Repellent Weekly Cycles of Interleukin 2: Effect of Outpatient Treatment with a Lower Dose of Interleukin 2 on Non-Major Histocompatibility Complex-restricted Killer Activity


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ABSTRACT

Fifteen patients with advanced malignancy who had failed conventional therapy were entered into a protocol consisting of 1 inpatient mo of repetitive weekly cycles of interleukin 2 (IL-2) at 3 x 10^6 units/m2/day by constant infusion for the first 4 days of each week. This was followed by IL-2 administered on an outpatient basis at the same schedule but at a dose of 1 x 10^6 units/m2/day for the next 1 to 6 mo. Nine patients had renal carcinoma, four had melanoma, and two had lymphoma. Thirteen patients completed the induction month, and ten patients completed >1 mo of outpatient therapy.

Only one patient had therapy discontinued because of toxicity due to IL-2. No major toxicities occurred during outpatient therapy. After 1 mo of IL-2 at 3 x 10^6 units/m2/day, profound changes similar to those previously documented were seen in peripheral blood lymphocyte (PBL) counts (4.7-fold increase), lymphokine-activated killer activity (16-fold increase), and the percentage of PBL with natural killer-associated markers including a 3.6-fold increase in the percentage of PBL expressing the Leu 19 (NKH-1) marker, a 3.7-fold increase in Leu 11 (FcIgGR), and a 3.0-fold increase in Leu 17 (OKT10).

These indicators of IL-2 effect all remained elevated relative to the baseline at the end of 1 and 2 mo of outpatient therapy at the lower dose. However, lymphokine-activated killer activity and Leu 17 percentage were significantly reduced relative to the effect of the higher induction dose. PBL taken from patients while receiving maintenance therapy showed strong and rapid responses to IL-2 in vitro, confirming the in vivo effects of prolonged IL-2 treatment. Nevertheless, there were no complete or partial antitumor responses seen.

This study demonstrates that an immunologically active dose of IL-2 can be given long term as outpatient therapy with tolerable toxicity and complete or partial antitumor responses seen.

INTRODUCTION

The use of IL-2 in the treatment of advanced malignancies has received considerable investigation in the past few years. Demonstrable antitumor activity has been seen in advanced renal cancer, melanoma, and lymphoma (1–6). Although the initial responses were seen following a treatment combination of in vitro-cultured LAK cell infusions and in vivo high-dose IL-2 administration (1), responses have been noted with high-dose IL-2 alone (2, 3).

The mechanism of the antitumor effects following IL-2 treatment remains uncertain, but murine studies indicate a correlation between in vivo antitumor responses, LAK susceptibility of the tumor, and the degree of LAK activation (7). We and others have demonstrated that continuous infusion of IL-2 can generate considerable LAK activity in vivo (8). IL-2 treatment at intermediate doses can be given on a repetitive weekly basis with tolerable toxicity, and has not required the routine use of vasopressors or intensive care unit support (9). This regimen induces dramatic increases in lymphocyte number and in vitro antitumor reactivity (LAK activity), and this effect increases with both dose and duration of treatment (9).

In our previous study of 23 patients receiving 4 weekly cycles of IL-2 at 3 x 10^6 units/m2/day, by 3 different administration schedules, 3 patients showed partial responses with shrinkage of >50% of all measurable tumor (10). After the initial month of IL-2, all patients were observed without IL-2 for the next 28 days and showed a gradual decrease to near baseline of all parameters that had been activated by the initial 4 wk of IL-2 (lymphocyte count, LAK activity, lymphocytes expressing NK, or activation antigens). Seven of these patients were then retreated with a second 28-day course of IL-2 (4 repetitive weekly cycles) at the same dose as their initial month and showed clinical tolerance and immune activation that were nearly identical to those obtained with their first month of IL-2 (10). These data demonstrated that the immune activation that was achieved after the first month of IL-2 (“induction” treatment) was lost over the next 28-day observation period, but could be restimulated upon exposure to IL-2.

Although these results suggested that continuing IL-2 treatment at that same dose, rather than stopping after 4 wk, might continue to increase the state of immune activation, such doses are best tolerated only on an inpatient basis, and we (and our patients) desired to use treatment that could be safely administered at home. We therefore evaluated whether continuing repetitive weekly cycles of IL-2, at a lower dose that could be tolerated at home, would promote continued immune activation.

