EXHIBIT P

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Recombinant Leukocyte A Interferon: Pharmacokinetics, Single-Dose Tolerance, and Biologic Effects in Cancer Patients

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Sixteen patients with advanced cancer were treated with recombinant-DNA-produced pure leukocyte A interferon (IFLrA) intramuscularly in doses ranging from 3 to 198 × 10^6 units, with interval periods of 72 to 96 hours between doses. At the two lowest doses of 3 and 9 million units, there was a cross-over evaluation between IFLrA and partially pure leukocyte interferon (IFN-C) produced from human cells. The maximum observed serum concentration of IFLrA measured by enzyme immunoassay and bioassay increased with increasing doses. The mean serum concentrations of IFLrA and IFN-C were similar. Clinical effects produced by IFLrA and IFN-C were similar, including fever, chills, myalgias, headache, fatigue, and reversible leukopenia and granulocytopenia. Eight patients had transient and mild numbness of the hands or feet, or both. Three patients developed low titers of antibody to IFLrA. Seven of 16 patients showed objective evidence of tumor regression during the study.

INTERFERON, a naturally occurring protein discovered in 1957 (1), has potent antiviral, antiproliferative, and immunomodulating properties (2-4). Clinical experience in treating viral and neoplastic diseases has been limited due to the small quantities of species-specific human interferon (5). Leukocyte interferon of approximately 1% purity produced from human buffy coat cells has shown clinical activity against selected human viral diseases and malignancies (6-11). Leukocyte interferon induces tumor regression in a number of malignant tumors, including breast cancer, multiple myeloma, and malignant lymphoma (9-11), and may prolong the disease-free survival of patients with osteogenic sarcoma (8). Recently, biological synthesis of several species of leukocyte interferon in *Escherichia coli* by recombinant DNA techniques has been achieved (12, 13). Recombinant leukocyte A interferon (IFLrA), the first of a series of biosynthetic interferons produced for clinical investigation, has been purified to homogeneity and has biologic activity in preclinical testing (13-15).

We present the results of the first clinical study with a recombinant interferon. We report on the pharmacokinetics, single-dose tolerance, and biologic activity of IFLrA in cancer patients. In addition, a comparative analysis of the pharmacology and tolerance of recombinant interferon and partially purified buffy coat leukocyte interferon was made at selected doses.

Materials and Methods

The isolation, expression, and purification of IFLrA were done by Hoffmann La Roche and Genentech and have been described (13-15). The plasmid containing an entire leukocyte interferon gene was identified and engineered in *E. coli* in collaboration with Genentech (13, 14). The purification of IFLrA was done using a specific monoclonal antibody column to leukocyte A interferon (15, 16). The purified protein was homogeneous by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Material was more than 95% pure; the specific activity was 2 to 4 × 10^8 units/mg of protein when tested on AG1732 (human fibroblast) or MDBK (bovine kidney) cell lines. Molecular weight was estimated to be 19 200 daltons (17). Cultures of the material for bacteria and mycoplasma were sterile, and a limulus test for endotoxin was negative.

Several biological activities of this purified recombinant interferon were seen; the recombinant interferon showed equivalent in-vitro antiviral and antiproliferative activity compared to crude and purified natural leukocyte interferons (13, 18). In addition, IFLrA stimulated natural killer cell activity in vitro (ORTALDO J, HERBERMAN R, KUNG H, HOBBS D, STAEHELIN T, PESTKA S. In preparation.) and inhibited hematopoietic colony formation (VERMA DS, SPITZER G, GUTTERMAN JU,

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et al. Homogeneous preparations of natural and recombinant human leukocyte interferon block granulopoietic differentiation. In preparation.). Preclinical in-vivo testing showed antiviral activity comparable to human leukocyte interferon and was safely used in rabbits and squirrel monkeys (13). The interferon was provided in ampules of 3×10^6 units/mL and 18×10^6 units/mL. The final freeze-dried preparation contained human albumin and was reconstituted immediately before use.

Partially pure human leukocyte interferon (IFN-C) was prepared as described by Cantell and Hirvonen (19). The specific activity was approximately 1×10^6 units/mg protein.

A total of 16 patients were treated, eight at The University of Texas Cancer Center, M. D. Anderson Hospital and Tumor Institute, and eight at Stanford University Medical Center. Each patient received an initial intramuscular injection of 3×10^6 units of interferon with doses escalating to 198×10^6 units, as tolerated. A minimal interval of at least 72 hours followed each dose to permit clinical and laboratory values to return to baseline and to evaluate each dose independently. At the two lowest doses, there was a cross-over evaluation of IFLrA and IFN-C to permit comparative analysis of pharmacokinetics, single-dose tolerance, and biologic effects.

