ABSTRACT: Clinical trials with high doses of interleukin 2 (IL-2) have shown antitumor responses, but many of the patients have experienced severe and occasionally life-threatening toxic effects. Preclinical studies indicate that modifications in IL-2 dose, route, and schedule can influence both immune activation and antitumor effects. This study evaluated the clinical tolerance to and immunologic modifications induced by four repetitive weekly cycles of IL-2, with two dose levels (1X10<6> and 3X10<6> U/m<2> per day) of IL-2 and three different daily administration schedules [bolus, continuous, or combined (bolus and continuous)], with and without indomethacin treatment. Patients in all treatment groups experienced acceptable, non-life-threatening toxic effects and immune system stimulation characterized by rebound lymphocytosis with increased numbers of natural killer and lymphokine-activated killer cells and enhanced direct cytolytic function. These immune changes were significantly enhanced by the repetition of IL-2 cycles beyond the first week of therapy. At an IL-2 dose of 3X10<6> U/m<2> per day, bolus IL-2 was less immunostimulatory than continuous-infusion IL-2. The combined regimen (with half of each daily dose given as a bolus and half as a 24-hr infusion) was as stimulatory as continuous-infusion IL-2 and also induced antitumor effects. Finally, the addition of indomethacin to this regimen did not significantly modify in vitro or in vivo immune response parameters but appeared to worsen the systemic toxic effects of renal dysfunction and capillary leakage. These results suggest that continuous or combined infusion of IL-w at 3X10<6> U/m<2> per day on this schedule should be considered for further testing in phase II trials or in combination with other therapeutic modalities.

TEXT:

Interleukin 2 (IL-2), a lymphokine secreted by activated T cells, has the ability to activate, in vitro and in vivo, a heterogeneous population of nonspecific cytotoxic lymphocytes [lymphokine-activated killer (LAK) cells], which can lyse a variety of neoplastic and nonneoplastic tissues [n1-n7]. Regression of experimental murine tumors has been demonstrated as a result of administration of high doses of IL-2 alone or in combination with exogenously activated LAK cells [n8-n12]. In these models, IL-2 appears to activate either exogenously added or endogenously induced LAK cells, which may then mediate the in vivo antitumor effects [n4,n6,n13]. The role of tumor- or antigen-specific cytotoxic T cells (CTL) in this process has not been well defined but may be significant in some murine tumor systems [n14-n16].

Clinical trials of high-dose IL-2 regimens have documented reproducible antitumor effects (with or without exogenous LAK cells) in some neoplasms (especially renal cell carcinoma and metastatic melanoma) [n17-n20]. However, these trials have been complicated by severe toxicity, with life-threatening impairment of renal, hematologic,
pulmonary, and cardiac function making intensive-care unit (ICU) monitoring necessary [n19,n21-n25]. Despite the overall low mortality (2%-4%) and rapid reversibility of these toxic effects after discontinuation of IL-2 treatment, the severity of these side effects has led some to question and usefulness of IL-2 therapy as an approach to cancer treatment [n26].

Phase I trials have examined the toxicity, pharmacokinetics, and immunomodulatory effects of IL-2 administered by single or repetitive schedules via bolus or continuous infusion [n27-n30]. We have previously demonstrated that IL-2 administered by continuous or bolus infusion at 1X10<6>-3X10<6> U/m<2> per day over 4-7 days causes in vivo immune activation with acceptable toxicity [n30-n32]. The dose-limiting side effects at an IL-2 dose of 3X10<6> U/m<2> per day were a decline in performance level, systolic hypotension, and fever. These toxic effects were managed on a general medical ward and were not life-threatening [n30]. Patients treated at these doses had a decrease in lymphocyte counts and LAK cell precursors in their peripheral blood after initiation of IL-2 treatment [n31]. After 4-7 days of IL-2 treatment, lymphocyte counts rebounded, with an increase in cells bearing activation markers. Lymphocytes obtained during the rebound period demonstrated enhanced natural killer (NK) and direct LAK cell activity [n32]. These immune effects were not sustained; the augmented lymphocyte counts and cytotoxic function returned to baseline within 7 days after therapy was completed.

These results suggested that reinitiation of IL-2 treatment shortly after toxic effects had reversed (3 days after cessation of IL-2 administration) might re activate, and possibly enhance, the IL-2-induced immune activation. The present trial was designed to test this hypothesis and evaluate further the immunologic and clinical effects of variations (bolus, schedule, and indomethacin addition) in the administration of IL-2 given by repetitive weekly 4-day infusions.

