In Vivo Administration of Purified Jurkat-derived Interleukin 2 in Mice

John H. Donohue, Michael T. Lotze, Richard J. Robb, Maury Rosenstein, Rita M. Braziel, Elaine S. Jaffe, and Steven A. Rosenberg

Surgery Branch, Division of Cancer Treatment [J. H. D., M. T. L., M. R., S. A. R.] and Hematopathology Section, Laboratory of Pathology [R. M. B., E. S. J.], National Cancer Institute, Bethesda, Maryland 20205, and the E. I. duPont deNemours and Co., Glenolden Laboratory [R. J. R.], Glenolden, Pennsylvania 19036

ABSTRACT

Pure human interleukin 2 (IL-2), produced by the T-cell lymphoma Jurkat, was injected in mice to study the serum half-life, toxicity, and in vivo immunological effects of IL-2. The serum half-life (t1/2) of Jurkat IL-2 in mice appeared to have two components: (a) a rapid initial phase with t1/2 of approximately 2 min during which most of the exogenous IL-2 was cleared from the serum; and (b) a second, slower component with t1/2 of about 9 min. Mice given injections i.p. or i.v. with pure Jurkat IL-2, at doses comparable on a µg/kg basis to contemplated doses for humans, showed no signs of toxicity on the basis of serial measurements of weight, serum liver and kidney chemistries, or histology of lymphoid and vital organs. Jurkat IL-2 had no effect on the rate of growth or survival of mice with an established s.c. methylcholanthrene-induced fibrosarcoma, but Jurkat IL-2 used in conjunction with in vitro sensitized and IL-2-expanded specific immune splenocytes prolonged survival of mice with disseminated FBL-3 tumor. This survival prolongation was highly significant when compared to treatment with Jurkat IL-2 alone (p < 0.001) or an equivalent number of in vitro-resensitized and expanded cells alone (p = 0.004). Treatment of mice with i.p. Jurkat IL-2 subsequent to secondary immunization with allogeneic tumor enhanced by more than 5-fold the splenocyte cytotoxicity for alloantigen measured 7 days later. Thus, purified human IL-2 derived from the Jurkat cell line has a short half-life in mice with no apparent toxicity at large doses. In vivo efficacy of human IL-2 was demonstrated in increasing alloantigen responsiveness and in increasing the efficacy of transferred expanded immune lymphocytes in the FBL-3 lymphoma model.

INTRODUCTION

Since Morgan et al. (29) first described a T-cell-derived factor capable of maintaining long-term T-cell growth in culture, much has been learned about the biochemistry, production, and in vitro effects (reviewed in Refs. 10, 17, 20, and 40) of the responsible glycoprotein, IL-2. In our investigations of IL-2 and its potential use in adoptive immunotherapy, we have demonstrated its usefulness in cloning (27) and expanding (43) effector lymphocytes, and shown that lymphoid cells exposed to IL-2 are lytic for fresh autologous and allogeneic tumor (19, 25). These results have given promise to the idea of using immune cells, treated in vivo with IL-2, to augment or restore immune function in vivo. Initial studies in humans have shown the feasibility and safety of such treatment on a limited scale (26).

In addition to the in vitro use of IL-2 in producing effector cells for immunotherapy, several studies have demonstrated the in vivo effects of IL-2 by itself or with adoptively transferred cells. Among the in vivo activities noted have been enhanced natural killer function (23), augmentation of alloantigen responsiveness (5, 23, 37), improved recovery of immune function in acquired immunodeficient states (18, 28, 47), induction of immune functions in nude mice (34, 42, 46), and antitumor effects, either alone (2, 31) or with immune lymphocytes (4). A serious problem with all but 2 of these studies (4, 23) has been that the IL-2 preparations have been crude or partially purified supernatants. Attributing the in vivo results to IL-2 alone when multiple other lymphokines are present may be premature.

A human T-cell leukemia line, designated Jurkat (16, 24, 39), has been shown to produce large quantities of IL-2 when stimulated with phytohemagglutinin and PMA under serum-free conditions (18). More recently, Jurkat IL-2 has been purified, and large-scale production has begun (35). We have studied the toxicity and potential in vivo effects of this purified IL-2 in mice prior to beginning in vivo infusions of pure Jurkat IL-2 in humans.