Patient Selection and Experimental Design

Patients in the study had advanced metastatic cancer and were considered incurable. They were ambulatory and had not had anticancer therapy for at least 4 weeks before entering the study. Criteria for entrance into the study included a life expectancy of at least 12 weeks and preserved renal (serum creatinine, $\leq$ 2 mg/100 mL), hepatic (bilirubin, $\leq$ 1.4 mg/100 mL), and hematologic functions (leukocyte count, $\geq$ 4000/mm^3; granulocytes, $\geq$ 1500/mm^3; and platelets, $\geq$ 150 000/mm^3). All patients signed informed-consent forms.

Recombinant leukocyte A interferon was given intramuscularly into the deltoid muscle with a minimal interval of 72 hours between injections. Individual doses of 3, 9, 18, 36, 54, 72, 90, 108, 144, and 198 million units were given. At the two lowest doses, IFLrA was alternated with 3 and 9×10^6 units of IFN-C. Patients were given alternating sequential doses; thus, eight patients received IFLrA followed by IFN-C, and eight received IFN-C followed by IFLrA.

One of the initial objectives was to treat each patient with the maximal dose tolerated. Patients were treated on Mondays and Thursdays of each week. A minimal period of 72 to 96 hours was used as an interval between doses. Longer intervals were used depending on biologic effects and at the discretion of the senior investigators.

Patients were seen daily and monitored closely. Vital signs including heart rate, blood pressure, respiration, and temperature were monitored at 1, 2, 4, 6, 12, 18, and 24 hours after each dose. Each intramuscular site was examined locally at 1/2, 1, 3, 6, 12, and 24 hours after injection. A physical examination was done 6 days a week until the end of the study. An electrocardiogram and chest roentgenogram were done before the study and after the final dose. Urinalyses were done at baseline and every

Table 1. Phase I Pharmacokinetic Study of Recombinant Leukocyte A Interferon: Clinical Features of 16 Patients

Patient	Diagnosis	Age/Sex	Previous Treatment	Cumulative Dose of IFLrA	Number of Injections	Maximal Dose	Days on Interferon
		yr		*units × 10⁶*		*units × 10⁶*	
1	Nodular poorly differentiated lymphocytic lymphoma	37/F	Leukocyte interferon (IFN-C)*	498	9	108	47
2	Nodular poorly differentiated lymphocytic lymphoma	39/M	Chemotherapy, leukocyte interferon (IFN-C)	408	8	108	47
3	Multiple myeloma	45/M	None	750	10	198	92
4	Multiple myeloma	50/M	Leukocyte interferon (IFN-C), chemotherapy	390	8	108	47
5	Multiple myeloma	56/F	None	282	7	90	29
6	Nodular poorly differentiated lymphocytic lymphoma	46/F	Chemotherapy	732	10	198	70
7	Multiple myeloma	49/M	Chemotherapy	534	9	144	56
8	Adenocarcinoma of the breast	54/F	Chemoimmunotherapy, radiotherapy	642	9	198	49
9	Adenocarcinoma of the ovary	65/F	Chemotherapy	642	9	198	38
10	Malignant melanoma	36/M	Hormonal therapy	444	8	144	34
11	Adenocarcinoma of the breast	64/F	Chemotherapy, radiotherapy, hormonal therapy	300	7	108	32
12	Chronic myelogenous leukemia	32/M	Chemotherapy	390	8	108	42
13	Adenocarcinoma of the colon	65/M	Chemotherapy	390	8	108	50
14	Adenocarcinoma of the breast	49/F	Chemotherapy	192	6	72	36
15	Nodular poorly differentiated lymphocytic lymphoma	54/M	Leukocyte interferon (IFN-C)	444	8	144	37
16	Diffuse and nodular poorly differentiated lymphocytic lymphoma	36/F	Chemotherapy, radiotherapy	732	10	198	62

* IFN-C = partially pure human leukocyte interferon.

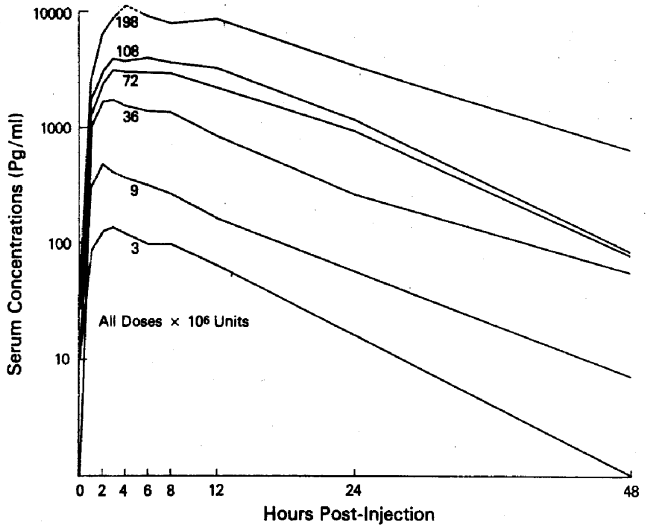

Figure 1. The arithmetic mean serum concentrations of interferon as measured by the enzyme immunoassay. The numbers of patients measured at 3, 9, 36, 72, 108, and 198 million units are 16, 16, 16, 16, 14, and 5, respectively.

