Phase I Trial of Subcutaneous Interleukin 3 in Patients with Refractory Malignancy: Hematological, Immunological, and Pharmacodynamic Findings

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ABSTRACT

We conducted a Phase I trial of s.c. recombinant human interleukin 3 (rhIL-3) to evaluate the toxicity, maximal tolerated dose, pharmacokinetics, and in vivo biological effects of this cytokine. Thirty-one patients with refractory cancer were entered into the study between November 1991 and June 1993. Therapy consisted of s.c. rhIL-3 daily for 15 days administered to cohorts of three to nine patients at dose levels of 60–4000 μg/m²/day. Cycles were repeated at intervals of 28 days.

Seventy-five cycles of rhIL-3 were administered (median, two per patient) and the maximal tolerated dose was 2000 μg/m²/day. Toxicity was moderate, with most patients developing chills, fever, and myalgia. Dose-limiting toxicity consisted of diarrhea (two patients) and headache (one patient). Hematological effects of rhIL-3 included significant dose-related increases of WBC (P < 0.001), neutrophils (P < 0.001), and eosinophils (P < 0.001). Platelet counts and absolute lymphocyte numbers also increased. Various CD3+ lymphocyte subsets increased; however, lytic activity (natural killer and lymphokine-activated killer) of peripheral blood lymphocytes was not enhanced. Serum levels of the soluble IL-2 receptor increased in a dose-related fashion, and IL-2-induced lymphocyte proliferation also was increased variably. Pharmacokinetic studies were performed in 13 patients, and area under the curve and maximal concentration values increased with increasing rhIL-3 dose levels (P < 0.001) and correlated with maximal changes from baseline in WBC, neutrophils, and eosinophils. rhIL-3 antibodies were detected in 8% of patients by day 29 of cycle 1 but were not neutralizing.

rhIL-3 is well tolerated when administered s.c. and has reproducible hematological and immunological effects. The pleiotropic effects of this cytokine on various in vivo biological parameters were demonstrated clearly. Further studies of its immunoregulatory effects are warranted.

INTRODUCTION

IL-3 is one of a family of glycoproteins that are responsible for regulation of hematopoietic and immunological functions (1). It was described originally as a factor found in conditioned media from mitogen-stimulated splenic lymphocytes that promoted induction of 20α-hydroxysteroid dehydrogenase in spleen cells from athymic mice (2). Subsequently, it has been shown to have multiple functions and to affect the activities of a wide variety of cell types.

IL-3 promotes the survival and proliferation of early multipotential erythroid and myeloid hematopoietic cells (3, 4), with activity broader than most colony-stimulating factors (5). The cDNA of IL-3 has been isolated from human lymphocytes and expressed in yeast (6) and Escherichia coli (7). Sufficient quantities of rhIL-3 are now available, and clinical trials with this cytokine in patients with bone marrow failure and chemotherapy-induced myelosuppression are under way.

In addition to these activities, rhIL-3 has been reported to support the growth of murine helper T cells (8) and to promote IL-2-related proliferation of human T lymphocytes (9) and the proliferation of cloned human T cells (10). Cannistra et al. (11) noted that human monocytes incubated with rhIL-3 developed enhanced endotoxin-associated cytotoxicity, and enhanced tumor necrosis factor-α gene expression and secretion. Additionally, Thomassen et al. (12) reported that rhIL-3 modulated the functions of human mononuclear phagocytes both in vivo and in vitro.

In view of the data indicating that rhIL-3 has pleiotropic activities and may enhance potential antitumor effector mechanisms, a Phase I clinical trial to investigate the clinical, immunological, and pharmacodynamic effects of this cytokine in patients with refractory cancer was performed. The results of this trial demonstrate significant hematological effects as well as...
Table 1  Phase I trial of IL-3: study schema and patient entry

<table>
<thead>
<tr>
<th>Dose level</th>
<th>rhIL-3 (µg/m²/day s.c.)</th>
<th>Patients entered</th>
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<tr>
<td>I</td>
<td>60.0</td>
<td>3</td>
</tr>
<tr>
<td>II</td>
<td>125.0</td>
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<td>9</td>
</tr>
<tr>
<td>VII</td>
<td>4000.0</td>
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</tbody>
</table>

pleiotropic activities on various mononuclear cell effector populations.

PATIENTS AND METHODS

Patients. Patients ≥18 years of age with refractory cancer and evaluable or measurable disease, having Eastern Cooperative Oncology Group performance statuses of 0 and 1 were eligible. Prior chemotherapy (one regimen), immunotherapy (one regimen, excluding rhIL-3), hormonal therapy, or radiotherapy were allowed and must have been completed >21 days prior to study entry (28 days for radiation and 42 days for mitomycin C- or nitrosourea-containing regimens). Exclusion criteria included any of the following: requirement for corticosteroids, pregnant female, positive studies for HIV or hepatitis, active infection, significant effusions or ascites, central nervous system metastases, or a history of asthma requiring ongoing treatment. Additionally, adequate cardiac function (New York Heart Association class ≤2) was required.

