Phase I Study of Pharmacological and Immunological Effects of Human Lymphoblastoid Interferon Given to Patients with Cancer


ABSTRACT

An extensive Phase I evaluation of human lymphoblastoid interferon has been completed which, in addition to describing its clinical and pharmacological effects, emphasized a broad-scale evaluation of the immune response as a function of interferon dosage. Dose-limiting toxicity was generally due to constitutional symptoms which are remarkably similar to those produced by influenza, although transient peripheral and central neurotoxicity (including deterioration in cognitive and behavioral functions) is observed at higher doses. It is difficult to establish "clean" dose-response effects except for fever and bone marrow suppression, neither of which is a major dose limitation. Enhancement of the immune system was limited to natural killer cells which had a complex dose-response relationship, whereby low interferon concentrations were less stimulatory (than were high doses) following a single dose but gave more sustained stimulation over a 5-week course of 3 times per week i.m. administration. The effects on various measures of monocyte function and of nonspecific immunity (hypersensitivity, immunoglobulins, complement) were negative. We suspect that in practice it may be difficult to exploit the narrow dosage window of immunostimulation, but it is important to note that the nontoxic lower doses were more stimulatory than were the very high doses which are being used in numerous clinical trials.

INTRODUCTION

A quarter century has elapsed since Isaacs and Lindenmann discovered the inducible secretory glycoproteins produced by eukaryotic cells in response to viral infection or viral products (17). Because of the interest in this unique natural substance for the treatment of cancer, and because of the scarcity, impurity, and expense of available human leukocyte interferon, other sources of natural and synthetic interferons have been sought. The National Cancer Institute has sponsored a series of investigations with human leukocyte interferon and with human lymphoblastoid interferon (Wellferon), both α-interferons. Some of the early pharmacological studies with the latter type of interferon were reported by Priestman (27).

This report described an extensive study of the pharmacological effects of human lymphoblastoid interferon as well as its effects on the immune system in patients with cancer. Since anticancer effects of interferon are potentially due to stimulation of the immune system and/or to its nonspecific antiproliferative effects, it is important to determine not only the maximal tolerated dose and blood levels achieved, but also the dose that provides optimal effects on the immune system. Without knowing which aspects of the immune response will be affected by lymphoblastoid interferon, it was necessary to study monocyte functions, NK cells, lymphocyte subpopulations, immunoglobulins, complement, and skin test responsiveness.

MATERIALS AND METHODS

Patient Selection. Nineteen patients participated in the full immunopharmacological studies; 17 completed the course of treatment. In a supplementary study, 4 patients were also given a more rapid dose escalation of interferon in order to determine the clinical effects. Patients were eligible for the study if they had (a) microscopically proven cancer for which no cure was available or with which standard approaches had been unsuccessful, (b) good general health (Karnovsky's performance status, >70%), (c) no chemotherapy or radiation therapy for at least 1 month prior to study, (d) normal granulocyte and platelet counts, and (e) no evidence of impaired hepatic or renal function. Oral and written informed consent was obtained after detailed explanations were given to patient and family.

Drug and Dose Schedule. Human lymphoblastoid interferon (Wellferon) is an α-interferon preparation produced by the Wellcome Foundation, Ltd., Beckenham, Kent, England. It is prepared from cells of the human B-lymphoblastoid line, Namalwa, stimulated with Sendai virus, and contains at least 8 proteins with interferon antiviral activity. The concentration of human lymphoblastoid interferon used in this trial was 14.12 megaunits (1 megaunit = 1 x 10^6 units)/ml (Lot CIN/10) as provided by the National Cancer Institute. This preparation had been purified to a level of 2 x 10^6 IU of human α-interferon per mg of protein and was supplied frozen in tris-glycine-buffered 0.9% NaCl solution. This lot was evaluated for pyrogen content by both the Limulus amebocyte lysate method as well as the official U.S.P. pyrogen test. The Limulus amebocyte lysate test equaled 0.4 ng/ml (endotoxin equivalent), and the U.S.P. pyrogen test equaled 0.5, 0.6, 0.75, 0.9, 1.0, and 1.4 (rabbit temperature rises), thus defining this lot as pyrogenic by U.S.P. criteria. Interferon was stored at -20° and was thawed and prepared for administration before each dose. Doses less than 2 megaunits were diluted with buffer to bring the volume to amounts that were easily measurable.

