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Clinical and Immunological Studies of Human Fibroblast Interferon

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Summary. *Human fibroblast interferon (HFIF) was used in 26 patients with various malignant diseases, most of whom had received previous chemotherapy. The dosages used were 3 × 10^6IU or 6 × 10^6IU HFIF IV daily. Of 24 evaluable patients, two attained partial remissions (one with chronic lymphocytic leukemia and one with multiple myeloma), and seven (stomach Ca two, multiple myeloma two, CLL one, malignant melanoma one, and non-Hodgkin's lymphoma one) attained stable disease. The majority of patients experienced fever with body temperature exceeding 38° C and chills, which became uncommon after several days of treatment. Other side-effects included myelosuppression, general malaise, anorexia, hepatic dysfunction, and renal dysfunction, which were mild and tolerable.*

Lymphocyte natural killer (NK) activity against culture cell lines was measured before and at various times after HFIF treatment. The majority of patients reached the highest NK activity at 18–24 h, mostly at 24 h after the initiation of HFIF therapy. In one group of patients NK activity subsequently remained at the highest level during HFIF treatment, while in another group of patients NK activity declined even with daily infusion of HFIF but usually remained above the pretreatment level. There seemed to be no correlation between NK activity and clinical activity. In contrast to NK activity against culture cell lines, no increase in lymphocyte cytotoxic activity against autologous tumor cells was observed following HFIF treatment. Mixed lymphocyte tumor cell reactions were tested in six patients and showed a slight increase of ^{3}H-thymidine uptake in one patient, but the others had no change. In vitro sensitization to assess the in vitro generation of cytotoxic cells was negative in all six patients. Lymphocyte blastogenic responses to non-specific mitogens showed no significant change. The delayed-type hypersensitivity reaction to recall antigens was increased in about half the patients after HFIF treatment.

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Introduction

The antitumor effects of interferon have been established in spontaneous and transplantable animal tumor systems of both viral and nonviral origins. Since the report of activity against osteogenic sarcoma by Strander et al. [12] there has been much interest in the use of interferon in the treatment of human malignant diseases. Subsequent reports have indicated significant antitumor effects against malignant lymphoma [1, 3, 8], multiple myeloma [3, 9], acute leukemia [4], and breast cancer [3].

The mechanism of antitumor activity of interferon has been interpreted both as a direct cytotoxic effect on tumor cells and as an effect mediated through immune system.

In the present paper, we describe our clinical experience with human fibroblast interferon (HFIF) in various malignant diseases, and immunological evaluations in patients receiving treatment with HFIF.

Materials and Methods

Interferon was produced from human fibroblast cell line at Toray Co., Japan, and offered to us for clinical study. The specific activity was 1 × 10^7 IU/mg protein. Safety testing for use in human beings was carried out at the Institute of Microbial Chemistry, Tokyo, Japan. Since the supply of HFIF was limited, it was decided that malignant lymphomas and multiple myeloma were to be accepted as the highest-priority target tumors for systemic administration. Thereafter, as the supply increased, acute leukemias, stomach cancer, breast cancer, ovarian cancer, and others were included in the group of target tumors.

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The eligibility conditions for patient selection were as follows: (1) histological or cytological proof of malignancy; (2) measurable lesions; (3) failure of response to standard treatments, with a minimum of 4 weeks from cessation of prior treatment and life expectancy more than 2 months; (4) Karnofsky performance status greater than 50%; (5) adequate physiologic functions; (6) age less than 75 years; and (7) no active double cancer. All patients were treated with HFIF alone. Most of the patients received 3×10^6 IU HFIF daily, and in some patients the dose was escalated to 6×10^6 IU either at the start of treatment or in the middle of the course of treatment. HFIF was dissolved in 250 ml 5% glucose and infused IV over 1 h. This was repeated every day for a minimum period of 4 weeks in the majority of cases. However, in patients who showed definitive tumor progression, the treatment was discontinued even before 4 weeks had elapsed. Responders received maintenance treatment either daily or according to a 3-times-a-week schedule.

Response Criteria

Solid Tumors. Complete remission (CR) indicated disappearance of all known disease. Partial remission (PR) was a decrease of at least 50% in the product of diameters in measurable lesions, providing also that no lesion had progressed and no new lesions appeared. Stable disease (SD) was defined as a decrease of less than 50% or an increase of less than 25% in the size of measurable lesions. Progressive disease (PD) was defined as an increase of over 25% in the size of any lesions or the appearance of new lesions.

Multiple Myeloma. Responses were evaluated in accordance with changes in myeloma protein production. PR was defined as a decrease of myeloma protein by at least 50% from the pretreatment value. Hgb level had to improve or to be maintained at over 9 mg/dl for a case to be classed as responsive. SD was defined as a less than 50% reduction or a 25% increase in myeloma protein.

Chronic Lymphocytic Leukemia (CLL). PR indicated a decrease in peripheral blood lymphocytes to less than 15,000/mm^3, peripheral neutrophils above 2,000/mm^3, and a reduction of lymph node and spleen size by at least 50% compared with pretreatment values. Hgb and platelet counts had to be maintained in the normal range. SD was defined as a reduction in peripheral blood lymphocytes and in lymphnode and spleen size by less than 50% compared with the pretreatment values.