Adequate hematological, renal, pulmonary, and hepatic function were required and were defined as follows: hemoglobin, ≥9.5 g/dl; WBC, >3500/µl; granulocytes, ≥1500/µl; platelets, ≥100,000/µl; prothrombin and partial thromboplastin times, within normal institutional limits; serum creatinine, ≤1.5 mg/dl; bilirubin, ≤1.5 mg/dl; carbon monoxide diffusing capacity, ≥50% predicted; and forced expired volume in 1 s and forced vital capacity, ≥80% predicted. The protocol was activated following Institutional Review Board approval, and informed consent was obtained from each patient in accordance with institutional and federal guidelines.

rhIL-3. Nonglycosylated rhIL-3 was obtained from Sanofi Pharmaceuticals Corp. and supplied by the National Cancer Institute. The specific activity of the rhIL-3 was 1.8–2.2 × 10⁶ units/mg protein. Vials containing 250 or 750 µg rhIL-3 were reconstituted with 1 ml nonbacteriostatic sterile water and administered immediately after preparation.

Study Design. This was an open label, Phase I trial of s.c.-administered rhIL-3. The cytokine was administered by study nurses to cohorts of three to nine patients on days 1–15, with escalation of dose levels from 60 to 4000 µg/m²/day (Table 1). Patients were observed for 6–8 h after the first dose of rhIL-3. Cycles were repeated at intervals of 28 days in stable or responding patients. Pretreatment studies to define eligibility were performed within 14 days of registration, with the exception of selected radiological examinations or end organ function studies not required for tumor assessment (performed within 28 days). The schema outlined in Table 1 was used, and cohorts of patients received increasing dose levels of rhIL-3 until the MTD was determined. Dose escalation was permitted when all patients within a cohort completed cycle 1. This dose was defined as one dose level below which three of six patients experienced grade III or IV toxicity (National Cancer Institute toxicity criteria), excluding fever. Once the MTD was determined, an additional six patients were treated at this level to characterize toxicity further.

Dose modifications of rhIL-3 were permitted for grade III toxicity. A dose reduction of 50% was required, and therapy was held until there was a return to grade I or less. If the identical toxicity recurred, or grade IV toxicity developed, treatment was terminated. Dose modifications for leukocytosis and/or thrombocytosis were also used. For WBC counts ≥100,000/µl or platelets ≥600,000/µl, rhIL-3 was held and re instituted with a 50% dose reduction when the levels returned to ≤50,000 and/or <600,000/µl, respectively.

Clinical Study Parameters. Complete blood counts with differential counts and biochemical studies were obtained before therapy, three times a week during rhIL-3 administration, and on days 15, 21, and 29 of each cycle. The biochemical studies included creatinine, blood urea nitrogen, sodium, potassium, chloride, albumin, total bilirubin, aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase, calcium, phosphorus, glucose, uric acid, carbon dioxide, and total protein. Prothrombin and partial thromboplastin times were obtained at baseline and on days 5 and 12 of cycle 1. Toxicity was assessed daily during cycle 1, and vital signs (blood pressure, pulse, and temperature) and physical examinations were performed once weekly. National Cancer Institute clinical toxicity criteria were used to grade side effects, and standard response criteria were used (13). Bone marrow aspirations and biopsies were obtained before treatment and on day 11. Finally, tumor measurements in patients with objectively measurable disease were performed every 4 weeks.

Peripheral Blood Immunoeytometry. Three-color immunoeytometric analysis of PBLs was performed two to three times prior to therapy and on days 4, 11, and 29 of cycle 1, as described previously (14). FITC, phycoerythrin, and peridinin chlorophyll protein-conjugated monoclonal antibodies were used to identify and quantitate lymphocytic subsets, which included CD2, CD3, CD4, CD8, CD11a, CD11b, CD14, CD25, CD16c, CD34, CD45RA, CD45RB, CD45RO, CD56, Leu8, and HLA-DR (Becton Dickinson, San Jose, CA; Coulter, Hialeah, FL; and Gentrac, Plymouth Meeting, PA), phenotypically. Isotypic controls for each particular subclass of immunoglobulin were used to allow for the most accurate delineation of positive and autofluorescent populations and to control for nonspecific binding by a particular subclass of immunoglobulin compared with the autofluorescent background. Live gating of the forward and orthogonal scatter channels, as determined by fluorescence (CD45 "CD14"+) back gating, was used to acquire events for lymphocytes selectively.

Immunohistology. Patients with accessible tumors underwent biopsy prior to study entry and, if possible, again on day 4 or 11 of cycle 1. MHC expression and effector cell phenotyping within the tumor bed were performed by immunohistology as described previously (15). Baseline pretreatment and follow-up biopsy tissue was frozen rapidly in isopentane,
precooled to −130°C in liquid nitrogen, and stored at −70°C until sectioning. Mouse monoclonal antibodies to the following determinants were incubated with the sections at dilutions optimized for tonsillar tissue sections: CD3, CD4, CD8, CD14, CD56, CD16b, HLA-DR, CD22 and HLA class I determinants. The chromogenic substrate 3-amino,9-ethylcarbazole H₂O₂ was used to visualize antigen localization. Equivalently diluted non-immune mouse IgG was used as a negative control. Results were expressed using a semiquantitative scoring system as reported previously (16), with an increase over baseline of at least 50% positive cellular staining considered significant.

