Phase I Trial of Intraperitoneal Recombinant Interleukin-2/Lymphokine-activated Killer Cells in Patients with Ovarian Cancer


Vermont Regional Cancer Center (J. A. S., J. L. B., A. L. M., B. W. G., J. D. R., R. J. A., R. F. B.), Department of Medicine (J. A. S., B. W. G., J. D. R., J. A. D., R. J. A., R. F. B.), Department of Obstetrics and Gynecology (J. L. B.), and Department of Mathematics and Statistics (L. D. H.), University of Vermont, Burlington, Vermont 05405

ABSTRACT

Ten patients with ovarian cancer refractory to conventional therapy were treated with intraperitoneal (i.p.) recombinant interleukin-2 (rIL-2) and lymphokine-activated killer cells (LAK). The 28-day protocol consisted of 6 priming i.p. rIL-2 infusions on days 0, 4, 6, 8, 10, and 12. Leukopheresis was performed for mononuclear cell collection on days 15, 16, 17, and 18 and lymphokine-activated killer cells were given i.p. with the rIL-2 on days 19 and 21. Three additional i.p. rIL-2 infusions were given on days 23, 25, and 27. Three dose levels of rIL-2 were tested: 5 x 10^6, 2 x 10^6, and 8 x 10^6 units/m^2 body surface area. The dose-limiting toxicity was abdominal pain secondary to ascites accumulation with significant weight gain. Other toxic effects included decreased performance status, fever, nausea and vomiting, diarrhea, and anemia. Peripheral lymphocytosis and eosinophilia were seen at all dose levels. The maximum tolerated dose is 8 x 10^6 units/m^2/dose.

Peripheral and peritoneal IL-2 levels were measured with a bioassay using an IL-2-dependent cell line. At the highest dose level, serum IL-2 was >10 units/ml for 18 h. After the first infusion, a 2-log dilution of the i.p. IL-2 was measured in the serum. In the postleukopheresis i.p. IL-2-dosing period less IL-2 was detected in the serum than in the earlier i.p. IL-2-priming period.

The induction and persistence of LAK activity were studied. Peritoneal LAK activity was detected as early as 4 days after the first i.p. infusion, by day 11 in all evaluable patients, and persisted for the 6-day interval between priming IL-2 and LAK/IL-2 infusion. Peritoneal lymytic activity persisted until day 28 in 5 tested patients. These peritoneal cells retained lytic activity 48 h in culture medium without rIL-2 present. Peritoneal LAK activity correlated with the percentage of mononuclear cells and the percentage of CD56-positive mononuclear cells in the peritoneum. The yield of peripheral lymphocytes after the six i.p. priming doses of rIL-2 correlated with the dose level of rIL-2 infused. Peripheral blood LAK activity showed a minimal, however progressive, increase during the treatment protocol. LAK activity could be enhanced if rIL-2 was present during the 4-h assay. These studies indicate that i.p. rIL-2 infusion induced durable regional LAK activity and primes peripheral blood cells for LAK activity if exposed briefly to additional IL-2.

INTRODUCTION

Current approaches to the treatment of ovarian cancer include cytoreductive surgery, radiotherapy, and the administration of systemic agents given p.o., i.v., or i.p. A theoretic advantage of the i.p. route is the increased ratio of peritoneal to systemic exposure of the drug or agent used. This advantage may be exploited in a disease such as ovarian cancer which for most of its natural history is confined to the abdominal cavity. Using this rationale in numerous clinical trials investigators have administered i.p. immunotherapy to patients with regional tumors using single-agent cytokines such as IL-2 (1-7), interferons (8-13), or combinations of cytokines (14).

Interleukin-2 activates a population of lymphocytes which is then capable of lysing natural killer-resistant tumor cells without major histocompatibility complex restriction (15). This LAK phenomenon has been utilized in adoptive immunotherapy with rIL-2 to produce antitumor responses in animal models (16-18) and in patients with advanced metastatic cancer (19). High-dose intermittent bolus rIL-2 given i.v. has yielded durable responses in renal cancer and melanoma but is associated with significant hypotension and fluid retention as treatment-limiting toxicities (19). The administration of LAK along with rIL-2 may enhance the therapeutic effect of systemic rIL-2 alone (20).

Comparison of i.v. and i.p. routes of IL-2 administration in murine models showed that i.p. administration of IL-2 and LAK was more effective than systemic treatments in reducing intraperitoneal tumors (21). The bioavailability of IL-2, determined by the in vivo growth of adoptively transferred T-cells, correlates better with the duration of time IL-2 is detectable in serum than the peak IL-2 levels achieved (22). Initial clinical trials of i.p. rIL-2 have resulted in in vivo development of LAK as well as toxicities of weight gain and diarrhea (1). To better understand the rIL-2 and cellular kinetics of i.p. administered rIL-2 and LAK we conducted a phase I trial in patients with advanced ovarian cancer. The objectives were to determine the maximum tolerated dose of i.p. rIL-2, to evaluate the feasibility of peripheral LAK harvest using i.p. rIL-2 priming, to describe serum and peritoneal IL-2 kinetics, and to study the induction and persistence of peripheral and regional LAK activity.