A total of 28 patients received repetitive weekly 4-day infusions of IL-2. Six distinct treatment groups were studied, and differences among the treatment groups allowed comparison of the effects of two dose levels of IL-2 (1X10<6> and 3X10<6> U/m<2>/day), three different modes of daily iv IL-2 administration (bolus injection, continuous infusion, and combined bolus and continuous infusion), and the addition of indomethacin to the IL-2 regimen. This report confirms our preliminary observations that repetitive cycles of IL-2 can be tolerated with acceptable toxicity and produce antitumor effects [n33,n34] and details the toxicity of, tolerance to, and immunologic effects of these six different treatment regimens. The toxicity of four repetitive weekly cycles of IL-2 was not appreciably different from that of a single week of IL-2 therapy. The prolonged 4-week course of IL-2 treatment had a striking effect on immune activation compared with a 1-week course. For all six treatment groups, lymphocyte count, number of lymphocytes with NK and activation markers, and lymphocyte-mediated LAK activity all increased after 4 weeks of IL-2 treatment to levels higher than after a single 4-day cycle. Continuous-infusion IL-2 was more effective in inducing these changes than was bolus IL-2. Finally, the use of indomethacin did not significantly alter these immune responses but did increase the spectrum of IL-2-associated toxicity.

Patients and Methods
Patient Selection

From August 1986 through November 1987, 28 eligible patients with recurrent or metastatic, biopsy-proven renal cell carcinoma or melanoma were entered into this phase I trial [National Cancer Institute-Biological Response Modifier Program (NCI-BRMP) Protocol B85-013]. Eligibility criteria included an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, adequate bone marrow function (wbc count >/= 4,000/mu L and platelet count >/= 100,000/mu L), adequate renal function (serum creatinine <= 2.0 mg/dL and BUN <= mg/dL), adequate hepatic function (total serum bilirubin <= 2.0 mg/dL and serum alkaline phosphatase and transaminase levels <= 2 times the upper limits of normal), and disease that can be evaluated or measured. Criteria for patient exclusion included treatment with cytotoxic agents within 3 weeks or with biologic response modifiers within 4 weeks or radiation therapy within 2 weeks of entry onto this trial. Patients who had serious infections or central nervous system metastases or who were receiving corticosteroids, nonsteroidal anti-inflammatory agents, or aspirin were ineligible. All patients signed consent forms approved by the University of Wisconsin Human Subjects Committee.
Study Design

All patients were scheduled to receive four consecutive 96-hour cycles of IL-2, separated by 72 hours of observation. Six different treatment groups (table 1) were based on two iv administered dose levels, three different daily IL-2 administration schedules, and the addition of oral indomethacin. IL-2 was administered as (a) a continuous 24-hour infusion, (b) a single daily 15-minute bolus, or (c) a combination of schedules a and b in which half the daily dose was given by bolus and half was given by 24-hour continuous infusion. A minimum of three patients were enrolled in each treatment group. Patients were assigned sequentially, not randomly, to the six groups. Additional patients were enrolled in certain treatment groups in order to clarify the toxicity of each regimen and to have sufficient complete immunologic monitoring.

Acetaminophen (650 mg) and meperidine (25-50 mg) were used to treat fever and rigors, respectively. Furosemide was administered for excessive fluid retention. Corticosteroids, other immunosuppressants, or nonsteroidal antiinflammatory agents (except indomethacin in group 6) were not given during the treatment period. All patients were evaluated 28 days after completion of the 4-week course of IL-2. A subsequent 28-day course of IL-2 was administered only if stable disease or a tumor response (partial or complete) was observed. Response evaluation followed ECOG criteria for partial response, complete response, and progressive disease [n35]. Patient enrollment and all clinical monitoring were quality controlled both by the University of Wisconsin Clinical Cancer Center Clinical Trials Unit and by the NCI clinical trials data monitor (Theradex, Princeton, NJ).

Interleukin 2

Recombinant IL-2 (rIL-2) was provided through the NCI-BRMP by Hoffmann-La Roche (Nutley, NJ). The NCI-BRMP standard for dosage was used, with a specific activity of $1.2-1.5 \times 10^7$ U/mg of protein. IL-2 was provided in lyophilized form and reconstituted with sterile preservative-free normal saline.

Clinical and Immunologic Monitoring Schedule

Physical examinations and standard clinical hematology and chemistry assessments were performed at three baseline time points (within 14 days of initiation of IL-2 therapy), throughout the 28 days of therapy, as well as 7 and 28 days after completion of the 28-day treatment period. Lymphocyte and eosinophil counts on days 5, 12, 19, and 26 were determined for blood samples obtained before cessation of the IL-2 infusion. Surface marker analysis of peripheral blood mononuclear cells (PBL) was performed before therapy began (two specimens), 24 hours after the initiation of each weekly cycle of IL-2, upon completion of each cycle of IL-2, and 24 and 48 hours after the first and fourth cycles of IL-2. Direct lymphocyte-mediated cytotoxicity (as described below) was assayed with blood samples obtained before therapy (three specimens), immediately after completion, and 24 and 48 hours after completion of each weekly cycle. In vitro proliferation assays (as described below) were performed with blood obtained from patients before the beginning of therapy (three specimens) and then approximately 24 hours after the completion of each weekly IL-2 infusion.