72 hours thereafter until completion of the study.

Laboratory Studies

A complete blood count with differential, leukocyte count enumeration, reticulocyte count, and platelet count was done every 24 hours. Sequential multiple analyses of alkaline phosphatase, aspartate transaminase (formerly called serum glutamic-oxalacetic transaminase), serum bilirubin, fasting blood sugar, blood urea nitrogen (BUN), serum cholesterol, total protein, uric acid, phosphorus, calcium, serum albumin, and lactic acid dehydrogenase (LDH) were done before the study and 24 hours after each dose. Alanine transaminase (formerly serum glutamic-pyruvic transaminase), serum creatinine, electrolytes, and prothrombin time were measured before each dose.

Quantitative changes in tumor size were evaluated by physical examination, radiologic examination, and pertinent laboratory studies such as serum protein electrophoresis and measurement of Bence Jones protein (11). The criteria for responses have been described previously (11).

Serum samples were collected at 0, 1, 2, 3, 4, 6, 8, 12, and 24 hours after doses of 3, 9, 36, 72, 108, and 198 $\times$ 10^6 units. Urine samples were collected 2 hours before the dose and at 0 to 2, 2 to 4, 4 to 8, 8 to 12, and 12 to 24 hours after treatment. Serum interferon titers were measured at Hoffmann-La Roche by a modified bioassay using MDBK cells as targets and vesicular stomatitis virus as described previously (20). Interferon titers are expressed as reciprocals, with the dilutions producing a 50% reduction of virus cytopathic effect. All samples were corrected to the standard G023901-527 reagents from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.

An enzyme-linked immunoassay using two monoclonal antibodies to IFLrA was done at Hoffmann-La Roche (SPIEGEL H. Unpublished data.). In principle, the assay depends on stoichiometric binding to interferon by two monoclonal antibodies (16), one of which is covalently linked to peroxidase. The peroxidase reacts with *o*-phenylenediamine in the presence of peroxide. The color produced was read in a spectrophotometer at 492 mm; details of the methods used will be reported separately. The development of antibodies to IFLrA and IFN-C was tested using a modification of the bioassay in which neutralization of the antiviral effect of interferon containing samples was measured.

Results

Table 1 shows the clinical features of the 16 patients in the study, including their diagnosis, age, sex, previous therapy, cumulative recombinant interferon dose, number of injections, maximal dose, and number of days on interferon. The first eight patients are from The University of Texas, M. D. Anderson Hospital, and the second eight from Stanford University Medical Center. The patients ranged in age from 32 to 65 years; eight were men and eight, women. Five patients had malignant lymphoma, three had adenocarcinoma of the breast, four had multiple myeloma, and one each had adenocarcinoma of the ovary, malignant melanoma, adenocarcinoma of the colon, and chronic myelogenous leukemia. Twelve of the patients had previously received chemotherapy or hormonal therapy or both. Four patients had previously received partially purified buffy coat leukocyte interferon, and two previously had not had systemic therapy. The number of IFLrA injections ranged from 6 to 10, with a median of 8. The maximum dose given ranged from 72 to 198 million units, and the period of treatment ranged from 29 to 92 days. The cumulative dose of IFLrA ranged from 192 million units to 750 million units.

The pharmacokinetics and biopharmaceutical measures of IFLrA were evaluated in 16 patients. Bioavailability of IFLrA was the primary pharmacokinetic variable evaluated in this study. Due to the limited sampling and variability of data, the clearance rate and the volume of distribution could not be evaluated. Not all subjects received each dose, but five to 16 patients were included at each dose level. Figures 1 and 2 show the mean serum concentrations as measured by the enzyme immunoassay (in picograms per millilitre) and the bioassay (in units per millilitre) respectively, up to 48 hours after representative doses. The enzyme immunoassay is a research tool and direct correlation with the bioassay results remain to be proved.

