Clinical and Immunological Effects of Recombinant Interleukin 2 Given by Repetitive Weekly Cycles to Patients with Cancer

Paul M. Sondel, Peter C. Kohler, Jacquelyn A. Hank, Karen H. Moore, Nancy S. Rosenthal, Jeff A. Sosman, Robin Bechhofer, and Barry Storer

ABSTRACT

Eleven patients received four consecutive weekly cycles of human recombinant interleukin 2 (IL-2) by continuous infusion for 4 days/week. Two dose levels were tested, 1 and 3 × 10^6 units/m²/day. Toxicities experienced by most patients included fever, rigors, fatigue, anemia, eosinophilia, and liver function abnormalities. All side effects from treatment reversed and no severe or life-threatening problems occurred. A marked lymphocytosis was seen following the 4 weeks of therapy. Fresh lymphocytes obtained during this lymphocytosis mediated enhanced destruction in vitro of a natural killer cell-resistant tumor cell line (Daudi). The increase in the absolute number of circulating lymphocytes and their enhanced ability to mediate direct lysis of Daudi targets resulted in a >100-fold mean increase in cytotoxic potential by the end of IL-2 treatment. One patient, with renal carcinoma, who was treated at 3 × 10^6 units/m²/day experienced a sustained measurable response with >50% regression of pulmonary and hepatic metastases. Five patients were retreated with a second course of IL-2, lasting 4 weeks. This therapy was well tolerated in four of these five patients, with similar immunological changes occurring. No further antitumor responses were seen in these patients. Thus, a relatively well tolerated immunotherapy regimen using IL-2 can induce dramatic increases in lymphocyte number and augment their in vitro antitumor reactivity.

INTRODUCTION

The therapeutic potential of interleukin 2 for the treatment of human cancer has been studied in murine models and in vitro with human lymphocytes (1–11). In vitro, IL-21 augments the activation of lymphocytes which mediate antigen-specific recognition of certain target cell populations and directly activates a separate population of lymphocytes that lyse tumor cells. The latter, termed “lymphokine-activated killer cells,” result from the culturing of peripheral blood lymphocytes or splenocytes in vitro with IL-2 (5). PBL activated in this manner have the capacity to lyse a wide range of malignant cells and virally transformed normal tissues with minimal destruction of normal tissue (6, 7).

The transfer of tumor reactive T-cells or LAK cells to tumor-bearing mice can be curative, provided that the tumor burden within the animal is not sufficiently great to "escape" immunemediated destruction and that the immune cells provided are able to maintain their function in vivo (8, 9). IL-2 given as part of such adoptive immunotherapy regimens can maintain the in vivo function of in vitro activated cells (10). In addition, IL-2 given alone has the capacity to activate an endogenous popu-

lalion of tumor specific T-lymphocytes or IL-2 responsive "LAK-like" cells present in vivo, resulting in an antitumor effect (11, 12).

Clinical trials have shown that antitumor responses can be attained in patients with disseminated malignancies using infusion of in vitro activated T-cells, combinations of LAK cells plus IL-2, or high doses of IL-2 alone (13–15). At the doses of IL-2 used in many of these studies, severe toxicity has resulted (15–17). While these toxic effects have been shown to be rapidly reversible with the discontinuation of therapy, intensive care unit support has often proved necessary (14–17). This level of toxicity has led some to question the applicability of this approach (18).

Our prior clinical studies have shown that, at doses of 1–3 × 10^6 units/m²/day, IL-2 given as a continuous infusion is tolerated with acceptable toxicity (not requiring vasopressors or intensive supportive care) for at least 4 days (19). Patients treated at these doses had a decrease in the number of circulating PBL and LAK cell precursors upon initiation of IL-2. Following completion of therapy, a rebound in the number of circulating PBL was documented (19). The magnitude of this rebound was dose dependent and the lymphocytes circulating were shown to bear markers of lymphocyte activation (Ia+ and OKT-10+). In addition, functional assessment of these lymphocytes demonstrated an enhancement of natural killer cell cytotoxicity and direct LAK activity (20).

