Immunomodulatory Properties and Toxicity of Interleukin 2 in Patients with Cancer


ABSTRACT

We performed a phase Ia/Ib study of interleukin 2 (IL2) in patients with cancer. Single doses of IL2 from 10⁵ units/m² to 10⁷ units/m² were well tolerated but failed to induce significant immunological changes. Chronic IL2 treatment for 5 days out of 7 for 3 weeks was well tolerated at doses below 10⁶ units/m² and was accompanied by significant immunological changes. Following chronic treatment with intramuscular injections of IL2 at 1 × 10⁶ units/m², we observed augmentation of peripheral blood natural killer activity and induction of peripheral blood LAK activity. Induction of LAK activity was most evident when IL2 was included in the cytototoxicity assay. There was a marked increase in the number of peripheral blood mononuclear cells bearing the Leu-19 marker in association with the observed increases in natural killer and LAK activity. A small percentage of Leu-19⁺ cells coexpressed CD3. There was heterogeneous expression of the low affinity Fc receptor (CD16). In vivo induced Leu-19⁺ cells could be divided into two populations, dim and bright, based on the intensity of fluorescent staining with antibodies to Leu-19. The majority of Leu-19 bright cells were CD16⁺ while the majority of Leu-19 dim cells were CD16⁻. In addition, the intensity of CD16 staining was higher for Leu-19 dim cells than for Leu-19 bright cells. Increases in the amounts of CD38 and CD8 antigens were also observed. Significant increases in serum levels of the soluble IL2 receptor were observed during treatment. One partial remission was noted in a woman with non-Hodgkin’s lymphoma.

INTRODUCTION

IL2 is a 15,000 dalton protein that interacts with specific receptors on a variety of hematopoietic cells. It was originally described as T-cell growth factor because of its capacity to selectively support the proliferation of T-cells in cultures of human bone marrow (1). In addition to being a principal growth factor for T-cells, IL2 mediates numerous immunologic functions that suggest it might be useful as an anticancer agent. IL2 supports the growth of human cytotoxic T-cells, enhances the cytotoxicity of NK cells and monocytes, and is the principal factor required for the induction and growth of LAK cells.

Antitumor responses have been observed in humans with cancer when IL2 is used alone (2, 3) or in combination with LAK cells (4). The exact mechanisms of the antitumor effects of IL2 are unknown, but since IL2 appears to have a limited capacity to exert direct antitumor effects on solid tumors, it is felt to act by maximally stimulating the immune system to mount an attack on the tumor. The fact that the addition of LAK cells markedly augments the antitumor activity of IL2 in mice (5) suggests that the final mediator of tumor regression may be the IL2-activated killer cell. Further evidence to support the role of LAK cells in tumor regression comes from the study of Mitchell et al. (6) in which induction of LAK activity in the peripheral blood during IL2 therapy correlated with tumor response in patients with melanoma. These observations stress the importance of measuring immunological changes occurring in IL2-treated patients in defining an optimal therapeutic regimen. Recent reports have shown that i.v. administration of IL2 can lead to induction of LAK cells in the peripheral blood of treated patients (7–10). These are important initial findings, but to reproducibly enhance immune function using IL2, more data are required to determine the optimal dose, route, and schedule of IL2 administration. This report summarizes the toxicity and immunological effects of acute and chronic administration of IL2 at a variety of doses.

PATIENTS AND METHODS

Patient Population. All patients entering this trial were evaluated and treated at the BRMP, Frederick, MD. To be eligible to enter this study, patients must have had a histologically confirmed diagnosis of a malignancy refractory to standard therapy, or a neoplasm for which there was no standard therapy. All patients had measurable disease. Patients with T-cell malignancies were excluded. Pretreatment evaluation included physical examination, CBC and differential cell count, routine serum chemistry screen, chest radiograph, and CT scans as required to evaluate tumor. All patients were negative for HIV antibody and HBsAg. Patients must have had a life expectancy of at least 2 months and a Karnofsky performance status of greater than 60%. All patients had normal cardiac, renal, and hepatic function. Written informed consent was obtained from all patients, and the protocol was approved by the Institutional Review Boards of both the Frederick Cancer Research Facility and the Clinical Oncology Program of the National Cancer Institute.

