In Vivo Administration of Purified Human Interleukin-2 to Patients with Cancer: Development of Interleukin-2 Receptor Positive Cells and Circulating Soluble Interleukin-2 Receptors following Interleukin-2 Administration

Michael T. Lotze, Mary C. Custer, Susan O. Sharrow, Laurence A. Rubin, David L. Nelson, and Steven A. Rosenberg

ABSTRACT

Recent studies have demonstrated efficacy of immunotherapies including interleukin-2 (IL-2) in the treatment of malignancies in rodents and humans. High levels of IL-2 receptor-positive cells were found in the peripheral blood of patients receiving recombinant IL-2 in these Phase I clinical trials. This was demonstrated both in patients receiving i.v. IL-2 who had detectable circulating levels of IL-2 as well as in patients receiving i.p. IL-2 who did not. Up to 100% of the anti-Tac binding could be inhibited by preincubation with IL-2 indicating that this was indeed an IL-2 receptor that was identified. Two-color experiments demonstrated that few Leu 2-positive cells (<5-10%) but over 30% of the Leu 3-positive cells bore Tac antigen. Most of the M3-positive monocytes were Tac-positive (83.7%) and negative for other T-cell (Leu-4) and non-specific murine markers (Ly-2 and Thy-1.2). Although normal individuals had a mean of only 186 units/ml (range, 83-335 units/ml) of soluble IL-2 receptor, patients receiving IL-2 had as much as 20,000 units/ml of soluble IL-2 receptor line in their serum. The physiological role of the IL-2 receptor identified on the cell surface of Leu 3 and M3-positive cells as well as in the serum is unclear. Soluble IL-2 receptors appeared in the circulation early following IL-2 administration, approximately 1 week prior to the detection of circulating IL-2 receptor-bearing cells. Further studies will be needed to assess the role of IL-2 in monocyte function, the precise function of IL-2 receptor-bearing Leu 3-positive cells, and the relationship of these findings to the toxicity and success of this immunotherapy in humans.

INTRODUCTION

Interleukin-2 is a glycoprotein with a molecular weight of 15,000 which is essential to the proliferation of antigen-stimulated T-cells. The gene encoding this protein has recently been cloned and expressed in bacteria (1, 2). The administration of purified human interleukin-2 to both humans and mice is associated with significant immunological effects including the ability to increase natural killer or lymphokine-activated killer cells and the production of γ-interferon in vivo (3-6). In addition, in both established murine metastatic sarcoma (4) and melanoma (7) models, the adoptive transfer of IL-2-activated cells in combination with IL-2 has been demonstrated to be efficacious. Previous studies carried out in humans with both Jurkat-derived IL-2 (3) and recombinant IL-2 (6) have demonstrated a serum IL-2 half-life of approximately 6.9 min with a later (30-60 min) delayed clearance consistent with a two-compartment model of IL-2 distribution. In these studies a marked redistribution of lymphoid cells in the periphery was noted with an early depletion of all lymphoid cells. A 2-12-fold expansion of circulating lymphoid cells in vivo could be demonstrated with continuous administration of IL-2. In those earlier studies, as many as 25-40% of these circulating peripheral blood mononuclear cells were noted to express IL-2 receptors using flow cytometric analysis with an antibody to the IL-2 receptor. Since IL-2 receptor expression is thought to be a requirement prior to IL-2-driven expansion of lymphoid cells, its evaluation was critical in these patients receiving this therapy.

The current report presents results of further study of the consequences of in vivo administration of IL-2 in humans. Circulating Tac-positive cells were further analyzed by two-color immunofluorescence and were noted to consist of both T-cells and macrophages. In addition to lymphocyte-associated IL-2 receptors circulating soluble IL-2 receptors were found in the serum of these treated patients.

MATERIALS AND METHODS

Patient Selection and Treatment. All patients were seen at the Clinical Center, NIH. A clinical protocol utilizing recombinant IL-2 was approved by the Clinical Research Committee, National Cancer Institute. An individual New Drug application was submitted by the Investigational Drug Branch, NCI, and approved by the Food and Drug Administration. Informed consent was obtained from all patients. Patients offered treatment with recombinant IL-2 had failed standard therapy for their underlying malignancy and were treated with either IL-2 alone or IL-2 in combination with in vitro IL-2-activated lymphocytes (LAK cells). Patients were treated with IL-2 administered i.v. in 5% human serum albumin (Cutter Pharmaceuticals, Baltimore, MD) as a bolus or by continuous infusion. In addition, two patients were treated i.p. with IL-2 through a Tenckhoff catheter. LAK cells for infusion were generated with autologous leukopherosed cells activated for 3 days of in vitro activation with IL-2.