We report here the results of a clinical trial administering repetitive doses of IL-2. This study is based on the findings that doses of IL-2 which could be tolerated for 4 days induced detectable immunological changes and that recovery from the side effects induced by IL-2 was prompt. We present the clinical tolerance, immunological effects, and antitumor activity from 11 patients who were treated with IL-2 at 1 and 3 × 10^6 units/m²/day by continuous i.v. infusion.

MATERIALS AND METHODS

Patients. From August 1986 through March 1987, 11 patients were enrolled in this phase I trial (NCI-BRMP protocol B85-013). All patients signed approved informed consent forms. Patients treated had locally recurrent or metastatic renal carcinoma or malignant melanoma which was incurable with surgery. Eligibility requirements included ECOG performance status of 0 or 1, normal hematological parameters, a serum creatinine of <2.0 mg/dl, alkaline phosphatase and serum transaminases of <2 times the upper limits of normal, and normal clotting parameters. In those patients who had had previous therapy, resolution of all toxicity was required. Ineligibility criteria included the presence of intracranial metastases and severe infection or the need to receive corticosteroids.

Recombinant Interleukin-2. Recombinant human IL-2 was provided through the National Cancer Institute-Biological Response Modifiers Program by Hoffmann-LaRoche Inc. (Nutley, NJ). The NCI-BRMP standard for unitage was used for all laboratory and clinical testing; the specific activity of the IL-2 used was 15 × 10^6 units/mg. IL-2 was provided in a lyophilized form and reconstituted daily with sterile...
normal saline. The IL-2 was given through a peripheral vein unless central access was available.

Study Design. Patients were scheduled to receive 4 consecutive cycles of IL-2 over 28 days. Each cycle consisted of 4 days of treatment with IL-2 given by continuous infusion, followed by 3 days of observation. The first 3 patients were treated at $1 \times 10^6$ units/m$^2$/day and the next 8 patients at $3 \times 10^6$ units/m$^2$/day. Patients were evaluated daily during treatment and at 1 and 4 weeks after completing the 28-day course of IL-2 treatment. All treatment was given on the general oncology unit of the University of Wisconsin Hospital.

Patients were evaluated for antitumor response 4 weeks after completing the IL-2 treatment, using the standard ECOG criteria (21). Those patients with evidence of response or stable disease were retreated with a second 4-week course of IL-2 given at the same dose and schedule as their first course, provided that they still met eligibility criteria.

In Vitro Immunological Testing. In vitro analyses of lymphocytes obtained from patients were performed utilizing the chromium release cytotoxicity assay described previously (7). In brief, fresh lymphocytes were harvested from peripheral blood, washed, and suspended in tissue culture medium containing 10% human serum. Fresh patients' peripheral blood lymphocytes were cultured in medium alone or with 200 units of IL-2 for 1 h prior to the addition of target cells (in equal volume), resulting in an IL-2 concentration of 100 units/ml during the $^{51}$Cr release assay. [We have previously shown that LAK cells generated in vitro in suboptimal concentrations of IL-2 corresponding to levels that can be maintained in vivo (unlike LAK cells induced with high concentrations of IL-2 in vitro) mediate significantly augmented killing when IL-2 is present during the $^{51}$Cr release cytotoxicity assay.4] $^{51}$Cr-labeled Daudi Burkitt's line target cells (American Type Culture Collection, Rockville, MD) were added with 4 separate concentrations of patients' cells into round-bottomed microwells. Radiolabel released into the supernatant was measured with results expressed as percentage of cytotoxicity of the indicated target cell, at 17.7 effectors/target cell or as lytic units/10$^7$ PBL (where 1 lytic unit causes 20% cytotoxicity) as previously described (22). The "cytotoxic potential" was calculated by multiplying the lytic units/10$^7$ PBL by the total lymphocyte count per ml of blood at the time the cells were obtained from the patient.