Fifteen patients were entered into this treatment protocol consisting of 1 mo of inpatient repetitive 4-day weekly cycles of IL-2 at 3 x 10^6 units/m2/day by constant infusion. This was followed by outpatient IL-2 administration using the same schedule, but at 1 x 10^6 units/m2/day for the next 1 to 6 mo, depending upon tumor status.

Our results suggest that outpatient IL-2 is well tolerated at 1 x 10^6 units/m2/day with continued immune activation at a level that is greater than baseline, but less than the peak value obtained after the initial induction month of IL-2.

MATERIALS AND METHODS

Patients.

From December 1987 to June 1988, 15 patients were enrolled onto this pilot study. Eligible patients had metastatic renal carcinoma or malignant melanoma, which was incurable with surgery, or non-Hodgkin's lymphoma, which had failed conventional therapy. Eligibility requirements included Karnofsky performance status ≥70%,
normal hematological parameters, a serum creatinine of <2.0 mg/dl, alkaline phosphatase and serum transaminases of less than twice the upper limits of normal, and normal clotting parameters. Treatment with cytotoxic agents within 4 wk, biological response modifiers within 4 wk, radiation therapy within 4 wk of entry onto this trial, or any prior IL-2 treatment was not permitted. Patients with serious infections and central nervous system metastases or those receiving corticosteroids, nonsteroidal antiinflammatory agents, or aspirin were ineligible. All patients signed consent forms approved by the University of Wisconsin Human Subjects Committee.

Recombinant Interleukin 2. Recombinant human IL-2 was provided by Hoffmann-LaRoche Inc. (Nutley, NJ). The National Cancer Institute-Biological Response Modifiers Program standard for unitage was used for all laboratory and clinical testing; the specific activity of the IL-2 used was 15 to 20 x 10^6 units/mg. IL-2 was provided in a lyophilized form and reconstituted with sterile normal saline solution. The IL-2 was given through a peripheral vein unless central access was available.

Study Design. Patients who entered received an induction course of four consecutive weekly cycles of IL-2 given over 28 days. Each cycle consisted of 4 days of treatment with IL-2 (3 x 10^6 units/m^2/day) given by continuous infusion, followed by 3 days of observation. All patients were treated for the first 28 days on the Clinical Oncology Inpatient Unit or the General Clinical Research Center of the University of Wisconsin Hospital. Patients were evaluated daily during the 28-day course of IL-2 treatment.

Acetaminophen (650 mg) and meperidine (25 to 50 mg) were utilized to treat fever and rigors. Furosemide was administered for excessive fluid retention. The IL-2 dose was halved, or treatment was stopped for Grade III or IV toxicities. Neither corticosteroids or other immunosuppressants nor nonsteroidal antiinflammatory agents were given during the treatment period because of possible confounding immunomodulatory effects and our previous experience of renal toxicity with IL-2 and nonsteroidal antiinflammatory agents (11). No vaspressors were used.

Patients were evaluated for antitumor response and toxicity at the end of the initial 4wk induction period, using the Eastern Comparative Oncology Group criteria (12). Those patients with evidence of response or stable disease commenced a "maintenance" course of 4 wk of IL-2 at one-third of that dose tolerated during the fourth cycle of the induction course (usually 1 x 10^6 units/m^2/day) given by the same schedule as their "induction" course. Patients were subsequently evaluated at monthly intervals and continued on maintenance until progression. The daily IL-2 infusion was given in a small volume (30 ml) of heparinized saline solution via a portable constant infusion pump (Cormed, Medina, NY). For the first few weeks of maintenance therapy, patients remained within 50 miles of Madison, WI, to allow close monitoring of their outpatient therapy with weekly clinical visits, laboratory testing, and physical examination. Subsequent months of therapy were given to patients while living at home, with weekly visits to their local physician, weekly phone calls to our nurse coordinators, and one outpatient visit to our clinic on Day 27 of each maintenance month. Patient enrollment and all clinical data monitoring were quality controlled by both the University of Wisconsin Clinical Cancer Center's Clinical Trials Unit and Hoffmann-LaRoche data monitors.