MATERIALS AND METHODS

Animals. Female C57BL/6 mice were obtained from the Animal Production Colonies of the NIH and were used between 12 and 20 weeks of age.

Tumors. MCA-102 is a fibrosarcoma induced in our laboratory by i.m. injection of 0.1 ml of 1% 3-methylcholanthrene in sesame seed oil and serially passaged i.m. in C57BL/6 mice (32). A tumor cell suspension of the third transplant generation was prepared by mincing the harvested tumor and passing the cells through a double layer of No. 100 nylon mesh. FBL-3, a Friend-virus-induced lymphoma/leukemia of C57BL/6 origin (gift of G. Ting, National Cancer Institute), was maintained in ascites form in C57BL/6 female mice. P815, a mastocytoma syngeneic to DBA/2 mice, was serially passaged in female DBA/2 mice in an ascites form.

IL-2 Preparations. Murine LF-IL-2 production has been described previously (41). In brief, BALB/c splenocytes were incubated with concanavalin A (10 µg/ml; Miles Laboratories, Elkhart, IN) for 2 hr, washed 3 times in HBSS, and resuspended in CM. CM consisted of RPMI 1640 (Biofluids, Rockville, MD), supplemented with 10% fetal calf serum (Biofluids), 0.03% glutamine (NIH Media Unit), 0.1 mM nonessential amino acids (M. A. Bioproducts, Walkersville, MD), 1 mM sodium pyruvate (M. A. Bioproducts), 5 × 10−5 M mercaptoethanol (Aldrich, Milwaukee, WI), penicillin (100 units/ml; NIH Media Unit), gentamicin (50 µg/ml; Schering, Kenilworth, NJ), and Fungizone (0.5 µg/ml; Flow Laboratories, McLean, VA). After incubation for 24 hr at 37°, the supernatants were centrifuged, passed through 0.45-µm filters (Millipore Corp., Bedford, MA), and refrigerated until use.

Murine EL-4 IL-2. Murine EL-4 IL-2 was prepared as described previously (7), using an EL-4 thymoma cell line originally obtained from J. Farrar (NIH). EL-4 cells at 106/ml in RPMI 1640 (Biofluids) with 0.03%
glutamine (NIH Media Unit) and glutamine (50 µg/ml; Schering) were stimulated under serum-free conditions with PMA (10 µg/ml; Sigma Chemical Co., St. Louis, MO). After 48-hr incubation at 37°, the supernatant was collected, concentrated 100-fold using a Pellicon-Millipore apparatus with an M. 10,000 cutoff (Millipore), filtered twice, and refrigerated until use.

Human Jurkat IL-2. Human Jurkat IL-2 was produced and purified using the human Jurkat lymphoma line. This procedure has been described in detail elsewhere (35). Jurkat IL-2 was produced in large volume, under serum-free conditions, by adding phothemagglutinin (1.5 µg/ml) and PMA (50 µg/ml) to 4 × 10⁷/ml Jurkat cells in Dulbecco’s Modified Eagle’s Medium with high glucose, penicillin, and streptomycin. The cells were incubated for 15 to 20 hr at 37° and removed by centrifugation. The supernatants were concentrated 20-fold on an HIPS Amicon hollow-fiber cartridge, then slowly passed through an immunoaffinity column bearing a specific monoclonal antibody for IL-2. The column was copiously washed with 10 mM Tris buffer, 1 M NaCl, and 1% NP-40 detergent before eluting the IL-2 with 1.5% acetic acid. The IL-2 solution was dialyzed against phosphate buffered saline using a long-term dependent cell line (CTLL-2, subclone 15H1). Protein content was determined using the Lowry technique. Biochemical purity was assessed using 2-dimensional isoelectric focusing/sodium dodecyl sulfate-polyacrylamide gel electrophoresis and amino acid sequencing of an amino-terminal sequence using automated Edman degradation with a vapor phase sequanator.

Splenocyte Suspension. Spleens were removed, mashed with the plunger of a plastic syringe, passed through a No. 40 wire mesh, and spun in HBSS. Erythrocytes were lysed by resuspending the cells in buffered ammonium chloride solution for 1 min. Cells were washed 3 times in HBSS, resuspended in CM, and passed through a No. 100 nylon mesh.