In general, mean maximum observed serum concentra-

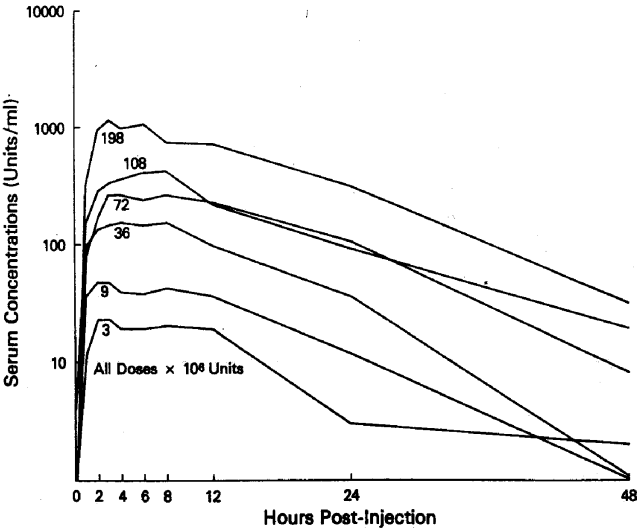

Figure 2. The arithmetic mean serum concentrations of interferon as measured by the bioassay with MDBK cells as target cells. The numbers of patients measured at 3, 9, 36, 72, 108, and 198 million units are 16, 16, 16, 16, 14, and 5, respectively.

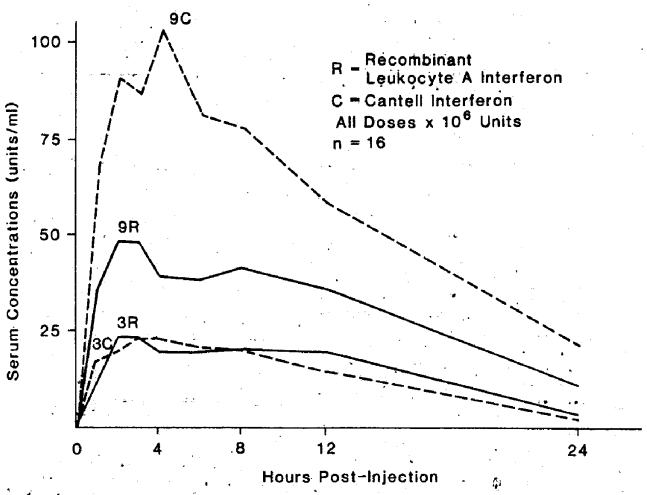

Figure 3. The comparative arithmetic mean serum levels of 16 patients treated with IFLrA and IFN-C as measured by a modified bioassay at 3 and 9 million units.

tion (C_{max}) and area under curve (AUC) values increased with increasing doses. When C_{max} and AUC were normalized for the dose given and represented as pg/mL $\cdot 10^6$ unit dose or units/mL $\cdot 10^6$ unit dose, there was dose proportionality. The time of the maximum observed serum concentrations (t_{max}) tended to increase slightly with increasing doses up to 72×10^6 units. The t_{max} varied from 4.6 ± 2.5 hours at the 3 million units IFLrA dose to 6.1 ± 3.6 hours at the 72 million unit IFLrA dose. Above this level, two injection sites were used and the trend disappeared. This result is consistent with a depot effect on the intramuscular route of the injection site. The half-life ranged from 6 to 8 hours regardless of the dose.

The constancy of the pharmacokinetic activity across the dose range is noteworthy. As long as the injection volume at a single site was limited to 2 mL, linear pharmacokinetics were observed. The mean serum concentrations of IFLrA did not differ in patients with solid tumors compared to patients with hematologic malignancies as had been reported previously for IFN-C (11). Recombinant leukocyte A interferon was not detected in the urine as measured by the enzyme immunoassay after doses ranging from 3 to 72×10^6 units.

Figure 3 shows the arithmetic mean serum concentrations of IFLrA and IFN-C at doses of 3×10^6 units and 9×10^6 units, respectively, as measured by the modified bioassay. The only statistically significant difference between the IFLrA and IFN-C value is the AUC at 9×10^6 units, where the IFN-C value is significantly larger than the IFLrA value, $p < 0.05$. The half-lives of elimination were calculated at 9×10^6 unit doses and were 7.3 and 8.2 hours for IFN-C and IFLrA, respectively.

Figure 4 shows the individual doses given to each of the 16 patients. (The reasons for discontinuing the interferon dose for each of the patients are explained in the footnotes.) The maximal tolerated dose varied from 72 to 198 million units. Five patients reached the maximal level of 198 million units. Interferon was discontinued in four patients because of severe and prolonged fatigue and in three patients because of numbness of the hands or feet, or both. Interferon was discontinued in the other four patients due to personal reasons unrelated to side effects.

The clinical side effects associated with IFLrA and IFN-C are shown in Table 2. In general, the side effects were similar to those reported previously for natural leukocyte interferon (11). As shown in the table, IFLrA and IFN-C at 3 and 9 million units, respectively, produced virtually the same clinical side effects. The symptoms of fever, chills, myalgias, and headache occurred after almost all doses. Fever occurred after virtually 100% of interferon injections, but there was no correlation between increasing febrile response and increasing interferon doses. Fever generally began 2 to 6 hours after an injection of interferon and peaked at 6 to 12 hours; in almost all patients, it resolved spontaneously within 24 hours. Some patients received acetaminophen for fever during the first 8 to 12 hours after a dose of interferon. Headache, chills, and myalgias occurred frequently after a dose of interferon. These symptoms usually resolved spontaneously within 24 hours and did not show a relation to dose response. Antihistamines frequently relieved the headaches.