Interleukin 2 Preparation. Recombinant IL2 was produced by Hoffmann-LaRoche, Nutley, NJ, using standard recombinant DNA technology. This clinical grade material had passed pyrogenicity testing and had no detectable endotoxin. The IND number for this IL2 was BBIND-2050. The material was shipped lyophilized at 10⁶ units/vial (>95% pure) and was reconstituted with sterile saline. IL2 activity was measured as growth-promoting activity using an IL2-dependent T-cell line and quantitated in BRMP units.

Study Design. The study was performed in two parts. During part I, patients were divided into three groups based on the route of administration (i.e., i.v., and intramuscular [i.m.]) and then each patient was treated once weekly with escalating doses of IL2 according to the following two schedules: Schedule A, 10⁵, 10⁶, 10⁷, and 10⁸ units/m²; Schedule B, 10⁴, 10⁵, 3 × 10⁶, and 10⁸ units/m².

Blood was drawn at baseline (+2–3) and at 24 and 48 h following each dose. Each sample of peripheral blood was tested for the following...
immune parameters: NK activity, LAK activity, proliferation to Con A, PHA, or mixed allogeneic lymphocytes, serum levels of soluble IL2 receptor and expression of cell surface markers as described below. The original study design called for examination of immunomodulatory effects after each dose of IL2 and calculation of an OID which was defined as the minimal dose of IL2 that resulted in a significant and sustained or repeated elevation of at least one parameter of immune function with minimal depression of other functions. Part II of this study, which was to begin 1 month after completion of part I, called for each patient to receive daily i.m. injections of IL2 at their individual OID from Monday through Friday for 3 weeks. When it became clear that measurement of immunological changes after a single dose of IL2 would not permit determination of an OID, the dose of IL2 for part II was chosen based on toxicity during part I. Patients received the highest dose considered tolerable during part I. Responder patients were eligible for further treatment as were selected patients with stable disease who tolerated IL2 treatment with minimal toxicity.

Toxicity Assessment. Subjective toxicities were graded and recorded by nurses on a daily basis during the first 4 weeks of treatment. Thereafter, patients were asked to complete a toxicity scale checklist three times per week. Physical examinations were performed on the day of and 24 h and 48 h after IL2 administration during part I of the study and then weekly during part II. A complete blood count, chemistry panel, and urinalysis were performed weekly during the study.

For purposes of dose modification, toxicities were graded as being I–IV. Dose escalation was permitted in the absence of grade III toxicity. For grade III toxicity during the chronic treatment phase, patients could be treated at the next lower dose once toxicity had returned to baseline.

Response Assessment. Tumor measurements were recorded at the end of part I and monthly thereafter. Complete response was defined as complete disappearance of all lesions for at least 4 weeks with no new lesions developing. Partial response was defined as a 50% or greater decrease in the sum of the two largest perpendicular diameters of all measurable lesions maintained for at least 4 weeks with no new lesions developing. PD was considered a 25% or more increase in any measurable lesion or the appearance of new lesions. Stable disease was defined as disease not meeting the above criteria for response or progression.

Cell Preparation and Cryopreservation. To eliminate day-to-day variability in assay and staining procedures and instrumentation, all assays except for NK assays were performed on cryopreserved cells and at least two pretreatment samples were assayed. At the completion of each treatment phase (parts I and II), all samples from one individual were thawed and assayed in a single assay. Peripheral blood mononuclear cells were separated from heparinized blood by Ficoll-Hypaque density gradient centrifugation (LSM, Litton Bionetics, Rockville, MD); washed three times in RPMI 1640 medium supplemented with 20% fetal calf serum, 0.1% gentamicin, 1% l-glutamine, and 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer. The PBMC to be frozen were suspended in complete media containing 15% dimethyl sulfoxide and placed into the precooled phase of a liquid nitrogen storage freezer until needed.

Cytotoxicity Assay. Freshly isolated mononuclear cells were used for determination of natural killer (NK) activity because the recovery of NK activity from cryopreserved cells obtained at different times shows great variability. The assay was a standard 4-h 51Cr-release cytotoxicity assay using the cell line K562 as target (11). Measurement of LAK activity was performed in the same manner except the relative NK-resistant Daudi cell line was used as the target. The percentage of specific cytotoxicity was calculated as the percentage 51Cr released in the experimental group minus the percentage released in the medium control. Data are expressed as lytic units, calculated from the specific cytotoxicity at each of the effector:target ratios using an exponential fit equation (12). One lytic unit is defined as the number of effecter cells required to lyse 1000 target cells (LU[20%]) and the data are expressed as lytic units per 10^6 effector cells.