Effect of Mouse Serum on Jurkat IL-2 Activity. Jurkat IL-2 was diluted to 0.3 µg/ml in RPMI 1640 with 10% fresh C57BL/6 serum. This solution was then diluted 1:3 and 1:100 with fresh C57BL/6 serum (final serum concentrations were 70 and 99%, respectively) or RPMI 1640 (300 µl total volume) and incubated for 2.5 hr at 37°. Duplicate serial 2-fold dilutions in CM were done with each sample on 96-well culture plates (Costar No. 3596; Costar, Cambridge, MA), and 3 × 10⁵ cells of an IL-2-dependent line, initiated and maintained long-term in our laboratory, were added per well. Plates were incubated for 24 hr at 37°, pulsed 4 hr with 2 µCi of [³H]thymidine (New England Nuclear, Boston, MA) per well, and harvested on a multiple automated sample harvester (MASH II) unit (M. A. Bioproducts). Mean cpm were calculated for each dilution.

Long-Term Growth of a Murine Lymphocyte Line in Jurkat IL-2. Clone 14-11, a C57BL/6 subclone, generated as described previously, was grown over a 2-month period in various concentrations of Jurkat IL-2. This clone was formed by depletion of fresh C57BL/6 splenocytes with Lyt-2 monoclonal antibody and newborn rabbit complement, in vitro sensitization with irradiated DBA/2 splenocytes, cloning by limiting dilution (1000 cells/well), and subcloning at 1 cell/well, as reported by Rosenberg et al. (38). Subclone 14-11 had a strong proliferative response to H-2d antigen but had no cytotoxic activity against DBA/2 or P815 targets; 10⁵ 14-11 cells with 5 × 10⁴ irradiated DBA/2 splenocytes (3300 rads) were grown in various concentrations of Jurkat IL-2 diluted in CM for 7 days in 24-well culture plates (Costar No. 3524). The wells were harvested, cell counts were done to determine cell growth, and a proliferative assay in triplicate was set up with 10⁵ clone 14-11 and 10⁵ irradiated DBA/2 splenocytes (3300 rad)/well in 96 flat-well plates (Costar No. 3596). The plates were pulse for 4 hr with 2 µCi of [³H]thymidine/ (New England Nuclear, Boston, MA) or 40 hr after 40-fold incubation at 37°. The wells were harvested using a MASH II apparatus (M. A. Bioproducts) and counted. Means for the triplicate samples were determined, and the stimulatory index was calculated by

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Clone 14-11 cells were replated at 10⁵ cells of clone well in 24-well plates in Jurkat IL-2 dilutions with DBA/2-irradiated feeders on the same day that they were counted. These cells were maintained for a total of 9 weeks with no source of exogenous growth factors other than Jurkat IL-2.

Serum Half-Life of Jurkat IL-2. The original Jurkat IL-2 solution was diluted to 0.5 µg/ml in RPMI 1640 with 2.5% or 10% fresh C57BL/6 serum. One ml was injected i.v. and the mice were heated and bled from the tail vein at specified times after injection. Sera from 2 mice/time point were pooled, and the IL-2 bioassay described above was performed on duplicate samples of each. The serum IL-2 titer was defined as the reciprocal of dilution at which 50% of the maximum cpm were present.

Toxicity of Jurkat IL-2 in Mice. After dilution of the original Jurkat solution to 0.2 and 2.0 µg/ml in RPMI 1640 with 3.3% normal C57BL/6 serum, 0.25 ml (0.05 or 0.5 µg) of either solution was administered i.p. twice daily for 7 days. Control mice were given the same volume of a solution lacking IL-2 at the same times. All mice were weighed daily during injections. On day 2 after the last injection, half of the mice were bled and sacrificed. Serum blood urea nitrogen, creatinine, total bilirubin, glutamine oxaloacetate transaminase, lactate dehydrogenase, and alkaline phosphatase were measured for each mouse. The liver, lungs, kidneys, spleen, and thymus of each mouse were weighed, and specimens of each of these organs, as well as peripheral lymph nodes, bowel mesentery, and bone marrow, were fixed in B5 preservative. The histological appearance of each organ was examined with slides stained with hematoxylin and eosin. Surviving mice were weighed one to 3 times weekly for the next 4 months.