Clinical and Immunological Monitoring Schedule. Physical examinations and standard clinical hematology and chemistry assessments were performed at 2 baseline time points (within 14 days of starting IL-2) and throughout the 28 days of inpatient therapy. Lymphocyte counts on Days 6 and 27 of the induction course and at the end of each subsequent monthly course (Days 55 and 83) were determined on blood obtained 24 h after the cessation of the fourth 4-day IL-2 infusion for that month. Surface marker analysis of PBL and direct lymphocyte-mediated cytotoxicity by these PBL were performed prior to therapy (two specimens), on Days 6 and 27 of the first course and on completion of subsequent maintenance courses. The presence of antibodies to IL-2 was not tested at any time point.

Phenotyping of PBL Cells. Flow cytometric analyses were performed on whole blood specimens after lysis of red blood cells with NH4Cl and then gated for the lymphocyte fraction using a standard indirect immunofluorescent technique on an Ortho 50 Cytofluorograph (Ortho Diagnostic Systems, Inc., Raritan, NJ). Monoclonal antibodies OKT3, directed against the mature T-cell receptor-associated CD3 molecule, and OKT4, directed against the HLA-DR Class II MHC molecule, were obtained from Ortho Diagnostic Systems, Inc. (13). The Leu-11 (Fc-Ig) and Leu-19 (NKH1-1) monoclonal antibodies directed against NK cell markers (14, 15) and the Leu7 (OKT10) antibody associated with lymphocyte activation (16) were obtained from Becton Dickinson (Mountain View, CA); the anti-Tac (directed against the p55 IL-2 receptor) was a gift of Dr. T. Waldman (17).

Direct Cytotoxic Assays. Direct cytotoxicity of patients' fresh PBL was performed as previously described (18). In brief, fresh PBL were cultured at 37°C in 5% CO2 in medium alone or supplemented with 200 units/ml of IL-2 for 1 h prior to the addition of target cells. Target cells were added in equal volume, resulting in an IL-2 concentration of 100 units/ml during the 51Cr 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 51Cr release cytotoxicity assay (8, 9). Target cells were labeled with 250 μCi of 51Cr for 2 h at 37°C, and then 5 x 10^4 cells were added to quadruplicate U-bottomed microwells with four separate concentrations (effector:target ratio = 50, 25, 12, 6) of patients' PBL. Plates were centrifuged and then incubated for 4 h. Radiolabeled 51Cr release into the supernatant was measured. The percentage of cytotoxicity was calculated using the formula

\[
\text{% of cytotoxicity} = 100 \times \frac{(\text{test cpm} - \text{spontaneous cpm})}{(\text{maximal cpm} - \text{spontaneous cpm})}
\]

51Cr release data from all 4 effector:target ratios were converted to lytic units (8). One lytic unit was defined for these studies as the number of effectors resulting in 20% lysis of 5 x 10^4 target cells. Lytic units are expressed as lytic units/10^6 cells. Target cells were the K562 erythroleukemia cell line or the Daudi, Burkitt's lymphoma, cell line obtained from the American Type Culture Collection. Although the destruction of the Daudi and K562 targets may reflect lytic function by similar cell populations, these assays will be referred to as functional assessment of LAK and NK activity, respectively. Cryopreserved lymphocytes from 2 leukapheresed healthy control donors (see below) were used in each assay to allow detection of abnormal laboratory variation.

IL-2 Responsiveness of Posttherapy PBL. Patient PBL were obtained on Days 27, 55, and 83 of therapy corresponding to 24 h after the completion of the first, second, and third months of treatment with IL-2. These cells were cryopreserved by controlled rate freezing in 10% dimethyl sulfoxide and stored in liquid nitrogen. The cells were subsequently thawed and incubated with or without 200 units/ml of IL-2 for 24 h. Then cells were assayed for cytotoxicity against 51Cr-labeled Daudi targets as described above. Killing by these cells was compared with killing by cryopreserved PBL from two healthy individuals cultured identically to the patients' PBL. Comparisons were also made to the cytotoxicity mediated by fresh PBL from these same patients assayed on the day of isolation following 1-h preincubation with IL-2 as described above. Only 3 patients had sufficient cells cryopreserved at each time point for subsequent analysis.