Lymphocyte Assays. The proliferative response of PBLs was measured by the uptake of [³H]thymidine prior to therapy and on days 4, 11, and 29. PBLs were isolated using ficoll-hypaque gradients from peripheral blood collected in sodium heparin, and platelets were removed with a fetal bovine serum gradient. The PBLs were cultured at a density of 5 x 10⁶ cells/well in U-bottom, 96-well plates in triplicate and stimulated with the following: (a) RPMI medium; (b) 10, 50, 250, and 1000 units/ml IL-2 (Hoffmann-LaRoche Inc., Nutley, NJ); and (c) 10 µg/ml phytohemagglutinin. At 48 h, cells were pulsed with 1 mCi [³H]thymidine (6.7 Ci/mmol; DuPont New England Nuclear, Boston, MA) and harvested 24 h later using a MASH harvester (Cambridge Technology Inc., Watertown, MA). Filters were added to scintillation fluid (Ecocrin; National Diagnostic, Manville, NJ) and counted.

The cytolytic activity of PBLs was studied prior to therapy and on days 4, 11, and 29 of cycle 1. A 4-h ⁵¹Cr release assay as described previously (14) was used to assess NK and LAK activity. Cytolytic activity was expressed as lytic units per 10⁶ mononuclear cells tested as determined from linear regression analysis of dose-response curves, in which the natural log of the number of effector cells was plotted against the percentage specific lysis. One lytic unit was defined as the number of effector cells required to produce 15% specific lysis of 5.0 x 10⁵ target cells in a 4-h period. NK and LAK activity were determined versus the K562 and Daudi cell lines, respectively.

Pharmacokinetic Studies. Pharmacokinetic studies were performed in one to two patients, who were selected at random, per dose level. Venous blood samples were collected in 10-ml vacutainer tubes containing sodium heparin and placed on ice, and plasma was collected for determination of IL-3 levels. Samples were obtained before cycle 1 of rhIL-3 and at 15, 30, 60, 120, 240, 360, and 1440 min on day 1 and before each rhIL-3 injection on days 3–5, 8–12, and 15. Thirteen patients were studied, including two at each dose level, except for the cohort receiving 1000 µg/m²/day (one patient). IL-3 concentrations were determined using an ELISA. Ninety-six-well plates were coated overnight with 2 µg/ml of an anti-IL-3 monoclonal antibody (designated F15-371-12; Sandoz Pharmaceuticals) at 4°C. Uncoated sites were blocked by adding TBS buffer containing 1% BSA at room temperature for 2 h. After washing, 100 µl/well IL-3 standards, quality control samples, and unknown serum samples were added in triplicate to the coated microtiter wells. The plates were incubated for 1 h at 37°C and washed with TBS buffer containing 0.05% Tween 20. A biotin-labeled monoclonal anti-IL-3 recognizing a different epitope than that recognized by the first antibody (biotinylated F14-746-1-16; Sandoz Pharmaceuticals) was added, and the plates were incubated for 1 h at 37°C. After washing, streptavidin conjugated to alkaline phosphatase (Jackson ImmunoResearch Laboratories, West Grove, PA) was added, and the plates were incubated for 1 h at 37°C. The enzyme substrate nitrophenyl phosphate (Kirkegaard and Perry Laboratories, Gaithersburg, MD) was added after washing the plates, and the absorbance of the colored product was measured on a Microtiter Plate Reader (Molecular Devices, Sunnyvale, CA). The concentration of IL-3 was calculated from the standard curve using a four-parameter logistic curve fit. The lower level of detection in normal human serum is 80 pg/ml. The pharmacokinetic characteristics of s.c. rhIL-3 were derived by using noncompartmental methods. The Cmax was obtained from the concentration/time data, as was Tmax of its occurrence. The AUC was calculated by the trapezoidal rule over one dosing interval for s.c. rhIL-3. The apparent CL was calculated as CL (ml/min/kg) = rhIL-3 dose × 10⁻³/AUC × 60. The elimination rate constant (Kel) was derived from linear regression analysis, and the corresponding elimination t½ was calculated as log₂/elimination rate constant.