Fatigue was a common complaint when interferon was given in higher doses. With increasing doses, fatigue became more severe and persisted from 7 to 21 days after individual interferon doses. In four of 16 patients, fatigue was the dose-limiting factor, usually after escalation of the dose to 108 million units (Figure 4).

The development of mild numbness and paresthesias of the hands or toes or both occurred in eight patients. In general, these effects occurred at doses of 72 million units

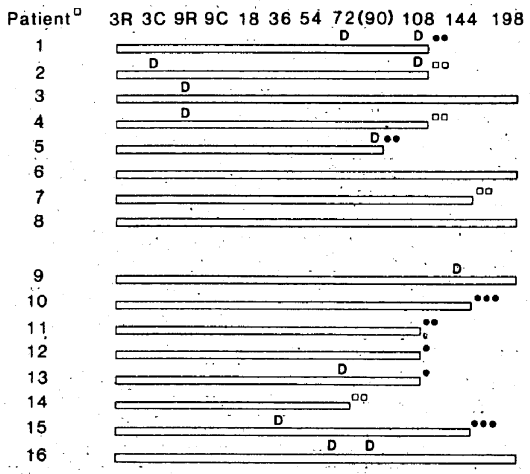

Figure 4. The individual maximal doses given per patient. R = recombinant leukocyte A interferon; C = partially pure leukocyte interferon. All doses $\times 10^6$ units. D = caused a delay in the next dose. □ = Odd-number patients received recombinant interferon first, even-number received partially pure leukocyte interferon first. □□ = Discontinued; in the judgment of the principal investigator, patient reached maximum tolerated dose on basis of clinical symptoms—fatigue. Patient 14 received radiotherapy. ●● = Discontinued, numbness. ● = Discontinued due to reasons unrelated to side effect. ●●● = Discontinued for personal reasons. ( )—optional.

Table 2. Major Side Effects Associated with Interferon

	Dose of Interferon*											
	3R	3C*	9R	9C	18R†	36R	54R	72R	90R	108R	144R	198R
	% of patients											
Fever	94	94	100	100	94	100	100	100	100	100	100	100
Chills	44	43	75	50	63	81	94	69	63	100	75	100
Myalgias	44	25	56	57	50	50	44	50	63	50	50	40
Headache	63	33	44	31	44	50	50	63	88	64	88	80
Fatigue	6	0	0	13	44	50	69	88	63	93	75	80
Gastrointestinal disturbances	13	13	25	13	31	19	38	19	38	29	50	60
Numbness	0	0	0	0	0	6	6	13	50	29	13	0
Number of patients	(16)	(16)	(16)	(16)	(16)	(16)	(16)	(16)	(8)	(14)	(8)	(5)

* C = partially pure leukocyte interferon dose × 10^6 units; R = recombinant interferon dose × 10^6 units.

and above, although two patients experienced transient symptoms at lower doses. The experience at The University of Texas, M. D. Anderson Hospital, and Stanford University Medical Center was different. In five patients at M. D. Anderson Hospital, numbness and paresthesias occurred in the hands and toes. These symptoms persisted for 2 to 14 days after individual doses in four of the five patients. In the other patient, the symptoms lasted for a few minutes only. In the three patients at Stanford, numbness and paresthesias were present only in the hands. Only one patient had this sensation on more than one occasion, and it lasted for seconds.

Objective decrease in sensory perception of the fingertips was noted by a neurologist in two patients. This finding was attributed to a mild sensory peripheral neuropathy. Nerve conduction studies and electromyograms were done at The University of Texas, M. D. Anderson Hospital; findings were normal in all instances. Interferon was discontinued in three patients because of this side effect. All cases of numbness and paresthesias resolved completely after discontinuation of the interferon. There was no correlation between these symptoms and previous treatment with agents known to cause neurotoxicity.

Occasional side effects reported in one or two patients with a few doses were tightness of the chest or throat, lightheadedness, dizziness, nasal congestion, cold or pale hands, dryness of the neck and face, bad taste in the mouth, and trembling. Gastrointestinal symptoms, including anorexia, nausea, and diarrhea, tended to occur at the higher dose levels. Minimal alopecia developed in one patient. Weight loss ranging from 1.4 to 7.3 kg (median value, 3.1 kg) was a common occurrence in this study. Lesions indicative of recurrent herpes simplex developed in three patients after the initial one to three injections.