Immunological Studies Included. (1) Natural killer (NK) activity of lymphocytes; (2) lymphocyte blastogenic response to nonspecific mitogens: phytohemagglutinin (PHA), concanavalin A (Con A), and pokeweed mitogen (PWM); (3) skin tests for delayed-type hypersensitivity to nonspecific antigens; Varidase, *Candida,* and PPD. Furthermore, the following studies were also performed when autologous tumor cells were available: (4) lymphocyte cytotoxicity to autologous tumor cells; (5) lymphocyte blastogenic responses to autologous tumor cells treated with mitomycin C (MMC) (mixed lymphocyte tumor cell reaction: MLTR); and (6) in vitro sensitization to assess the generation of cytotoxic lymphocytes induced by autologous tumor cells. Tumor cells were collected either from bone marrow or from carcinomatous effusions before the start of treatment. The Cryo-Med programmable freezing system was used to freeze tumor cells, and cells were stored at −180° C in liquid nitrogen.

In vitro lymphocyte studies were performed before starting HFIF treatment and every 6 h until 24 h after treatment, and then repeated every 3−7 days. Skin tests were performed before HFIF treatment and then every 2−3 weeks.

NK Activity and Lymphocyte Cytotoxicity. Peripheral blood lymphocytes from patients were purified from heparinized venous blood by Ficoll-Conrey gradient and suspended in RPMI 1640 medium supplemented with 10% AB(+) male serum. The lymphocyte concentration was adjusted to 1×10^6/ml and 2×10^6/ml, and 0.2 ml was added to triplicate wells of Nunc round-bottomed plates. A ^{51}Cr-release assay was used to detect lymphocyte NK activity and cytotoxicity. Both K562 cells, derived from chronic myeloid leukemia in blast crisis, and Daudi cells, derived from Burkitt's lymphoma, were used as target cells for NK activity. For target cell preparation, K562, Daudi, and autologous tumor cells were adjusted to 2×10^6 in 0.2 ml medium and 50−100 μCi in 0.1 ml $Na_2{}^{51}CrO_4$ (New England Nuclear, 200−300 mCi/mg) was added to each cell suspension, which was then incubated at 37° C for 1.5 h. After incubation, the cells were washed three times and were added to different concentrations of lymphocytes in triplicate wells, to give effector-to-target cell ratios of 20:1 and 40:1. After 6 h of incubation, the supernatant in the wells was harvested with Titer tec supernatant collection system and the radioactivity (cpm) of ^{51}Cr released into the supernatant was measured in a well-type gamma counter. Percent cytotoxicity was calculated as:

$$\frac{\text{cpm experimental release} - \text{cpm spontaneous release}}{\text{cpm maximal release} - \text{cpm spontaneous release}} \times 100 .$$

Lymphocyte Blastogenic Response. A microculture lymphocyte stimulation assay was employed to monitor lymphocyte blastogenic responses. The lymphocyte concentration was adjusted to 5×10^5/ml for mitogen-treated cultures and 0.2 ml was added to triplicate wells of Nunc flat-bottomed plates. For MLTR cultures, the cell concentration was adjusted to 1×10^6/ml and 0.1 ml was added to triplicate wells. Nonspecific mitogens were added in 0.02 ml, to give the following concentrations: PHA 1 : 100, PWM 1 : 30, Con A 1 : 200 of the stock solutions. For MLTR cultures, autologous tumor cells were thawed, washed, and treated with 100 μg MMC/ml for 1 h. After three washes, the cell count was adjusted to 1×10^6/ml and 0.1 ml was added to an equivalent number of autologous lymphocytes in culture plates. Mitogen-treated cultures were terminated after 5 days and MLTR cultures after 7 days of incubation. During the final 7 h of incubation, 1 μCi ^{3}H-thymidine well (New England Nuclear, Boston, USA, 11.5 Ci/mmole) was added to the cultures. Labeled cells were harvested onto glassfiber filters by MASH (multiple automated sample harvester) and processed for liquid scintillation counting. Lymphocyte blastogenic responses were taken as cpm per 1×10^5 lymphocytes. The stimulation index (SI) was taken as the cpm in the stimulated culture divided by the cpm in the appropriate unstimulated control.

In vitro Sensitization. For in vitro sensitization, MLTR cultures were performed with a minor modification to the lymphocyte-to-tumor cell ratio. MMC-treated tumor cells were adjusted to 1×10^6/ml, 1×10^5/ml, and 1×10^4/ml, and 0.1 ml was added to Nunc round-bottomed plates to give lymphocyte-to-tumor cell ratios of 1 : 1, 10 : 1, and 100 : 1. After 7 days' incubation, the well contents were gently pipetted to disperse cell clumps and 1×10^4 ^{51}Cr-labeled tumor cells were added to the wells, after which the ^{51}Cr-release assay was performed as described previously.

Delayed Hypersensitivity Skin Test Reactions. Delayed hypersensitivity was evaluated by a battery of skin test antigens including Varidase, *Candida,* and PPD. The induration response was recorded in millimeters at 48 h.