Preparation of IL-2. Recombinant IL-2 was generated and purified to apparent homogeneity (>99% pure) by the techniques previously described (2) and was kindly supplied by the Cetus Corporation, Emeryville, CA. Endotoxin content was below the limits of detection (0.05 ng/ml) and the sodium dodecyl sulfate concentration was 197 ng/mg IL-2 protein. Lyophilized IL-2 was received by the Clinical Pharmacy, NIH, and stored at –20°C. The IL-2 was reconstituted with sterile water and diluted with 5% human serum albumin (Cutter Pharmaceuticals) to the appropriate concentration prior to administration. Final container testing, general safety testing, and culture were carried out at Cetus. IL-2 activity was measured in the standard assay using the CTLL dependent cell line as described previously (6). One unit of activity was defined as that quantity of IL-2 which gave half maximum activity in the bioassay using an 18-h incubation and 4 × 10⁵ cells/well. The unit defined by the Biological Response Modifiers Program (BRMP) standard (8) is approximately equal to 30-50 units in our assay. 1 BRMP unit is equal to approximately 2.3 Cetus units. Assay of IL-2 in the serum and peritoneal cavities of patients was carried out with the same assay. All sera and ascites specimens were heated to 56°C for 30 min to remove inhibitory activity in this assay.

Media and Reagents. All assays were carried out in complete medium consisting of RPMI 1640 medium (Biofluids, Rockville, MD) with 10% FCS, fetal calf serum; FITC, fluorescein isothiocyanate; LAK, lymphokine activated killers; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; TAC, antibody defining T-cell activation antigen; IL-2R, IL-2 receptor.
human AB serum (KC Biological, Kansas City, MO) or 15% fetal calf serum (GIBCO, Grand Island, NY) with 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.03% glutamine (NIH media unit). PHA (GIBCO) was reconstituted with distilled water and used at 0.1% final concentration for use in proliferation assays.

**Patient Sample Collection and Preparation.** Single cell suspensions of fresh human tumors were obtained by incubating tumor with an enzyme mixture as previously described (3) following initial mincing and frozen in 90% AB serum and 10% dimethyl sulfoxide until used. Venous blood was obtained in serum separation Vacutainers (Becton-Dickinson, Rutherford, NJ) clotted and serum separated and stored at -20°C until used. Human plasma was similarly obtained in citrate-containing tubes and kept chilled, centrifuged at 450 x g and separated. Heparinized venous blood was obtained from patients and fractionation of PBM cells carried out on lymphocyte separation medium gradients (Litton Bionetics, Kensington, MD) with 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.03% glutamine (NIH media unit). PHA serum (GIBCO, Grand Island, NY) with 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.03% glutamine (NIH media unit). PHA serum (GIBCO, Grand Island, NY) was reconstituted with distilled water and used at 0.1% final concentration for use in proliferation assays.

**IL-2 Activation of LAK Precursors.** Cells tested for generation of LAK activity were incubated at 1 x 10^6 cells/ml in 2-ml wells in 24-well plates (Costar, Cambridge, MA) for 3 days with 1000 U/ml RIL-2 or with timed sera from patients receiving IL-2, harvested and washed two to three times prior to assay.

**Cytotoxicity Assay.** A 4-h 51Cr release assay was used to measure cytotoxicity against fresh tumor or K562 targets as previously described (3). Cryo-preserved lymphocytes were thawed and assayed in parallel at effector-to-target ratios of 40:1 to 2.5:1 with 5 x 10^5 Cr labeled targets per well. Following a 4-h incubation, the plates were harvested with the Skatron harvesting system (Flow, McLean, VA) and counted.

**Monoclonal Antibody Staining and Flow Cytometry.** Serial assays of PBM were performed on frozen cells with biotin or FITC-conjugated monoclonal antibodies. Monoclonal reagents purchased from Becton-Dickinson (Mountain View, CA) included Leu 1 (pan T reagent, CD5, p67, IgG2a), Leu 2 (class I-reactive T-cells, CD8, p32-33, IgG1), Leu 3 (class II-reactive T-cells, CD4, p55, IgG1), Leu 4 (pan T reagent, CD3, p19-29, IgG1), Leu 7 (natural killer, IgG), Leu 10 and 12 (B-cell, IgG1), Leu DR (Ia analogue, IgG2a), Leu-M3 (macrophage, CDW14, IgG2b), and Thy 1.2 (rat anti-T-cell, IgG2b) and Lyt 2 (rat anti-mouse, IgG2b), versus negative controls were used to stain PBM. Antibodies obtained from Ortho Pharmaceuticals (Ranton, NJ) included OKT3 (pan T reagent associated with T-cell antigen receptor, IgG2a and OKT11 (sheep red blood cell receptor, IgG2a). Anti-Tac (human IL-2 receptor, IgG2a) antibody was obtained from Dr. Thomas Waldmann and directly fluoresceinated or biotinylated following partial purification from ascites fluid. 7G7 is an IgG2a murine anti-human IL-2 receptor whose binding is not blocked by IL-2.3 Cell staining procedure involved mature cell surface protein of M, 55,000.