Cell Surface Markers. Enumeration of leukocyte subpopulations was performed by flow cytometry using a panel of monoclonal antibodies directed against cell surface antigens. Cryopreserved Ficol-Hypaque separated mononuclear cells were used in all determinations and, where possible, all samples obtained serially from each patient were tested on a single day. The following monoclonal antibodies were obtained from Becton-Dickinson Immunocytometry Systems (Mountain View, CA): anti-Leu-4 (CD3), anti-Leu-3a (CD4), anti-Leu-2b (CD8), anti-Leu-11 (CD16), anti-Leu-12 (CD19), anti-Leu-10 (HLA-DQ), anti-Leu-M3 (CD14), anti-Leu-19, and anti-HLA-DR. The anti-Tac antibody was the gift of Dr. Thomas Waldmann of the National Cancer Institute. Anti-OKT10 (CD38) was obtained for Ortho Diagnostic Systems, Inc. (Raritan, NJ). All antibodies except anti-Leu-19, anti-OKT10, and anti-Tac were directly conjugated with FITC or PE. Reactivity with anti-Leu-19, anti-OKT10, or anti-Tac was detected through a two-step staining procedure in which incubation with primary antibody was followed by incubation with the F(ab')2 fragment of goat anti-mouse IgG that was FITC labeled (Tago, Inc., Burlingame, CA). For two-color analysis, cells were stained with a mixture of monoclonal antibody pairs directly conjugated with FITC or PE. The cells were analyzed on an Ortho Cytofluorograf 30-H equipped with a model 2150 computer (Ortho Diagnostic Systems, Inc., Westwood, MA). Viable cells were selected for analysis based on propidium iodide (10 μg/ml) exclusion, as detected by red fluorescence. Cells were gated by low angle forward and right angle light scatter properties as lymphocytes while a second analysis gate included all mononuclear cells. The percentage of cells reactive with each monoclonal antibody was determined by comparison of fluorescently labeled cells with cells that had been incubated with labeled isotype controls. Logged single color fluorescence was plotted as a linear 1000 channel histogram.

Proliferation Assay. Lymphoproliferative responses to Con A, PHA, and mixed allogeneic leukocytes were performed in a miniaturized version of a standard assay which has been described in detail previously (13).

Soluble IL2 Receptor Assay. Serial serum samples were collected during the course of IL2 therapy. Normal laboratory volunteers served as controls. sIL2R levels were determined as described previously (14). Briefly, serial dilutions of each serum specimen were added to wells of a 96-well Immulon microtiter plate (Dynatech Laboratories, Alexandria, VA) previously coated with the monoclonal anti-IL2 receptor antibody anti-Tac (generously provided by Dr. T. Waldmann, NIH). Plates were incubated for 2 h, washed, and incubated 2 h further with antibody 7G7/B6 conjugated with FITC. This antibody recognizes an epitope of the IL2 receptor distinct from that recognized by anti-Tac (15). The plate was then washed and an alkaline phosphatase-conjugated rabbit anti-FITC antibody was added and the mixture was incubated for 1 h, washed, and then p-nitrophenyl phosphate (1 mg/ml; Sigma) was added. The absorbance of each well was determined after 30 min at 405 nm by using a Titertek ELISA plate reader (Flow Laboratories, Rockville, MD). A reference reagent, consisting of the cell-free supernatant of an IL2-dependent normal human T-cell line 4 days after stimulation with 10% IL2 (Cellular Products, Buffalo, NY) was used in all these assays. The undiluted supernatant was assigned an arbitrary value of 1,000 IL2 receptor units/ml, and the absorbance values as determined by ELISA assay of serial dilutions of this supernatant were used to generate a reference curve. The absorbance of the test wells was then compared with the standard curve and was converted to a numerical value. Receptor levels in this study are expressed in units/milliliter.

Statistical Methods. Differences from baseline for each immune parameter were assessed using a paired sample t test to incorporate the pair-to-pair variability of the patients' data. Patients served as the pairing factor. Values were log transformed and differences were computed by subtracting average baseline measurements and peak treatment values (16). The Student's t test was used to analyze pre- and posttreatment group means. Linear trends were detected using regression analysis on parameters regressed over time on study (17). Significance was determined on all statistical tests at the 0.05 level. All the statistical tests were performed using the Statistical Analysis System available on the IBM system 370 at the National Institutes of Health Division of Computer Research and Technology.
RESULTS

Patient Summary

The pretreatment characteristics of all 14 patients are listed in Table 1. Thirteen of 14 patients completed all four weekly doses of IL2 in part I of the study; nine according to schedule A and four according to schedule B. Two patients developed progressive disease during or shortly after part I, so only 11 patients received chronic IL2 therapy during part II of the study. Eight of these 11 patients completed all of the planned (3 weeks) chronic therapy; two developed dose-limiting toxicity and one died suddenly.