Results

Twenty-six patients with various malignant diseases were entered in the study: non-Hodgkin's lymphoma (NHL) 5, multiple myeloma 5, stomach cancer 4, acute myelocytic leukemia (AML) 2, chronic lymphocytic leukemia (CLL) 2, breast cancer 2, hepatoma 2, ovarian cancer 2, malignant melanoma 1, and common duct cancer 1. Table 1 summarizes the clinical responses of all patients to the treatment with HFIF. One of the multiple myeloma patients and one of the hepatoma patients died within 7 days of treatment and both were considered to be inevaluable because of early death. Of the 24 evaluable patients, 2 achieved PR (one patient each with CLL and multiple myeloma) and 7 were considered to have SD (multiple myeloma 2, stomach Ca 2, CLL 1, NHL 1, and malignant melanoma 1).

The duration of response in the two PR patients was rather short, 8 weeks and 9 weeks. Furthermore, the disease progressed during HFIF treatment in these patients. Three of the seven SD patients are still in SD with daily or intermittent HFIF treatment, and these patients have been progression-free for 52+ weeks, 15+ weeks, and 9+ weeks. Of ten patients who received 6×10^6 IU HFIF either from the start of treatment or from the middle of the course of treatment, one patient attained PR, two patients had SD, and the others had PD.

Among five previously untreated patients two achieved PR, but the rest had PD. All seven instances of SD occurred in previously treated patients. This result suggested that there is no correlation between the response to HFIF and previous chemotherapy.

Brief summaries of the clinical history are presented for two PR patients and one SD patient:

Case 1: 73-year-old female, CLL (Fig. 1). The patient was admitted to hospital because of generalized lymphadenopathy. The initial WBC count was 100, 300/mm^3, most of the cells being matured lymphocytes with B cell marker. Hepatomegaly was also observed. The diagnosis of CLL was made and daily

Table 1. Clinical evaluation of HFIF in cancer patients

Case	Age	Sex	Diagnosis	Prior chemotherapy	Dose		Response	Response duration[a] (weeks)
					IU	Days		
YM	52	M	NHL	+	3×10^6 6×10^6	$\times$ 30 $\times$ 12	PD	
HY	67	F	NHL	+	3×10^6	$\times$ 23	PD	
NY	34	F	NHL	+	3×10^6 6×10^6	$\times$ 14 $\times$ 14	PD	
FA	71	F	NHL	+	3×10^6	$\times$ 15	PD	
SY	33	F	NHL	+	3×10^6	$\times$ 63	SD	9+
SN	56	F	Multiple myeloma	−	3×10^6	$\times$ 15	PD	
ST	57	M	Multiple myeloma	+	3×10^6	$\times$ 125	SD	52+
IH	54	F	Multiple myeloma	+	3×10^6	$\times$ 7	NE	
IK	46	F	Multiple myeloma	−	6×10^6	$\times$ 81	PR	9
KY	46	F	Multiple myeloma	+	3×10^6	$\times$ 38	SD	7
AK	59	F	Stomach Ca	+	3×10^6	$\times$ 65	SD	8
SU	44	F	Stomach Ca	−	3×10^6	$\times$ 37	PD	
KK	56	M	Stomach Ca	+	3×10^6	$\times$ 70	SD	15+
OS	53	F	Stomach Ca	+	6×10^6	$\times$ 22	PD	
YY	71	F	AML	−	3×10^6	$\times$ 10	PD	
SH	16	M	AML	+	6×10^6	$\times$ 10	PD	
NT	73	F	CLL	−	3×10^6 6×10^6	$\times$ 62 $\times$ 24	PR	9
YM	54	M	CLL	+	6×10^6	$\times$ 27	SD	6
KS	52	F	Breast Ca	+	3×10^6 6×10^6	$\times$ 21 $\times$ 12	PD	
KR	43	F	Breast Ca	+	3×10^6	$\times$ 10	PD	
LK	32	M	Hepatoma	+	6×10^6	$\times$ 16	PD	
IT	48	M	Hepatoma	+	3×10^6	$\times$ 5	NE	
YK	37	F	Ovarian Ca	+	6×10^6	$\times$ 30	PD	
TS	44	F	Ovarian Ca	+	6×10^6	$\times$ 41	PD	
ON	51	M	Malignant melanoma	+	6×10^6	$\times$ 102	SD	10
KF	62	F	Common duct Ca	+	3×10^6	$\times$ 14	PD	

[a] Response duration: Remission duration in PR cases and progression-free time in SD cases