IL-3 Antibodies. The presence of IL-3 antibodies in sera was investigated prior to therapy and on days 4, 11, and 29 of cycle 1. These time points were chosen for consistency to obtain these studies in all patients. Antibody formation was assessed by using a series of assays. First sera were screened with an ELISA as follows. Microtiter plates (Maxisorp; Nunc, Roskilde, Denmark) were coated with 100 µl 5 µg/ml rhIL-3, covered with plate sealers (Dynatech, Inc., Chantilly, VA), and incubated overnight at 4°C. The plates were washed three times with 300 µl wash buffer (TBS and 0.05% Tween 20), and 200 µl blocking buffer (TBS and 1% BSA) were then added. The plates were incubated for 1 h at 37°C and washed again three times. One hundred microliters of positive and negative antibody serum controls and the unknown serum samples were diluted 1:100 in serum diluent buffer (PBS, 1% BSA, and 0.05% Tween 20) and then added to the plates in triplicate. Plates were ressealed and incubated for 1 h at 37°C. After washing three times, 100 µl alkaline phosphatase-conjugated, goat antihuman IgG, IgM, and IgA (H+L; Kirkegaard and Perry Laboratories) were then added, and plates were ressealed and incubated at 37°C for 1 h. After washing three times, 100 µl substrate solution (p-nitrophenyl phosphate in diethanolamine) were added to each well. The plates were incubated at room temperature for 15–25 min and measured on the UMax Microtiter Plate Reader at 405–650 nm, and the screening absorbance was reported. If positive (A, ≥0.363), the serum sample was retested in the split-plate ELISA. The split-plate ELISA served to estimate the antibody titers. A similar approach was used in this assay, except half of the microtiter plates were coated with 100 µl buffer, and the other half were coated with 100 µl 5 µg/ml rhIL-3. Threefold serial dilutions (starting at 1:100 dilution) were performed on antibody controls and serum samples. These serial dilutions were performed identically on both the rhIL-3 coated and noncoated sides of the plates. The plates were then measured on the UMax Microtiter Plate Reader at 405–650 nm. In the positive antibody controls, A of ≥0.800 was required. A specific absorbance for each serum sample was determined by subtracting the absorbance of the well not containing rhIL-3 from the well coated with this cytokine. Samples were considered positive if the specific A was
Samples positive in the split-plate assay were assessed for neutralizing activity. rhIL-3 (5 units/ml) was incubated with increasing concentrations of the serum sample for 1 h at 37°C in a microtiter plate. A positive goat antiserum was included in each assay. M-07e cells were added to give a final concentration of 2 × 10^5 cells/ml. The assay mixture, in a total volume of 200 μl/well, was incubated for 72 h at 37°C in a 10% CO2 humidified incubator and pulsed with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Promega, Madison, WI) for a final 5 h to evaluate the proliferation of the M-07e cells. A solubilization solution was then added before the absorbance of the wells was determined. A serum sample was considered positive if it inhibited the proliferative response to 5 units/ml IL-3 by at least 50%, and the highest percentage inhibition was twice the percentage inhibition of the pretreatment serum sample at the equivalent dilution.

Miscellaneous Studies. Plasma and total histamine levels were assessed prior to therapy and on days 4, 11, 15, and 29. Blood was collected in EDTA and placed immediately on ice, and plasma samples were collected, aliquoted, and frozen. Total histamine levels were measured in whole blood collected in sodium heparin, which was lysed by dilution in distilled water followed by two freeze-thaw cycles. Histamine levels were determined (sensitivity, 0.2 nm) using a RIA kit (AMAC, Inc., Westbrook, ME).

Serum sIL-2R levels were determined prior to treatment and on days 4, 11, and 29. The samples were batched and frozen at −70°C, and sIL-2R levels (sensitivity, 50 units/ml) were determined using an enzyme immunoassay kit (T-Cell Diagnostics, Cambridge, MA). Similarly, IL-6 serum levels were determined prior to therapy and on days 4 and 11 in 19 patients. Immunoreactive IL-6 levels (sensitivity, 0.35 pg/ml) were measured with an enzyme immunoassay kit (R&D Systems, Inc., Minneapolis, MN).

Statistical Analysis. Two-sided P values were computed for the absolute and percentage changes from baseline to selected on-study days for the various hematological and immunological variables. In some instances, the dates of laboratory tests were shifted by 1 day to coincide with the time points analyzed. The methods used to compute the P values were the Wilcoxon signed rank test (17) for an overall change (increasing or decreasing) from baseline (raw or percentage changes) and the Jonckheere-Terpstra test (18) for a monotone-increasing or -decreasing dose effect in the change from baseline. Spearman rank correlation coefficients (19) were used to assess the correlation between the pharmacokinetic and hematological parameters.

RESULTS

Thirty-one patients were registered on this trial, and all were eligible. Entry by dose level is outlined in Table 1. The median patient age was 64 (range, 32–76) years, and the male:female ratio was 19:12. Patients had a variety of tumor types, including 13 colorectal, 9 melanoma, 3 renal cancer, 2 pancreatic, 1 soft tissue sarcoma, 1 mesothelioma, 1 peripancreatic tumor, and 1 unknown primary site. Thirteen (42%) of the patients had an Eastern Cooperative Oncology Group performance status of 0, and the remainder had a status of 1. The majority of the patients (74%) had received prior systemic therapy (chemotherapy with or without other, 21 patients; immunotherapy with or without other, 3 patients; and radiation with or without other, 9 patients).

Two of 31 patients had minimal tumor regression during rhIL-3 therapy, although not sufficient to be termed a partial response. Both individuals had melanoma and cutaneous lymph node disease. In a third patient with malignant melanoma, a decrease in lactate dehydrogenase was seen without changes in the sizes of measurable metastases.

The toxicity of rhIL-3 was moderate and dose related, and the MTD was determined to be 2000 μg/m² s.c. day 1–15. The frequency of selected toxicities at higher dose levels (≥500 μg/m²) of rhIL-3 is outlined in Table 2. Dose-limiting (grade III) side effects developed in three of six patients receiving 4000 μg/m² rhIL-3. Severe diarrhea unresponsive to diphenoxylate and atropine occurred in two patients and cleared within 24–48 h when rhIL-3 was held. In one patient, severe headaches unresponsive to analgesics or propranolol developed. Seventy-five cycles of rhIL-3 were administered, with a median of two (range, one through seven) cycles per patient. Toxicity was moderate at most dose levels of rhIL-3, and dose modification...
Fig. 1. Peripheral blood levels of WBC, neutrophils, lymphocytes, monocytes, eosinophils, and platelets in patients receiving rhIL-3. Patients are separated into those receiving lower doses (60–500 μg/m²/day, □) and higher doses (1000–4000 μg/m²/day, △) of rh-IL-3.

during cycle 1 was required in 6 of 31 patients. Reasons for dose modification included thrombocytosis in one patient (dose level V), nausea, vomiting, and grade III alkaline phosphatase elevation related to disease progression in two patients (dose level VI), grade III headaches in two patients, and diarrhea in a third treated at dose level VII. More than 90% of patients developed grade I or II fatigue and fever and chills, with two patients having temperature elevations ≥40°C. Anorexia (77%) and myalgia (81%) were likewise common and resulted in a typical flulike syndrome in most patients.