Phenotyping of PBL Cells

Flow cytometric analyses were performed on whole-blood specimens. Rbc were lysed with NH[4]Cl, and the lymphocyte fraction was evaluated by 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; OK1a, directed against the HLA-DR class II major histocompatibility complex (MHC) molecule; and OKB1, directed against a B-cell marker] were obtained from Ortho Diagnostic Systems Inc. [n36,n37]. The Leu-11 and Leu-19 monoclonal antibodies directed against NK cell markers [n38,n39] were obtained from Becton Dickinson (Mountain View, CA), and the anti-Tac monoclonal antibody, directed against the p55 chain of the IL-2 receptor [n40], was a gift of Dr. T. Waldmann.
In Vitro Immunologic Assays

For functional assays, PBL were isolated by fractionation on Ficoll-Hypaque gradients (Pharmacia Fine Chemicals, Piscataway, NJ).

Proliferative assays. PBL (10<5>) were incubated in 0.2-mL U-bottomed 96-well microplates (Costar, Cambridge, MA) in tissue culture medium supplemented with 10% human serum. Either rIL-2 at a final concentration of 100 U/mL, phytohemagglutinin M (PHA) (Difco Laboratories, Detroit, MI) at a final dilution of 1% of the stock solution, or medium alone was added to the cultures. Cultures were incubated at 37 degrees C in 5% CO[2] for 72 or 144 hours, and for the last 18 hours 1 mu Ci of tritiated thymidine (New England Nuclear Corp., Boston, MA) was added per well. Cultures were harvested with a MASH harvester (Otto Hiller, Madison, WI), and radioactivity was counted by liquid scintillation. Means of median counts per minute are reported [n31].

Direct cytotoxicity assays. The direct cytotoxicity of patients' fresh PBL was determined as described previously [n32]. In brief, fresh PBL were cultured at 37 degrees C in 5% CO[2] in medium alone or medium supplemented with 200 U of IL-2 per mL for 1 hour before target cells were added. Target cells were labeled with 250 mu Ci of <51>Cr for 2 hours at 37 degrees C, and then 5X10<3> cells were added to quadruplicate U-bottomed microwells at effector-to-target cell (E/T) ratios of 50, 25, 12, and 6 (patients' PBL were used as effector cells). Plates were centrifuged and then incubated for 4 hours. Release of the radiolabel into the supernatant was measured. Percent cytotoxicity was calculated by using the formula: % cytotoxicity = [(test cpm - spontaneous cpm)/(maximal cpm - spontaneous cpm)] X 100. <51>Cr release data for all four E/T ratios were converted to lytic units (LU) [n41]. One LU was defined for these studies as the number of effector cells resulting in 20% lysis of 5X10<3> target cells. Lytic units are expressed per 10<7> cells. Target cells were the K562 erythroleukemia cell line or the Daudi Burkitt's lymphoma cell line, obtained from Dr. A. Maluish of NCI-BRMP (Frederick, MD).

Statistical Analysis

Statistical tests of treatment effects within each of the six treatment groups (or within pooled groups) were made by using paired t-tests. Comparisons between treatment groups were made with a multiple-regression analysis, which permitted simultaneous evaluation of several comparisons of interest by using a pooled estimate of variance for all six groups. These comparisons included, for example, high-dose continuous-infusion IL-2 with and without indomethacin, the continuous versus combined schedules (adjusted for dose), high dose versus low dose (adjusted for schedule), and a test of the interaction between dose and schedule. Direct P values obtained for each analysis are shown and were not corrected for the number of comparisons made.

For most assays and clinical parameters, treatment effect was expressed as the difference in parameter values over a fixed period of time.

Results

Patient Summary

A total of 29 patients were enrolled in this trial. The treatment groups are outlined in table 1, and the clinical characteristics of all 28 eligible patients are summarized in table 2. Eighteen patients received a single 4-week course of IL-2 treatment, seven patients received two separate 4-week courses, two patients received three courses, and one patient received four courses of IL-2 therapy. A total of 42 courses of IL-2 were initiated as part of this protocol. [One patient (No. 24) was enrolled but was deemed ineligible due to central nervous system (CNS) involvement with acute nonlymphocytic leukemia and inadequate bone marrow function (based on a low wbc count at the initiation of therapy). This patient's clinical course was characterized by rapid progression of leukemia requiring alternative chemotherapy after 2 weeks of IL-2. This patient did not demonstrate any IL-2-associated toxic effects distinct from those experienced by the other 28 patients. Because he was ineligible for entry on several counts, data for this patient were not included in the clinical or immunologic analyses.]
Clinical Toxicity