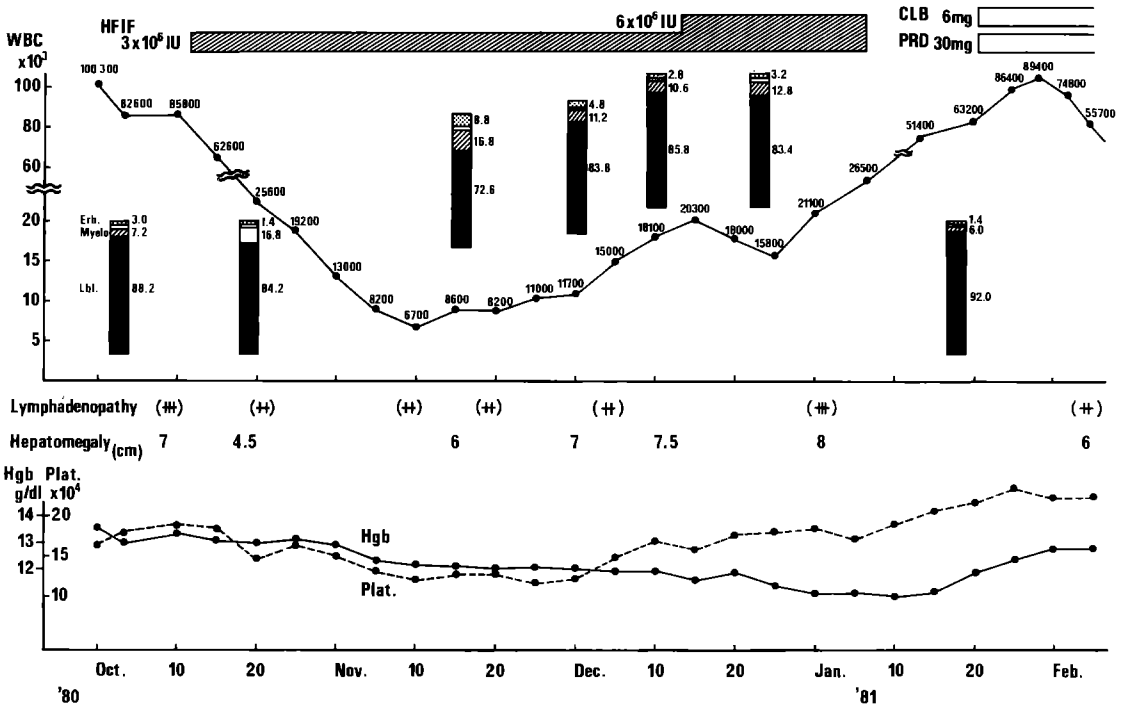


Fig. 1. Clinical course of NT, 73-year-old female, CLL

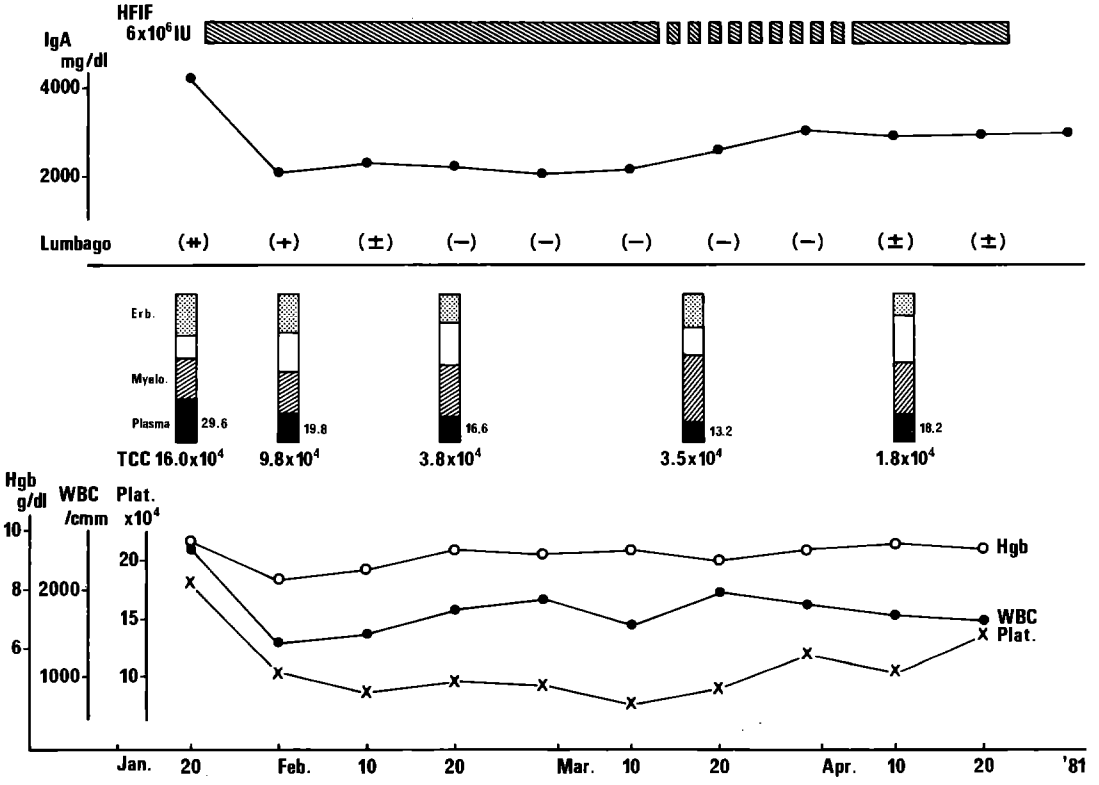


Fig. 2. Clinical course of IK, 49-year-old female, multiple myeloma (IgA K)