Flow cytometry analyses were performed on whole blood specimens, with red blood cells lysed by NH$_4$Cl and then gated for the lymphocyte fraction analysis using standard indirect immunofluorescent technique on an Ortho Cytofluorograph (Ortho Diagnostic Systems, Inc., Raritan, NJ). Antibodies OKT3, OKT10, OKIa, and OKB1, directed against T-cells, thymocytes, the HLA-DR molecule, and B-cells, respectively (23, 24), were obtained from Ortho Diagnostic Systems, Inc.; the Leu-11, Leu-19, and NK-1 monoclonals directed against NK cell determinants (25) were obtained from Becton Dickinson; the anti-TAC (directed against the IL-2 receptor) and the anti-NKH-1A (directed against an NK associated determinant) were gifts of Drs. T. Waldmann (26) and J. Ritz (27).

RESULTS

Patient Summary

Eleven patients were treated in this study, all of whom met eligibility requirements. Patient characteristics are summarized in Table 1. Six of the patients treated had metastatic or locally recurrent renal cell carcinoma, and 5 patients had metastatic malignant melanoma. The mean age was 47 years, 8 of the 11 patients were male, and 7 were ECOG performance status 1.

<table>
<thead>
<tr>
<th>Disease</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal cell carcinoma</td>
<td>6</td>
</tr>
<tr>
<td>Melanoma</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 1 Patient characteristics

Eleven patients received IL-2 in this protocol. The number of patients in each category is given. The mean age of all patients was 47 (range 29–62) years.

<table>
<thead>
<tr>
<th>No. of patients</th>
</tr>
</thead>
</table>

Clinical Toxicity

Listed in Table 2 are the clinical toxicities which occurred during therapy. Eight patients developed temperatures >39.5°C at some point during treatment. Acetaminophen (650 mg every 4 h) was given whenever temperatures rose to ≥38.5°C, with minimal change in the fever pattern. Rigors did not develop in any of the patients treated at $1 \times 10^6$ units/m$^2$/day but did occur in 6 of the 8 patients treated at $3 \times 10^6$ units/m$^2$/day. Meperidine provided prompt relief for these rigors. No patients received systemic corticosteroids, indomethacin, or other antiinflammatory/immunosuppressive agents.

Cutaneous reactions were common, occurring in 5 of the 8 patients treated at $3 \times 10^6$ units/m$^2$/day. As described above, patient 5 developed urticaria following the first 4-day cycle of IL-2. Furosemide had been given shortly before the development of the urticaria, and it was felt that sensitivity to this medication was possible. However, shortly after starting the second 4-day cycle of IL-2 treatment a widespread urticarial rash was noted. The IL-2 was stopped and diphenhydramine was given with resolution of the urticaria. A series of intradermal skin tests with escalating concentrations of IL-2 was negative with a positive histamine control. No further treatment with IL-2 was given to this patient.

Patient 7 developed significant abdominal bloating during his first 2 cycles of IL-2 at $3 \times 10^6$ units/m$^2$/day. An evaluation revealed no evidence of colonic obstruction or ascites. For the third and fourth cycles the IL-2 dose was reduced to $1 \times 10^6$ units/m$^2$/day and was tolerated without further problem.

Fluid retention occurred in all patients at both dose levels tested. Weight gain over the 28 days of treatment ranged from 3.2 to 9.1 kg. Six patients received furosemide in an attempt to

control this problem. No patient developed evidence of pulmonary edema or required assisted ventilation. Patient 7 did require supplemental oxygen during his initial treatment at 3 × 10^6 units/m^2/day. At the lower dose he had no respiratory complaints and oxygen was not used.

Six patients had intermittent episodes of systolic hypotension with blood pressures decreasing >20 mm Hg from pretreatment recordings. Evidence of decreased organ perfusion did not occur in any patient, and vasopressors were not required to treat hypotension. Upon completion of treatment the blood pressure returned to baseline levels within 24 h.

Nausea and vomiting controlled with antiemetics was seen at some point during treatment in 6 patients. Mild nonbloody diarrhea developed in 6 of the 11 patients and one additional patient had persistent diarrhea which required treatment with diphenoxylate hydrochloride and atropine sulfate. With completion of treatment all gastrointestinal problems resolved within 48 h.