The toxicity of Jurkat IL-2 when administered i.v. in a bolus was assessed by injecting i.v. 1 ml of Jurkat IL-2 (0.5 µg/ml) in RPMI 1640 with 10% C57BL/6 serum once weekly for 4 consecutive weeks. All mice were weighed 3 times weekly for 5 weeks, then twice weekly for 4 weeks, and weekly for 2 months. The same serum chemistries described above were measured at 3 days subsequent to the last i.v. IL-2 inoculation. These doses and methods of administration were selected to correspond with the doses (on a mg/kg basis) to be used in human studies of this IL-2.

Effect of Jurkat IL-2 on MCA-102 Growth. MCA-102 tumor was induced in C57BL/6 mice by injection of 10⁶ tumor cells i.d. on the abdomen. Five days later, when a palpable tumor was present, twice-daily i.p. injections of the control or Jurkat IL-2 solutions (0.05 and 0.5 µg/injection) were started. These injections were continued for 7 days, with the mice being weighed and tumor diameters recorded daily. Weights and tumor measurements were recorded every other day thereafter until death of the mice.

Combined Immune Lymphocyte and Jurkat IL-2 Therapy in an Intrafootpad FBL3 Model. Immune C57BL/6 splenocytes were obtained by inoculation of 10⁷ live FBL-3 cells in 0.05 ml i.m. in the hind leg. Six to 12 weeks after immunization, after regression of the resultant tumor, spleens were harvested and used for in vitro sensitization or direct adoptive transfer. IVS lymphocytes were prepared as described previously (9). Briefly, 6 × 10⁷ immune splenocytes and 10⁶ irradiated (15,000 rad) FBL-3 cells were incubated for 5 days at 37° in 50 ml of flasks (Costar No. 3050) containing 20 ml of CM. Cells were washed twice in CM and replated at 10⁵ cells/well in 24-well plates (Costar No. 3524) in a 1:1 mixture of CM and LF-IL-2. After 6 or 7 days of growth (1.6 to 10.8-fold expansion in these experiments), the cells were harvested, washed twice in CM, and passed through a No. 100 nylon mesh before being adoptively transferred i.v. into tumor-bearing mice. FBL-3 tumor was inoculated into C57BL/6 mice as reported by Eberlein et al. (8); 10⁷ live FBL-3 in the right rear footpad shortly after the mice received 500-rad total body irradiation from a ⁹⁰Sr source. Adoptive transfer of cells was performed at 5 days after tumor irradiation at which time disseminated FBL-3 is known to exist (8). Jurkat IL-2, 0.5
μg either in 1 ml of CM alone or a 1:1 mixture of CM and 30% (w/w) gelatin (ICN Biochemicals, Cleveland, OH), was injected i.p. daily for 3 or 4 days subsequent to the adoptive transfer of cells. Footpad thickness was measured 3 times weekly with dial calipers, and survival was checked daily.

Enhancement of Allosensitization with Jurkat IL-2. C57BL/6 mice were immunized with an i.p. injection of 1.5 to 2.0 x 10⁷ live P815 tumor cells and reimmunized 2 weeks later with the same dose of liver tumor cells. One ml of CM, EL-4 concentrated supernatant, or Jurkat IL-2 in CM (0.5 μg Jurkat IL-2/ml) was injected i.p. twice daily for the 3 days following the secondary immunization. The EL4 solution used in these experiments had approximately half of the IL-2 titer (14,700 units/ml versus 34,500 units/ml) as Jurkat IL-2 (1.0 μg/ml), using our bioassay. On the seventh day following immunization, the spleens were harvested, and single-cell suspensions were prepared. Cytotoxic activity of the splenocytes were assessed at 4 and 20 hr later by a ⁵¹Cr release of labeled EL-4 and P815 targets. Percentage of lysis (mean to triplicate samples) was calculated by:

\[
\text{Sample counts} - \text{background counts} \times 100
\]

\[
\text{Total counts} - \text{background counts}
\]

Total counts were released by 0.05 N HCl, and background counts were in CM. Lytic units were defined such that one lytic unit was the number of effectors needed to lyse 50% of 10⁸ target cells.

Statistics. Comparison of survival between groups with intrafootpad FBL-3 were performed using a 2-sided Mantel-Haenszel test (31).