The site of the intramuscular injection was closely monitored. Minimal local tenderness and induration rarely occurred during the first 48 hours. No significant changes in vital signs occurred. Blood pressure tended to decrease from baseline during the first 24 hours after an injection and was consistent with that of a patient at bedrest. Moderate elevation of blood pressure occasionally occurred 2 to 6 hours after injection but returned to normal a few hours.

The patient with chronic myelogenous leukemia was excluded from hematologic analysis. Total leukocyte count in 13 of the 15 patients decreased in comparison to the baseline value measured before each dose (Figure 5). The progressive decrease in the total leukocyte count was analyzed for each patient after each dose was given. A

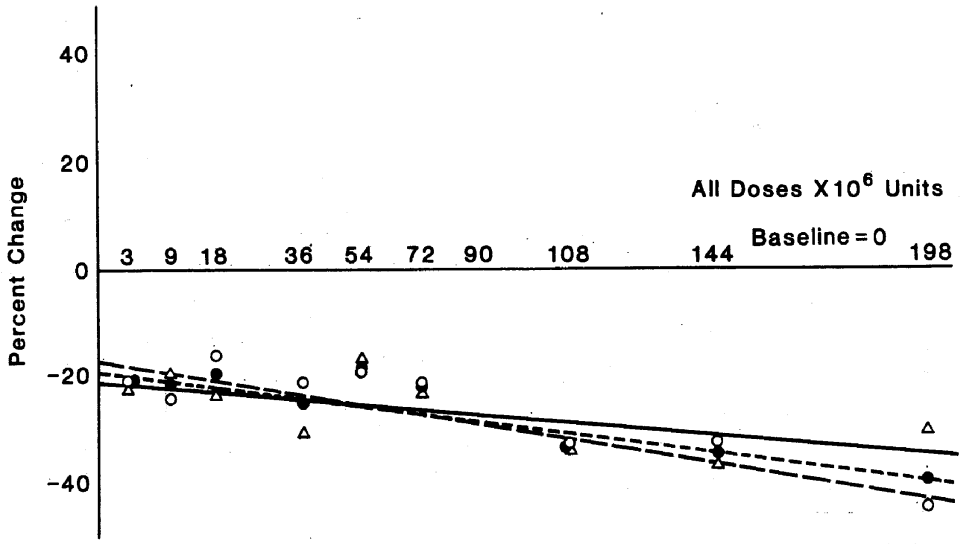

Figure 5. Mean levels of leukocyte count at each dose level for patients with hematologic malignancies and nonhematologic malignant solid tumors. The plot is carried out by a least square regression analysis. Δ—Δ = nonhematologic malignancies; o—o = hematologic malignancies; ●—● = both.

proportionately greater decrease from the baseline value was seen with higher doses. When patients were divided into hematologic and nonhematologic groups, only patients with hematologic malignancies showed this dose response ($p < 0.05$) (21). Because of the design of the study, the decrease could be related to the cumulative effect of many doses or to increasing dose levels or both.

A relative lymphocytosis was frequently noted in the patients in this study. This condition was due both to an increase in the absolute number of lymphocytes and a decrease in the absolute number of neutrophils. Of 152 injections evaluated for hematologic values, the absolute lymphocyte count rose 98 times and fell 52 times, while the neutrophil count rose 11 times and fell 141 times. Recombinant leukocyte A interferon and IFN-C produced similar effects on hematologic values at either 3 or 9 million units.

A minimal decrease in the hematocrit values and platelet counts was observed; however, the decrease in hematocrit values (average of 6% per patient during the study) could have been accounted for, in part, by the phlebotomy requirements.

The effects of interferon on blood chemistries were minimal. Seventeen percent of the patients had elevated lactic dehydrogenase (LDH) levels during the study, but these levels returned to their baseline values before the next dose was given. Levels of alanine transaminase rose after only 6% of the injections. Most of these effects were caused by one patient who was abusing alcohol during the study. No effects on serum creatinine and BUN were seen. Mild pyuria (5 to 10 leukocytes per high-power microscopic field) developed in five patients during the study. This condition often resolved spontaneously despite persistent use of interferon and was considered unrelated to interferon administration.

Three of 16 patients (Patients 1, 7, and 14) developed antibodies to IFLrA. Antibody was not present before the study and was undetected while the patients were receiving interferon. Patient 1 had a detectable antibody level of 1:7.5 10 days after receiving her last dose of interferon. Six months later, the antibody titer had risen to 1:30; however, during this period the patient was treated for 4 weeks with IFN-C. Patient 7 had a titer of 1:20 10 days after the end of study; this titer has remained stable 2 months after the end of study. Patient 14 developed a titer of 1:30 3 weeks after her last dose, and this titer has remained stable 1 month after study. Titers of 1:7.5, 1:20, and 1:30 are sufficient to neutralize in vitro 150, 400, and 600 units/mL of interferon, respectively. Preliminary evidence indicates that the antibody is of the IgG class.