Fatigue was common, with a decrease in performance status of 2 ECOG grades occurring in 6 patients. Although the performance level of all patients improved once treatment was completed, some level of fatigue persisted in most patients for 7–10 days after discharge.

One patient developed atrial fibrillation during treatment which required the temporary use of digoxin. No evidence of cardiac ischemia occurred in any patient during or after treatment. Neither confusion nor any other evidence of neurological change occurred in any of the patients treated.

**Laboratory Changes Associated with Treatment**

Changes in hematological parameters and serum chemistries during treatment are summarized in Table 3. A decrease in hemoglobin occurred in all patients during the 28 days of treatment, with 6 patients' values decreasing below 10 g/dl. The lowest hemoglobin noted was 7.1 g/dl. Two patients received packed red cell transfusions, while the remainder had a recovery in their hemoglobin following the completion of therapy. Neutropenia occurred in 3 patients. This developed during the first 28-day course of IL-2 in patients 7 and 8; both had a nadir neutrophil count of 610. Patient 10, whose neutropenia developed during a second 28-day course of IL-2, had a nadir neutrophil count of >10,000/μl at some point during therapy. The mean maximum for the 11 patients treated was 11,300 eosinophils/μl, and the most extreme value was 19,000/μl (the leukocyte count at that time was 26,700/μl). One patient with a history of asthma noted increased nasal congestion and wheezing which required more frequent use of his bronchodilator.

Table 2 Clinical toxicities occurring with IL-2

<table>
<thead>
<tr>
<th>Patient</th>
<th>IL-2 (units/m2/day)</th>
<th>Temp max. (°C)</th>
<th>Rigors</th>
<th>Rash</th>
<th>Weight gain (kg)</th>
<th>Hypotension (≥20 mmHg)</th>
<th>Gastrointestinal toxicity</th>
<th>Decline in performance</th>
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<tr>
<td>1</td>
<td>1 × 10^6</td>
<td>38.2</td>
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<td>0</td>
<td>3.2</td>
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<tr>
<td>2</td>
<td>1 × 10^6</td>
<td>38.5</td>
<td>0</td>
<td>0</td>
<td>4.8</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>3</td>
<td>1 × 10^6</td>
<td>38.0</td>
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<td>4</td>
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<td>39.6</td>
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<td>+</td>
<td>3.4</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>5</td>
<td>3 × 10^6</td>
<td>39.7</td>
<td>+</td>
<td>+</td>
<td>7.3</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>3 × 10^6</td>
<td>39.6</td>
<td>+</td>
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<td>3 × 10^6</td>
<td>39.8</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>8</td>
<td>3 × 10^6</td>
<td>39.8</td>
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<td>9</td>
<td>3 × 10^6</td>
<td>39.6</td>
<td>+</td>
<td>+</td>
<td>5.8</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>10</td>
<td>3 × 10^6</td>
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<tr>
<td>11</td>
<td>3 × 10^6</td>
<td>40.2</td>
<td>0</td>
<td>0</td>
<td>2.8</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

An increase in the eosinophil count was seen during the course of treatment in all patients; 6 patients had an eosinophil count of ≥10,000/μl at some point during therapy. The mean maximum for the 11 patients treated was 11,300 eosinophils/μl, and the most extreme value was 19,000/μl (the leukocyte count at that time was 26,700/μl). One patient with a history of asthma noted increased nasal congestion and wheezing which required more frequent use of his bronchodilator.

Unlike regimens utilizing “high-dose” IL-2 in which severe renal and hepatic dysfunction are routinely observed (13, 16), the highest bilirubin noted at any time in this study was 1.2, the highest creatinine was 1.9, and the highest blood urea nitrogen was 28. Increases in liver function tests (alkaline phosphatase and serum transaminases) were seen in the majority of patients during treatment. In all cases these abnormalities
were not clinically significant and reversed when treatment was completed.