The study consisted of 2 phases, A and B, for each patient. In Phase A, a single dose (0.1 to 4.0 megaunits/sq m) was administered deep into the gluteal muscles. After a 2-week rest period, Phase B, the phase of chronic administration, began; in this phase, 15 injections were given over a 5-week period, using an escalating dose schedule as patients were added to the study. Injections were given on Monday, Wednesday, and Friday of each week. The dose progression schedule began with 0.5 megaunit/sq m for the first series of patients and reached a maximum of 15 megaunits/sq m for the last 5 patients during their final week of the escalation schedule (Table 1). After the goals of Phases A and B were achieved, a small supplementary series of experiments was performed with buffer to bring the volume to amounts that were easily measurable.

Received March 17, 1983; accepted June 10, 1983.
**Table 1**

*Dose i.m. and tumor response*

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Phase A (megaunits/sq m)</th>
<th>Phase B (megaunits/sq m)</th>
<th>No. of doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>M</td>
<td>Prostate</td>
<td>0.1</td>
<td>0.5</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>M</td>
<td>Nasopharyngeal</td>
<td>0.5</td>
<td>0.5</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>M</td>
<td>Nasopharyngeal</td>
<td>1.0</td>
<td>0.5</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>55</td>
<td>M</td>
<td>Metastatic colon</td>
<td>1.0</td>
<td>0.5</td>
<td>15</td>
</tr>
<tr>
<td>5</td>
<td>46</td>
<td>F</td>
<td>Metastatic melanoma</td>
<td>2.0</td>
<td>2.5</td>
<td>15</td>
</tr>
<tr>
<td>6</td>
<td>60</td>
<td>F</td>
<td>Pancreas</td>
<td>2.0</td>
<td>2.5</td>
<td>15</td>
</tr>
<tr>
<td>7</td>
<td>31</td>
<td>F</td>
<td>Thymoma</td>
<td>2.0</td>
<td>2.5</td>
<td>15</td>
</tr>
<tr>
<td>8</td>
<td>59</td>
<td>M</td>
<td>Waldenstrom's</td>
<td>2.5</td>
<td>2.5</td>
<td>15</td>
</tr>
<tr>
<td>9</td>
<td>60</td>
<td>M</td>
<td>Prostate</td>
<td>2.5</td>
<td>2.5</td>
<td>15</td>
</tr>
<tr>
<td>10</td>
<td>52</td>
<td>F</td>
<td>Histiocytoma</td>
<td>2.5</td>
<td>2.5</td>
<td>15</td>
</tr>
<tr>
<td>11</td>
<td>30</td>
<td>F</td>
<td>Metastatic melanoma</td>
<td>2.5</td>
<td>2.5</td>
<td>15</td>
</tr>
<tr>
<td>12</td>
<td>45</td>
<td>F</td>
<td>Colon</td>
<td>3.0</td>
<td>2.5</td>
<td>5</td>
</tr>
</tbody>
</table>

| 13      | 65       | F   | Colon                      | 3.0                      | 2.5                      | 5            |
| 13A     | 38       | M   | Metastatic melanoma        | 3.0                      | 3.0                      | 3            |

| 14      | 44       | F   | Metastatic breast          | 3.0                      | 2.5                      | 3            |
| 15      | 51       | F   | Metastatic breast          | 4.0                      | 2.5                      | 3            |
| 16      | 55       | F   | Metastatic colon           | 4.0                      | 3.0                      | 3            |
| 17      | 36       | F   | Neurofibrosarcoma          | 4.0                      | 3.0                      | 3            |

using a more rapid dose escalation program in 4 patients, 3 of whom were fully recovered at more than 4 weeks after completing Phases A and B (Studies 1 to 3) and one new patient (Study 4), beginning with 5 megaunits/sq m and increasing each subsequent injection by 5 megaunits/sq m (5, 10, 15, 20, 25, 30, and 35 megaunits/sq m) on a schedule of 3 times per week up to the maximum tolerated dose (Table 2). This was primarily a toxicological study to determine the maximum tolerated dose of rapid escalation.