Headaches were seen in 68% of patients and were severe in one patient receiving 4000 μg/m² rhIL-3. These were relieved by acetaminophen in patients at dose levels I–III and acetaminophen with or without propranolol in patients receiving higher doses. Facial flushing occurred in 84% of patients and generally appeared 2–4 h after rhIL-3 administration. Forty-eight percent of patients also developed conjunctival injections, which cleared following discontinuation of the cytokine.

Cardiovascular toxicity was mild. Hypotension was uncommon; it developed in one patient (250 μg/m² rhIL-3) and was transient and mild. Edema was seen in 39% of patients, was also mild, and resolved when rhIL-3 was discontinued.

Gastrointestinal toxicity included anorexia (77%), nausea and vomiting (71%), and diarrhea (52%). The nausea was mild and responded to symptomatic therapy with prochlorperazine. Severe diarrhea was seen in three patients and in one instance was unrelated to rhIL-3. One patient developed gastrointestinal bleeding unrelated to therapy. Hepatic toxicity was also seen
Table 3  Pharmacokinetic parameters of s.c. rhIL-3

<table>
<thead>
<tr>
<th>Dose rhIL-3 (µg/m²/day s.c.)</th>
<th>60</th>
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<th>1000</th>
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<th>4000</th>
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<tr>
<td>No. patients studied</td>
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<td>2</td>
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<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mean AUC ± SE (ng/h/ml)</td>
<td>4.76 ± 1.15</td>
<td>16.0 ± 1.01</td>
<td>29.05 ± 2.53</td>
<td>43.39 ± 16.08</td>
<td>126.45</td>
<td>154.58 ± 28.06</td>
<td>635.64 ± 169.0</td>
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<tr>
<td>Mean C_max ± SE (ng/ml)</td>
<td>0.847 ± 0.29</td>
<td>3.375 ± 0.03</td>
<td>5.77 ± 0.03</td>
<td>7.37 ± 2.56</td>
<td>31.48</td>
<td>33.03 ± 2.56</td>
<td>120.0 ± 14.0</td>
</tr>
<tr>
<td>Mean T_max ± SE (hr)</td>
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<td>3.0 ± 1.0</td>
<td>3.0 ± 1.0</td>
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<td>8.0</td>
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</tr>
<tr>
<td>Mean CL/f ± SE (ml/min/kg)</td>
<td>4.6 ± 1.12</td>
<td>2.61 ± 0.17</td>
<td>2.89 ± 0.25</td>
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<td>4.46 ± 0.81</td>
<td>2.25 ± 0.60</td>
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**Fig. 2**  Peripheral blood levels of selected mononuclear cell subsets during rhIL-3 determined by flow cytometry. Populations include total CD3+, CD4+, CD3+/CD25+/HLADR+, and CD14+/CD45RB+/HLADR+. Patients are divided into those receiving lower doses (60–500 µg/m²/day, □) and higher doses (1000–4000 µg/kg/day, △) of rhIL-3.

and was generally mild. In a single patient (dose level V), mild elevation of bilirubin (2.2 mg/dl) developed, which resolved during continued rhIL-3 administration.

Metabolic abnormalities that developed included hyperglycemia [18 (58%) of 31 patients] and hypocalcemia [22 (71%) of 31 patients] and were generally mild to moderate in severity. When calcium levels were corrected for albumin concentrations (20), the frequency of hypocalcemia was only in 3 of 31 patients, which may reflect hypoalbuminemia secondary to cytokine administration. Miscellaneous toxicities included insomnia (32%), dizziness (29%), cutaneous toxicity characterized as localized erythema and stinging at the injection site (26%), visual changes (6%), paresthesias (3%), urticaria (3%), and mild creatinine elevation (3%). Finally, confusion, which appeared secondary to concomitant medication, was noted in three patients during rhIL-3 administration.

Selected hematological data are summarized in Fig. 1. Increases from baseline to day 15 in total WBC ($P < 0.001$), neutrophils ($P < 0.001$), and eosinophils ($P < 0.001$) related to increasing rhIL-3 doses were seen. Platelet and absolute lymphocyte counts also increased significantly (both $P < 0.001$) but were not related to the dose of rhIL-3. Bone marrow specimens were obtained in 23 of 31 patients before therapy and again on days 9–11 of rhIL-3. Increased cellularity during therapy was noted in 14 of 23 specimens and seemed more common at dose levels V–VII (8 of 10 patients) compared with I–IV (6 of 13 patients). Differential counts did not demonstrate consistent changes.