Immunological Assessment

Lymphocyte Count. Fig. 1 shows the mean daily lymphocyte counts, by dose level, for the 8 patients who finished 4 cycles of IL-2 at their starting dose. Twenty-four hours after beginning each cycle of IL-2 treatment (days 2, 9, 16, and 23), a significant decrease in total lymphocytes occurred (P < 0.003 for each cycle). These lymphocyte nadirs showed a progressive increase for sequential cycles, the nadir in cycle 4 being significantly greater than the nadir in the first cycle (P < 0.001).

During each of the 4 separate 7-day cycles of IL-2 treatment, there was a significant rebound in lymphocyte counts 24 h after stopping the IL-2 (days 6, 13, 20, and 27), compared to the value just prior to starting IL-2 for that cycle (P < 0.002, 0.03, 0.006, 0.005 for cycles 1-4, respectively). Compared to pretreatment values (day 0), a significant (P < 0.003) rise in the total lymphocyte count was seen following the first 4 cycles of therapy (day 27). With the completion of each 4 days of treatment, the rebound lymphocyte counts showed a progressive increase. In the 9 patients completing the 4 cycles of IL-2, the mean lymphocyte count at day 27 was 5390 lymphocytes/mm³ higher than that recorded on day 6, 24 h after completing the first cycle of treatment (P < 0.006), and 6840 lymphocytes/mm³ greater than that obtained prior to starting IL-2 treatment (P < 0.003).

As seen in Fig. 1, the mean lymphocyte "rebound" values are higher for the 5 patients receiving 3 x 10⁶ units/m²/day than for the 3 patients receiving 1 x 10⁶ units/m²/day. However, the mean rebound values at 3 x 10⁶ units/m²/day were not significantly greater than those at 1 x 10⁶ units/m²/day, by statistical testing. In previous studies, when larger changes in IL-2 dose were tested, a significant IL-2 dose-related effect in lymphocyte rebound values was noted following a single 4–7-day cycle of IL-2 (19, 20).

Lymphocyte Phenotype. Table 4 shows the percentage and absolute number of circulating peripheral blood lymphocytes that express the indicated cell surface markers (as detected by monoclonal antibodies). Corresponding to the decrease seen in total lymphocyte count on day 2 (24 h after initiation of the first cycle), there was a significant drop in the number of lymphocytes bearing each of the 6 tested markers. However, the overall makeup of the lymphocyte population (based on the percentage of PBL positive for each marker) did not change except for a slight increase in the percentage of cells bearing the Ia marker.

Functional Assessment

Lymphocytes were obtained at multiple times during the course of treatment and tested in a chromium release assay (Table 5). Prior to initiating therapy with IL-2, patients' lymphocytes demonstrated minimal capacity to kill the Daudi target. Twenty-four h after the first 4-day cycle of treatment (day 6), lymphocytes tested against the same target cells showed a significant increase over the baseline level of cytotoxicity (8.2% versus 1.7%, P = 0.005, in the absence of IL-2, and 49.9% versus 6.2%, P = 0.0001, in the presence of IL-2). This level of increased cytotoxicity was maintained after each 4-day cycle. The mean percentage of cytotoxicity by PBL obtained 24 h after 4 cycles (day 27) was also significantly increased over pretreatment cytotoxicity (55.6% versus 6.2%, P = 0.0001, in the presence of IL-2, and 11.7% versus 1.7%, P = 0.0007, in the absence of IL-2). Although the values for mean percentage of cytotoxicity obtained on days 26, 27, and 28 are all greater than those obtained on the corresponding days after the first cycle (days 5, 6, and 7), these differences are not statistically significant. Following each cycle of IL-2, cytotoxicity by PBL obtained 24 h after stopping the IL-2 was slightly greater than by PBL obtained 48 h after stopping the IL-2 (P < 0.005).