The toxic effects observed during this trial are summarized in tables 3 and 4. These toxic effects occurred while patients were receiving IL-2 or shortly after treatment cessation. The time at which maximal toxicity appeared was quite variable, but effects appeared to be most extreme during days 3 and 4 of each 4-day treatment cycle. The 3-day interval of rest allowed most toxic effects to resolve completely before the next cycle of IL-2 was begun. Toxicity was not greater during weeks 2-4 than during the first week. Some patients appeared to tolerate subsequent weekly cycles with greater ease, but this was not uniformly true.

Table 3. Clinical toxicity -- objectivity findings

Table 4. Clinical toxicity -- laboratory abnormalities

Fever was the most prevalent side effect, being observed in nearly all patients. Only three patients, two in group 5 and one in group 6, had temperatures above 40.0 degrees C. Rigors were present among patients in all treatment groups except those receiving IL-2 by continuous infusion at $1 \times 10^6$ to $1 \times 10^6$ U/m2/day (group 2). Severe rigors requiring parenteral meperidine were observed among patients receiving bolus IL-2 as part of their regimen (groups 1, 3, and 4). Skin rash, nausea, vomiting, and diarrhea were frequent toxic effects of IL-2 therapy and were mild to moderate in all cases. No episodes of diarrhea led to dehydration; all episodes of nausea and vomiting were medically controllable. No skin rash resulted in ulceration or necrosis. Table 3 shows that these toxic effects were more commonly seen in patients receiving the higher dose of IL-2.

Hypotension with a decline in systolic blood pressure of $>40$ mm Hg was observed in all treatment groups; the greatest decline for any single patient was 75 mm Hg. However, these changes were transient, and no vasopressor support was required at any time. Fluid retention with a concomitant weight gain of $>5.0$ kg was most prevalent in the patients receiving indomethacin (treatment group 6). No patient in the study had $>10.0$ kg of weight gain, and no definite symptoms of pulmonary edema were observed throughout the study.

CNS toxic effects, including disorientation, difficulty concentrating, and insomnia, were infrequent and mild. These effects were observed particularly in patients in group 6, who were also receiving indomethacin, which may have played a role in these symptoms.
Other clinical toxic effects not described in the tables included one episode of atrial fibrillation requiring digoxin (patient 1 in group 1) and central-line sepsis, which developed in patient 8 (group 5). Both of these events resolved with proper management without interruption of IL-2 therapy.

Hematologic toxicity is outlined in table 4. Hemoglobin levels decreased in many patients, reflecting shifts in vascular volume, diagnostic phlebotomy, and possibly a myelosuppressive effect of the IL-2 treatment [n24]. Only six patients required transfusions of packed rbcs, and no patient received more than 2 units of blood. Transient declines in neutrophil counts occurred in association with a rebound lymphocytosis during the 3 days of observation between cycles of IL-2. These declines were more frequently observed in treatment groups 5 and 6. No patient had prolonged neutropenia (<500 cells/mu L for >48 hr). Platelet counts were minimally decreased by IL-2 therapy, and no patient developed significant thrombocytopenia (<90,000 cells/mu L) or any bleeding complications.

Liver function tests revealed only mild abnormalities in most treatment groups. No patient demonstrated hyperbilirubinemia (>2 mg/dL). Only two patients (one in group 5 and one in group 6) had elevations in SGOT or alkaline phosphatase of >5 times normal values. Extravascular fluid and albumin shifts, perhaps from capillary leakage mediated by IL-2-induced LAK cells [n42], were evident from the frequent decline in serum albumin levels during therapy. However, severely depressed albumin levels (<2.0 g/dL) were observed only in the indomethacin-treated group (group 6).

Kidney function was only mildly affected in most treatment groups. However, in the group treated with indomethacin, three of six patients had elevation in serum creatinine to >2.0 mg/dL; one of these patients had severe azotemia, with a creatinine elevation to 5.5 mg/dL. These elevations reversed rapidly after cessation of IL-2 therapy. Oliguria (urine output <600 mL/24 hr) was observed in most treatment groups, but more severe oliguria (<250 mL/24 hr) was seen predominantly in the patients receiving indomethacin. These results suggest that the addition of indomethacin to an IL-2 regimen of 3X10<6> U/m<2> per day may cause more rather than fewer toxic effects, as measured by weight gain, oliguria, and changes in serum albumin, BUN, and creatinine levels.