Pharmacokinetic studies were performed in 13 of 31 patients, and the various pharmacokinetic parameters are summarized in Table 3. Pretreatment IL-3 plasma levels were below the level of detection in 10 of 13 patients. On days 2–15, preinjection plasma IL-3 levels generally remained undetectable (4 of 6 patients) at the lower dose levels (60, 125, and 250 µg/m²/day), whereas at higher levels (500, 1000, 2000, and 4000 µg/m²/day) increases were noted in 7 of 7 patients at various time points.
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Dose I (pt 2) (60 μg/m²)

Pre Day 4 Day 11 Day 29

Dose II (pt 5) (125 μg/m²)

Pre Day 4 Day 11 Day 29

Dose VI (pt 18) (2000 μg/m²)

Pre Day 4 Day 11 Day 29

Fig. 3 PBL proliferative response at 72 h to varying concentrations of rIL-2 (0, 10, 50, 250, and 1000 units/ml) in vitro during rhIL-3 treatment (before and days 4, 11, and 29). Phytohemagglutinin (10 μg/ml) was used as the positive control (data not shown).

Both the Cₘₐₓ (P < 0.001) and AUC (P < 0.001) increased with increasing rhIL-3 dose levels, and the AUC:dose ratio was comparable over the dose levels administered. The T₁/₂ of rhIL-3 was estimable in only three patients because of limited sampling times. Values of 3.44, 5.18, and 23.86 h were found. The Tₘₐₓ varied from 2 to 8 (median, 3) h. The CL values varied from 2.5 to 4.5 ml/min/kg and were similar at all dose levels. Neither AUC nor Cₘₐₓ values were found to be related to liver function abnormalities or calculated creatinine CL.

The relationships between various hematological changes and the AUC or Cₘₐₓ of rhIL-3 were also investigated. The maximal change from baseline (days 2-15) for absolute numbers of WBC (r = 0.84; P < 0.001), neutrophils (r = 0.78; P = 0.002), and eosinophils (r = 0.97; P < 0.001) were correlated highly with the AUC values. Likewise, Cₘₐₓ was also correlated with the maximum change from baseline (days 2-15) for absolute numbers of total WBC (r = 0.82; P < 0.001), neutrophils (r = 0.76; P = 0.003), and eosinophils (r = 0.94; P < 0.001). In contrast, no significant correlations with changes in platelet levels were found.

Immunocytometry of peripheral blood mononuclear cells was performed in 30 of 31 patients during rhIL-3 treatment. Panlymphocytosis was noted, with significant increases from baseline values at day 11 in the CD3⁺ (P = 0.006), CD56⁺ (P < 0.001), and CD19⁺ (P < 0.001) subsets found. Examination of the CD3⁺ subsets revealed significant increases in CD3⁻/CD25⁻/HLADR⁺ (P = 0.002) and CD3⁺/CD25⁻/HLADR⁺ (P < 0.001) subsets, with the increases in the CD3⁺/CD25⁻/HLADR⁺ subset (P = 0.01) also related to increasing rhIL-3 dose levels (P = 0.015). CD45RB⁺/CD14⁺/HLADR⁺ cells (monocytes) were also increased from baseline (P < 0.001) in a dose-dependent fashion (P < 0.001). Changes in selected subsets during rhIL-3 therapy are illustrated in Fig. 2. Finally, CD34⁺ mononuclear cells were also studied, but no significant changes were detected.

PBL cytolytic activity, including LAK and NK activity, was studied serially in 19 of 31 patients. LAK activity was not detected, and no consistent or significant increases in NK activity were seen (data not shown). The proliferative responses of PBLs to IL-2 from patients receiving rhIL-3 were examined in vitro in 27 of 31 patients. IL-2-induced proliferation seemed to be increased variably during and following rhIL-3 administration in approximately 61% of patients studied. This was most apparent at lower dose levels of rhIL-3 (≤1000 μg/m²) and was seen with either low (10 units/ml) or high (1000 units/ml) in vitro concentrations of rIL-2. Fig. 3 illustrates these findings in a selected group of patients. The impact that IL-3 had on serum levels of sIL-2R was also investigated. Elevated serum concentrations of sIL-2R were seen with all doses of rhIL-3 administered, and data are summarized in Fig. 4. The greatest change from baseline occurred on day 11 of cycle 1, and significant dose-dependent increases compared with baseline (P < 0.001) were present. Levels of sIL-2R seemed to plateau at dose level V or VI.

As expected from its biological activity in vitro, IL-3 also induced increases in proteins associated with the inflammatory response. Serum concentrations of IL-6 increased during therapy, but in a more variable manner. Increased levels were seen in the majority of patients studied but were not related to the rhIL-3 dose. On day 11, the mean serum IL-6 levels were 21.3 ± 5.8 pg/ml compared with baseline values of 6.7 ± 1.6 pg/ml (P = 0.006). Plasma and total blood histamine levels were also determined in 25 of 31 patients (Fig. 5). Interestingly, total histamine levels seemed to decrease on day 4 (P < 0.001). Variable elevations of plasma and total histamine content were seen, which were significant for the latter (P < 0.001) on day 15. No relationship to rhIL-3 dose levels could be demonstrated, however.

Twenty-five patients were assessed for the development of antibodies to rhIL-3. Eleven patients were identified as potentially positive with the screening assays, and using the split-plate
Fig. 4 Mean serum levels of sIL-2 receptor prior to and during rhIL-3 treatment expressed as units/ml ± SE. At dose level V, data on only one patient were available.