**RESULTS**

**IL-2 Administration in Patients Treated i.v. or i.p.** IL-2 was administered to patients by a variety of protocols including continuous administration i.v., intermittent i.v., or bolus. Four patients with sufficient available cells and sera were studied in detail and are presented here. Continuous administration at doses up to 3000 U/kg/h were associated with barely detectable levels of 1-5 IL-2 units/ml serum (6). Bolus i.v. administration of IL-2 was associated with transient circulating levels of IL-2 for 1-4 h depending on the dose administered (3, 6). When the IL-2 at comparable doses was administered i.p. to the two patients presented no circulating levels of IL-2 in the serum could be demonstrated (assay sensitivity < 0.2 U/ml). In spite of the lack of demonstrable serum levels of IL-2 in these patients receiving IL-2 by continuous infusion or i.p., significant changes in circulating mononuclear cells were demonstrated (see below). Three subsequent patients receiving i.p. IL-2 did demonstrate circulating IL-2 in the serum (13). Clinical efficacy of IL-2 alone (14) at high doses or in combination with the adoptive transfer of cells (15) was demonstrated to cause significant tumor regressions in patients with melanoma, renal cell cancer, and colorectal carcinoma. None of the patients reported here were thought to have responded to this therapy.

**Patients Receiving Long-Term Continuous RIL-2 Develop Tac-Positive Cells, and Increased Total T-Cell Numbers.** As demonstrated previously with both Jurkat-derived (3) and recombinant IL-2 (6), there is a rapid and reproducible decrease in all mononuclear cells within the first few hours following IL-2 administration with gradual recovery within 3-7 days of continuous administration. Following this period there is a recovery and then an increase in the total number of T-cells with a preferential increase in Leu 3-positive cells. Four patients selected for special study because of the availability of sequential samples of cryopreserved peripheral blood and/or peritoneal cells are presented in Table 1. Tac positive cells accounted for up to 35% of the circulating mononuclear cells in the peripheral blood following prolonged IL-2 administration. Similar percentages of cells were demonstrated in the peritoneal cavity of patients receiving IL-2 at that site. Patients 1 and 2 received an

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3 William Biddison, personal communication.

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Table 1: Patients treated with RIL-2

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Dose/route, IL-2 diagnosis</th>
<th>Cells</th>
<th>WBC</th>
<th>% Tac +</th>
<th>% Tac +</th>
<th>% Tac +</th>
<th>% Tac +</th>
<th>% Tac +</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$10^4$–$10^5$ U/kg t.i.d. i.p. x 21 days (colorectal carcinoma)</td>
<td>PBMC</td>
<td>7.0</td>
<td>24</td>
<td>0.5</td>
<td>7.5</td>
<td>46</td>
<td>12.5</td>
</tr>
<tr>
<td>2</td>
<td>$1$–$3$ x $10^3$ U/kg t.i.d. i.p. x 14 days (colorectal carcinoma)</td>
<td>PBMC</td>
<td>8.4</td>
<td>19</td>
<td>ND</td>
<td>5.3</td>
<td>13</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>$3$ x $10^2$ U/kg i.v. 3 x/wk x 14 days (sarcoma)</td>
<td>PEC</td>
<td>1.2 x $10^3$</td>
<td>ND</td>
<td>ND</td>
<td>7 x $10^2$</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>$10^3$ U/kg i.v. x 21 days (melanoma)</td>
<td>PBMC</td>
<td>5.8</td>
<td>15</td>
<td>0.3</td>
<td>6.5</td>
<td>28</td>
<td>7.6</td>
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* T.i.d., three times a day.

† PEC, peritoneal exudate cells. Cells/ml following Ficoll-Hypaque separation.

ND, not done.