Following a 28-day observation period after the initial 4-week course of IL-2, 11 patients showed no evidence of progressive malignancy and received a second 4-week course of IL-2. Patient 7 received his second course at the same lower dose (1X10<6> U/m<2> per day continuous infusion) of IL-2 that was tolerated for days 15-28 of the first treatment course (see above). Two patients had their second course of IL-2 terminated after <28 days. Patient 26 (group 6) developed neurologic symptoms secondary to brain metastases while receiving the second course of IL-2. Patient 10 (group 5) developed severe transient neutropenia (absolute neutrophil count, 0), leading to cessation of therapy after the third week of IL-2 therapy. This resolved within 48 hours without any infectious complications. Bone marrow biopsy and aspirate samples taken at the time of neutropenia revealed normal myelopoiesis with a predominance of lymphocytes bearing NK markers (Leu-19 and Leu-11). After an additional month of more observation, three patients received a third 4-week course of IL-2 treatment (one received a fourth course) and tolerated these with toxic effects similar to those seen in the initial 4 weeks of treatment.

Immunologic Monitoring

Lymphocyte and eosinophil counts. As reported in previous studies [n27,n30,n31], a significant (P<.001) drop in circulating lymphocyte counts occurred within 24 hours of IL-2 therapy initiation for all 28 patients analyzed (table 5). These counts remained low during the 4 days of IL-2 treatment but then increased dramatically 24 hours after IL-2 was stopped (fig. 1). Each subsequent weekly cycle of IL-2 caused a rapid decrease in circulating lymphocytes. The lymphocyte nadirs increased with each subsequent IL-2 cycle. The mean (=/- SD) lymphocyte count 24 hours after the fourth cycle was initiated (1,372 +/- 811 lymphocytes/mu L) was similar to the mean pretherapy count (1,422 +/- 673/mu L). The lymphocyte counts obtained 24-48 hours after the first 4-day cycle of IL-2 therapy showed a striking increase over baseline values (P<.001) when data for the entire group of 28 patients were analyzed. The rebound lymphocyte counts experienced after the fourth week of IL-2 were significantly increased by a mean of 2,882/mu L over the rebound counts after the first week of IL-2 (P<.001 for the 21 patients completing 4 weeks of therapy). As shown in
Table 5, patients receiving $3 \times 10^6$ U/m$^2$ per day showed a greater rebound in lymphocyte counts if IL-2 was given by continuous rather than by bolus infusion (group 5 compared with group 3, $P<.009$). Although the dose of IL-2 appeared to affect the degree of change in lymphocyte counts (group 2 vs. group 5), this was not statistically significant ($P=.052$) in this study. This lack of a significant difference may reflect the small size of the treatment groups or the relatively small difference in the doses of IL-2 being compared.

Table 5. Lymphocyte and eosinophil counts

[SEE ORIGINAL SOURCE]

Eosinophil counts increased throughout the 28 days of therapy, with a decline during the observation periods between IL-2 cycles (fig. 1). By the end of treatment, patients exhibited a striking eosinophilia, with a mean peripheral eosinophil count $>5,000$ mu L (table 5). The eosinophil counts increased during the month but did not exhibit the same drop upon initiation of IL-2 or rebound upon cessation of IL-2 that was observed with lymphocyte counts.

Changes in phenotype of circulating lymphocytes. Table 6 shows the changes observed among the different treatment groups for lymphocyte phenotype for six different surface markers. When all patients were analyzed, a significant increase in the number of lymphocytes expressing the CD3 marker was found after 4 weeks of IL-2 treatment. At the same time, the actual percentage of CD3$<$+ cells declined from a mean of 62% before therapy to a mean of 44% at the end of the fourth week. The number of cells expressing class II HLA MHC molecules (Ia$<$++) was also higher after IL-2 therapy. All treatment groups demonstrated an increase in the mean number of Ia$<$+ cells. The mean percentage of Ia$<$+ cells also increased after 4 weeks of IL-2 treatment, from 17% to 26% ($P<.001$). While the number of lymphocytes expressing the TAC chain of the IL-2 receptor increased significantly ($P<.05$) after 4 weeks of IL-2 therapy, the percentage of these cells increased minimally (from 12% to 16%).