RESULTS

Lack of Serum Inhibition on Jurkat IL-2 Activity. We examined the effect of increasing concentrations of mouse serum, incubated with dilutions of Jurkat IL-2 for 2.5 hr, on the thymidine uptake of an IL-2-dependent cell line. As seen in Chart 1, similar dilutions of IL-2 in markedly different concentrations of C56BL/6 mouse serum, had identical activities. The original Jurkat IL-2 solution (0.3 μg/ml) in 10% serum had a titer of 980 units/ml using this assay. Dilutions of 1:3 in RPMI 1640 (3.3% serum) and serum (70% final serum concentration) had titers of 430 and 360 units/ml, respectively. Finally, 1:100 dilutions of the same original IL-2 solution in RPMI 1640 (0.1% serum) and serum (99% serum) had measured titers of 5.3 and 5.0 units/ml. These findings corroborate previous findings by our laboratory (7) of no serum-inhibitory effect on IL-2 activity, using our proliferative assay.

Maintenance of Murine Clone Growth and Antigen-specific Responsiveness in Jurkat IL-2. Growth of the proliferative murine clone 14-11 was supported by Jurkat IL-2 at 0.03 μg/ml as seen in Chart 2. More than 10²-fold expansion occurred over a 2-month interval. The specific proliferative activity to H-2⁰ alloantigens was maintained throughout this period as well. In separate experiments using Jurkat IL-2 (0.005 μg/ml), the proliferative activity was well-maintained over the 3 weeks tested (Table 1). Maximum cell growth and maintenance of proliferation index for this cell line was obtained with IL-2 concentrations between 0.03 and 0.012 μg/ml (data not shown). Thus, an IL-2-dependent murine cell line can maintain its specific proliferative response to antigen and be grown to large numbers in pure human IL-2.
Table 2

**Jurkat IL-2 toxicity: effect on hepatic and renal function**

<table>
<thead>
<tr>
<th>Serum chemistry</th>
<th>Group</th>
<th>Blood urea nitrogen (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Glutamine oxaloacetate transaminase (nanounits/ml)</th>
<th>Lactic dehydrogenase (nanounits/ml)</th>
<th>Alkaline phosphatase (nanounits/ml)</th>
<th>Total bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>34.5 ± 0.7</td>
<td>0.7 ± 0.1</td>
<td>48.5 ± 6.6</td>
<td>164.0 ± 66.5</td>
<td>183.0 ± 66.5</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>0.05 µg i.p.</td>
<td></td>
<td>41.5 ± 8.4</td>
<td>0.9 ± 0.2</td>
<td>58.0 ± 0.0</td>
<td>304.0 ± 75.0</td>
<td>229.0 ± 75.0</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>0.5 µg i.p.</td>
<td></td>
<td>37.0 ± 7.1</td>
<td>1.0 ± 0.1</td>
<td>55.0 ± 2.8</td>
<td>220.5 ± 15.3</td>
<td>156.5 ± 30.4</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>0.5 µg i.v.</td>
<td></td>
<td>28.6 ± 6.6</td>
<td>0.6 ± 0.1</td>
<td>56.8 ± 10.8</td>
<td>149.6 ± 87.6</td>
<td>152.0 ± 76.7</td>
<td>0.2 ± 0.1</td>
</tr>
</tbody>
</table>

Control group given i.p. injections with 1 ml of RPMI 1640 with 3.3% mouse serum twice daily for 7 days; serum was drawn on the day after the last injection. Mean ± S.E. There was no significant difference between any group.

Injections i.p. of 0.05 or 0.5 µg of Jurkat IL-2 in 0.25 ml of RPMI 1640 with 3.3% mouse serum twice daily for 7 days; blood was drawn on the day after the last injection.

Injections i.v. of 0.5 µg of Jurkat IL-2 in 0.25 ml of RPMI 1640 with 10% mouse serum weekly for 4 weeks; sera were collected 3 days after the last injection.

Table 3

**Jurkat IL-2 toxicity: effect on weight**

<table>
<thead>
<tr>
<th>Group</th>
<th>Control*</th>
<th>0.05 µg i.p.</th>
<th>0.5 µg i.p.</th>
<th>0.5 µg i.v.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treat</td>
<td>20.3 ± 2.6</td>
<td>21.5 ± 1.1</td>
<td>20.7 ± 1.7</td>
<td>21.9 ± 2.2</td>
</tr>
<tr>
<td>1 wk</td>
<td>20.4 ± 2.0</td>
<td>22.0 ± 1.8</td>
<td>21.1 ± 2.6</td>
<td>21.7 ± 1.6</td>
</tr>
<tr>
<td>1 mo</td>
<td>20.3 ± 1.8</td>
<td>23.1 ± 0.8</td>
<td>20.8 ± 0.9</td>
<td>22.9 ± 3.2</td>
</tr>
<tr>
<td>4 mo</td>
<td>22.1 ± 0.4</td>
<td>22.7 ± 0.3</td>
<td>21.4 ± 1.3</td>
<td>23.4 ± 3.4</td>
</tr>
</tbody>
</table>