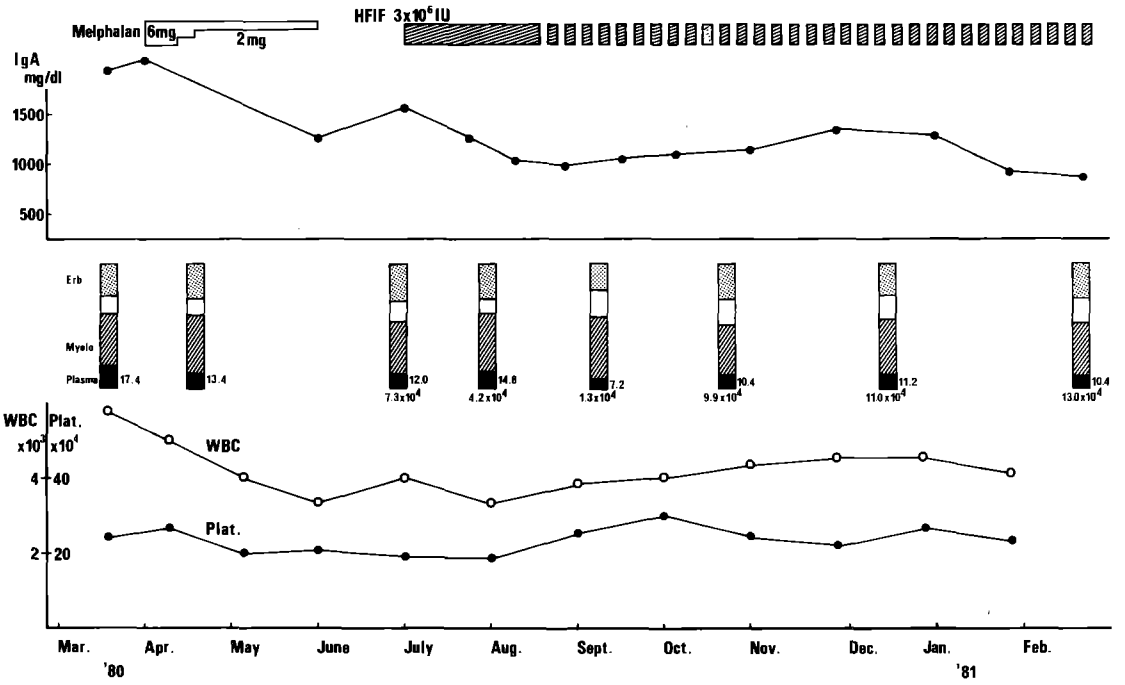


Fig. 3. Clinical course of ST, 57-year-old male, multiple myeloma (IgA K)

IV infusions of 3×10^6 IU HFIF were prescribed. The WBC count dropped dramatically after HFIF treatment was started, falling to less than 15,000/mm^3 on day 14, with an increase of more than 30% in neutrophils. The nadir of the WBC count was 6,700/mm^3 on day 28. During the treatment, no significant change was observed in the Hgb level or platelet count. Although the lymphoid series in the marrow were still dominant, the erythroid and myeloid series became more prominent. Hepatomegaly and lymphadenopathy were slightly attenuated. After 9 weeks of PR, lymphocytosis recurred during continuing daily treatment. The WBC count dropped transiently when the dose of HFIF was increased to 6×10^6 IU/day, but then rose again to as high as 51,000 and HFIF treatment was discontinued.

Case 2: 49-year-old female, multiple myeloma (IgA) (Fig. 2). Initially the patient complained of lumbago. Serum IgA was 4,300 mg/dl and the proportion of plasma cells in the marrow was 29.6%. With the diagnosis of IgA myeloma, the patient was scheduled to receive HFIF 6×10^6 IU daily. On day 10, the serum IgA level fell to 2,100 mg/dl and remained around this level thereafter. The lumbago improved gradually and had almost subsided 4 weeks later. Plasma cells in the marrow also decreased to 13% in 5 weeks. After 8 weeks of PR, the administration of HFIF was switched to an every-other-day schedule. Thereafter the serum IgA level started to rise and mild lumbago reappeared. Daily HFIF treatment was resumed but the serum IgA level remained rather high and HFIF treatment was discontinued in 12 weeks.

Case 3: 57-year-old male, multiple myeloma (IgA) (Fig. 3). Hyperproteinemia was found at a regular check-up and the diagnosis of IgA myeloma was made in March, 1980. The serum IgA was 1,980 mg/dl. The Bence-Jones test for protein in the urine was negative, and a bone survey was normal. Plasma cells in the marrow was 17.4%. Initially he was scheduled to receive melphalan treatment, which was continued until the end of May. The serum IgA was then down to 1,250 mg/dl; however, about 1 month later the IgA went up to 1,500 mg/dl and HFIF 3×10^6 IU daily IV was started. IgA went down to 1,000 mg/dl in 30 days and remained at this level during HFIF treatment. The proportion of plasma cells in the marrow was stable in the range of 12%–14%. After 43 days of HFIF treatment the patient was discharged and received maintenance therapy with HFIF, initially three times a week and then twice a week in the out-patients clinic. He has now had 52 weeks of the treatment and has been maintained in a good clinical condition with a serum IgA level of 1,000–1,200 mg/dl.

With regard to side-effects of HFIF treatment (Table 2), the majority of patients experienced fever with temperature exceeding 38° C, and chills. These

symptoms usually became uncommon after several days' treatments. General malaise not related to disease and probably related to HFIF itself appeared in ten patients. Anorexia due to HFIF treatment was not frequent. Mild and reversible hepatic and renal dysfunctions were observed in seven patients and one patient, respectively. Myelosuppression, mainly leukopenia and anemia, was observed in seven patients, although the degree was mild. In five patients, leukopenia appeared in 1–2 weeks from the start of HFIF treatment. Both neutrophils and lymphocytes decreased in number in these cases. These side-effects were all tolerable and did not make it necessary to discontinue HFIF treatment.