MATERIALS AND METHODS

Patient Eligibility. Patients with persistent or recurrent unresectable epithelial ovarian cancer were candidates for this trial. Eligibility requirements included age ≤70 years, Eastern Cooperative Oncology Group performance status 0 or 1, lack of other serious medical illnesses, adequate organ function defined by WBC count >4,000/mm^3, platelets >100,000/mm^3, serum creatinine <1.4 mg/dl, forced vital capacity >70% of predicted, forced expiratory volume in 1 s >70% predicted, and serum bilirubin <1.2 mg%. Prior immunotherapy, chemotherapy, or radiation therapy within the previous 4 weeks and prior rIL-2 were not permitted. All patients provided written informed consent approved by the University of Vermont Institutional Review Board.

Treatment Plan. Single-cuff Tenckhoff intraperitoneal catheters were placed using an incision large enough to lyse adhesions. In some cases, the catheters were placed at the time of the laparotomy documenting recurrent or residual disease. Isotope or other imaging studies to determine the distribution of fluid in the peritoneum were not done; however, the relatively large volumes instilled resulted in apparent

Received 2/5/90; accepted 7/2/90.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by grants from the National Cancer Institute (P30 CA22435), The Division of Research Resources (MO1-RR-109), the American Cancer Society, VT Division, Lake Champlain Cancer Research Organization (ACS Institutional Research Grant IN-156C), and the Vermont Regional Cancer Center.

2 To whom requests for reprints should be addressed, at Vermont Regional Cancer Center, One South Prospect Street, Burlington, VT 05401.

3 The abbreviations used are: IL-2, interleukin-2; rIL-2, recombinant IL-2; LAK, lymphokine-activated killer cells; AUC, area under the curve; MNC, mononuclear cells; HBSS, Hanks' balanced salt solution; LU, lytic unit; PBS, phosphate-buffered saline.
symmetric distribution in the abdomen as assessed by physical exami-

Fig. 1 describes the treatment protocol. Baseline serum and peri-

We collected as described above. In addition, i.p. fluid samples were

IL-2 Pharmacokinetics. Recombinant IL-2 was measured in a stan-

dard bioassay using an IL-2-dependent cell line, CTLL-2 (American

type of 13.1 × 10^6 reference units/mg (23). Three dose levels were

Given the difficulties of analysis of intraperitoneal therapy kinetics

A comparison of AUC values across days and among dosage groups

Lytic Assay. LAK activity was assayed in a standard 4-h chromium

Lytic activity was assayed in a standard 4-h chromium release assay as we have previously described (24) using the tumor cell line Daudi as the target cell. Effector cells were resuspended in RPMI 1640 medium with 10% heat-inactivated fetal calf serum. Percentage of specific lysis was calculated for each effector:target ratio and expressed as LU (numbers of effectors in 10^6 MNC lysing 30% of 5000

6303
RESULTS

Ten patients were enrolled in this trial and all were evaluable for toxicity. Patient characteristics including dose level and total rIL-2 given are shown in Table 1. Patient 9 received only six infusions of rIL-2 because of intraperitoneal infection prior to leukapheresis.

Toxicity. The dose-limiting toxicity was the accumulation of ascites. All patients gained weight (Table 2). The median percentage increase in weight was 9% (range, 1–42%). Abdominal girth was measured daily and increased in all patients with a median increase of 12% (range, 7–42%). The clinical impression was that weight gain was primarily from ascites and not peripheral edema. Weight gain was greater for higher doses of rIL-2. Subjectively, abdominal fullness and discomfort from ascites was the most distressing toxicity.

Given the duration and intensity of the treatment period general fatigue and confinement to bed were common complaints. Eastern Cooperative Oncology Group performance status was determined on a daily basis (Table 2). Ascites, fatigue, nausea, and diarrhea were the major toxicities resulting in a decreased performance status. A common but relatively minor complaint was of nasal congestion after the initial infusion of rIL-2. Hypotension occurred but was not dose or schedule limiting.

RBC transfusion was needed in all patients. The first 6 patients underwent phlebotomy for the collection of serum needed for LAK culture. With the availability of serum-free medium the phlebotomy procedure prior to treatment was eliminated. Patients received a median of 5 units of packed RBCs (range, 2–10). An effort was made to keep the hemoglobin >9 g/dl. Patients 7–10, who did not undergo phlebotomy for serum collection, required 5, 5, 4, and 2 units of RBCs, respectively.

Thrombocytopenia occurred as a result of both i.p. rIL-2 and the leukapheresis procedure. The lowest platelet counts tended to occur on days 17 and 18 just after apheresis and were thought to be due to the leukapheresis procedure. Two patients received platelet transfusions during this period. Platelet counts greater than baseline were recorded toward the end of therapy. Five patients had platelet counts >400,000/mm³ at the end of the trial on days 26–28.