**Study Plan.** After initial clinical and laboratory evaluation, patients were monitored according to a closely scheduled protocol. All patients were hospitalized on the Clinical Cancer Research Unit for Phase A of the study and during such times in Phase B when they were uncomfortable enough to request hospitalization or were in need of medical supervision. Interferon injections were always given at 7 a.m. when the patients were in the hospital and between 8 and 9 a.m. when they were outpatients. Following interferon injection in Phase A, vital signs were measured hourly for the initial 12 hr and then every 2 hr 12 times for 24 hr. Outpatients were instructed to record temperatures every 2 hr for 6 times following each interferon injection (Monday, Wednesday, Friday) and list all side effects. During the outpatient phase (B) of the study, the nurse clinician evaluated the patient 3 times a week, before giving the injection, and one of the oncologists evaluated the patient on a weekly basis.

**Blood Counts and Blood Chemistry.** Automated blood counts were performed, and reticulocyte counts and differential leukocyte counts were performed manually. Routine blood chemistries including electrolytes, liver function tests, creatinine, blood urea nitrogen, and protein measurements were performed in the Central Laboratories.

**NK Cell Studies.** Heparinized blood was separated according to the method of Boyum (2) and depleted of monocytes by adherence to plastic. The resultant mononuclear cell preparation contained <1% monocytes as determined with peroxidase and nonspecific esterase stains. NK assays against the human erythromyeloid leukemia cell line K562 (23) were performed as described previously (19). All assays were conducted in triplicate at effector:target cell ratios of 2.5:1 to 20:1. The S.E. is equal to or less than 5%. To minimize variability, all NK assays were performed with frozen K562 tumor cells (in dimethyl sulfoxide at −70°), using a programmed freezer (Cryo-Med. Model 700). Such cells have a susceptibility to NK lysis approximately equal to that of fresh target cells. In order to distinguish between assay variability and biological effects, mononuclear cells from one normal individual were frozen and stored in aliquots, and for each test performed with fresh patient cells, frozen cells from the control donor were used as a baseline. The variability of control effector cells in the NK assays against frozen K562 target cells did not exceed 10%. To determine the preinterferon, base-line activity of each patient, blood samples were drawn at 72 hr and at 1 hr prior to the first interferon injection and immediately tested for NK levels against K562 target cells. The interval from blood acquisition to assay did not exceed 2 hr.

**Monocyte Effector Function.** Forty ml of heparinized venous blood were separated using lymphocyte separation medium (Litton Bionetics, Kensington, Md.), the mononuclear leukocytes were washed twice using

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4 H. Koren, unpublished observation.
Monocyte chemotaxis to \(10^{-9}\), \(5 \times 10^{-9}\), or \(10^{-8}\) M \(N\)-formyl-N-methylleucylphenylalanine or to 1 or 3% zymosan-activated \(N\)-formylmethionylphenylalanine was performed as described by Pike et al. (26), except that 48-well microchemotactic chambers were used. Each well contained \(5 \times 10^4\) monocytes (50 \(\mu\)), and chemoattractants were tested in triplicate. Chemotactic response (cells migrated per 10 oil immersion fields) was expressed as the highest obtained response to any of the chemoattractants tested.

Monocyte polarization to \(10^{-9}\) or \(10^{-8}\) M \(N\)-formyl-N-methylleucylphenylalanine was performed as described by Clanciolo and Snyderman (5). Each of duplicate tubes contained 60 \(\mu\)l of buffer or chemotactic and 240 \(\mu\)l of cell suspension (1.88 \(\times 10^5\) monocytes).

Monocyte phagocytosis was performed as described by Pike et al. (26). One-half ml of cell suspension (4 \(\times 10^5\) monocytes) was added to each of triplicate 2.0-sq cm wells in a 24-well tissue culture plate, the (26). One-half ml of cell suspension (4 \(\times 10^5\) monocytes). Every 30 min prior to the injection of interferon served to determine endogenous (background) interferon activity. Immediately after clotting, serum was separated from the clot by centrifugation and stored frozen at —20° until assayed.

Interferon assays were performed using VERO cells by microtiter assay as described by Henderson and Joklik (13). Minimal detectable activity by this assay was 20 units/ml. The assay reference interferon was the same material (Wellferon) used in the clinical trial.