* Control mice given i.p. injections twice daily for 7 days of 0.25 ml of RPMI 1640 with 3.3% mouse serum.
+ Mice given i.v. injections of 0.05 or 0.5 µg of Jurkat IL-2 in 0.25 ml of RPMI 1640 with 3.3% mouse serum twice daily for 7 days.
* Mice given i.v. injections of 0.5 µg of Jurkat IL-2 in 1 ml of RPMI 1640 with 10% mouse serum weekly for 4 weeks.
* Mean ± S.E. There was no significant difference in any group.

0.1% of the initial serum IL-2 concentration remained at 1 hr after the injection.

**Toxicity of Jurkat IL-2 in Mice.** In preparation for a protocol examining the effects of Jurkat IL-2 infusions in humans, toxicity studies were performed in normal C57BL/6 mice. No ill effects of twice-daily i.p. injections of 0.05 or 0.5 µg of Jurkat IL-2 for 7 days or weekly injections of 0.5 µg of Jurkat IL-2 for 4 weeks (comparable to planned doses in humans) were detected by gross appearance of the mice, weights, and serum chemistries measuring renal and hepatic function. Table 2 displays the serum values on the day following the last i.p. injection and 3 days after the final i.v. infusion. No trend in renal or hepatic dysfunction was apparent in comparing mice given injections of RPMI 1640, of serum alone, and of the same solutions containing Jurkat IL-2. Similarly, the weights of mice in all groups (Table 3) were unaffected by treatment, either with i.p. or i.v. IL-2. The histological appearance of lymphoid organs (thymus, spleen, peripheral lymph nodes, and mesenteric lymph nodes), bone marrow, liver, lungs, and kidneys showed no gross or microscopic abnormalities in mice that received the Jurkat IL-2.

**Measurement of natural killer cell activity after 7 days of Jurkat IL-2 injections,** given twice daily i.p., showed no enhancement in activity, compared with serum-injected controls using a 4-hr lysis of 51Cr-labeled YAC cells (data not shown).

**IL-2 Treatment of Mice Bearing a Syngeneic Methylcholanthrene-induced Fibrosarcoma.** A methylcholanthrene fibrosarcoma syngeneic in C57BL/6 mice and described previously (32) was inoculated i.d. in 12 mice. Five days later, when a palpable tumor was present, twice-daily injections of normal mouse serum, 0.05 or 0.5 µg of Jurkat IL-2 i.p., were started and continued for 7 days. Tumor growth was similar for all groups, as seen in Chart 4. Most animals died of tumor by 35
days after injection, with all animals dead by Day 42. The rate of tumor growth and survival of mice were unaffected by Jurkat IL-2 given by this route and schedule.

Combined IL-2 and Immune Lymphocyte Immunotherapy of the FBL-3 Lymphoma/Leukemia. FBL-3 is a highly immunogenic tumor which can be cured by adoptive transfer with either in vivo immunized (12) or secondary IVS (4) murine lymphocytes when given together with cyclophosphamide. Recent work from our laboratory has demonstrated the ability of adoptively transferred immune lymphocytes, expanded in IL-2-containing supernatants, to enhance cure of disseminated (9) and intrafootpad (8) FBL-3 tumors. We attempted to determine whether the exogenous administration of IL-2 could enhance the therapeutic effects of sensitized expanded lymphocytes in this tumor model. To maximize the duration of serum IL-2 levels (7), Jurkat IL-2 was injected i.p. in 15% gelatin in mice daily for 3 days, starting 6 days after tumor inoculation and 1 day after adoptive transfer of 1 x 10^7 immune lymphocytes, secondarily sensitized to FBL-3 in vitro, and expanded for 7 days in LF-IL-2.