While the measurement of percentage of cytotoxicity corre-
complications resulted. A bone marrow biopsy done at the time of 0/mm³ 21 days after starting the second course of IL-2 neutrophil count rose to 660 within 24 h and no infectious No further IL-2 was given. Upon stopping therapy her clinical and laboratory findings were similar for each subsequent 28-day course in 4 of these 5 patients. Lymphocyte counts for the first course (patient 7 received his second 28-day course of therapy were retreated after a 28-day rest period. Retreatment from 27 lytic units/ml on day 1 to 3473 lytic units/ml on day 27 (P = 0.002). When lysis was measured in IL-2, the cytotoxic potential rose from 1.9 to 409.9 (P = 0.025). These differences in lytic units between baseline and finishing 1 month of IL-2 are shown in Fig. 2. The cytotoxic Lytic units/10⁷ PBL Medium 1.9 21 Medium + IL-2 6.8 26 5.8 23.4 1.7 6.2 4.7 19.9 6.7 36.7 25.9 242.0 8.2 49.9 25.6 247.3 3.7 35.7 11.3 172.1 4.8 44.0 14.9 247.1 6.0 43.2 18.1 234.1 5.3 33.5 18.0 156.0 7.9 54.1 24.7 471.6 10.2 47.9 33.6 308.9 7.9 40.9 26.3 214.0 8.4 52.9 24.9 372.2 11.7 55.6 37.0 409.9 10.3 48.0 33.1 294.8 7.0 32.3 21.9 149.1 Table 5 Lysis of Daudi target cells by fresh PBL Lysis was measured by fresh PBL in a 51Cr release assay in medium alone or in a final concentration of 100 units of IL-2/ml (see text for statistical analysis). Retreatment Five of the 9 patients who completed the first 28-day course of therapy were retreated after a 28-day rest period. Retreatment was given at the same dose and schedule as was tolerated for the first course (patient 7 received his second 28-day course of IL-2 at 1 × 10⁶ units/m²/day). The spectrum and severity of IL-2 on lysis mediated by circulating lymphocytes can thus be demonstrated by calculation of the cytotoxic potential (the number of lytic units of cytotoxic activity per ml of whole blood). The mean values of cytotoxic potential for all 9 patients are shown in Table 5. The mean values for lytic units of cytotoxic potential, measured in lytic units per ml of whole blood. Mean values for the 9 patients finishing 1 month of IL-2 are plotted for lytic units measured in medium alone and lytic units measured in IL-2.

Fig. 2. Cytotoxic potential. The number of lytic units/10⁷ fresh PBL was determined at multiple times, as measured on Daudi target cells in medium alone or in IL-2 (Table 5). The mean values for lytic units/10⁷ PBL was then multiplied by the patient's absolute lymphocyte count (lymphocytes per ml of blood) in the same blood specimen. This yielded the parameter of circulating cytotoxic potential, measured in lytic units per ml of whole blood. Mean values for the 9 patients finishing 1 month of IL-2 are plotted for lytic units measured in medium alone and lytic units measured in IL-2.

Fig. 3. Lymphocyte counts during a second month of IL-2 treatment. Of the 11 patients receiving IL-2, 4 patients received and completed a second month of treatment, begun 28 days after completing the first (patients 2, 6, 7, and 8). The mean of daily lymphocyte counts for patients 2, 6, and 7 are plotted for the first (Course 1) and second (Course 2) month of IL-2 treatment; patient 8 was excluded from this analysis because a catheter-related bacterial infection (the only one for any of these 11 patients) delayed his IL-2 therapy for the third cycle of the second month by 2 days, thus moving his pattern of lymphocyte peaks and nadirs out of synchrony with the other 3 patients. The IL-2 dose used for patient 2 was 1 × 10⁶ units/m²/day for each cycle; the dose for patient 6 was 3 × 10⁶ units/m²/day for each cycle; and the dose for patient 7 was 3 × 10⁶ units/m²/day for the first and second cycle of treatment in the first month and 1 × 10⁶ units/m²/day for the last 2 weeks of the first month and the entire second month of IL-2 treatment.
of neutropenia showed all three cell lines to be present. Surface marker phenotyping of mononuclear cells from her marrow demonstrated a striking increase in the number of cells bearing Leu-11 (50%) and Leu-19 (39%).