Fig. 1. Binding studies of anti-Tac antibody. A. PHA-activated normal PBM bind anti-TAC antibody but media-incubated cells do not. Normal PBM were incubated in complete media or complete media with 0.1% PHA-P for 60 h prior to assay in flow cytometry with antibody to Tac conjugated to biotin. A second incubation for 10 min with Texas red avidin was carried out prior to determining emitted fluorescence at 630 ± 11 nm following excitation at 590 nm. Net voltage fluorescence units are defined as the (mean voltage of Tac-positive cells x % positive cells) – (mean voltage of Thy 1.2-positive cells x % positive cells). B. Binding curve of patient's cells with anti-Tac antibody but media-incubated cells demonstrated no binding of anti-Tac when compared to the Thy 1.2-positive cells. C. Binding of anti-Tac to cells of a patient receiving RIL-2 is inhibited by RIL-2 but not recombinant insulin. PBMC were preincubated for 0.5 h and then maintained in various concentrations of either inhibitor throughout staining.

i.p. bolus of IL-2 three times a day for 2–3 weeks. Patient 3 received bolus RIL-2 three times a week and patient number 4 continuous infusion of RIL-2 for 3 weeks. The relative percentage of T-cells in the peritoneal cavity was measured at various time points and varied between 30–80%, similar to that in the peripheral blood following i.p. IL-2 treatment. Very few macrophages (<5%) were identified in the peritoneal cavity following IL-2 treatment. The detailed results in 20 patients receiving RIL-2 i.v. has been reported elsewhere (6) and only the two patients receiving i.p. IL-2 that have been further studied in detail in this report are presented in Table 1. In addition a 2–12-fold expansion of T-cell numbers was reported in all patients receiving continuous i.v. IL-2. Further analysis of Tac-positive cells were carried out in these four patients.

The Binding of Anti-Tac Antibody Determined by Flow Microcytometry on Circulating Cells Is Inhibitible by Preincubation with RIL-2. To determine whether the molecule detected by anti-Tac binding on the surface of circulating cells was capable of binding IL-2, a series of experiments were carried out. The biotinylated anti-Tac was titrated on 3-day, PHA-P (0.1%)-activated PBM and on similarly incubated but nonactivated PBM (Fig. 1A). As shown, when expressed in net voltage fluorescence units, a sigmoid binding curve was generated, in part related to the bivalent characteristic of the whole anti-Tac antibody. Concentrations of 0.025 µg/ml and 0.006 were selected as occurring in a linear area of the binding curve. Virtually no binding occurred with PHA nonactivated cells and essentially no binding of anti-Tac has been demonstrated on PBMC of cancer patients prior to receiving IL-2. In cancer patients treated with IL-2, similar binding curves of Tac-biotin to freshly isolated PBMC could be demonstrated (Fig. 1B) as PHA-stimulated normal PBM. Precise correlation between VFU and numbers of affinity of receptors is not possible but results from IL-2 radioligand evaluation revealed lower total numbers of receptors (1,500/cell) compared to 20,000–30,000 receptors on PHA blasts with 20% high affinity sites (17) compared to approximately 10% high affinity sites usually demonstrated on PHA-activated cells.

The binding of anti-Tac to PHA-activated PBM can be inhibited by preincubation with IL-2 (16, and data not shown). Preincubation of PBM with graded concentrations of IL-2 from a patient receiving continuous infusion RIL-2 for 3 weeks (patient 4) as well as a second patient (patient 2) demonstrated marked inhibition (>90%) of subsequent anti-Tac binding (Table 2) at two time points during treatment. Recombinant insulin did not inhibit binding of anti-Tac even when used at higher concentrations (Fig. 1C). This demonstrates that the binding of anti-Tac is inhibitable by IL-2 and is presumptive evidence that anti-Tac is specifically binding to the IL-2 receptor on these cells. A patient who received i.p. IL-2 for 3 weeks demonstrated no binding of anti-Tac when compared to the Thy 1.2 control (Fig. 2) at either 0.006 or 0.025 µg/ml of anti-Tac prior to treatment. Significant binding occurred at both anti-Tac concentrations (only 0.025 µg/ml shown in Fig. 3) while on treatment and were blocked completely by preincubation with 3 x $10^3$ U (approximately 1 mg/ml) of RIL-2 which was best demonstrated at the 0.006 µg/ml level (data not shown).

The Majority of Tac-Positive Cells in the Peripheral Blood Are Leu 3-Positive, Leu 2-Negative Lymphocytes or M3-Positive Cells (Monocytes). The distribution of Tac on subsets of mononuclear cells were examined in several patients. In two-color experiments designed to analyze the presence of a second antigen on cells positive (or negative) for the first antigen, Tac-
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Table 2 Inhibition of anti-Tac binding on patient cells by RIL-2

<table>
<thead>
<tr>
<th>Week of treatment</th>
<th>Block</th>
<th>Tac concentration (g/ml)</th>
<th>Net VFU</th>
<th>% of control</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 2</td>
<td>0</td>
<td>0.025</td>
<td>26,783</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.006</td>
<td>10,663</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>IL-2</td>
<td>0.025</td>
<td>2,810</td>
<td>10.5</td>
<td>89.5</td>
</tr>
<tr>
<td></td>
<td>IL-2</td>
<td>0.006</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Week 3</td>
<td>0</td>
<td>0.025</td>
<td>28,637</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.006</td>
<td>28,370</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>IL-2</td>
<td>0.025</td>
<td>2,973</td>
<td>10.4</td>
<td>89.6</td>
</tr>
<tr>
<td></td>
<td>IL-2</td>
<td>0.006</td>
<td>0</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

a Patient no. 4 PBMC obtained at the end of 14 and 21 days of treatment.

b Blocking was performed with media alone or 3 x 10^6 units RIL-2 for 1/2 h prior to and during the assay.