Fig. 5 Total blood histamine levels in patients receiving rhIL-3 (n = 25). Data are expressed as nM/ml at various time points before and during therapy.

method, 2 (8%) of 25 patients were identified as positive. One patient received 500 μg/m²/day, and the second received 4000 μg/m²/day. Both had antibody titers of 1:2700 on day 29 of cycle 1; however, the bioassay did not demonstrate the presence of neutralizing activity.

Finally, tumor biopsies were obtained in five patients prior to and during (day 4 or 11) rhIL-3 therapy, and in an additional patient during therapy (day 11). Immunohistological examination did not reveal alterations in mononuclear cell infiltrates or HLA class I or II expression on tumor cells. (data not shown)

DISCUSSION

The major objectives of this trial were to examine the toxicity of rhIL-3 in patients not receiving chemotherapy and, secondarily, to investigate the hematological and immunological effects of this cytokine. Our results demonstrate that s.c.-administered rhIL-3 is well tolerated at doses that exceed those administered previously. The MTD in our trial was 2000 μg/m²/day administered s.c. for 15 days. The major side effect seen was the development of a flulike syndrome seen commonly with recombinant cytokines. Additionally, hypocalcemia, the etiology of which is unclear, was noted in a significant proportion of patients. Dose-limiting effects included diarrhea and headaches. The etiologies of these toxicities are uncertain, but the elevations of total blood histamine content seen suggest a possible etiology. The accompanying facial flushing was also consistent with a histamine effect. Overall, significant elevations of basophils were not seen, but in selected patients, definite increases

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in this cell population occurred. Correlations in patients developing severe headaches, diarrhea, and/or flushing with histamine levels were, however, not apparent. Previous studies have demonstrated that various hematopoietic cytokines, including IL-3, can modulate the release of inflammatory mediators such as histamine from human basophils (21, 22). In a previous Phase I trial of rhIL-3, Lindemann et al. (23) did not find elevations of plasma histamine levels during s.c. administration of this cytokine. Okayama et al. (24) have noted, however, that IL-3 not only produces histamine release but also primes basophils and augments the actions of other agents such as anti-IgE antibodies. Previous in vivo studies of histamine release by human basophils (25) obtained from patients receiving various doses of rhlL-3 demonstrated increases in total basophil numbers, with an initial decrease in histamine content per basophil during the initial 5 days of therapy and a subsequent return to baseline. The results in the present study in which total blood histamine levels were measured may be consistent with these data. The initial decrease in total histamine levels and subsequent variable increases of circulating basophils and total blood histamine levels suggest that this as an explanation for the changes seen in our trial. Our findings address the histamine content of whole blood and not individual basophils.

Previous Phase I trials of rhIL-3 (23, 26–32) have used continuous i.v. infusion (4 or 24 h) or s.c. administration. Dose levels up to 16 µg/kg/day or 1000 µg/m² were administered, and variable toxicity profiles were noted. In the reports by Ganser et al. (26) and Biesma et al. (27), headaches refractory to acetaminophen occurred at doses of 500 µg/m² and 15 µg/kg/day, respectively. These were not reported by Kurzrock et al. (30), who administered up to 1000 µg/m² rhIL-3 daily as a 4-h infusion for 28 days. In the trial reported by D'Hondt et al. (31) in patients with small cell lung cancer, doses up to 10 µg/kg/day rhIL-3 were administered by continuous infusion without development of dose-limiting toxicity. In a multi-institutional study (32) of lymphoma patients undergoing autologous bone marrow transplant, doses of 5.0 and 10.0 µg/kg administered as 2-h infusions produced unacceptable toxicity, which was described as malaise, headache, and fever. The use of acetaminophen and propranolol in our patients seems to have permitted escalation to 4000 µg/m², with diarrhea then becoming dose limiting. The reasons for differences between the various trials in the observed toxicity profiles may relate to routes of rhIL-3 administration, different patient populations, or administration of rhIL-3 after chemotherapy.

This is the first study to assess antibody formation in patients receiving rhIL-3. The presence of serum antibodies was noted in a small number of patients (8%; 95% confidence interval, 1–26%) by the end of cycle 1; however, the bioassay did not demonstrate rhIL-3 neutralizing activity in these patients. Patients were not assessed after multiple cycles of therapy, and it is possible the frequency of antibody formation may increase with continued administration of rhIL-3.

The hematological effects of rhIL-3 in this trial were more dramatic than in previous reports with WBC ≥30,000/µl seen in 11 (73%) of 15 patients receiving ≥2000 µg/m²/day rhIL-3. The effects on platelets were less pronounced, with 8 of 22 patients treated with ≥500 µg/m² demonstrating thrombocytosis (platelets ≥600,000/µl). The dose-response relationship noted with rhIL-3 and WBC and neutrophil counts demonstrates rhIL-3 was directly responsible, and the absence of this relationship for platelet elevations suggests another mechanism such as secondary cytokine production may be involved. Previous reports have noted (23, 29, 31) increased serum levels of IL-6 during rhIL-3 administration, which were confirmed in the present study. IL-6 has significant effects on platelet counts (33) regularly producing thrombocytosis. The source of IL-6 may be secondary production by monocytes, as indicated in the study by Thomassen et al. (12), which demonstrated elevated levels of IL-6 secretion by monocytes from patients who were receiving rhIL-3. In the report by D’Hondt et al. (31), it was noted that day 15 platelet levels were correlated with the dose level of rhIL-3 administered to untreated cancer patients following infusion of the cytokine for 7 days. Reasons for the differences seen in our trial and in this report are not clear; however, the schedules of rhIL-3 administration were different.