Seven of the 16 patients showed objective evidence of tumor regression during this study. Among the five patients with nodular poorly differentiated lymphocytic lymphoma, one had a partial remission, three had minor responses, and one had a mixed response. The partial remission occurred in Patient 15, who had previously had a complete remission using IFN-C. Two of the minor responses occurred in Patients 1 and 2 at The University of Texas, M. D. Anderson Hospital; they had previously been treated with a 28-day course (total of 84 million units) of IFN-C without response. Patient 1 had shown progression 1 month before entering the study and the condition of Patient 2 had remained stable for 1 year before the study. Both patients showed objective evidence of tumor regression on radiologic examination of abdominal lymph nodes: Patient 1 showed a 30% to 40% decrease in tumor-involved lymph nodes; in Patient 2, several pathologically involved lymph nodes disappeared, while others remained stable or decreased less than 50%. One patient with chronic myelogenous leukemia showed a reduction of peripheral leukocyte count from 22 000 to 12 300/mm^3 and a reduction of more than 50% in spleen size. These results will be reported in more detail (HORNING S, LEVINE J, MILLER R, ROSENBERG S. Clinical and immunologic effects of recombinant leukocyte A interferon in eight patients with advanced cancer. Submitted for publication).

Discussion

Recent progress in recombinant DNA technology and production of monoclonal antibodies has yielded a single purified species of leukocyte interferon (13-16). This study is the first clinical investigation of a purified recombinant-DNA-produced interferon; previous clinical studies of interferons have used preparations of approximately 1% purity. With recent knowledge gained from recombinant DNA techniques, we know that these preparations contained a mixture of eight or more species of leukocyte interferon (22, 23).

This investigation has described the pharmacologic profile, single dose tolerance, and biologic effects of IFLrA. Recombinant leukocyte A interferon is well absorbed after intramuscular injections, resulting in serum concentrations similar to those previously reported for buffy-coat-derived leukocyte interferon (5, 6, 11). For the first time in clinical investigation, an enzyme immunoassay has been used to measure a single antigenic species of leukocyte interferon with concentrations expressed in a weight per volume basis. The development of monoclonal antibodies to leukocyte interferon (16) has led to the production of an enzyme immunoassay for the detection of IFLrA. The enzyme immunoassay is still a research tool, and a direct correlation between IFLrA and the bioassay is not possible at this time. However, the ability to measure serum levels on a weight per volume basis was useful for monitoring interferon levels in this study and should improve the specificity, sensitivity, and reproducibility of measuring interferon levels.

Blood levels 10 to 20 times higher than those previously reported for buffy-coat-derived leukocyte interferon were achieved at the higher dose ranges (2, 5, 11). The pharmacologic profile of IFLrA was similar to that produced by the mixture of natural leukocyte interferon. Thus, the inability of *E. coli* bacteria to glycosylate the interferon protein may not be an important determinant in the pharmacologic behavior of recombinant DNA-derived interferon. In addition, recent evidence indicates that the major, naturally occurring, leukocyte interferon species are largely devoid of carbohydrate (24).

Antibody to IFLrA developed in three of 16 patients.

Although no apparent subjective or objective clinical abnormalities were seen that could be attributed to this development, the precise significance of these antibodies must be evaluated by future clinical studies. The degree of neutralization detected in vitro in the current study indicates that the in-vivo neutralization of pharmacologic doses of interferon by an antibody is possible. Antibodies to partially pure fibroblast interferon (25) as well as partially purified leukocyte interferon (TROWN P, KRAMER M, GUTTERMAN JU. In preparation) have been detected in patients treated with these agents.

The clinical and laboratory side effects induced by IFLrA were similar but not identical to those previously recorded with buffy coat interferon (5, 6, 9, 11). Hematologic toxicity was frequent, mild, and not dose limiting. The decrease in circulating leukocytes and granulocytes was rapidly reversible during interval-dosing periods. The kinetics of leukocyte recovery indicate that interferon may produce or cause cell margination or redistribution rather than affect maturation of myeloid stem cells (26). However, in-vitro effects on myeloid differentiation have been shown with both IFN-C and IFLrA (26) (VERMA DS, SPITZER G, GUTTERMAN JU, et al. Homogeneous preparations of natural and recombinant human leukocyte interferon block granulopoietic differentiation. In preparation.)

In contrast to the frequent increase in the alanine transaminase in most patients treated with daily doses of crude leukocyte interferon, the alanine transaminase of patients in this study rarely rose. The lack of effect on liver enzymes noted in this study may be due to the intermittent schedule or the qualitatively and quantitatively different effect of this particular pure interferon species.