Statistical Analysis. Treatment effects for a given parameter over time were assessed using repeated measures analysis of variance; Fisher's protected Least Significant Difference method was used to determine significant pairwise differences between specific time points (19). To account for likely violation of the assumption of equicorrelation, Box's conservative correction was applied to both the overall F test and pairwise t tests by dividing the degrees of freedom for error by one less than the average number of time points available per individual (19). Cited P values reflect the larger of the significance level of the overall F test and the pairwise comparison. Analyses for lymphocyte counts and lytic units were based on log-transformed data. Lytic unit values close to zero were recoded to value 5.0 to avoid inflated comparisons. For clarity of presentation, tables and figures are derived from untransformed data.

A separate statistical analysis using paired t test comparisons, as
used previously (8, 9), generated qualitative conclusions similar to those
provided by this repeated measures analysis of variance; these separate
$P$ values are not shown.

Surface markers were analyzed for change in the percentage of cells
positive for that marker. Twelve patients were available for analysis at
the end of the induction month, 10 at the end of the first maintenance
month, and 8 at the end of the second maintenance month.

In order to assess the effects of the outpatient therapy, a comparison
was made with patients treated with the same IL-2 dose and induction
schedule but with no maintenance IL-2 in an earlier trial (11). Com-
parisons of treatment effects between 9 patients in the current trial
receiving 1 mo of induction therapy and 1 mo of maintenance therapy
and 5 patients in our previous trial receiving similar induction therapy
and 1 mo of observation were made using two-sample t tests.

Each patient is used as his own control which compensates to some
extent for the problem of potential variation in laboratory procedure
over time. This does not eliminate but does mitigate the likelihood of
laboratory changes leading to false conclusions. Even in a randomized
trial, there can be changes over time which cannot be corrected in these
assays. Since we are unaware of any demographic characteristics which
influence LAK activity, the historical controls are not matched to those
in the current study. Nevertheless, the patients in the historical group
had the same malignancies, renal and melanoma, and a similar age
distribution (29 to 61 yr), and all had had previous therapy (all five had
prescribed dose. One patient with metastatic renal carcinoma
were listed in Table 1. Only three patients were previously
untreated, and all had metastatic disease. Three patients failed
to complete the first month of induction therapy at the
prescribed dose. One patient with metastatic renal carcinoma
progressed during the induction, one patient had therapy discon-
tinued because of Grade 3 pulmonary toxicity, and one patient who developed pulmonary toxicity had his dose reduced
50% and was given steroids. Because of his steroid treatment, all laboratory data from this patient were excluded from the
immunological analyses. He received maintenance therapy for
2 wk prior to pulmonary progression, and his clinical data are
included in the toxicity and response analyses. Of the 13 pa-
patients who began maintenance IL-2, 3 failed to complete the
first month of maintenance therapy. One patient with melana-
oma progressed, one patient with renal carcinoma required
radiotherapy for a lesion in his clavicle, and one patient with
melanoma had pulmonary progression. Two patients pro-
gressed by the end of the first month of maintenance. One
patient with renal carcinoma had progression of his renal mass,
and one patient with renal carcinoma had progression of lung
metastases. Two patients with renal carcinoma and one with
lymphoma had progressed by the end of the second month of
maintenance.

Overall there were no complete or partial responses in this
study. One patient with renal carcinoma did show >50% reduc-
tion in total tumor size at the end of the induction month but
showed a slight increase in tumor size after 1 mo of maintenance
(back to only a 30% reduction from pre-IL-2 dimensions) and
clear progression after 2 mo of maintenance. Five patients with
renal carcinoma had stable disease for 10, 6, 3, 2, and 2 mo,
respectively. One patient with melanoma was stable for 4 mo,
and one of 2 patients with lymphoma was stable for 4 mo but
stopped treatment because of recurrent infection in his central
line and went off study.

