5	8	0	6	0	14	0	0	0	0	7
PET	1170	1310	1350	1230	1150	1550	1330	1350	1470	1470	1870	1690	1875	1790	1860	1785	1880	2000
FAB	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0
PIC	0	0	0	ND	0	ND	0	0	0	0	ND	ND	ND	0	ND	ND	ND	0
PIA	0	0	0	ND	0	0	0	0	0	0	ND	ND	ND	0	ND	ND	ND	0
TCH	0	0	0	ND	0	ND	0	0	0	0	ND	ND	ND	0	ND	ND	0	ND
BER	73	68	ND	89	66	ND	66	ND	78	74	76	ND	ND	81	ND	86	ND	64

*For specification of age, sex, diagnosis and treatment schedule, see Table 1.
†Level of CEA within or lower than blank controls.

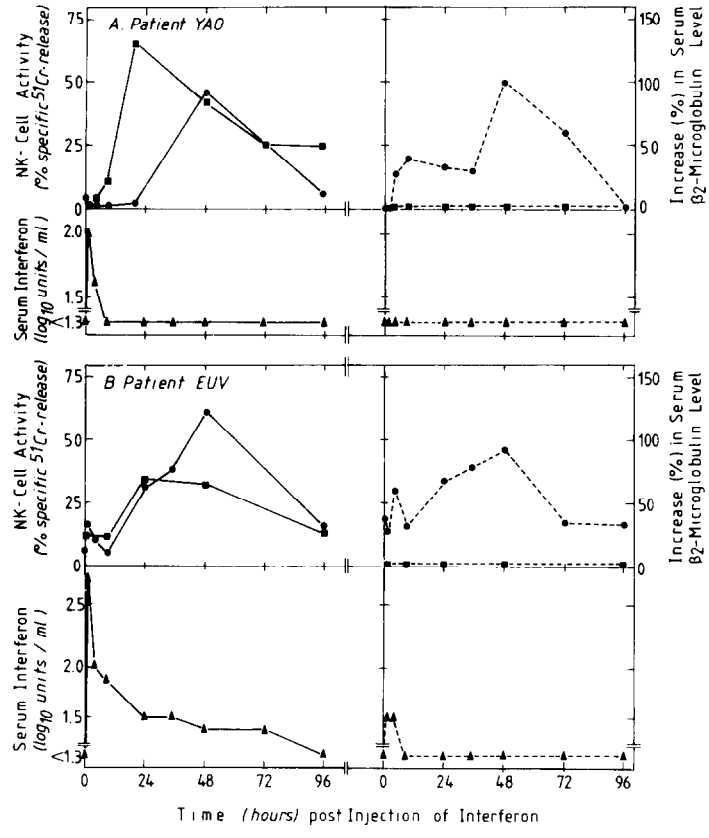

Fig. 1. Serum levels of IFN (▲) and β_2-microglobulin (■), and NK cell activity (●) in two patients (YAO and EUV) given a single i.m. injection of 10^7 units of leukocyte IFN (full lines), followed one week later by a same dose of fibroblast IFN (dashed lines).

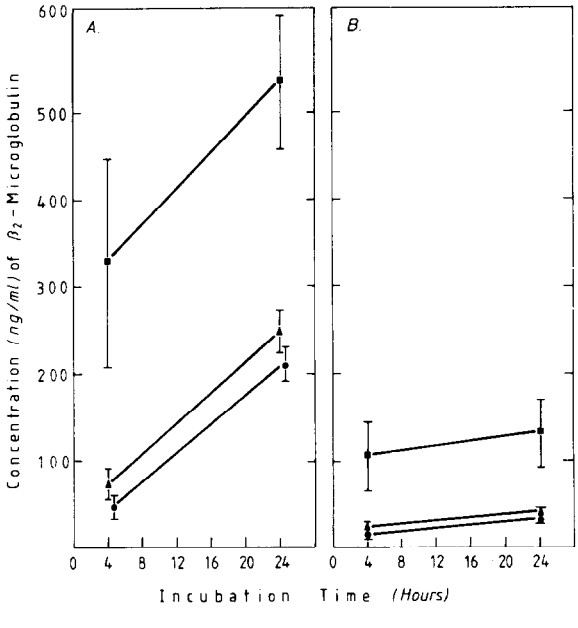

Fig. 2. Release of β_2-microglobulin by lymphocytes (A) and fibroblasts (B) incubated with leukocyte or fibroblast IFN. Suspension of lymphocytes (5×10^6 cells) or monolayers of fibroblasts (2.5×10^6 cells) incubated in 1 ml of control medium (●) or medium with 10^4 units of leukocyte (■) or fibroblast (▲) IFN. Bars indicate ± 1 standard error of mean.

both control and fibroblast IFN-treated cultures, while leukocyte IFN treament induced an increased release of β_2-microglobulin.