Figure 2. Tac-positive cells are Leu 2 negative, Leu 3 positive. Slice analysis of a patient's (no. 4) cells gated on all cells, Leu 2-positive, 2-negative, 3-positive, or 3-negative cells were examined for Tac or Thy 1.2 (negative control positivity). 64.58% of cells were Leu 3 positive, 10.66% Leu 2 positive, and 37.54% of all Tac-positive cells are Leu 2 negative, Leu 3 positive. Slice analysis of patient 1's cells presented in Fig. 4. The monocytes represented 15.74% of all cells evaluated (Fig. 4A). Tac was present on 33.41% of all cells (Fig. 4B). M3-positive cells (Fig. 4, C and D) were stained (83.73%) with anti-Tac but Leu 4, Lyt 2, and Thy 1.2 were negative (<10%). No Tac, Thy 1.2, Lyt 2, or Leu 2 binding could be demonstrated on M3-positive cells in normal individuals (data not shown). In addition, overnight incubation of M3-positive cells in media did not cause shedding of Tac nor did incubation of normal cells in serum containing soluble IL-2 receptor cause generation of Tac-positive, M3-positive cells.

The present studies demonstrate that IL-2 receptor-positive cells appear in large numbers in the peripheral blood of some positive subsets could be quantitated by slice analysis of the correlated two-color data. A panel of FITC-conjugated monoclonal antibodies were analyzed on Tac-positive and Tac-negative cells as determined by reactivity with biotinylated-Tac followed by Texas red avidin. These data are presented in Fig. 3 in patient 1. These data show that a portion of Tac-positive cells in PBMC and all Tac-positive cells from the peritoneal cavity in a single patient are Leu 1 positive, Leu 4 positive, characteristic of classic T-cells. As can also be seen in Fig. 3 by slice analysis, these Tac-positive cells are Leu 2 negative, Leu 3 positive. 7G7 is an antibody which recognizes an epitope distinct from the IL-2 and Tac binding sites of the IL-2 receptor molecule and appears to stain all the Tac positive cells in the peritoneal cavity. At higher concentrations of this antibody all Tac-positive cells of the PBMC were also positive (data not shown). The expression of Tac antigen on Fc receptor-positive cells (Leu 11, 3G8, or B73) cells is currently being evaluated. Consistent binding of the antibody to M3-positive cells (monocytes) was seen. Slice analysis of patient 1's cells is presented in Table 3. Approximately one third of the Leu 3-positive cells are Tac positive whereas only 6% of the Leu 2-positive cells expressed Tac antigen. As shown in Table 4, most of the M3-positive or dull Leu 3-positive cells (monocytes) were Tac positive (80-90%) following 2 weeks of IL-2 treatment. A display of slice information obtained from patient one is presented in Fig. 4. The monocytes represented 15.74% of all cells evaluated (Fig. 4A). Tac was present on 33.41% of all cells (Fig. 4B). M3-positive cells (Fig. 4, C and D) were stained (83.73%) with anti-Tac but Leu 4, Lyt 2, and Thy 1.2 were negative (<10%). No Tac, Thy 1.2, Lyt 2, or Leu 2 binding could be demonstrated on M3-positive cells in normal individuals (data not shown). In addition, overnight incubation of M3-positive cells in media did not cause shedding of Tac nor did incubation of normal cells in serum containing soluble IL-2 receptor cause generation of Tac-positive, M3-positive cells.