Toxicity. All significant toxicities occurred during the induc-
tion month and were comparable with those described previ-
ously for the same 4-wk induction regimen (9, 11). The most
severe toxicity related to IL-2 induction therapy was respira-
tory distress. Of the 15 patients treated, dyspnea requiring supple-
mental oxygen occurred in two patients and resolved rapidly
upon cessation of IL-2; one of these patients discontinued
therapy, and the other had a 50% dose reduction. No ventilatory
support was required. Both of these patients were elderly and
had prior respiratory problems associated with allergy to dust
and paint, respectively. One patient had a dramatic increase in
alkaline phosphatase to 1550 units, but this was not associated
with any hepatic toxicity or biliary obstruction. Therapy was
interrupted until this laboratory value returned to normal;
treatment was then resumed at 3 × 10^6 units/m^2/day, and no
further elevation was seen. One patient had a transient elevation
in bilirubin to 5.2 mg/dl. As with the previous patient, he was
also successfully retreated at the same dose without further
bilirubin elevation. Five patients had a decline in systolic blood
pressure of >40 mm of Hg. None was symptomatic. The range
of nadir blood pressure was 70 to 100 mm of Hg. Except for
one patient who developed atrial fibrillation requiring digitali-
zation, the other four patients required increases in p.o. fluid
intake only. Decreases in blood pressure were always transient
(2 to 5 h). Other clinical and laboratory toxicities occurring
during the induction month are summarized in Table 2. Toxi-
cities, although tolerable, were cumulative with each of the 4
wk of induction, but did not usually require cessation of treat-
ment.

In general, maintenance therapy was tolerated well on an
outpatient basis without major toxicities. No patients had ther-
apy discontinued because of IL-2 toxicity during maintenance
therapy, although 2 patients had catheter-related infections for
which IL-2 therapy was interrupted. Two patients required
transfusions for hemoglobin <9.5 g/dl, and 3 had transient
declines in neutrophil count below 1000, one of whom had a
catheter-related infection. No patient required a dose reduction

<table>
<thead>
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<th>Table 1 Patient characteristics</th>
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<td>Total patients</td>
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<tr>
<td>Male</td>
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<td>Female</td>
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<td>Age (yr)</td>
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<td>Karnofsky performance status (%)</td>
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<td>Prior therapy</td>
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4.7-fold (P < 0.0001) by the end of the induction month. For samples obtained at baseline, 24 h after completing the first week's 4-day cycle of IL-2 (Day 6); 24 h after completing the second maintenance month (Day 83) (see Table 3). The lymphocyte count was still elevated over baseline 2.5-fold (P < 0.0001) but was significantly lower than at the end of the induction month (P = 0.001). At the end of the second maintenance month, the count remained stable at 3.3-fold over baseline (P < 0.0001) which was not significantly different from the first maintenance month.

These results after 1 month of induction therapy followed by 1 mo of maintenance IL-2 therapy were compared with data obtained in our previous study (11). In that study, a group of five patients completed an identical month of induction treatment (same dose and schedule of IL-2) and were evaluated after the induction month (Day 27) and after 1 mo of observation without IL-2 treatment (Day 55). The 2.5-fold increase in lymphocyte count after maintenance IL-2 in this present study appeared greater than the 1.5-fold elevation seen on Day 55 in the prior study but showed only a trend toward statistical significance (P = 0.075).

Non-MHC-restricted Killing. Twelve patients who completed 1 mo of therapy at the starting dose of 3 × 10^6 units/IL-2/m^2/day showed significant increases in PBL-mediated cytotoxicity of Daudi and K562 targets (Fig. 1), particularly when measured in the presence of in vitro IL-2 (16- and 11.6-fold increases, respectively). There were no significant differences in the LAK response of patients who had received prior therapy and those who had not.

Ten of these patients completed 1 mo of maintenance therapy at one-third of their induction dose. Of these, 9 had data collected and showed a statistically significant decrease in IL-2-dependent LAK activity compared with that seen after 1 mo of induction (P = 0.009). However, both LAK and NK activity remained substantially elevated compared with baseline (P < 0.0001) with increases of 6.9- and 8.5-fold, respectively (Fig. 2).

Eight patients completed 2 mo of maintenance therapy. There was no significant difference in the level of effector cell activation at that time compared with the previous month (Fig. 2).


during maintenance therapy. Of the 13 patients who received maintenance therapy, 5 patients had nausea or vomiting, only one of which was Grade 2. Three patients experienced Grade 1 diarrhea and 4 had Grade 1 rashes. Several patients conducted normal activities while receiving this outpatient IL-2 treatment, and those who had not.