Table 2. Side-effects observed in HFIF treatment

Fever (> 38° C)		24/26
Chills		22/26
General malaise		10/22
Loss of appetite[a]		1/19
Myelosuppression[b]		7/24
Leukopenia	5	
Anemia	2	
Hepatic dysfunction[c]		7/24
Renal dysfunction		1/24

[a] Excluding the patients with AML and stomach cancer, who were anorectic before HFIF treatment

[b] Excluding the patients with AML and CLL. Cases with WBC counts more than 50% decrease or Hgb more than 3 g/dl decrease

[c] Cases with either GOT or GPT escalated to more than three times pretreatment value

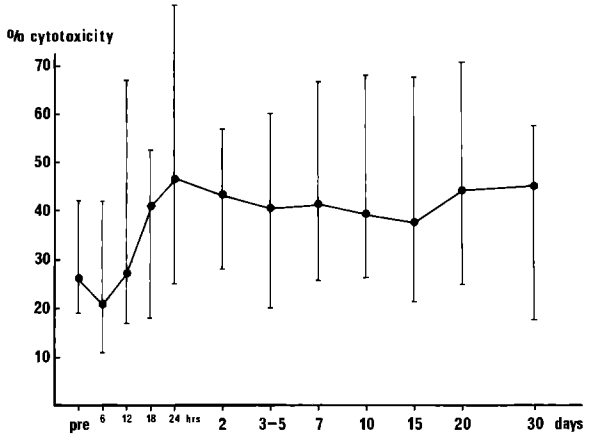
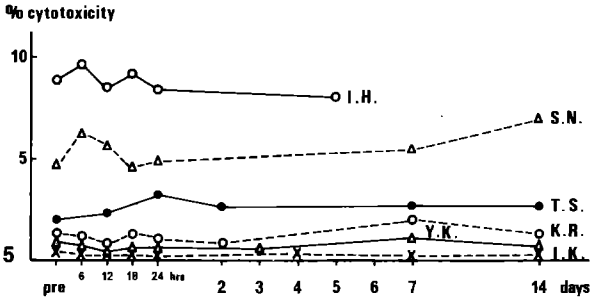


Fig. 4. Median lymphocyte NK activity against K562 following HFIF treatment in 16 patients. Target: effector = 1 : 40

Figure 4 summarizes NK activity against K562 cells in all 16 patients at various times following HFIF treatment. NK activity against Daudi cells had a similar response pattern, although the response was smaller than that against K562 cells. Of the two target-to-effector ratios used, 1 : 40 always gave the higher response and so the data presented are those obtained with this ratio. After HFIF injection there was an initial decrease at 6 h in some patients, followed by an increase above the pretreatment levels, and in the majority of the patients the highest NK activity was reached at 18–24 h, most often at 24 h. Thereafter, two types of response pattern were observed. In one group of patients, NK activity remained at the highest level throughout HFIF treatment and sometimes reached a value even higher than that at 18–24 h. In another group of patients, NK activity decreased but usually remained above the pretreatment level. There seemed to be no correlation between NK activity and clinical efficacy. Both responders (including SD patients) and nonresponders showed a similar response pattern of NK activity and there was no significant difference between them in pretreatment values or the highest values.

Autologous tumor cells were stored for six patients and lymphocyte cytotoxic activity against autologous cells was done in these patients (Fig. 5). Two of them had 5%–8% cytotoxicity before HFIF treatment but the rest showed almost no cytotoxicity.



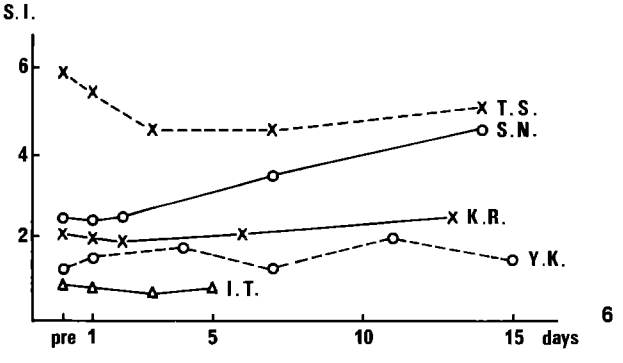


Fig. 5. Lymphocyte cytotoxicity against autologous tumor cells following HFIF treatment in six patients. Target: effector = 1 : 40

Fig. 6. Mixed lymphocyte tumor cell reaction following HFIF treatment in six patients