Soluble (Nonmembrane Bound) IL-2 Receptor Is Found in the Sera of Patients Receiving IL-2. A recently developed enzyme-linked immunosorbent assay for the quantitative measurement of IL-2 receptor molecules in solution was employed to study serum IL-2 receptor levels in patients receiving IL-2. This assay is very sensitive and reproducible (10, 11) with results expressed as units per milliliter. The cell-free supernatant of an IL-2-dependent T cell line serves as the reference standard, having been assigned a level of 1,000 IL-2 receptor units/ml. Serum IL-2 receptor levels in 33 normal individuals averaged 186 U/ml with a range of 83-335. The results of serum levels in nine patients treated with RIL-2 alone or RIL-2 plus lymphokine activated killer cells is presented in Table 5. As can be seen, the mean level of IL-2 receptor in these cancer patients (700) was somewhat higher than our group of normal controls. With treatment, serum levels increased up to 30,000 U/ml with subsequent decrease after completion of treatment. The time course in one of these patients receiving 300,000 units/kg for 3 weeks times a week as a bolus infusion for 21 days is presented in Fig. 5A. An increase in serum IL-2 receptor levels is seen within 1 h following the first dose and subsequently following each later dose resulting in a progressively increasing level of over 9,000 units/ml. With administration of the last dose, levels transiently decreased within 1-5 min and then increased by 15-60 min with a subsequent decrease following discontinuation of IL-2.

The appearance of soluble IL-2 receptor in the serum preceded by several days (7-10 days) the detection of Tac-positive cells in the circulation. Subsequent experiments designed to evaluate the physiological role of these high levels of circulating IL-2 receptor were unable to demonstrate an effect of graded doses of these patient's serum incubated with the CTLL cells used in the IL-2 assay over a range of IL-2 concentrations or in a LAK generation assay (data not shown). In some patients a fall in IL-2 receptor levels occurred despite continued administration of RIL-2 (Fig. 5A). In this patient, Tac-positive cells continued to increase in the PBMC over the period of IL-2 administration.

DISCUSSION

The present studies demonstrate that IL-2 receptor-positive cells appear in large numbers in the peripheral blood of some...
in IL-2 receptor number with augmentation of transcription of the IL-2 receptor gene within 4 h of its addition to late-cultured PHA lymphoblast (26).

We, as well as others, have been unable to demonstrate appreciable numbers of IL-2 binding or Tac antibody binding cells in the peripheral blood of normal individuals. This is also true in our patients with cancer who were evaluated. None of our patients had more than 5-6% positive peripheral blood lymphocytes on repeated observations and most were below the detectable limits of approximately 1%. Following immunization with tetanus toxoid, Yachie and colleagues demonstrated that as many as 10% of peripheral T-cells become positive by conventional antibody binding techniques and 25% using a rosetting method (27). In that study the appearance of Tac-positive cells appeared to peak at approximately 12 h and clear by 24 h following immunization. This is different from the expression of Tac antigen in patients receiving IL-2 which required approximately 1 week of continuous administration to find measurable levels of Tac antigen expression. Other recent studies evaluating Tac antigen expression on cells obtained from synovial fluid tissue in patients with rheumatoid arthritis (28) have demonstrated that as many as 40% of these cells express Tac antigen and as many as 80% express la determinants. However, no patients expressed levels of Tac antigen on peripheral blood mononuclear cells at levels higher than 20% and most were significantly below this level. Increased proliferative response to IL-2 in in vitro cultures of PBL and cells from synovial fluid was noted. They also found a separate subpopulation of T-cells which expressed both la and Tac antigen. The present study also demonstrates la-positive, Tac-positive cells in the periphery and in the peritoneal cavity of our treated patients. The physiological role of Tac-positive cells

### Table 3 Slice data PBM, patient 1 receiving IL-2

<table>
<thead>
<tr>
<th>Antibody</th>
<th>% all cells</th>
<th>% Tac positive</th>
<th>Thy 1.2 positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cells</td>
<td>100</td>
<td>33.41</td>
<td>1.25</td>
</tr>
<tr>
<td>Leu 1 positive</td>
<td>33.75</td>
<td>26.94</td>
<td>1.84</td>
</tr>
<tr>
<td>Leu 2 positive</td>
<td>4.78</td>
<td>6.44</td>
<td>0.74</td>
</tr>
<tr>
<td>Leu 3 positive (bright)</td>
<td>26.04</td>
<td>39.10</td>
<td>2.35</td>
</tr>
<tr>
<td>Leu 3 negative</td>
<td>55.33</td>
<td>14.39</td>
<td>1.77</td>
</tr>
<tr>
<td>Leu 4 positive</td>
<td>33.56</td>
<td>26.14</td>
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Table 4 Slice data PBM, patients receiving RIL-2