Toxic Effects

Part I. Single doses of IL2 spaced 7 days apart were well tolerated and all but one patient successively completed part I of the study. One patient with melanoma developed thrombocytopenia (72,000/mm³) and hyperbilirubinemia (2.9 mg/dl) after a single i.v. dose of IL2 (10⁸ units/m²) and was removed from study. There were insufficient numbers of patients treated by different routes (i.m., s.c., i.v.) and doses of IL2 to make it possible to prevent meaningful toxicity information broken down according to both dose and route of administration. Therefore, Table 2 lists the toxicities according to the dose of IL2 administered regardless of the route. At 10⁷ and 10⁸ units/m² there was minimal toxicity. At doses ≥10⁹ units/m² there was a dose-related increase in the incidence and severity of fever, chills, headache, and fatigue. There was also a dose-related decrease in blood pressure and urine output, although with single doses of IL2 aggressive supportive measures usually were not required.

Part II. Table 3 lists the toxicities associated with chronic treatment with IL2 for 5 days out of 7 for 3 weeks at various doses. All patients received chronic treatment with IL2 by i.m. injection. One patient received 3 weeks of treatment at 10⁸ units/m² and had minimal toxicity. Seven patients completed all 15 scheduled doses of IL2 at 10⁶ units/m² with the toxicities as shown in Table 4. None of the three patients scheduled to receive 10⁷ units/m² of IL2 were able to complete this phase of therapy because of unacceptable side-effects. At this dose level, one patient had the fourth dose withheld because of azotemia, oliguria, and diffuse pulmonary infiltrates. Additional doses were accompanied by recurrent Grade III renal toxicity despite significant treatment delays, 50% dose reduction and addition of indomethacin and dexamethasone. The patient eventually developed a small bowel obstruction from progressive disease and was removed from study. A second patient received the initial five doses of IL2 at 10⁷ units/m² but required significant medical support including dopamine for blood pressure support, furosemide, and supplemental O2 for hypoxemia secondary to bilateral interstitial pulmonary infiltrates and pleural effusions. These symptoms recurred with retreatment and therapy had to be discontinued. An additional patient died suddenly approximately 24 h after receiving his third dose of IL2 at 10⁷ units/m². He was unable to be resuscitated after a witnessed cardiac arrest just moments after his blood pressure and pulse had been measured and found to be normal. Autopsy showed diffusely metastatic malignant melanoma with disease in the brain, lungs, liver, bone marrow, pericardium, and membranous interventricular system.

Antitumor Effects. No tumor responses were noted after weekly single injections of IL2. Two patients had PD after part I and three patients had PD after part II. Two patients received extended therapy beyond part II of the protocol. One patient with renal cell carcinoma and one with an indolent non-Hodgkin's lymphomas received 21 months and 19 months of IL2 at the 10⁶ units/m² dose level, respectively. Both patients received IL2 by i.m. injection 5 days out of 7 for 3 weeks with 4 weeks off in between cycles. Chronic long-term treatment was well tolerated. The patient with renal cell cancer never achieved an objective response and was removed from study when he eventually developed progressive disease. The woman with non-Hodgkin’s lymphoma had a >90% reduction in iliac and retrocaecal lymphadenopathy during IL2 therapy. She developed progressive disease 6 months after IL2 was discontinued.

Immunomodulatory Effects

In addition to determining the toxicity of single and multiple doses of IL2, this study was designed to establish, within an individual patient, an optimal immunomodulatory dose and route of IL2 that could be used for chronic therapy. Insufficient numbers of patients were treated to examine the immunomodulatory effects of IL2 by dose and route, so all subsequent data are grouped according to dose of IL2.