Fresh uncultured immune lymphocytes (10^6 lymphocytes) routinely cured mice of FBL-3 tumor (Table 4), while the mean survival of untreated mice was 19.8 days. Treatment with 15% gelatin alone or Jurkat IL-2 in gelatin had no effect in prolonging survival compared to no treatment (mean, 19.8 and 20.3 days). Injection of 1 x 10^7 IVS and expanded cells increased mean survival to 24.8 days. Jurkat IL-2 in gelatin injected daily for 3 days after transfer of 1 x 10^7 IVS cells prolonged mean survival to 37.8 days. This was a highly significant prolongation when compared to the administration of IVS cells with gelatin but no IL-2 (p = 0.004). Thus, IL-2 enhanced the therapeutic effect of IVS cells using this system. Similar results were obtained in 3 additional experiments using Jurkat IL-2 and expanded lymphocytes for the adoptive immunotherapy of the FBL-3 tumor.

Enhancement of Allosensitization with Pure Jurkat IL-2. Previous studies have demonstrated increased cytotoxic activity of splenocytes after alloimmunization when EL-4 supernatants (37) or purified murine IL-2 (23) was administered subsequent to the allostimulus. Using purified human IL-2, we have demonstrated a similar enhancement of allosensitiveness (Table 5). In both of these experiments, the EL-4-pretreated mice showed greater cytotoxicity, but 3 to 4 times the IL-2 activity, as measured by our bioassay, were administered in the Jurkat group. In both of these experiments, Jurkat IL-2 injections increased splenocyte cytotoxicity for the relevant target (P815) compared to animals treated with CM alone. Splenocytes from animals not immunized, but given injections of Jurkat IL-2 at the same doses and schedule, showed no lysis of ^51Cr-labeled P815 targets (data not shown). In addition, no mice in either the EL-4 or Jurkat IL-2 group survived.
2 treatment groups showed any lytic activity for syngeneic EL-4 tumor targets, in either a 4- or 20-hr assay (data not shown). Two subsequent experiments using a similar immunization scheme but without an EL-4 IL-2 control also demonstrated enhancement of allosresponsiveness by Jurkat IL-2.

DISCUSSION

Pure IL-2 has previously been available in only small quantities and, consequently, most studies reporting IL-2 effects have used crude or partially purified preparations containing IL-2. Robb et al. (35) have recently described the large-scale purification of human IL-2 from a tumor source, and mg quantities of this material have been prepared for human studies. Because of our plan to administer this material systemically to humans, we undertook initial studies to examine the toxicity, metabolism, and immunological effects of pure IL-2 in mice.

Serum inhibition of IL-2 activity, as reported previously (21), could limit the usefulness of in vivo IL-2 administration. Our laboratory (7) has reported previously a lack of inhibition of IL-2, using a proliferative IL-2 assay, at concentrations of up to 75% normal mouse serum. Concentrations of up to 99% serum likewise showed no inhibitory effect on Jurkat IL-2 after incubation for 2.5 hr. Differences in IL-2 assay systems (cytotoxic versus proliferative) and responding cells (lectin-stimulated blasts versus our long-term line) may account for the discrepancy between our results and those reported by Harnd et al. (21).

Although human IL-2 differs from murine IL-2 in molecular weight and other biochemical properties, mouse T-cells are responsive to human IL-2 (40). Long-term maintenance of T-cell lines in crude conditioned media has been attributed to the presence of IL-2, although other factors present could be responsible for maintaining cell growth and antigenic specificity. A cloned T-cell line, 14-11, which proliferates to a specific alloantigen (H-2k) was grown for 2 months in medium containing no lymphokines other than Jurkat IL-2. Throughout this period, this cell line grew well with 4.6- to 13.8-fold expansion each week. At the same time, line 14-11 retained proliferation in response to the H-2k alloantigen. Thus, it is possible to grow murine effector clones to large numbers, with maintenance of function, in dilute solutions of pure human IL-2.

Murine IL-2 has a serum half-life in mice of 3 to 4 min after i.v. injection (7, 21, 31). A large component of this clearance appears to be related to renal clearance (7), similar to the elimination of other small proteins from serum (44). Jurkat IL-2 was cleared from mouse serum in 2 phases. The initial component had a half-life of approximately 2 min, similar to that seen with murine IL-2, and this accounted for the removal of more than 90% of the serum IL-2. The final 10% of injected IL-2 was cleared with a slower half-life of approximately 9 min. Assuming the initial short half-life represents renal clearance of Jurkat IL-2, this second component may represent IL-2 bound to serum proteins and, thus, not filtered by the glomerulus. Competitive binding for these sites by other proteins in unpurified supernatants could explain the lack of this slower phase in our previous work (7). An alternate explanation for this difference is that human IL-2 was metabolized after injection in mice in a manner distinct from murine IL-2, although we consider this unlikely.