Table 2: Supplementary interferon clinical trials

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient</th>
<th>Age (yr)</th>
<th>Cancer</th>
<th>Dose (mega-units/sq m, single injection)</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>44</td>
<td>Breast</td>
<td>5.0</td>
<td>Fever, chills, severe headache, lethargy, malaise, fatigue, myalgia, arthralgia, anorexia, weight loss, nausea and vomiting, dry mouth, loss of memory, light-headed, dizziness, diaphoresis, depression, fear, pain at injection site, abdominal cramping</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>51</td>
<td>Breast</td>
<td>5.0</td>
<td>Severe chills, headache, fever, myalgia, arthralgia, fatigue, malaise, anorexia, depression, paresthesias, chest pains, alopecia</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>31</td>
<td>Melanoma</td>
<td>2.5</td>
<td>Chills, arthralgia, headache, myalgia, malaise, fatigue, feeling of disorientation, anorexia, diarrhea, paranoid ideation, fear, lightheadedness, depression, paresthesias</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>46</td>
<td>Multiple myeloma</td>
<td>1.0</td>
<td>Mild chills, myalgia, arthralgia, severe nausea and vomiting, anorexia, fever, hot flashes, diaphoresis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Nonspecific Measures of Immunity. Serum concentrations of IgG, IgA, IgM, and IgD were measured in relation to age-, race-, and sex-matched controls (3) in order to deduce the percentile ranking of the values detected in each patient. The quantitative Mantoux-type skin tests were measured at 0.25, 6, 24, 48, and 72 hr and evaluated in relation to observations in responder patients and normal subjects. Epicutaneous tests with histamine and mecholyl were done as described by Buckley et al. (4).

In order to deduce the effect of interferon on nonspecific attributes of immune function, all observations were evaluated by multivariate analysis of variance for longitudinal dose-dependent trends in relation to appropriate cohort and time-dependent effects.
A second set of antiviral assays was carried out on 3 patients receiving doses of interferon in the range of 20 to 30 x 10^6 units/sq m. These assays, performed by the interferon manufacturer, used microtiter evaluation of cytopathogenic effects produced by vesicular stomatitis virus on human foreskin fibroblasts. A NIH interferon reference standard was used to standardize the assays.

Data Management and Analysis. The research protocol required 1006 separate clinical and laboratory results for each of the 17 patients in the major portion of the study (Phases A and B). Data collection was coordinated by a nurse clinician and analyzed using the on-line data management system of the Duke Comprehensive Cancer Center, the Time-Oriented Record for Oncology (6).

RESULTS

Clinical Studies. Seventeen of 19 patients completed Phases A and B of the study; supplementary studies were then done on 4 patients using a more rapid dose escalation schedule. One patient was dropped from the study during the initial evaluation in Phase A when it was discovered that he had major bone involvement with tumor, requiring palliative radiation therapy. Another (patient 13) was dropped from the study midway through Phase B because of an episode of aphasia and hemiparesis. This patient had a history of numerous transient cerebral ischemic attacks, although none in the year prior to this study. At the time of her stroke, she was receiving low doses of interferon and experiencing few side effects; careful evaluation at another hospital showed occlusive changes in cerebral arteries (angiography) and no evidence of organ toxicity. The episode was thought to be unrelated to the interferon. She recovered completely but was not studied further. Both of these patients completed the pharmacokinetic studies of Phase A, and those data were used. The remaining patients completed the course of study. The age, sex, and type of tumor are listed for each patient in Tables 1 and 2, together with the dosages and schedule of interferon. The major toxicities encountered during chronic drug administration (Phase B) were the constitutional findings of fever, chills, fatigue, anorexia, malaise, myalgia, and weight loss. These are summarized in Table 3. (The toxicity seen in Phase A was less dramatic, except for fever and chills, but qualitatively so similar that it is therefore not presented.) Constitutional findings clearly represented the dose-limiting toxicity, which in this escalation program was 15 megaunits/sq m; one of the 5 patients who received this dose in the final week of the 5-week escalation schedule in Phase B just managed to complete her course of treatment by willing herself not to "quit" prematurely. Unconvinced that the subjective symptoms which patients had experienced at 15 megaunits/sq m could be justified as an end point in a toxicity study, this willing patient received 2 additional doses of 18 and 23 megaunits/sq m according to the same injection schedule. At that point, she refused further treatment because of lethargy, malaise, fatigue, myalgia, numbness of the fingers, aversion to the smell and taste of food, and severe aching in her buttock and legs.

The small supplementary study used a more rapid dose escalation scheme (5, 10, 15, 20, megaunits/sq m, etc.). Two patients tolerated 30 megaunits/sq m, and 2 tolerated 35 megaunits/sq m according to the same injection schedule. At that point, she refused further treatment because of lethargy, malaise, fatigue, myalgia, numbness of the fingers, aversion to the smell and taste of food, and severe aching in her buttock and legs.
fear that one additional dose would be fatal; they found it difficult to be more precise in this description. Two patients also experienced neurological changes including disorientation, difficulty in calculating, and paranoid ideation.