Part I. Single doses of IL2 failed to cause significant changes in spontaneous proliferation or proliferative responses of patients' PBMC to Con A, PHA, or pokeweed mitogen. Similarly

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Table 1 Patient characteristics

| Number | 14 |
| Median age | 58 (range, 37–54) |
| Male/female | 6/8 |
| ECOG performance status | 0 |
| Prior chemotherapy | 11 |
| Prior radiation | 4 |
| Prior immunotherapy | 5 |
| Diagnosis |  |
| Adenocarcinoma of colon | 4 |
| Melanoma | 4 |
| Lymphoma | 3 |
| Renal cell carcinoma | 1 |
| Adenocarcinoma of breast | 1 |
| Malignant schwannoma | 1 |

Table 2 Toxicity of single doses of IL2

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<th>Dose (units/m²)</th>
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<th>Chill/fever</th>
<th>Fatigue</th>
<th>Headache</th>
<th>JBP</th>
<th>N/V</th>
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<td>2</td>
<td>0/2</td>
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<tr>
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<td>12</td>
<td>2 (99.1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>6 (100)</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>10⁹</td>
<td>13</td>
<td>11 (100.7)</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>2</td>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>3 x 10⁷</td>
<td>4</td>
<td>3 (101.2)</td>
<td>3</td>
<td>0</td>
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</tr>
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</table>

Other toxicities included H. Zoster, rash, diarrhea, oliguria, nodal tenderness, anorexia.

a Data, number of patients experiencing the indicated toxic effect.
b JBP, decrease in blood pressure.
c N/V, nausea/vomiting.
there were no significant changes in percentage of positivity or MFC of any of the cell surface markers examined. There were no significant changes in serum soluble IL2 receptor (sIL2R) levels and only minor changes in NK activity were observed in an occasional patient. The minimal changes in immune function observed after a single dose of IL2 made determination of an individual OID impossible. Therefore, the selection of IL2 dose for part II was made based on tolerance to treatment during part I. This was usually the highest dose received by the patient during part I. One patient received chronic treatment of $10^5$ units/m², seven patients at $10^6$ units/m², and three patients at $10^7$ units/m².

Part II. In contrast to single dose therapy, patients receiving chronic treatment with IL2 showed multiple immunomodulatory effects.

Cytotoxic Activity. None of the three patients scheduled to receive IL2 at $10^7$ units/m² completed therapy and are not included in our analysis of chronic IL2 treatment. The single patient receiving $10^5$ units/m² experienced an increase in NK activity from 175 LU/10⁶ cells at baseline to 805 LU/10⁶ cells after chronic IL2 therapy. Table 4 summarizes the immunological changes observed in seven patients receiving chronic IL2 therapy at $10^6$ units/m². All seven patients receiving IL2 for 3 weeks at $10^6$ units/m² exhibited IL2-induced increases in peak NK activity compared to baseline ($P = 0.002$ by paired t test). When the mean of on-treatment NK activity for all seven patients ($285 ± 36$ LU/10⁶ cells) was compared with baseline values ($114 ± 18$ LU/10⁶ cells), analysis by the t test revealed a significant increase in NK activity ($P < 0.0001$). Linear regression analysis revealed an increasing trend over time for all patients when they were examined together ($P < 0.0001$) and in three of the seven patients examined individually. NK activity in the two patients who received IL2 for more than 1 year continued to increase during IL2 therapy even after 18 months of treatment. NK activity was observed to return to baseline during the month off therapy and then increase with resumption of chronic IL2 therapy.

The peripheral blood of two patients receiving chronic IL2 therapy was examined for the presence of cells that could kill Daudi cells in vitro (LAK cells), either directly or when IL2 at 100 units/ml was included in the cytotoxicity assay. Each patient received IL2 ($1 \times 10^6$ units/m²) by i.m. injection on Days 0–4, 7–11, and 14–18. Table 5 contains the results of cytotoxicity assays performed on cells obtained from two patients just before the first and last dose of each 5-day treatment cycle for each of 3 successive weeks. Patient 3 had high levels of NK activity but low levels of LAK activity at initiation of therapy. There was a modest increase in the ability of this patient's cells to kill Daudi cells but the increase was most evident when IL2 was included in the cytotoxicity assay (see Days 11 and 18 in Table 5). Patient 4 had low levels of LAK activity at the beginning of each week of IL2 therapy that increased during the 5 days of IL2 therapy. LAK activity was even more evident when IL2 was included in the cytotoxicity assay (see Days 11, 14, and 18). Even on Day 14 where there appears to be only a minimal increase in LAK activity, the simple addition of IL2 to the cytotoxic assay indicates that significant numbers of activated cells are present.