Table 6. Phenotypes of circulating lymphocytes

[SEE ORIGINAL SOURCE]

Both Leu-11 and Leu-19 surface markers, associated with NK cells and more recently with in vivo-activated LAK cells [n43,n44], showed increased expression as a result of IL-2 therapy. This increase appeared most striking in groups 5 and 6, receiving continuous IL-2 at $3 \times 10^6$ U/m$^2$ per day. For the 16 patients analyzed, the mean percentage of Leu-19$<$+ cells increased from 21% to 45% after 4 weeks of therapy. Most of the phenotypic changes noted after 4 weeks of IL-2 therapy were apparent after the first week (not shown), except for the Leu-19 and Leu-11 markers. The percentages of Leu-19$<$+ and Leu-11$<$+ cells noted after 4 weeks of IL-2 therapy were far greater than those noted after 1 week of IL-2 therapy (an increase from 28.9% at 1 week to 44.6% at 4 weeks for Leu-19 and from 18.9% to 29.6% for Leu-11; $P<.01$ for both markers), documenting that prolonged IL-2 treatment was influencing in vivo expansion of cells with these markers. Finally, the number of cells expressing B-1, a B-cell surface marker, was essentially unaffected by IL-2 therapy. This contrasts sharply with the changes observed for the other lymphocyte markers and suggests that IL-2 treatment selectively expands certain lymphocyte subpopulations without influencing others.

Proliferative capacity of lymphocytes activated by in vivo IL-2. Before therapy and weekly during each rebound period, PBL were assayed for their ability to respond to a variety of stimuli (medium, IL-2, PHA, and several allogeneic cell populations) in an in vitro proliferation assay ([3H]thymidine incorporation). PBL proliferation induced by 72 hours of culture with IL-2 was markedly increased by in vivo IL-2 treatment, while background proliferation (in medium alone) remained essentially unchanged (for all groups combined: mean proliferation in the presence of IL-2 increased from 6,538 cpm for pretherapy to 25,483 cpm for week 4 PBL, and proliferation in medium alone was 459 cpm pretherapy and 406 cpm on week 4). The 72-hour proliferative response of PBL obtained after 4 weeks of IL-2 treatment was significantly greater ($P<.05$) than that of pretherapy PBL for most treatment groups (2, 3, 4, and 5); this difference was highly significant when data for all patients were analyzed together ($P<.001$). This degree of
enhancement was not significantly affected by variations in the treatment regimens. In contrast, the in vitro proliferation induced by 1% PHA, a T-cell mitogen, was not increased by 4 weeks of IL-2 therapy (data not shown).

Induction of cytotoxic cells during IL-2 therapy. Table 7 shows the results of cytotoxicity assays for all six treatment groups. After 4 weeks of IL-2 therapy, fresh PBL showed a striking increase in lysis of K562 cells (P<.001 when data for all patients were included). Furthermore, individual groups (3, 4, and 5) each demonstrated significant (P<.05) increases in NK cell activity.

Table 7. Generation of direct cytotoxic cells

[SEE ORIGINAL SOURCE]

Evaluation of direct LAK activity (table 7), assessed by the ability of fresh PBL to destroy Daudi cell targets, revealed insignificant pretherapy activity (<10 LU/10<7> cells). When data for all six treatment groups were pooled, the LAK activity by PBL after 4 weeks of IL-2 therapy was significantly (P<.001) increased. However, the degree of this cytotoxic activity was still quite low (35 LU/10<7> PBL). In contrast, the enhancement in LAK activity after 4 weeks of IL-2 treatment was much more apparent when the 4-hour assay was performed in vitro with IL-2 present. While lysis of the Daudi target cells by pretherapy PBL was increased minimally by the presence of IL-2 in vitro (mean values for all 21 patients increased from 4 to 18 LU), IL-2 dramatically increased the ability of PBL obtained after 4 weeks of IL-2 therapy to lyse Daudi cells in the <51>Cr release assay (mean values increased from 35 to 389 LU). The increased LAK activity (comparing baseline and week 4 PBL samples) in the presence of IL-2 in vitro was significant when data for all patients were pooled and for patients in group 5 alone (P<.001). The marked enhancement of Daudi cell-directed cytotoxicity by inclusion of IL-2 in the 4-hour assay was not observed for PBL from normal subjects [n32].

Further analysis of these data documented that NK and LAK cell activities (both with and without IL-2 in the in vitro assay) were significantly (P<.01) greater after the fourth week of therapy than after the first week, as measured by LU/10<7> PBL, when data for all patients were analyzed. NK cell activity increased from 120 LU after week 1 to 251 LU after week 4; LAK cell activity in the absence of IL-2 increased from 16 LU after week 1 to 35 LU after week 4, and LAK activity in the presence of IL-2 increased from 199 LU after week 1 to 389 LU after week 4. These results indicate that additional IL-2 treatment after the first week further increased both the number of lymphocytes and their cytotoxic activity. Since PBL counts increased after 4 weeks of IL-2 treatment and the LU of cytotoxicity/10<7> cells also increased, it was not unexpected that the "cytotoxic potential" (LU of LAK activity/10<7> cells) for individual groups increased dramatically. The cytotoxic potentials for the six treatment groups after 4 weeks of IL-2 therapy increased 40-295-fold (fig. 2). Although cells from patients receiving IL-2 by continuous infusion (groups 2, 5, and 6) appeared to attain greater cytotoxic potential than those from the groups receiving bolus or combined IL-2 therapy (groups 1, 3, and 4), these differences were not statistically significant.