The maximum fever following the initial dose of interferon was related to the dose ($p < 0.005$). Tolerance to fever and chills occurred during the chronic phase of interferon administration like that reported previously by Priestman (27). However, during the 5 weeks of the Monday, Wednesday, and Friday schedule, the highest temperature of the week was invariably on Monday; the fever was lower on Wednesday and Friday (data not shown). The other constitutional symptoms did not abate over the course of time and in fact exerted a cumulative fatiguing effect.

The ratio of all blood counts before and after chronic administration of interferon is provided in Chart 1. The administration of the interferon produced a reduction in segmented neutrophils and monocytes ($p = 0.0001$ and $0.0064$, respectively). A small reduction was observed in platelets and lymphocytes ($p = 0.025$ and $0.072$, respectively). Slightly lower hemoglobins and hematocrits were attributed to blood drawing throughout the study. Despite the development of anemia, only one-half of the patients were able to mount a reticulocyte response during the course of interferon, although anemia did recover after cessation of interferon therapy.

The (absolute) lymphocyte counts consistently followed a well-defined pattern in Phase A. After injection of interferon, lymphocyte counts decreased steadily with nadir occurring at 6, 8, or 12 hr; thereafter, they recovered to preinjection levels by 48 hr. In Phase B, lymphocyte counts were checked prior to the next dose; the values remained near the prestudy levels with a small decrease over time.

**Effect of Lymphoblastoid Interferon on Erythropoiesis and Granulopoiesis.** The effects of interferon on bone marrow cultures were consistent with the suppressive effect on the formed elements of the blood. *In vitro* erythropoiesis and granulopoiesis were studied in all 17 patients who completed the study. Four bone marrow aspirations were performed on each patient: before interferon administration; 24 hr after the single injection of Phase A; 14 days after this injection (just before the Phase B period of the study); and on Day 50 at the conclusion of chronic interferon administration. As illustrated in Table 4, 14 days after a single injection of interferon (Phase A), a significant reduction in colony growth was noted in granulopoiesis (64% of pretreatment level) and erythropoiesis (45% for CFU-E growth and 54% for BFU-E growth). A smaller decline in granulopoiesis (28%) growth was observed at Day 50. In erythropoiesis, a further decline to 42% in CFU-E and 23% in BFU-E growth was noted on Day 50. The depression in growth of colonies was noted in all patients, although the magnitude was not statistically correlated with dose. Cellularity of 4 marrow aspirates (biopsy not performed) in each of these patients did not show any significant change throughout interferon treatment.

From these findings, we can conclude that interferon inhibits the colony growth of bone marrow precursor cells at 14 days following a single injection and 36 days (*i.e.*, Day 50 of the entire course of interferon administration) following chronic administration.

**Chemistries.** There were no major abnormalities in blood chemistries except for hyperchloremia and hypernatremia at higher doses, which were associated with anorexia and dehydration. Minor elevations of aspartate aminotransferase and lactate dehydrogenase occurred in 3 patients.

**NK Cell Studies.** In Phase A, assays were performed before and after the single dose of interferon. The 2 control (preinterferon) samples generally varied by <10%. Every patient showed a marked drop in NK activity at 12 hr; NK activity recovered by 24 or 48 hr and was stimulated above pretreatment values in 10 of 16 patients at 48 hr. We could not determine a dose-response curve after a single injection (Chart 2). With chronic administration of interferon, NK activity appeared to be related to the dose. Higher doses stimulated more NK activity by the end of the first week of administration, but this effect was lost thereafter, and

![Table 4](chart.png)
by the end of Weeks 4 and 5, patients receiving high doses had significantly less NK activity than did those receiving lower doses (Chart 2). Identical results were obtained regardless of whether the NK data were analyzed based on the mean NK volume for all 17 patients at -72 hr, as shown in Chart 2, or if analyzed for each patient based on individual baseline levels at the -72-hr time point (data not shown).

Lymphocyte Counts and NK Activity. The U-shaped pattern of initial drop and subsequent recovery applied to both lymphocyte counts and NK activity in Phase A. Moreover, profiles of lymphocyte counts and NK activity in Phase B were similar, although not parallel, for 8 patients (i.e., Patients 3, 4, 5, 7, 8, 9, 14, and 15).