Immunological Effects. The immunological effects seen after 1 mo of repetitive 4-day IL-2 cycles given weekly at 3 × 10^6 units of IL-2/m^2/day by continuous infusion have been reported previously (9). We found similar changes following the induction month in the patients treated in this study, with significant increases in lymphocyte counts, NK-associated markers, and LAK function. The effects of continuation of IL-2 treatment, at a lower dose, on these same parameters are presented separately for each of these parameters.

Lymphocyte Count. The lymphocyte counts were compared for samples obtained at baseline, 24 h after completing the initial week's 4-day cycle of IL-2 (Day 6); 24 h after completing 4 wk of high-dose induction with 3 × 10^6 units/m^2 (Day 27); at the end of the first maintenance month (4 wk after wk of maintenance IL-2 at 1 × 10^6 units/m^2 (Day 55)); and at the end of the second maintenance month (4 wk after wk of maintenance IL-2 (Day 83)) (see Table 3). The lymphocyte count was increased 2.4-fold over baseline (P < 0.0001) after 1 wk and 4.7-fold (P < 0.0001) by the end of the induction month.

At the end of the first month of maintenance therapy, the
In an analysis similar to that performed for lymphocyte counts, the 5.3-fold increase in LAK activity from baseline to Day 55 is greater than the 3.7-fold elevation over baseline seen on Day 55 after no maintenance IL-2, but was not statistically significant ($P = 0.45$).

We and others have demonstrated that a major component of LAK activity induced by IL-2 therapy is mediated by Leu19-positive, activated NK cells ($20 - 22$). The percentage of Leu19-positive cells remained elevated following 1 and 2 mo of maintenance therapy (Fig. 3), although there was a decrease in lymphocyte counts and in IL-2-dependent LAK activity compared with that seen after the induction month (Day 27) (see Fig. 2).

For the 3 patients who had sufficient cryopreserved cells available at all time points during maintenance therapy, we tested whether PBL obtained after maintenance IL-2 responded with a higher level of killing following 24 h of culture in IL-2 compared with resting PBL from control individuals. The increased level of LAK activity measured for PBL obtained on Days 27, 55, and 83 post therapy from all three patients tested (following 24 h in vitro incubation in IL-2) was far greater than the minimal killing mediated by control donors’ PBL (Table 4). Furthermore, the activity of patients' PBL following in vivo IL-2 was considerably boosted by 24-h incubation with IL-2 as compared with 1-h incubation with IL-2. Patient 3 did not show as marked an increase in cytotoxicity, following 24 h of incubation, as did Patients 1 and 2 during maintenance therapy, although killing was still considerably greater than that by control PBL. This indicates that outpatient maintenance IL-2 treatment maintains a circulating population of cells that respond strongly and rapidly to brief exposure to higher concentrations of IL-2, although variations from patient to patient in responsiveness to IL-2 may occur.

killer Cell-associated Surface Proteins. Three antigens associated with cells that can mediate non-MHC-restricted killing were measured in this trial, Leu 11 (FcγR), Leu 19 (NKH-1), and Leu 17 (OKT10). Leu 11 and Leu 19 are both antigens found on the surface of cells that can mediate non-MHC-restricted killing (14, 15). Leu 17 is an antigen found on activated T-cells (16), but it is also expressed on cells with LAK activity (20). Significant increases were seen in the percentage of PBL expressing each of these antigens (Fig. 3). Our statistical analysis focuses on changes in the percentage of cells expressing these markers, as this best describes the changes in distribution of lymphocyte phenotypes. It should be pointed out, however, that since the lymphocyte count rose substantially (described above) after IL-2 treatment, the increase in the absolute number of cells expressing each marker was much more striking than the changes in percentage of cells. For example, at Day 27, there was a 4.7-fold increase in total lymphocyte count and, thus, a 3-fold increase in the percentage of PBL expressing a given marker corresponds to nearly a 15-fold increase in the actual numbers of PBL with that marker.

At the end of the month of induction, the percentage of Leu 17-positive cells increased from 20.6 to 61.5%, Leu 11-positive cells increased from 4.1 to 15.5%, and Leu 19-positive cells increased from 13.9 to 49.4%. After 1 mo at the lower maintenance dose, the percentage of these cells remained similar (Fig. 3). Only Leu 17 had significantly decreased since the Day 27 value [from 59.9 to 47.7% ($P = 0.011$)]. Both Leu 17 and Leu 19 were still elevated ($P < 0.0001$) with 2.3- and 2.9-fold increases over baseline. Of note, Leu 11 remained elevated 2.6-fold on Day 55 over baseline, but this was not statistically significant ($P = 0.13$).