Table 3. Lymphocyte blastogenic responses following HFIF

	Lymphocyte blastogenic response (median net cpm)[a]					
	PHA (range)		PWM (range)		ConA (range)	
Pretreatment	144,674	(73,764–209,084)	237,605	(108,812–454,802)	206,870	(73,491–343,336)
12 h	139,510	(77,366–355,164)	243,869	(120,859–418,018)	194,315	(88,865–499,702)
24 h	144,059	(87,377–328,717)	253,892	(134,613–334,656)	246,556	(125,432–480,892)
2–3 days	149,860	(130,306–187,859)	252,685	(85,604–486,809)	216,563	(82,054–265,132)
5–7 days	121,070	(65,257–237,026)	268,725	(136,134–415,615)	257,451	(127,074–429,994)
11–14 days	163,887	(108,527–243,886)	226,311	(101,450–407,266)	194,080	(55,937–393,104)
20 days	125,631	(103,905–160,214)	295,129	(72,319–376,368)	235,551	(59,118–427,018)
30 days	158,167	(93,601–226,259)	279,716	(138,761–354,627)	304,508	(102,699–471,034)

[a] Median net cpm in 11 patients

Following HFIF treatment, none of the six patients showed any significant increase of cytotoxicity, although NK activity against K562 cells was enhanced in these patients.

Figure 6 shows the result of MLTR in six patients. One patient showed a slight increase in SI at 7 and 14 days after HFIF treatment, but none of the other patients had any significant change in SI after HFIF treatment. In vitro sensitization was performed in these six patients. Lymphocytes stimulated with MMC-treated autologous tumor cells were not significantly different from lymphocytes without stimulation in their cytotoxicity against autologous tumor cells after HFIF treatment. Even the patient who showed an increase of MLTR after treatment had no change in in vitro sensitization.

Lymphocyte blastogenic responses to nonspecific mitogens were tested in 11 patients. There were some fluctuations in tritiated thymidine uptake (net cpm) at various times of study, but in general, blastogenic responses remained unchanged before and after HFIF treatment (Table 3).

The changes in skin test reactivity to recall antigens are shown in Table 4. When skin test reactivity at 3–4 weeks after the initiation of HFIF treatment was compared with pretreatment reactivity, about one half of the patients studied showed an increase of 100% or more in reactivity to one of the three antigens.

Discussion

There have been several reports that leukocyte interferon (LI) is useful in various types of malignant diseases, such as malignant lymphoma, multiple myeloma, acute leukemia, and breast cancer. Because LI is relatively difficult to obtain in large quantities, the usual phase I–II studies of LI have not been performed and we cannot acertain the optimal dosage and optimal schedule for the antitumor effect. Nor do we know the maximal tolerated dose of this drug.

HFIF is produced from a human fibroblast cell line and can be relatively easily obtained in large quantities. There have been a limited number of clinical trials of HFIF. MacPherson and Tan [7] reported a phase I study of HFIF, and showed that four patients with colon cancer and one patient with renal cell carcinoma had stable disease. Niethammer et al. [10] observed tumor regression with HFIF treatment in patients with multiple myeloma and nasopharyngeal carcinoma.

Our present study showed two PR and seven SD in 24 evaluable patients. The administered dose of HFIF was 3×10^6 IU or 6×10^6 IU daily and there seemed to be no significant difference in response rate between these two treatment schedules. It might be coincidental that two patients who attained PR were previously untreated, because seven patients who had SD were all previously treated. As Gutterman et al. [3] stated, there is no correlation between previous chemotherapy and response rate. Of the two patients who obtained PR, one had multiple myeloma and the other, CLL. In one PR patient with CLL, the WBC count dropped rapidly after HFIF was started, and in another PR patient with multiple myeloma, the serum IgA level fell to less than 50% of the pretreatment value in 10 days' treatment. Among seven SD, four patients had CLL, multiple myeloma, or malignant lymphoma. Thus lymphoproliferative disorders, such as multiple myeloma, malignant lymphoma, and CLL, must be sensitive to HFIF. It is also interesting that two SD were obtained in four patients with stomach cancers. Since too few chemotherapeutic agents for control of stomach cancer are available it would certainly be worthwhile to evaluate the efficacy of HFIF in this disease.

In both PR patients, the response duration was rather short. Their disease became progressive while

Table 4. Delayed-type hypersensitivity reaction following HFIF treatment

Patient	Antigen	DTH reaction (mm induration)			
		Pre	2 weeks	3 weeks	4 weeks
SN	*Candida*	14 × 13	10 × 8	12 × 10	15 × 15
	PPD	7 × 5	8 × 7	15 × 13	8 × 7
	Varidase	8 × 8	35 × 33	33 × 25	25 × 23
AK	*Candida*	0 × 0	0 × 0		4 × 4
	PPD	0 × 0	2 × 2		4 × 4
	Varidase	0 × 0	10 × 11		18 × 14
ON	*Candida*	30 × 22	52 × 40		64 × 49
	PPD	34 × 42	52 × 57		48 × 38
	Varidase	23 × 25	80 × 50		107 × 61
KS	*Candida*	0 × 0			6 × 6
	PPD	0 × 0			7 × 7
	Varidase	19 × 18			40 × 32
ST	*Candida*	11 × 11		20 × 20	
	PPD	10 × 10		20 × 20	
	Varidase	23 × 20		43 × 34	
YK	*Candida*	9 × 8	0 × 0		0 × 0
	PPD	5 × 5	0 × 0		0 × 0
	Varidase	13 × 12	30 × 18		10 × 10
LK	*Candida*	10 × 11		10 × 10	
	PPD	8 × 8		10 × 10	
	Varidase	20 × 20		29 × 40	
TS	*Candida*	15 × 15	10 × 9		20 × 19
	PPD	26 × 26	15 × 14		28 × 24
	Varidase	50 × 40	63 × 44		50 × 40
KF	*Candida*	5 × 5	15 × 13		
	PPD	0 × 0	0 × 0		
	Varidase	0 × 0	0 × 0		
OS	*Candida*	0 × 0	7 × 6		
	PPD	0 × 0	3 × 3		
	Varidase	5 × 3	4 × 3		
KY	*Candida*	13 × 16	24 × 14		
	PPD	10 × 6	4 × 4		
	Varidase	27 × 21	10 × 11		
SU	*Candida*	17 × 12	6 × 8		5 × 3
	PPD	20 × 13	24 × 14		13 × 12
	Varidase	40 × 23	49 × 37		60 × 34
YM	*Candida*	45 × 38			50 × 50
	PPD	26 × 22			26 × 32
	Varidase	43 × 38			96 × 62
NT	*Candida*	7 × 10		15 × 13	
	PPD	6 × 6		8 × 7	
	Varidase	4 × 4		24 × 17	