Monocyte Function Studies. The results of the monocyte function assays are summarized in Table 5. Most patients were assayed twice before treatment and 8 times during treatment. There were no significant differences in any of the functions during the course of therapy, although the percentage of patient chemotaxis values which were below normal increased (from 19 to 32%), and the percentage of superoxide anion values which were above normal decreased (from 50 to 32%).

Nonspecific Measures of Immunity. No significant differences in nonspecific cutaneous sensitivity and responsiveness to methacholine and histamine, recall antigen responses, or serum levels of IgG, IgA, IgM, IgD, C1Q, C3, C4, and Factor B were detected during the course of interferon treatment.

Serum Interferon Concentrations. Chart 3A depicts the highest serum concentration of interferon and the time at which it was observed in each of the 19 patients who received the initial injection in Phase A. No detectable activity was found in the serum at any time in those patients who received 0.1 or 0.5 \( \times 10^6 \) units/sq m of body surface area. One patient who received 1.0 \( \times 10^6 \) units/sq m body surface area and one who received 2.5 \( \times 10^6 \) units/sq m had detectable activity at any point following the injection. None of the 8 patients receiving doses up to and including 2.0 \( \times 10^6 \) units/sq m had detectable activity in the 12-hr postinjection sample. The time at which the peak interferon activity was observed varied widely from patient to patient, and at times, no true peak value could be identified (data not shown). In general, the maximum concentration was observed in the sample drawn at the fourth hr. There was a correlation between dose and maximum serum concentration, although the linear regression coefficient of 0.58 reflected the wide scatter in the data. The serum concentrations observed in this study are in good agreement with those reported previously for human leukocyte interferon (11, 12), recombinant \( \alpha \)-interferon (11), and human lymphoblastoid interferon (27).

The serum concentrations of 3 patients who received higher doses of interferon in supplementary studies (20 to 30 \( \times 10^6 \) units/sq m body surface area) are shown in Chart 3B. For these patients, serum interferon activity was assayed independently in 2 laboratories, the first (Duke) using VERO cells as targets and the second (Burroughs Wellcome and Co.) using human foreskin fibroblasts. There was close concordance between the 2 laboratories, and such differences as existed were thought to be within the limits of each assay. The averaged result of the 2 assays is depicted in Chart 3B. The maximum serum interferon concentration in patients who received 20 to 30 \( \times 10^6 \) units/sq m was approximately 10 times that of the patients who received 2 to 3 \( \times 10^6 \) units/sq m. As in the patients receiving lower doses of interferon, peak serum concentrations were observed 4 to 5 hr following injection. There was then a rapid decline in activity to approximately the 8-hr postinjection point, after which levels dropped very slowly. At 24 hr, each of the 3 patients had >150 units/ml of activity remaining, and one patient who received 30 \( \times 10^6 \) units/sq m had 150 units/ml of activity persisting at 52 hr after injection.

**DISCUSSION**

This study describes the clinical, pharmacological, and immune responses of patients with incurable cancer who were observed during single and multiple injections of human lymphoblastoid interferon. Despite many reports of small series of cases treated by human leukocyte interferon, we do not yet have a clear picture of its toxic effects, particularly on the immune system. Nor do
we have dose-response data for any toxic or immune effects with which to make judgments about dosage for future clinical trials. In the absence of prior experience with human lymphoblastoid interferon, the question of which components of the immune system to study posed a major dilemma in protocol design, hence our decision to use a broad series of immune tests in this early phase of trials with this interesting substance. It is hoped that the information gained will be useful for the extensive clinical testing which this interferon preparation will undergo.

The toxic effects of interferon are generally clear and reproducible, although tolerance for any given dose may differ in different patients. Beyond a certain threshold (1 megaunit/sq m), all patients develop fever with the first exposure to interferon and, in general, the higher the dose, the greater the fever and its attendant chills and malaise. (The remarkable reproducibility of fever and of the constitutional symptoms is highly reminiscent of influenza.) The constitutional findings were similar to those reported by Priestman using human lymphoblastoid interferon with a different dose schedule (27). The maximum chronic dose administered by Priestman was 7.5 megaunits/sq m, and he suggested that 2.5 to 5 megaunits/sq m would be suitable for chronic administration. Like Priestman, we found that, upon repeated administration, a tolerance developed to the febrile response. However, we noted that on a 5-week Monday, Wednesday, and Friday schedule, while the general trend was toward tolerance, after each Monday dose, the temperature was higher than after the previous Friday injection, suggesting that the extra day of rest was sufficient to begin to erode tolerance. The implication is that injection of interferon only twice weekly might cause more problems with the febrile response. Our patients were denied the use of antipyretic agents during Phase A; during Phase B, they were allowed to use acetaminophen when fever exceeded 102°. It is clear that antipyretics can block the febrile response to interferon; what is not clear is whether they also modify the antitumor or the immune effects of interferon. Further studies are planned to answer this question.