At the end of the second month of maintenance, the percentages of cells expressing these 3 markers remained virtually unchanged (Fig. 3), and Leu 17 and Leu 19 continued to be significantly elevated over baseline. In an analysis similar to that used for the LAK and NK results, surface marker data obtained after 1 mo of induction therapy followed by maintenance IL-2 were compared with the data in our previous study 1 mo after the completion of induction therapy without maintenance. There appeared to be a greater increase from baseline in the percentage of Leu 17 cells with maintenance therapy (2.3-fold versus 1.3-fold), but this difference between the treatments was not significant ($P = 0.11$). Of note, there was a significant difference between these 2 treatment groups in the change from baseline to the total number of cells bearing the Leu 17 marker [8.2- versus 2.1-fold ($P = 0.04$)].

T-Cell-associated Surface Proteins. Resting T-lymphocytes
express the CD3 (T3) molecule but not the p55 (Tac) IL-2 receptor, nor Class II MHC antigen (Ia). Activation of T-cells can induce expression of both Tac and Ia. However, both Tac and Ia can be expressed by activated lymphocytes other than T-cells (13–17). At the end of the month of induction, the percentage of cells expressing the CD3 antigen was significantly decreased from 68.3 to 38.6% (P < 0.0001). Cells expressing the IL-2 receptor, however, increased 4.5-fold [from 2.4 to 10.7% (P = 0.0006)]. There was a slight increase (P = 0.02) in the percentage of cells showing Ia expression (16% versus 11%) (Fig. 4).

After 1 mo of maintenance therapy (Fig. 4), CD3 had increased slightly from the nadir at Day 27 (P = 0.04) but was still decreased relative to baseline by 30% (P < 0.0001). IL-2 receptor expression decreased 31% (P = 0.03) but was still significantly elevated over baseline by 3.1-fold (P = 0.008). Ia expression was significantly lower than after induction therapy (52% decrease, P = 0.008) and was no longer significantly different from baseline (P = 0.42). All of the changes from baseline seen at the end of the first month of maintenance were unaltered after a further month of maintenance IL-2 treatment (Fig. 4).

The results after 1 mo of induction therapy followed by maintenance IL-2 were again compared with the data obtained in our previous study 1 mo after the completion of induction therapy without maintenance. There were no significant differences between these 2 groups of patients in the change from baseline for these parameters.

**DISCUSSION**

This study was designed to determine whether the immunomodulatory effects induced by 4 wk of high-dose IL-2 could be maintained by continuing IL-2 treatment using the same schedule, but at a lower dose of IL-2 that could be tolerated on an outpatient basis. We have established that a dose of 1 × 10^6 units of IL-2 given by continuous infusion is well tolerated by most patients on an outpatient basis, although associated with significant fatigue for some patients. Patients received this therapy at home, and many found the treatment to be compatible with normal daily activity. Clinical monitoring during maintenance required monthly outpatient clinic visits, weekly telephone calls to discuss tolerance, and weekly clinical hematology and chemistry laboratory results.

This outpatient regimen of IL-2 maintains a heightened immune state. Following 1 mo of this outpatient IL-2 treatment, there were significant increases over baseline in lymphocyte counts, non-MHC-restricted cytotoxicity, and the percentage of cells expressing the Leu 17 and Leu 19 activation or NK-associated antigens. These immune response indicators were not only elevated relative to baseline levels, but the elevation over baseline was also greater in this study than in a group of 5 historical patients who received the same initial induction course but did not receive any subsequent IL-2 maintenance treatment as part of a previous clinical trial (11), although this was not statistically significant. We recognize that this conclusion must be qualified, as this was not a randomized study with matched controls. However, since we know of no demographic characteristics that determine response to IL-2, we feel that this comparison is valid, albeit of limited power. Furthermore, this increase in immune status remained stable over several months of treatment for those patients who received more than 1 mo of lower dose outpatient IL-2.