Injection of crude human IL-2-containing supernatants (1) into patients has resulted in rigors, pyrexia, and nausea and vomiting shortly after injection. Serum liver enzymes, antibody levels (including autoantibodies), and complement levels remained normal after these injections, but serum cortisol levels and coagulation times rose transiently. A recent report of the administration of purified human IL-2 to a single immunodeficient child mentions no similar toxicity, although bronchospasm was noted subsequent to s.c. IL-2 injections (15). In preparation for our own studies in humans using pure human IL-2, we examined its potential toxicity in mice. No mice showed adverse effects subsequent to Jurkat IL-2 administration. Weight gains were comparable to control mice, and no hepatic or renal injury was detectable by serum chemistry evaluation. No histological abnormalities or changes from controls were noted on examination of lungs, liver, kidneys, bone marrow, lymph nodes, spleen, or thymus. IL-2 had no apparent toxicity when given to mice and caused no histological changes in those organs. Of interest would be the examination of lymphoid organs from mice with congenital or acquired immunodeficiency to determine if histological changes and corresponding functional immune restoration occurred after IL-2 inoculation. A repopulation of regional lymph nodes with lymphocytes in a T-cell-deficient child (15) has been noted on postmortem examination following IL-2 administration.

The finding that human lymphocytes, cultured with IL-2 in vitro, leads to the generation of cells lytic for fresh human tumor (19, 25) has aroused interest in the use of IL-2 in vivo in an attempt to generate antitumor immune responses in cancer patients. The presence of IL-2 receptors on activated T-cells and their resultant proliferative response to IL-2 (33) has suggested that the administration of exogenous IL-2 may enhance the therapeutic effectiveness of the adoptive transfer of immune T-cells as a method of tumor immunotherapy. Several investigators have already shown the effectiveness of T-cell adoptive transfer (3, 4, 6, 13) in curing rodent tumors but, to date, only Cheever et al. (4) have shown an improved antitumor effect of both IL-2 and immune lymphocytes given together in mice with FBL-3 lymphoma. Pure IL-2 injections in our studies had no effect on methylcholanthrene fibrosarcoma growth when given i.v. and did not prolong survival. Other investigators (2), giving repeated doses of IL-2 locally with a similar tumor, have observed regression. The growth inhibition of murine sarcoma using crude supernatants could have been caused by a factor other than IL-2 or could reflect a differential effect of murine IL-2 and our human Jurkat IL-2 in this system. Using an intrafootpad model for disseminated FBL-3 in C57BL/6 mice, we have demonstrated a significant prolongation of survival using pure human IL-2 and IVS and expanded immune lymphocytes together. Less than curative doses of cells were used in an effort to dramatize any additional effect of IL-2.

Murine IL-2 has been demonstrated previously to enhance the response to alloantigen in mice (23, 37). We have shown the same effect using pure human IL-2 in mice. Preliminary results using divided doses of IL-2 (same total dose) given more frequently over 24 hr seem to increase the allosresponse over a single daily injection of IL-2. This enhancement of alloseactivity by in vivo IL-2 may have important applications in humans in the treatment of immunodeficient states, both congenital and acquired.

From the experiments reported in this paper, it appears that human Jurkat-derived IL-2 is cleared in mice with a serum half-life similar to murine IL-2 after i.v. injection. It remains to be shown whether short exposures to IL-2, as after i.v. infusion, have similar in vivo effects to more prolonged serum IL-2 levels. There was no evidence of toxicity of Jurkat IL-2 in mice, when
administered in doses comparable to amounts that will be injected into humans. Short-term use may avoid toxicity that may result from chronic endogenous local elevations of IL-2 as may exist in sarcoïdiosis (33). The demonstration of in vivo effects of pure human IL-2 in mice provides encouragement for future use of IL-2 in humans. Potential applications of this material may include the treatment of congenital conditions that have as a component a deficiency of IL-2, as well as the treatment of acquired immune-deficient states (e.g., postchemotherapy, post-trauma, chronic microbial diseases [22]), and in cancer immuno-therapy.

REFERENCES


In Vivo Administration of Purified Jurkat-derived Interleukin 2 in Mice


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