We found that with repeated administration of interferon pancytopenia resulted, and it was generally dose related. However, as observed by Priestman, the effects were usually not progressive despite rather high doses of interferon. This series of patients had normal blood counts at the beginning of the study, and none had received extensive prior radiation; more serious reactions might occur in patients who had severe myelophthisic anemia. The cytopenias apply to erythroid, myeloid, and platelet lines and are attributable to an inhibition of the bone marrow. One measure of this is the failure to raise an appropriate reticulocyte response in patients who are becoming progressively anemic; another is the inhibition of erythroid and granulocytic progenitor cells seen in bone marrow cultures from patients during the course of interferon treatment. It has been reported that interferon therapy may result in neutropenia, lymphopenia, and thrombocytopenia (27). Human leukocyte interferon has also been reported to have inhibitory effects on the GMFCF in vitro at concentrations as low as 10 units/ml (30). Clearly, bone marrow evaluation for cellularity and growth capacity is essential for determining toxicity and biological response. All of these inhibitory responses are reversible, as are the constitutional signs and symptoms.

We did not find any major abnormality of liver, kidney, or coagulation function, and the principal chemical changes seen were associated with dehydration.

With our escalation schedule, the maximum dose which could be tolerated was 15 megaunits/sq m or a total of 135 megaunits/sq m over 5 weeks. At the highest doses, the changes in cognitive and behavioral function (Table 2) are quite disturbing to patients and families; fortunately, they were fully reversible within 7 to 10 days after cessation of interferon. Given the symptomatic nature of many of the untoward effects, it was...
surprising that virtually all of the patients managed to reach approximately the same total dose (135 megaunits/sq m) when it was given using this schedule. Higher single doses (30 to 35 megaunits/sq m) were given with a more rapid dose escalation schedule, but the total dose was virtually identical. Again, it was remarkable that the maximum tolerated dose was approximately the same for all the patients taking that escalation course.

The toxic effects of new agents are generally determined by the degree of cytopenia or specific organ toxicity. It is very unusual to do a Phase I study in which the end point depends upon the patient telling the doctor when he or she has had enough. The doctors and nurses participating in this study were impressed by the commitment that these patients had to continue interferon for as long as they possibly could; yet with continued escalation over a 5-week period, the patients all reached a point when it became clear that they could take no more. This point was associated with severe depression, malaise, and a sense of impending doom. They became uncommunicative and disinterested in life but found it difficult to express their concerns. Within 2 to 3 weeks after discontinuation, the patients returned to their pretreatment status, pointing to the possibility of reinitiating courses of interferon at lower than maximum tolerated doses.

Because of expectations based on prior animal and in vitro data, it was somewhat disappointing that our evaluations of the effects of interferon on the immune system were largely negative with the exception of the NK results. Monocyte functions, immunoglobulin levels, complement levels, and measures of skin reactivity all failed to show any major alteration in patients treated with 5-week courses of interferon.