they were receiving HFIF treatment. One possible explanation for this early relapse is the rapid development of resistance. Another possibility is that the dose of HFIF might have been low, because in a CLL patient the WBC count went down again with the increased dose of HFIF. For evaluation of the efficacy of the treatment in previously treated patients, the progression-free time must be substantial. In this sense, it is encouraging that three patients are still in SD with HFIF treatment.

The clinical effect of HFIF in our present study was not so impressive. This might be due to the patients' status, as the majority of patients had advanced diseases when HFIF was started. We still do not know the optimal dose and schedule of HFIF, which might be another reason for our poor response. Further phase I–II trials will be required to resolve these problems.

With regard to side-effects, most patients experienced fever and chills, especially during the first week of the treatment. About one-third of the patients had elevated transaminase levels during treatment. We are now convinced that these side-effects are due to HFIF itself or to impurity of the material. Myelosuppression was usually mild and appeared in the first 1–2 weeks of the treatment.

Interferon has been shown to have an antitumor effect mediated by the immune system, in addition to its direct cytocidal effect. On the basis of the animal experimentation the immune-mediated antitumor effects of interferon are said to be due to enhancement of NK activity, enhancement of phagocytosis, and enhancement of the cytotoxicity of sensitized lymphocytes. In our present study enhancement of NK activity was observed shortly after HFIF administration, and the highest activity was seen at 24 h after administration. Einhorn et al. [2] and Huddlestone et al. [5] reported peak NK activity at 12 h and at 18 h after LI treatment, respectively. Our data with HFIF are similar to theirs, although the highest activity seems to be a little delayed in our study.

In some animal tumor systems, resistance to tumor grafts is found to be correlated with NK activity [11], but the significance of NK activity is not established in association with human tumor regression. The present study showed that most patients had increased NK activity after HFIF treatment, but they were not always in a good clinical condition. This may suggest that there is no obvious correlation between NK activity and clinical efficacy, and that the antitumor effect observed is due to a direct cytotoxic or cytocidal effect of HFIF. However, the majority of patients studied had advanced tumors and were bearing bulky tumor masses. It is possible that the enhancement of NK activity alone might not induce clinical improvement in patients with a large tumor burden. To ensure clinically significant NK activity, it is desirable to study how HFIF-induced NK activity correlates with the clinical course in patients with a small tumor burden.

In contrast to NK activity against culture cell line, lymphocyte cytotoxicity against autologous tumor

cell was not enhanced by HFIF treatment. The reason for this phenomenon is unknown, but there are several possible explanations. Firstly, frozen-stored tumor cells may have a lower NK sensitivity than culture lines. Vánky et al. [13] reported that the enhancement of NK activity against established cell lines was induced after the incubation of lymphocytes with LI in vitro, but that autologous lymphocyte-mediated cytotoxicity was not enhanced when fresh tumor cells or frozen tumor cells were used. These authors, however, observed that after 5–6 days' cultivation of tumor cells, the cytotoxic sensitivity was increased. Secondly, there may be only a small number of cytotoxic cells that can recognize tumor-specific antigens. Finally, HFIF may enhance cytotoxic activity only against allogeneic tumor cells, and not against autologous tumor cells, which means that HFIF will enhance the recognition of alloantigen.

A mild increase in the MLTR was observed in only one patient after HFIF treatment, but in vitro sensitization to assess the generation of cytotoxic cells was negative in all patients. These data suggest that HFIF has limited activity in stimulation of the tumor-specific immune reaction. However, the number of patients studied is small and we need more research to see the activation of tumor-specific immunity. In particular, it is important to see whether generation and/or activation of cytotoxic T cells are actually induced by HFIF in the human, as reported in a mouse tumor system [6].

The present study showed that HFIF had modest antitumor activity, and this would be useful particularly in the treatment of CCL, malignant lymphomas, and multiple myeloma. Interferons that have been used in cancer treatment have been far from pure, which will be the reason we still do not know the maximal tolerated dose and the optimal dose schedule. To establish the role of interferon as an antitumor agent clinical evaluation is mandatory, including a phase I–II study, with a more purified form of interferon. This will also make it possible to obtain more precise knowledge about immunological activity of interferon.

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