Nevertheless, the lowering of IL-2 dose at the end of the 4-wk induction did not enable maintenance of the same level of heightened immune status noted at the end of the high-dose induction month. The functional activation of non-MHC-restricted killing and the circulating lymphocyte counts were markedly reduced following maintenance outpatient IL-2 relative to the effect seen after 1 mo of high-dose induction therapy. In contrast, the percentage of cells expressing NK-associated markers of the IL-2 receptor (p55) after induction did not appreciably decrease from that level during maintenance therapy. The continued elevation of cells expressing NK-associated markers, despite a decrease in lymphocyte numbers and LAK activity, suggests that IL-2-responsive cells are still being maintained by the low-dose IL-2.

The rapid and magnified response of patients’ PBL following *in vivo* IL-2 treatment when incubated further with IL-2 *in vitro* suggests that augmented LAK activity by these PBL is dependent on continued IL-2 treatment (Table 4). We have examined the function of patients’ PBL after 1 mo of induction IL-2 therapy (8, 9) and will report separately on a detailed analysis of their rapid *in vitro* responses to IL-2.4 This rapid *in vitro* response is also observed for patients’ PBL following maintenance therapy. These cells are different from resting PBL in that they appear to be in an already activated or “primed” state, and their cytotoxic activity can be readily boosted by additional IL-2 given over a short period of time. This suggests that intermittent administration of higher doses of IL-2 for short periods of time during maintenance therapy might rapidly reinroduce the high levels of functional LAK activity seen at the end of the induction month. Preliminary data testing this concept are confirming this hypothesis.5 In this regard, during the induction month of high-dose IL-2, the rate of increase in the non-MHC-restricted killing in the first 4-day treatment period was greater than the rate of increase for any of the other subsequent 4-day infusions (Figs. 1 and 2), which further supports this concept that higher IL-2 doses during maintenance might be needed for short periods only. Testing of such a schedule is now in progress here and elsewhere (23).

This protocol also sought to test whether continued exposure to IL-2 could maintain the immunological responses which occurred during induction, thereby increasing the magnitude or incidence of antitumor responses. In 9 patients with renal carcinoma treated here, there were no complete or partial responses. The 95% confidence interval for this response rate

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*J. A. Sosman *et al.*, unpublished data.
is 0 to 34%. In our previous trial using this same induction regimen (4 wk of continuous infusion of IL-2 at 3 × 10^8 units/m^2/day) but without maintenance therapy, 1 of 9 patients with renal cell carcinoma had partial response. The 95% confidence interval for that response is 0.3 to 48.3%. Although patient numbers are small, it seems likely that the addition of the maintenance IL-2 therapy as used here did not improve the antitumor effect. For that reason, we feel further modifications of this regimen are required to try to better boost immune activation in order to achieve greater antitumor response rates in Phase II testing.

A variety of IL-2 regimens, either with or without infusions of in vitro-activated LAK cells, have real but modest activity in advanced disease (1–5, 11, 24, 25). Whether other modifications involving IL-2 dose and schedule, such as the intermittent boosting now being tested, will enable a greater antitumor response rate (measured in Phase II trials) with a well-tolerated regimen of IL-2 used alone remains uncertain. Testing of this immunologically active, well-tolerated regimen in the adjuvant setting, as suggested by some murine studies (26–29), may also merit further analysis.

Multiple preclinical studies indicate that additive or synergistic antitumor effects may be obtained by combining IL-2 treatment with a variety of other treatments (cytotoxic chemotherapy, other biological agents, monoclonal antibodies) (28, 30–34). Should these combinations prove effective in the clinical setting, then longer term sustained therapy such as that used in this trial may be required to overcome transient resistance of tumor cells to immunotherapy.

In summary, this trial has demonstrated that heightened immune status can be maintained by IL-2 given for extended periods. Patients received this treatment safely while at home with excellent tolerance. Modifications of this regimen could readily be tested in combination with other (potentially synergistic) treatments, or in the adjuvant setting, to maintain heightened immune activation on a prolonged basis.

## REFERENCES


Repetitive Weekly Cycles of Interleukin 2: Effect of Outpatient Treatment with a Lower Dose of Interleukin 2 on Non-Major Histocompatibility Complex-restricted Killer Activity


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