Proliferative Responses. Minor changes were noted in the response of patients' cells to various mitogens (data not shown). No patients' cells exhibited an increased proliferative response to PHA, Con A, or pokeweed mitogen. This was also true of spontaneous proliferation. Cells from three of five patients had a decreased response to Con A, one of five to PHA, and one of five to pokeweed mitogen. Two patients responded normally to all mitogens throughout treatment and one patient exhibited a decrease to all mitogens during IL2 therapy.

Cell Surface Markers. Significant changes were detected in a variety of cell surface markers. The most striking changes were the marked increases in the percentages of gated lymphoid cells bearing markers of NK or LAK phenotypes (Leu-19 and CD16, Table 4 and Fig. 1). During the chronic phase of IL2 treatment all patients tested had an increase in the percentage of lymphoid cells bearing the Leu-19 and CD16 determinants. Fig. 1 illustrates the magnitude of this increase in one patient with a malignant schwannoma who received $1 \times 10^6$ units/m² of IL2. Not only did the percentage of lymphocytes bearing Leu-19 increase from a baseline of approximately 30% to a peak of 74%, but the amount of antigen on the cell surface also increased as evidenced by an increase in MFC from 278 to 470 (Fig. 1). The increase in both percentage of positive lymphocytes and MFC was a consistent finding. Overall, in the five patients examined for Leu-19 expression during chronic IL2 treatment at $1 \times 10^6$ units/m², pretreatment Leu-19 on lymphocytes went from a mean of 19.8% positive with a MFC of 236 to mean posttreatment levels of 46% positive and MFC of 362. There appear to be two distinct populations of Leu-19+ cells; one "dim" population with a MFC of approximately 300
and a "bright" population with a MFC of approximately 600.

The major increase in this, and other, IL2-treated patients was in the "bright" population. An increased percentage of cells bearing other NK markers, CD16 (Fig. 1) and Leu-7 (data not shown), was also observed.

Two color flow cytometric analysis of the expanded lymphocyte population revealed that 97% of the Leu-19+ lymphocytes were negative for CD3 and only 2% were positive for both Leu-19 and CD3. The expanded lymphocyte population was heterogeneous for the low affinity Fc receptor (CD16) and for expression of the Leu-19 antigen. As shown in Fig. 2C, Leu-19 bright lymphocytes, generally defined by MFC >40, coexpressed low levels of CD16 while Leu-19 dim lymphocytes were generally CD16+ . Among the Leu-19 bright cells, only 22% coexpress CD16 and then only at low levels with a MFC of only 21. However, CD16 expression is much greater among the Leu-19 dull population in which 59% of the cells coexpress higher levels of CD16 (MFC = 32). Approximately 7% of the cells were CD16+ Leu-19+. A subpopulation of CD16+ cells did not express Leu-19. Other changes observed during treatment included small increases in the percentage of cells bearing CD25 (IL2R) (data not shown) and significant increases in the percentage of cells bearing the activation marker CD38 or OKT10 (Fig. 1). Although Fig. 2 only depicts the data obtained from a single patient, similar results were obtained in one additional patient for whom sufficient cells were available to perform two-color analysis.

Soluble IL2R. An additional finding that indicated in vivo activation of cells by IL2 was the observation that serum levels of the soluble IL2R increased during IL2 treatment (Table 4). Baseline serum levels of the sIL2R were found in two of five patients examined; both of these patients had non-Hodgkin's lymphomas. Increased serum levels of sIL2R were found during chronic IL2 therapy in all five patients in whom they were measured ($P = 0.0093$ by paired $t$ test). Increased levels were apparent by 48 h of initiation of IL2 and appeared to antedate the increases observed in NK activity (Fig. 3). Increased sIL2R coincided with the increase in Leu-19 positive cells seen during treatment. In the two patients receiving IL2 for more than 12 months, serum was examined by radioimmunoassay for the secondary induction of IFN-γ. No IFN-γ was detected in the serum of either patient ( assay sensitivity, 0.1 units/ml).