**Clinical Response**

At the time of initiating IL-2 treatment, all patients had evidence of tumor progression. Two months after starting therapy (day 56), 6 patients (patients 2, 4, 5, 6, 7, and 10) had stable disease and patient 8, with metastatic renal carcinoma, showed a measurable partial response (>50% shrinkage of all tumor, measured in the lungs and liver). Of the 5 patients treated with a second course of IL-2, all but patients 2 and 8 showed tumor progression 4 months after starting the IL-2.

Patient 8, who showed a measurable response, received a total of 3 courses of IL-2, each course separated by 1 month of observation. No further shrinkage was noted after the second or the third course of IL-2, with minimal abnormalities seen using computerized tomography of the chest and abdomen. However, 1 month after completing the third 28-day course of treatment, a measurable increase in a single pulmonary nodule was noted, indicative of disease progression. IL-2 was then reinitiated at the same schedule, but at an increased dose of 10^7 units/m^2/day; this proved to be too toxic, with severe malaise and renal dysfunction (creatinine of 2.6 mg/dl) after 2 weekly 4-day cycles. Patient 2 showed stable disease for 6 months of observation after completing 2 months of IL-2 at 1 x 10^6 units/m^2/day. Pulmonary metastases from her renal carcinoma then progressed. He was retreated by the same schedule at a higher IL-2 dose (3 x 10^6 units/m^2/day). Treatment was tolerated well but no response was documented.

**DISCUSSION**

Abundant animal data as well as clinical studies have shown that IL-2 given alone can activate a population of immune cells with antitumor activity (28). The mechanism for this response to IL-2 is presumably through activation of cytotoxic LAK cells the action of which, unlike cytotoxic T-cells, is not restricted by the major histocompatibility antigens (29-32). The major problem which has been encountered in both animal and patient studies is the severe toxicity associated with high doses of IL-2. Whether it is necessary to use doses of IL-2 which result in severe toxicity to induce endogenous LAK activity was the major question addressed in this study.

The short circulating half-life of IL-2 following i.v. injection and the need to expose responsive cells to sustained levels of IL-2 for maximal activation led us to explore the use of continuous infusion. Our initial studies comparing 7 daily bolus injections to a 7-day continuous infusion showed that IL-2 given by continuous infusion, at clinically tolerable doses, could activate a population of lymphocytes with enhanced lytic capacity against NK-resistant targets (22). Although no antitumor responses were seen in those 25 patients, the significant immune changes occurring at doses of IL-2 > 10^6 units/m^2/day suggested that this approach might, if extended, provide a means of administering IL-2 in a clinically tolerable yet efficacious fashion (19, 20). Similar data have now been generated from other centers (33).

In this present trial, 4 consecutive weekly cycles of 4 days of IL-2 treatment were tolerated at the doses tested. During treatment, patients remained on the general oncology service and most spent the days off treatment out of the hospital. No patient required intensive care unit support or vasopressors, and none experienced life-threatening toxicity.

Marked increases in the number of activated, IL-2-dependent, circulating lymphocytes were seen following the completion of each cycle of therapy. The sequential cycles of therapy maintained and possibly augmented the lytic activity of IL-2-dependent cells following each cycle. Overall a >100-fold increase in the circulating cytotoxic potential of the expanded systemic lymphoid mass was induced in the 9 patients completing treatment by this 1-month regimen. One of these 9 patients had a measurable antitumor response. More recently, 2 other patients with renal carcinoma, of 11 treated similarly, have also had measurable partial responses (34).

During the 28-day observation period which followed the 4 cycles of IL-2, the number and function of circulating PBL returned to pretreatment levels. Further treatment at this time was tolerated and similar immunological changes occurred. It is unclear whether continued therapy beyond 4 weeks (without a 28-day observation period) would have resulted in further enhancement in immune function. This issue is now being studied.

Quantitation of the immune effects induced by IL-2 in vivo, as shown here, can enable stepwise modifications in IL-2 treatment regimens to augment these immune changes. Continued efforts to use these immunological and clinical results to design safe, yet more effective, regimens using IL-2 (alone or in combination with other agents) are needed if this form of therapy is to have applicability to the general oncology population (17, 34-36).

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