Interestingly, the acute side effects associated with IFLrA are similar to those reported with viral infections. The preclinical studies done with IFLrA in subhuman primates did not predict a febrile response (13), suggesting that other species may have to be evaluated for animal toxicology studies of interferon. Although we cannot be absolutely certain that the fever induced by the recombinant preparation is due to the interferon molecule, there is no evidence indicating the presence of residual *E. coli* protein or any other contaminants. Similar pyrogenic as well as flu-like responses recently have been reported after intramuscular injection of a homogeneous interferon preparation purified from human buffy coat leukocytes (27). These data, therefore, support the conclusion that the interferon molecule is responsible for the side effects noted in treatment.

The dose-limiting side effect in several patients was attributed to fatigue, and further studies are needed to elucidate its mechanism. Although the frequency and severity of fatigue increased with higher doses, it is not possible to distinguish a true dose-response effect from cumulative effects of the interferon. The significance of the numbness and paresthesias in half the patients also must await further studies. In at least two of these patients, the symptoms were felt to represent a mild sensory peripheral neuropathy. It should be noted that Calvert and Gresser (28) reported that in-vitro interferon can increase neuronal activation.

The similar clinical and laboratory toxicities produced by IFLrA and IFN-C indicate that purification and synthetic production methods do not alter the basic biologic activities intrinsic to the interferon molecule itself.

Previous reports have clearly shown the ability of impure preparations of leukocyte interferon to induce regression of metastatic tumor in breast cancer, multiple myeloma, malignant lymphoma, and renal cancer (9-11, 29). Although this study was not designed primarily to measure antitumor activity, it is of great interest that patients receiving an intermittent schedule of increasingly high doses had clinical evidence of biologic activity. This activity suggests that the interferon molecule is responsible, at least in part, for the antitumor properties of crude or partially pure interferon preparations.

The development of recombinant technology to produce interferon proteins should greatly increase their use as potential antiviral and antitumor agents. Further phase I, II, and III studies are now necessary to establish the role of this as well as other species of leukocyte interferon in the treatment of human viral diseases and cancer.

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Occult Cancer in Patients with Acute Pulmonary Embolism

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An association between venous thrombosis and cancer was first suggested by Trousseau, and has been confirmed by multiple postmortem studies. Clinical studies have shown that thrombophlebitis migrans may occur before malignancies become clinically evident, and therefore serves as a clue to occult cancer. A relation between occult cancer and the commoner deep venous thrombosis and pulmonary embolism has not been established. We ascertained the incidence of cancer before and after pulmonary embolism was diagnosed by pulmonary angiography in 128 patients. The incidence of cancer before pulmonary embolism (12%) was essentially the same as that in a comparison group of patients without pulmonary embolism (10%). In the 2 years after pulmonary angiography, however, cancer was diagnosed in 13 patients with pulmonary embolism in contrast to no patients in the comparison group ($p < 0.001$). The most frequent cancers involved the lung, gastrointestinal tract, breast, and uterus. The malignancies were nearly always occult when pulmonary embolism occurred. These findings indicate that pulmonary embolism with or without overt deep venous thrombosis should alert the clinician to consider occult cancer.

AN ASSOCIATION between venous thrombosis and malignancy was first suspected in 1865 by Trousseau (1). This relation was documented by Sproul (2) who reported an increased incidence of venous thrombosis at the postmortem examination of patients who died of various malignancies, most notably carcinoma of the body or tail of the pancreas. Subsequent postmortem studies have shown an increased incidence of venous thrombosis and pulmonary embolism in patients with cancer of the lung, stomach, colon, uterus, breast, and prostate (3-6). Although these postmortem studies have documented an association between venous thrombosis and various malignancies, they have not clarified the time course of this relation.

Clinical studies have shown that a particular form of venous thrombosis, thrombophlebitis migrans, may appear months or even years before the signs and symptoms of malignancy emerge (4-5). Thrombophlebitis migrans, which involves superficial veins in sites that are rarely subject to deep venous thrombosis (such as the veins of the upper extremities), tends to be migratory and resistant to anticoagulation. This rare disorder is accepted as a clue to the presence of occult malignancy.

The time course of the relation between malignancy and the far commoner form of venous thrombosis, deep venous thrombosis, and its complication, pulmonary embolism, is not as clear. It is not known if the occurence of deep venous thrombosis or pulmonary embolism should lead clinicians to suspect occult malignancy. To ascertain if patients with acute pulmonary embolism have an increased incidence of occult cancer, we followed the course of a large group of patients with acute pulmonary embolism documented by pulmonary angiography.

Methods

From 1 July 1964 to 1 July 1975, nearly all patients on the medical or surgical services of the Peter Bent Brigham Hospital suspected of having acute pulmonary embolism were referred to the Cardiovascular Laboratory for evaluation. After a review of clinical and laboratory data, pulmonary angiography was done on 610 patients. The techniques for doing and interpreting these studies have been reported (7). Venography was done in a few of these patients.

A total of 142 patients were found to have unequivocal angio-

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