Stimulation of NK activity has been observed previously with interferon in vivo and in vitro (10, 14). Our work indicates that human lymphoblastoid interferon also stimulates NK activity in cancer patients. The initial response to a single dose of interferon was a drop in NK activity, beginning within a matter of hours and reaching a nadir at 12 hr; this is in accordance with other findings (8). The depressed NK activity 12 hr after the administration of interferon may not be entirely due to suppressor cells but could also be due in part to a transient depletion of lymphoid cells (20). This possibility is currently being tested by using a panel of monoclonal antibodies. NK activity generally returned to or exceeded base line within 24 to 48 hr and remained elevated for 1 week or more after a single injection. With injections 3 times per week, NK cell activity was stimulated by the end of the first week. During the course of 5 weeks, NK activity in some cases remained at the maximum level and in others gradually drifted down to or below base-line levels. Analyses of the data suggest that there is a dose-response relationship; the maximum stimulation achievable by the end of the first week is greater for patients receiving higher doses of interferon, but patients who receive lower doses are able to sustain this stimulation, whereas those who receive higher doses are not. Interestingly, very low doses such as 0.5 megaunit/sq m appear to be maximally efficient; this is important because these doses are very well tolerated, presumably for indefinite periods of time, and because the expense of interferon makes low doses a very attractive option. Interferon seemed to have very similar effects with this group of patients on antibody-dependent cellular cytoxicity against tumor target cells (20). The fact that the dose responses for the direct antineoplastic and immunostimulatory (e.g., NK activity) effects of interferon may vary should receive special attention and consideration in future studies. It will require larger clinical trials, comparing low- and high-dose interferon administration, to approach these important issues. It is noteworthy that Einhorn et al. (8) have reported recently that daily injections of human leukocyte-derived α-interferon stimulate NK activity in patients with multiple myeloma; however, over the course of 1 year, their lowest dose of 1.5 × 10^6 units gave greater stimulation than did 3 × 10^6, which in turn was more effective than was 6 × 10^6. Our own Phase II studies will avoid the use of high doses of interferon for reasons of both toxicity and NK activity, which are important considerations in longer therapeutic trials.

Although interferon has been shown to enhance both the phagocytic activity of macrophages in vivo and in vitro (7, 16) and the cytolytic activity of blood monocytes in vitro (15, 18), comprehensive studies of monocyte function in patients receiving interferon therapy have been undertaken only recently, and the results reported are usually of a preliminary nature. Neefe et al. (25), reporting on a Phase I trial of human leukocyte interferon involving 25 patients, noted that some patients had increased monocyte-mediated cytotoxicity at Day 3. In another recent abstract, Maluish et al. (24) stated that some of their 100 patients showed a consistent and sustained elevation of monocyte function as measured by a growth inhibition assay. Territo et al. (29) have reported in vivo activation of monocyte function, including chemotaxis, in patients receiving interferon treatment. Recently, Einhorn and Jarstrand (9) have reported that daily i.m. injections of α-interferon decrease the phagocytic capacity of peripheral monocytes for yeast particles by up to approximately 30%.

We observed no effect of interferon therapy on the 4 monocyte functions studied: chemotaxis and polarization to chemotactic stimuli; phagocytosis of opsonized sheep erythrocytes; and the ability to generate superoxide anion. Since phagocytosis was measured using an adhered monocyte population, it is possible that any stimulation or inhibition of phagocytic function by interferon was negated by a corresponding decrease or increase in adherence of the cells, although we consider this unlikely. We did not systematically examine the ability of the monocytes to kill tumor targets, although preliminary data which will be followed up in Phase II studies suggest that tumor killing by monocytes was modulated by interferon administration. It has been shown that monocyte chemotactic responsiveness is depressed in cancer patients and that the reversal of this depressed response by surgery or immunotherapy is indicative of a good prognosis (28). Thus, the lack of stimulation of chemotaxis or polarization by interferon treatment in our study is somewhat disappointing. Although some of our patients occasionally had a value elevated above base-line by more than the level of variation seen in normal controls, these elevations were transient and never sustained to the next sampling time. In future studies, care will have to be taken to differentiate between such transient, perhaps insignificant, episodes of activation and sustained periods of activation.

It is difficult to avoid commenting on the measurement of antitumor effects of interferon, even though these were incidental to this basic study, and the period of treatment and follow-up was too brief to use standard criteria. The effects seemed real but modest, with disease stabilization (no change in tumor size) being noted in 13 of 14 patients with measurable disease and one lengthy partial remission (>50% regression of s.c. nodules) occurring in a patient with metastatic melanoma. Since many of these patients were not observed by us prior to entry into this
study, we cannot interpret the frequency with which rapidly progressive (versus indolent) tumor remained in a stable state. Although a conjecture, we suspect that patients with large and bulky tumors are less likely to be favorably affected by interferon when given alone, suggesting not only that interferon might be more efficient in combination but also that its greatest usefulness will probably be with patients who may be incurable by other means but who have a more limited amount of disease.

ACKNOWLEDGMENTS

We are grateful to Sheri Dorenkamp for blood drawing, enumeration of cells, and differential counting; to the nurses on the Jordan Cancer Research Unit; and to Evelyn Morgan, R.N., and Patricia Deery who assisted with portions of this study.

REFERENCES

Phase I Study of Pharmacological and Immunological Effects of Human Lymphoblastoid Interferon Given to Patients with Cancer


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