Immunologic changes observed with subsequent 4-week courses of IL-2. Seven patients received, and completed on schedule, a second 4-week course of IL-2 at the same dose as their first course after a 4-week rest period. Clinical tolerance and the pattern of lymphocyte counts (both nadirs while receiving IL-2 and rebound lymphocytosis after completion of each 4-day treatment) were similar for each patient's first and second 4-week courses, as reported previously for the initial patients studied [n34]. Lymphocyte phenotyping (as in table 6) was performed on PBL from four of these patients during the second course, and the PBL showed similar changes in percentage and number of lymphocytes expressing the markers studied (data not shown). These results suggest that immune changes induced by IL-2 retreatment are not affected by a prior course of IL-2 followed by a 4-week rest period.

Antitumor Effects

Three patients achieved objective partial remissions of their metastatic tumors. Clinical details for these three patients (Nos. 8, 17, and 18) have been reported previously [n33]. All three had their primary renal cell carcinoma resected and showed transient responses of all metastases (three with lung nodules and one with liver involvement) after
IL-2 treatment. None of the responding patients received the lower IL-2 dose or bolus IL-2 treatment (groups 1, 2, and 3). There was one responder in group 5 (continuous) and two in group 4 (combined). These patient numbers are too small to calculate response rates. Furthermore, there is no statistical evidence that response to the combined IL-2 schedule, which produced responses in two of four patients in group 4, was different from that to the continuous-infusion IL-2 schedule at the same dose, which produced one response among 14 patients in groups 5 and 6 ($P=.11$). Evaluation of changes in lymphocyte counts, cytotoxicity assay results, and surface marker profiles did not reveal any obvious differences between the three clinically responding patients and the other patients treated. It is interesting that patient 18, who had a partial remission, and patient 26, who had a minor response (slightly less than 50% regression of pulmonary metastasis of malignant melanoma), both developed progressive brain metastases while their systemic disease was responding to IL-2 therapy.

Discussion

IL-2 with and without exogenous LAK cells has reproducibly demonstrated antitumor effects in a number of animal models of disseminated malignancy [n8-n12]. These antineoplastic affects have been observed with weakly immunogenic and nonimmunogenic tumors, suggesting that cells other than tumor-specific CTL may mediate this effect [n11,n13,n45]. These original murine studies emphasized that high doses of IL-2 and large numbers of exogenously activated nonspecific cytotoxic (LAK) cells were required to induce maximal tumor regression [n11,n13].

More recent murine studies describe ways to administer IL-2 that may enhance its antitumor activity while minimizing toxicity [n46,n47]. These studies suggest that IL-2 given in a manner that sustains serum levels for prolonged periods of time may be more immunologically effective. Furthermore, one study suggested that lower doses given continuously were more effective than higher doses of IL-2 [n47]. Clearly, it is still necessary to assess different clinical regimens of IL-2 administration for toxicity, immune modification, and antitumor effects based on these preclinical findings.

In this study of repetitive weekly cycles of IL-2, the treatment was tolerated with acceptable toxicity. Of 28 patients, only three had their first course of therapy stopped because of toxic effects and one other patient required a dose reduction. No patient required ICU monitoring or blood pressure support with vasopressors. No patient had severe hepatic or prolonged severe renal dysfunction. This is not to suggest that the treatment was without side effects. Most patients developed fever, malaise, rash, diarrhea, and nausea and vomiting, particularly at the higher dose. Interestingly, the addition of indomethacin appeared to increase toxicity by leading to greater fluid retention and more severe azotemia and oliguria. It was recently proposed that IL-2 and indomethacin interact to cause more severe renal insufficiency through inhibition of renal prostaglandin synthases [n48]. Indomethacin's moderating effects on systemic symptoms (fever, arthralgias, and myalgias) appeared to be minimal.

The immune effects of this regimen were striking. First, rebound lymphocytosis was observed after 1 week of therapy and increased over the next 3 weeks. This increase in lymphocytes was significantly greater when IL-2 was administered by continuous infusion than when it was given by bolus injection. Eosinophil counts increased progressively, but values appeared to be more erratic. The significance of this eosinophilia remains uncertain.

Phenotypic analysis of PBL from IL-2-treated patients revealed a selective stimulation, with predominant increases in the number of cells expressing the Leu-11<> and Leu-19<> NK-associated markers. Cells expressing the TAC (p55 IL-2 receptor) molecule were increased in number, but the percentage was only minimally increased. These findings are consistent with data suggesting that the bulk of in vivo-induced LAK cells are Leu-19<> and do not bear the CD3 marker or the TAC molecule [n43,n44,n49].