DISCUSSION

Single intramuscular injections of leukocyte IFN (10^7 units) in patients resulted in measurable levels of antiviral activity in the serum for several hours, with peak values at 1–4 hr, varying between 80 and 500 units/ml. With fibroblast IFN, at similar dosages, only very small amounts of antiviral activity were detected for only a very short period post-injection. This confirms previous findings of several authors who also found different pharmacokinetic behaviour of leukocyte and fibroblast IFNs in man [23], as well as in animals [17, 18]. Both IFNs have been shown to activate NK cell activity when added to cultures of peripheral blood lymphocytes *in vitro* [4, 21]. It has also been shown by others [24] that i.m. injections of leukocyte IFN in patients enhance NK cell activity detectable by testing spontaneous cytotoxicity of the patient's peripheral lymphocytes towards tumor cells *in vitro*. These results were confirmed in the present study. Moreover, we

also found that i.m. injection of fibroblast IFN, despite the low or undetectable serum levels of antiviral activity obtained, was able to activate the NK cell system *in vivo*. The level of activation obtained by fibroblast IFN was probably smaller than that obtained by leukocyte IFN. However, additional comparisons are needed to ascertain this point and, eventually, to determine the mechanism of this difference. In the preliminary dose–response experiment described here, it was found that the threshold dose to obtain activation of the NK cell system in patients was about 3×10^6 units for both types of IFN preparation, indicating that dosage itself was probably not responsible for the possible difference in levels of stimulation.

An important point made clear by the present study is the fact that the low blood levels obtained with intramuscular injections of fibroblast as opposed to leukocyte IFN do not preclude fibroblast IFN from reaching cells of the lymphoid system. It is unlikely, therefore, that these low blood levels are due to rapid destruction of fibroblast IFN before it reaches the blood stream. A more plausible explanation would be that fibroblast IFN is released more slowly from the injection site or is transferred more rapidly from the circulation to the tissues.

In general, our experiments and those of others seem to indicate that fibroblast and leukocyte IFNs have similar effects on the NK cell system *in vivo* as well as *in vitro* [4, 21]. This is of particular significance as it is believed that activation of the NK cell system is an important mechanism of the antitumor action of all IFNs.

In contrast to what was seen in the NK cell system, leukocyte and fibroblast IFNs differed from each other in their effects on the release of β_2-microglobulin. Leukocyte IFN, when injected intramuscularly, caused elevation of β_2-microglobulin levels in the serum, while fibroblast IFN failed to do so. This difference may have been due to different levels of IFN reaching the cells that produce β_2-microglobulin. Alternatively, it may be due to different tissue specificities or to inherently different capacities of leukocyte and fibroblast IFNs to affect the β_2-microglobulin system. To help distinguish between these possibilities, *in vitro* experiments were performed. Leukocyte IFN caused increased β_2-microglobulin release by lymphocytes and fibroblasts, while fibroblast IFN failed to do so. Therefore, failure of fibroblast IFN to cause release of β_2-microglobulin *in vivo* cannot be attributed to inability to reach the cells, since in the *in vitro* experiments both IFNs were applied in equivalent doses. Nor can differences in tissue specificity be invoked, since leukocyte IFN enhanced β_2-microglobulin release by both lymphocytes as well as fibroblasts. We are thus left with the hypothesis that the difference in effect on β_2-microglobulin release between leukocyte and fibroblast IFN preparations reflects inherently different properties of α- and β-type IFNs.

β_2-Microglobulin is linked to the products of the major histocompatibility complex, HLA in humans [25] and H-2 in mice [26]. These products are themselves modified by, or associated with, the virus-coded [27] or tumor-associated antigens [28] which act as targets for cytotoxic T-lymphocytes [29]. It has been shown by others that the expression at the cell membrane of β_2-microglobulin as well as of H-2 and HLA molecules is increased upon incubation of cells with IFN [6–8]. An increase of expression of Ia-antigens, on the other hand, was not seen [8, 30]. Increased serum levels of β_2-microglobulin in patients treated with leukocyte IFN could then be due to enhanced expression of the major histocompatibility complex in various cells in the body. The importance of such a phenomenon in mediating the antitumor effect of IFN is still unknown. However, a difference in effect of leukocyte and fibroblast IFN on the metabolism of β_2-microglobulin or HLA molecules may result in different therapeutic effects in virus-infected or tumor-bearing patients.

Preparations of IFN have been shown to enhance the expression of various cell membrane components, such as receptors for synthetic polypeptides [30], receptor for Concanavalin A [31], receptors for the Fc-fragment of IgG [9, 10] and also of CEA [32]. In the patients treated with IFN, no changes in serum levels of CEA were noted. Thus, it would appear that increased levels of β_2-microglobulin in patients treated with leukocyte interferon reflect a specific effect, rather than just nonspecific increase in release of various cell membrane components.

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