**DISCUSSION**

Interleukin 2 is responsible for the induction of LAK activity in peripheral blood cells in vitro (18). IL2-activated cells acquire the ability to recognize and kill a variety of fresh autologous tumor cells, as well as a number of NK-resistant tumor cell lines, in a nonantigen-specific and non-MHC-restricted manner. Significant antitumor effects have been noted when LAK cells are administered to tumor-bearing humans or animals in combination with IL2 (4, 19). Current data from mouse models suggest that the more LAK cells and more IL2 that is given, the better the antitumor response (20). No correlation between number of LAK cells or dose of IL2 with tumor response has been demonstrated in humans (4, 21). In fact, it is possible that exogenous administration of in vitro-activated LAK cells adds nothing to the antitumor effects of IL2 alone. Prospective randomized trials are in progress to address this issue. Significant antitumor responses have been seen when IL2 is used alone (2, 3) and it is possible that tumor regression might be due to the induction of LAK cells in vivo. This hypothesis is supported by the data of Mitchell et al. (6) showing that among the 24 patients with melanoma treated with low doses of IL2 and cyclophosphamide, there were no responses in the seven patients who failed to develop LAK cells and six responses in the 17 patients who developed peripheral blood LAK activity. These data suggest that a well-tolerated IL2 regimen optimized for immunological activity, particularly for induction of peripheral blood LAK activity, might have very potent antitumor effects.

**Fig. 1.** Flow cytometric analysis was performed on mononuclear cells obtained from the peripheral blood of one patient before and during IL2 therapy. Lymphocytes were gated by light scatter. Intramuscular IL2 was given on Days 0-4, 7-11, and 14-18. A, Leu-19; B, CD16; C, CD38.
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Fig. 2. Coexpression of Leu-19 on CD3* and CD16* lymphocytes. Peripheral blood mononuclear cells were stained with FITC-IgG, and PE-IgG, control antibodies (A); PE-Leu-19 and FITC-CD3 (B); PE-Leu-19 and FITC-CD16 (C); PE-CD3 and FITC-CD16 (D). Correlated measurements are displayed as contour plots. On the basis of the control sample, the contour plots were divided into quadrants to represent unstained cells (lower left), cells stained with both fluorochromes (upper right), or cells stained with only one fluorochrome (upper left and lower right). In this control sample (A), 100% of the cells were unstained (lower left quadrant). In this individual 2% of lymphocytes were CD3*, Leu-19*; 22% CD16*, Leu-19*; 36% CD16*, Leu-19*; and <1% CD16*, CD3*. In the CD16*, Leu-19* population, Leu-19*dim cells (<channel 50) exhibited higher intensity CD16 staining than Leu-19*bright* cells.

Fig. 3. NK activity and soluble IL2R levels in patient 6 during chronic IL2 therapy. —— NK activity; —— soluble IL2R, indicate time of IL2 administration.

To reproducibly induce in vivo LAK activity and other IL2-related changes, we need to acquire a better understanding of the biological effects of IL2 when administered to a patient with cancer. This phase I study was initiated before our current knowledge of IL2 and was designed to determine an optimal immunomodulating dose of IL2 for each individual patient and to use that dose for chronic therapy. Our study confirms the toxicity observed in other trials. Single doses up to 10^7 units/m^2 were well tolerated, however, chronic therapy was not possible at 10^7 units/m^2 because of intolerable side-effects in all three patients treated with this dose. In our study, 10^6 units/m^2 was well tolerated as an outpatient regimen when administered by i.m. injection. It is evident from our data that insufficient immunological changes were induced by single doses of IL2 to determine an individual patient OID. Other studies suggest that the earliest changes induced by IL2 consist primarily of a decrease in lymphocyte count with an associated loss of proliferative capacity, NK activity, and LAK precursors (10, 22, 23). These effects have been described for doses of IL2 \geq 10^5 units/m^2 when given i.v. by a variety of schedules. Thus, the first 24 h after IL2 administration is not the best time to examine patients for IL2-induced immune changes. Our observed lack of immunological effects after single doses of IL2 is most likely related to the low doses of IL2 used in some cases and the fact that monitoring was performed at 24 and 48 h when decreases in immune function might be expected.