Assays have shown major functional changes in PBL after IL-2 therapy. As noted in previous trials, in vivo-activated cells show early enhanced proliferation to IL-2 in vitro [n31]. This finding was more apparent in the higher-dose groups and was not seen for in vitro responses to PHA or alloantigens (data not shown). These results suggest that a subpopulation of PBL is activated by IL-2 therapy that can respond selectively with altered (more rapid)
kinetics to IL-2 in vitro. Analyses with cells separated by monoclonal antibody depletion techniques indicate that the cells proliferating to IL-2 in vitro and mediating LAK activity after a 4-week course of IL-2 are predominantly Leu-19<->Leu-11<-> lymphocytes (Hillman GW, Fisch P, Priewe AF, et al.: submitted for publication). NK and LAK cell activities by fresh PBL, in the presence or absence of IL-2 in vitro, were all significantly enhanced by in vivo IL-2 treatment. Cytotoxic activity was increased significantly beyond levels obtained with 1 week of IL-2 treatment by additional repetitive weekly cycles. Lympocyte function and phenotype data were obtained after each week of IL-2 treatment during the initial 4-week course of IL-2 given to each patient. Following the second and third weeks of IL-2 therapy, values for most of the parameters tested were nearly as great as those obtained after 4 weeks, indicating that continued IL-2 treatment at the same dose for 2-4 weeks maintains the heightened immune status at a level greater than that obtained with 1 week of IL-2 treatment. Measuring cytotoxic potential as total lytic capacity per microliter of blood allowed us to quantitate the immune stimulation and showed a 40-295-fold enhancement in cytotoxicity after therapy for the different treatment groups. Although the changes appeared larger in the high-dose groups, the differences between groups were not statistically significant. The lack of statistical significance may reflect the small numbers of patients per group and variability within each group, as well as the relatively small difference in dose levels used in the study.

We and others have previously shown an effect of IL-2 dose on immune change [n27,n30,n50]. Others have described induction of LAK activity by in vivo IL-2 therapy in cancer patients [n43,n44,n50]. However, these studies have described only isolated cases and not an entire study population. This study demonstrated stimulation of a variety of cytotoxic activities in an entire study group. These results demonstrate a consistency of immune activation by this type of IL-2 regimen that was not noted previously [n43,n44].

Antitumor effects were observed in three patients at the higher dose level [n33]. These responses were partial, not complete, and were short-lived (1, 3, and 5 mo), but the fact that they occurred in this trial of IL-2 at well-tolerated doses without need for infusion of exogenous LAK cells suggests that further testing is warranted. More recently, Mitchell et al. [n51] reported responses with low doses of IL-2 in patients with melanoma who had previously received suppressive doses of cyclophosphamide. In that study, and in the present trial, some patients who demonstrated systemic tumor regression with IL-2 simultaneously developed CNS metastases. These findings suggest that systemically administered IL-2 may not adequately penetrate the CNS.

It is our hope that this study and those of others trying to define more effective and less toxic schedules for IL-2 therapy may lead to improvements in our understanding and use of this form of immunotherapy. Despite the documented immune-mediated antitumor responses produced by this and other IL-2 regimens, the current response rates remain low, and there is a great need for further improvements. Preclinical data suggest that further modifications of these IL-2 regimens and the combination of IL-2 with other agents (chemotherapeutic and biologic) should now be pursued for the potential achievement of greater antitumor effects.

SUPPLEMENTARY INFORMATION: <2> Supported by Public Health Service contract CM-47669-02 from the Division of Cancer Treatment (National Cancer Institute) and grants CA-32685 (National Cancer Institute) and RR-03186 (Division of Research Resources), National Institutes of Health, Department of Health and Human Services; and by grant CH237C from the American Cancer Society.

<1> Received July 12, 1988; revised August 24, 1988; accepted August 24, 1988.

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We thank Drs E. C. Borden, P. P. Carbone, D. Goldstein, R. Hong, and D. Levitt. We also thank M. Pankratz and K. Blomstrom for preparing this manuscript.

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**GRAPHIC:** Figure 1, Mean daily lymphocyte and eosinophil counts for all 28 eligible patients. Counts are the combined mean values on each day for all patients receiving IL-2. The days on which IL-2 was administered during the 28-day period are shown by bars beneath the x axis.; Figure 2, Comparison of cytotoxic potential generated within each treatment group. Cytotoxic potential was calculated as the production of (lymphocyte count/µL of blood)X(LU/10⁷ PBL). Pretherapy values were based on three specimens taken before treatment. Posttherapy values were based on two specimens obtained 24 and 48 hr after IL-2 treatment was stopped (wk 4).