<table>
<thead>
<tr>
<th>Patient</th>
<th>Antibody</th>
<th>Tac + (%)</th>
<th>Thy 1.2 (%)</th>
<th>Lyt 2+ (%)</th>
<th>Leu 4+ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leu 3 dull positive (monocytes)</td>
<td>17.59</td>
<td>90.96</td>
<td>10.21</td>
<td>ND*</td>
</tr>
<tr>
<td></td>
<td>Leu M3 positive (monocytes)</td>
<td>15.74</td>
<td>83.73</td>
<td>7.88</td>
<td>6.58</td>
</tr>
<tr>
<td></td>
<td>Leu M3 negative (all others)</td>
<td>82.83</td>
<td>22.68</td>
<td>2.24</td>
<td>2.15</td>
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<tr>
<td>2</td>
<td>Leu M3 positive</td>
<td>36.25</td>
<td>90.96</td>
<td>11.71</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>M3 negative</td>
<td>61.67</td>
<td>34.11</td>
<td>4.99</td>
<td>ND</td>
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</tbody>
</table>

* ND, not determined.
It has been previously demonstrated that the IL-2 receptor can be modulated (31). Reexpression of Tac required mitogenic exposure (31) or exposure to phorbol esters or antigen (26). Whether the IL-2 receptor is internalized or shed following ligand-receptor interaction is unclear. An in vitro-release form of the Leu 2 molecule (32) and a soluble circulating form of the C3B/C4B receptor in human plasma (31) have been demonstrated. The precise immunological function of these soluble forms is difficult to determine. At least in one case, soluble plasma CR1 possesses function in vitro as a cofactor for the cleavage of C3B by factor I (33). Recent observations regarding soluble IL-2 receptors which are released in large numbers from activated human lymphoid cells has been presented (11). It has been speculated that such receptors may serve as an immunoregulatory role in cell growth and differentiation. The development of the assay for soluble IL-2 receptor was dependent on the development of a second antibody which binds to an epitope on the human IL-2 receptor separate from that recognized by either IL-2 or anti-Tac (10). Detection of soluble IL-2 receptor required two separate antibodies recognizing separate epitopes on the receptor molecule detected in the serum. The subsequent molecule characterization (11) indicated that this is indeed an IL-2 receptor. Increases in serum IL-2 receptor levels have not been observed in the serum of normal individuals treated with endotoxin or in cancer patients treated with γ-interferon (7). Although some increased levels of soluble IL-2 receptor could be demonstrated in the serum of some cancer patients the increase of approximately 10–20-fold in our patients receiving IL-2 was unexpected. Elevated serum levels of IL-2 receptor have been demonstrated in patients with a variety of lymphoreticular malignancies (11). It is possible that the release of this receptor might serve an important immunoregulatory role by competing for IL-2 with receptors present on cells and thus decrease the local or regional immune response. Our studies were unable to demonstrate significant inhibition by serum of patients following IL-2 administration in either biological assays for IL-2 itself or in LAK generation assays. It is possible that cells may have acquired (either specifically or passively) rather than synthesized the cell surface IL-2 receptor detected. For example, it has previously been demonstrated that class II molecules can be acquired by murine T-cells in vivo and in vitro (34—36).

The demonstration in the present study that continuous in vivo IL-2 administration leads to not only increased numbers of cells expressing IL-2 receptors but also to dramatic increases in amounts of soluble IL-2 receptor found in serum leads to difficulty in interpretation of the function and origin of this cell surface IL-2 receptor expression. Several lines of evidence suggest that at least a portion of the cells in these studies are synthesizing and subsequently expressing cell surface functional IL-2 receptors. First, increased message for IL-2 receptor in PBM could be demonstratable coincident with increased levels of IL-2 receptor-positive cells (data not shown). In addition, IL-2 receptor expression on T-cells consistently showed exquisite correlation with a subpopulation of Leu 3-positive cells. If these cells had acquired (rather than synthesized) this IL-2 receptor, then acquisition was by some mechanism specific for Leu 3-positive cells (and not all Leu 3-positive cells). Finally, in separate experiments, increases in spontaneous IL-2 induced proliferation and LAK activity following IL-2 administration and coincident with increased IL-2 receptor expression, suggest the presence of increased functional IL-2 receptors (data not shown).

was unclear in their studies but was thought to be related to the inflammatory response. Although a formal possibility exists that the IL-2 receptor bearing cells in our studies are related to the administration of products in the preparation other than IL-2 including sodium dodecyl sulfate, we believe that this is unlikely. A test of the hypothesis with in vivo administration of sodium dodecyl sulfate-free material would be required.