Our current understanding of IL2 suggests that chronic treat-
ment is the key to obtaining significant immunological effects. Investigators from Wisconsin have shown that daily i.v. administration of IL2 at doses of $\geq 10^6$ units/m2 for 4–7 days results in striking increases in lymphocyte number and augmentation of proliferative responses to IL2 and NK activity as well as LAK cell induction in the peripheral blood (7, 22). Induction of LAK activity was detected when IL2 was included in the cytotoxicity assay (22). They demonstrated that inclusion of IL2 in the cytotoxic assay increased killing of K562 by unactivated cells, but it did not induce killing of Daudi cells unless effector cells had been preactivated with IL2 (22). Their interpretation of this phenomenon is that IL2-activated cells are extremely dependent on the presence of IL2 and its absence, even for 4 h, during the cytotoxic assay could cause loss of cytotoxic activity. Our results support their findings in a number of ways. Increases in cytotoxic activity were observed only during chronic therapy, however, induction of cytotoxicity occurred at lower IL2 doses ($1 \times 10^5$ units/m2) and following i.m. rather than i.v. injection. Significant enhancement of NK activity and induction of LAK activity (particularly when IL2 was included in the cytotoxicity assay) were observed with this well-tolerated and relatively low daily dose of IL2. Thompson et al. (10) also described in vivo induction of cytotoxic activity but used higher daily doses of IL2 given i.v. by 2-h or 24-h infusion for 5 days every other week for 4 weeks. When given by 2-h infusion it took four cycles to see direct killing of NK-resistant targets by peripheral blood cells. McMannis et al. (9) were able to show induction of LAK activity within 4–6 weeks when IL2 was given by continuous i.v. infusion twice weekly at doses of $3 \times 10^5$ units/m2 or $1 \times 10^6$ units/m2. We were able to demonstrate LAK activity using a relatively low dose of IL2 when given by i.m. injection.

The LAK cells induced in our patients during chronic treatment at low doses of IL2 have a phenotype similar to those described by others (7, 10, 24). There was a marked expansion of Leu-19+ cells that, by two-color flow cytometric analysis, were predominantly CD3− and heterogeneous for CD16 (the low affinity Fc receptor). The Leu-19+ cells can be divided into four subpopulations based on the amount of the Leu-19 and CD16 (large molecules) or CD16 (small molecules) expression. The Leu-19− cells are mainly CD16− or express low levels of CD16 while Leu-19+ cells are predominantly CD16+. Whether both Leu-19 bright and dull populations participate in non-MHC-restricted cell-mediated cytotoxicity awaits studies performed after sorting cells into dim and bright populations. The IL2-induced cells acquired the CD38 activation antigen and also expressed low levels of the CD8 antigen similar to in vitro-activated LAK cells. There is also a modest increase in the number of cells bearing the IL2R although the increase appears much less than might be expected given the significant increases observed in soluble IL2R levels.

Elevation of serum sIL2R levels is another independent piece of evidence that IL2 has caused in vivo activation of cells. The source of sIL2R is not known and could be from NK cells, monocytes, T-cells, or B-cells. Lotze et al. (25) have described increased serum sIL2R levels after administration of high doses of IL2 and increased levels also have been noted in patients with adult-T-cell leukemia/lymphoma (26) and hairy cell leukemia (27). Although the significance of the increased sIL2R levels in our patients is not known, when used as a tumor marker in hairy cell leukemia and adult-T-cell leukemia/lymphoma, the level of sIL2R has been shown to correlate inversely with response to therapy. We would expect it to vary directly with degree of immune stimulation when it is used as a measure of immune activation and indeed studies in renal transplant patients who are acutely rejecting their allografts support this idea. A better understanding of the relationship of sIL2R to IL2 treatment may allow it to be employed as a surrogate marker of adequate IL2-induced activation rather than using the more cumbersome and poorly standardized cytotoxic assays.

Our data have important implications for future clinical trials using IL2 alone or in combination with cytotoxics, monoclonal antibodies, other lymphokines, or effector cells. We have shown that chronic outpatient administration of well-tolerated doses of IL2 results in the expansion of a population of lymphocytes of LAK cell phenotype whose appearance correlates with an enhancement of NK activity and the presence of LAK activity in the peripheral blood. Thus, this rather low, well-tolerated, and easily administered dose potentially could be employed long term to induce LAK activity in patients during a study to prospectively determine whether induction and long-term maintenance of LAK activity can induce tumor regression. If higher doses of i.v. IL2 are chosen to induce LAK activity, $1 \times 10^6$ units/m2 may be given by the i.m. route to try and maintain LAK activity. The finding that the majority of Leu-19+ cells induced are Leu-19 bright and therefore CD16− has implications for the design of trials in which IL2 and monoclonal antibodies are administered. Since CD16 is the low affinity Fc receptor, one would expect that to maximize ADCC it is the Leu-19 dim CD16− cells that need to be expanded to take advantage of this immunological mechanism. To answer these questions and to design future trials with combinations of agents, detailed immunological studies similar to those done here need to be performed.

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IMMUNOMODULATORY PROPERTIES OF IL2


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