A series of in vitro studies (10–12) have suggested that rhIL-3 also may have immunomodulatory effects on lymphocytes and mononuclear phagocytes. The present trial examined these effects in vivo and seems to confirm these findings. In a previous publication (12), increased cytokine production by monocytes from rhIL-3-treated patients was found in several patients. This was also reported by Maurer et al. (34), who noted enhanced secretion of IL-1β, IL-6, and tumor necrosis factor-α by lipopolysaccharide-stimulated monocytes obtained from IL-3-treated patients with myelodysplastic syndromes. In the present report, increased numbers of activated monocytes in peripheral blood were demonstrated, although no tissue or tumor infiltration of this cell population was noted.

The effects on PBLs are also of interest. Modest panlymphocytosis occurred, with no definite increase in cytolytic functions noted. Elevations of sIL-2R and the CD3+CD25+ lymphocyte subpopulations suggest expansion of an activated T-cell population. The variable enhancement of in vitro lymphocyte proliferation with rhIL-2 during rhIL-3 therapy is also consistent with these findings. The operative mechanisms are unclear, however. A report by Onishi et al. (10) noted down-regulation of the IL-2Rβ chain by rhIL-3 in vitro and no effect on IL-2Rα in cloned T cells. Nevertheless, rhIL-3 induced a proliferative response in the absence of IL-2. In our trial, the possibility that secondary cytokine secretion induced by rhIL-3 could be a factor exists; however, the clear dose-response relationship of rhIL-3 and levels of sIL-2R suggests that a direct effect on lymphocytes may be present.

The pharmacokinetics of rhIL-3 administered to patients not receiving chemotherapy were investigated in this trial. A linear relationship between the dose of rhIL-3 and the AUC or Cmax was demonstrated. CL seems to be independent of dose, with mean CL values similar for all dose levels. Limited data were available on the t1/2 of s.c. rhIL-3 in this trial. In previous reports (23, 35, 36), the t1/2 of s.c. rhIL-3 has varied from ~1.5 to more than 4 h at dose levels of 1.0–15.0 µg/kg, with peak serum levels reached at 2.8 ± 0.4 h (35). In the present trial, similar values were found at dose levels of 500 µg/m²/day.

Correlations of the pharmacokinetic parameters of rhIL-3 and its hematological effects were also investigated. Both the AUC and Cmax were correlated highly with the increases from baseline of total WBC, neutrophils, and eosinophils, in contrast
to increases in platelet counts. These findings are in contrast to those reported by Biesma et al. (36), in which no definitive dose-response relationship could be demonstrated at higher rhIL-3 doses (≥15 μg/kg/day). The differences observed may reflect variations in the patient populations treated.

The antitumor effects of rhIL-3, in view of its immunomodulatory actions, are also of interest. In our trial, two minor regressions of cutaneous disease were seen in the nine melanoma patients treated. In a recent study by Pulaski et al. (37), transfection of the IL-3 gene into line 1 (BALB/c alveolar lung cancer line) enhanced tumor rejection in syngeneic mice. Further studies suggested that IL-3 enhanced development of tumor infiltrating cytotoxic cells, and the antitumor effects required the presence of CD4+ cells. The antitumor responses in the present trial were brief, and tumor infiltration by lymphocytes was not observed. Enhancement of lymphocyte proliferation and elevated levels of various activated T-lymphocyte subsets were seen, however.

rhIL-3 is a well-tolerated cytokine that can be administered with acceptable toxicity at doses up to 2000 μg/m² to patients with cancer not receiving chemotherapy. Multilineage hematological effects were observed that were more impressive at the highest dose levels. The pharmacokinetics of rhIL-3 demonstrated the t1/2 and Cmax, were related to its hematological effects. Previous reports (29, 31, 32, 38, 39) also demonstrate the trilineage hematological effects of IL-3, with enhanced recovery from the myelosuppressive effects of chemotherapy. Randomized trials to demonstrate these effects conclusively are underway. Clear immunomodulatory effects were also seen in the present study, with alterations in both lymphocyte and monocyte numbers and function. Thus, in vivo studies demonstrate the pleiotropic nature of rhIL-3 clearly. It is unclear whether the immunoregulatory activities found are clinically significant and have a role in host-tumor cell interactions. The antitumor effects seen in this trial and the preclinical findings suggest that Phase II trials of this cytokine administered in combination with other cytokines (e.g., rhIL-6, rhIL-2, and rh-granulocyte-macrophage colony-stimulating factor) in cancers such as renal cell carcinoma and malignant melanoma may be of interest.

REFERENCES


22. Lindemann, A., Ganser, A., Herrmann, F., Frisch, J., Seipelt, G., Schulz, G., Hoelzer, D., and Mertelsmann, R. Biologic effects of re-


Phase I trial of subcutaneous interleukin 3 in patients with refractory malignancy: hematological, immunological, and pharmacodynamic findings.

R M Bukowski, T Olencki, H Gunn, et al.


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