Recent findings evaluating patients with aplastic anemia, have demonstrated circulating Tac-positive cells in a total of five out of five patients (29). A mean of 31% of the peripheral circulating T-cells were noted to be Tac positive and when sorted these cells demonstrated significant hematopoietic suppressor activity in in vitro assays. These activated suppressor T-lymphocytes were also IA positive and Leu 2 positive. The vast majority of Tac-positive cells in our system were Leu 2 negative, Leu 3-positive cells suggesting that they do not represent class I-restricted suppressors. Suppressor inducers which are Leu 2 negative, Leu 3 positive have been demonstrated in in vitro systems by Mohagheghpour and colleagues (30) and it is conceivable that the Tac-positive cells demonstrated by us could functionally be related to this class of cells. Upon in vitro culture our experience has been that cells which proliferate and predominate in culture with IL-2 stimulation of peripheral blood are Leu 2-positive cells. The Leu 3-positive, Tac-positive cells seen in our patients have unknown functional activity. If similar cells could be demonstrated in mice, adoptive transfer could determine if these cells were capable of suppressing a variety of immune responses.
causes proliferation but not differentiation. Our finding of Tac
tcells. An additional possibility that the antigen detected by M3
specific for a T-cell subpopulation (a fraction of Leu 3-positive,
blood of patients receiving IL-2 is more difficult to interpret.
antigen on the surface of circulating monocytes in the peripheral
cells, induces augmented immunoglobulin production, and
groups that the Tac antigen is expressed on activated human
patient developed many of the side effects related to RIL-2 administration
seen. A return to baseline is seen following conclusion of treatment. B, soluble

demonstrated in purified monocytes in the serum of these
receptors. It is possible that some or all of the monocyte IL-2
purified IL-2 is capable of up-regulating Tac expression on B-
shown). It has only recently been demonstrated by a number of
groups that the Tac antigen is expressed on activated human
B-cells (14, 37-39). It may be functionally important in that
purified IL-2 is capable of up-regulating Tac expression on B-
cells. A potential role of i.p. IL-2 administration for generating

tometry and Mary Ann Bodnar and Tyra Gulp for excellent assistance

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IN VIVO INTERLEUKIN-2 ADMINISTRATION

Table 5 Soluble IL-2 receptor appears in the serum of patients receiving RIL-2 or RIL-2 and LAK

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Treatmenta</th>
<th>Pre-RIL-2</th>
<th>On IL-2 (days)</th>
<th>On IL-2 + LAK (days)</th>
<th>RIL-2 post (days)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>i.p. 10⁶–10⁷ U/kg/h</td>
<td>408.5</td>
<td>4,198.2 (5)</td>
<td>5,390.5 (14)</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>C. i.v. infusion 3000 U/kg/h</td>
<td>583.7</td>
<td>5,913.2 (9)</td>
<td>476.99 (87)</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>C. i.v. infusion 1000 U/kg/h</td>
<td>347.2</td>
<td>6,164.4 (17)</td>
<td>2,194.1 (1)</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>i.v. bolus 3 x 10⁴ U/kg 3 x/week</td>
<td>1330.3</td>
<td>9,171.8 (21)</td>
<td>1,381.2 (75)</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>C. i.v. infusion 10⁵–10⁶ U/kg</td>
<td>620.9</td>
<td>9,502.3 (14)</td>
<td>4,441.0 (7)</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>i.v. 10⁴ U/kg t.i.d. + LAK</td>
<td>1837.6</td>
<td>16,263.7 (11)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>i.v. 1-3 x 10⁴ U/kg t.i.d. + LAK</td>
<td>774.9</td>
<td>5,702.9 (14)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>9</td>
<td>i.v. 10⁴ U/kg t.i.d. + LAK</td>
<td>219.2</td>
<td>30,863 (13)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>10</td>
<td>i.v. 10⁴ U/kg t.i.d. + LAK</td>
<td>(700)</td>
<td>6,178.7 (18)</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Range (mean) | 219.2-1837 | 4,198-16,263 | 5,494-30,863 |

* C, Days following completion of RIL-2 treatment.

Fig. 5. Soluble IL-2 receptor is found in the serum of patients receiving RIL-2. Coded serum samples were analyzed for IL-2 receptor content at various time intervals during a 21-day period of receiving IL-2. Somewhat higher levels than that seen in controls is seen in cancer patients (1200 units) prior to therapy. The fact that the total cell numbers increase as much as 100-fold over this period suggests that IL-2 is causing the ingress of cells from extraperitoneal sites or is allowing the direct in vivo expansion of T-cells. A potential role of i.p. IL-2 administration for generating large numbers of T-cells in vivo can now be considered. Future work will be designed to evaluate the potential therapeutic role of this material alone or in conjunction with passively transferred lymphokine activated killer cells both i.v. and i.p.
IN VIVO INTERLEUKIN-2 ADMINISTRATION


In Vivo Administration of Purified Human Interleukin-2 to Patients with Cancer: Development of Interleukin-2 Receptor Positive Cells and Circulating Soluble Interleukin-2 Receptors following Interleukin-2 Administration

Michael T. Lotze, Mary C. Custer, Susan O. Sharrow, et al.

Cancer Res 1987;47:2188-2195.

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