Significant WBC count changes occurred during the course of treatment (Table 3). A dose-related lymphopenia occurred after the first i.p. rIL-2 infusion. All patients experienced a lymphocyte rebound usually occurring on days 14–15 after the initial six infusions of rIL-2. Peripheral eosinophilia was dramatic with an approximately 100-fold increase in three patients at the highest dose level.

Liver and renal toxicities, as measured by serum chemistry were mild and transient. Only one patient had an aspartate aminotransferase >2 times normal and 5 patients had serum alkaline phosphatase >2 times normal. The highest bilirubin was 1.1 mg/dl on day 23, in a patient treated at the lowest dose level. Significant changes in serum creatinine did not occur.

One patient (patient 4) experienced abdominal wall extravasation of rIL-2 resulting in an area of skin hyperemia. Replacement of the catheter allowed intraperitoneal therapy without difficulty. One patient (patient 9) developed Staphylococcus aureus peritonitis after six infusions of i.p. rIL-2. Treatment with rIL-2 was stopped and i.v. and i.p. antibiotics were given.

Clinical Response. Nine patients had progressive disease despite rIL-2/LAK. One patient (patient 7) demonstrated reduction of a tumor nodule in the uterosacral ligament assessed by physical examination (from 0.5 to 0.2 cm) accompanied by a transient reduction of serum CA-125 (257 units/ml pretreatment, 155 units/ml immediately posttreatment, and 113 units/ml 1 month posttreatment). Disease progression in this patient occurred at 3 months posttreatment. The median survival of the 10 patients is >11 months (range, 2–27+ months). Two patients are currently alive with evidence of disease.

Two patients underwent laparotomy after therapy with rIL-2/LAK. One patient (patient 8) had placement of another Tenckhoff catheter for i.p. cisplatin treatment 8 months after rIL-2. Peritoneal adhesions more extensive than those seen at the earlier catheter placement were present. Another patient (patient 9) underwent exploratory laparotomy 8 months after rIL-2 for treatment of bowel obstruction. Obstruction was because of tumor and although adhesions were present they were not believed to be unusual in amount or quality.

rIL-2 Kinetics. The initial dose of rIL-2 was followed by 4 days without therapy allowing for prolonged sample collection so as to fully describe the kinetics of rIL-2 given i.p. IL-2 could be detected in the serum within 15 min in all patients except...
two receiving the lowest dose. Peak serum levels were usually reached within 3–6 h.

Fig. 2 shows the serum and peritoneal rIL-2 levels measured from two representative patients receiving the highest and lowest doses of rIL-2. Serum levels followed peritoneal levels over time. Generally, a 2-log dilution of the i.p. IL-2 level was measured in the serum. Peritoneal IL-2 levels were still detected at 72–96 h in patients receiving the highest dose level of rIL-2, but ascites was often not obtainable after 24–48 h in patients receiving lower doses.

Early in the treatment schedule there was a clear correlation between i.p. IL-2 dose level and peripheral IL-2 exposure as measured by the calculated AUC (Fig. 4). Interestingly, there was a dramatic decrease in peripheral IL-2 levels in the second half of the treatment, particularly in patients receiving the highest dose level of rIL-2. Most notably, patient 8 had no detectable serum IL-2 during the period of the last five infusions. This was accompanied by high i.p. IL-2 levels, reaching >25,000 units/ml, and ascites accumulation. Except for patient 5 there was a higher peripheral AUC for days 0–12 compared to days 13–27.

A repeated measures analysis of variance of the AUC values indicates that the three dose level groups do not follow the same pattern over days of administration, since there was a statistically significant interaction (P < 0.05) between dose level groups and days for either days 0–12 or 0–27. The only statistically significant differences among dose level groups in AUC levels at each day appeared at day 0 (even if nonconstant variances among the groups are compensated by an appropriate data transformation). At day 0 the high-dose level group had significantly higher mean AUC than the medium- or low-dose level groups. Fig. 4 indicates that the AUC on average tends to follow the expected pattern of higher AUC values with higher dose level given.

A contrast can be drawn between the rIL-2 levels of the first half of the study, in which rIL-2 was given on days 0, 4, 6, 8, 10, and 12, and the second half of the study, postleukapheresis. Fig. 4 illustrates that the AUC drops significantly from day 0 through day 12, particularly at high doses. There are statistically significant negative correlations between AUC and day for both the high and medium doses with r = −0.62 (P = 0.002) for high and r = −0.69 (P = 0.002) for medium. That correlation is not just due to the initial drop after day 0 but is also significant from days 4 through 12 for the high and medium doses (r = −0.46, P = 0.048, for high and r = −0.56, P = 0.036, for medium). The negative trend for high- and medium-dose levels also appears during the second half of the study (r = −0.42, P = 0.13, for high and r = −0.52, P = 0.045, for medium) although it is not as statistically significant.

In order to judge whether the overall level of AUC has decreased during the second half of the study the differences and percentage of differences in total AUC for the 5 doses (days 4–12) and the total AUC for the 5 doses (days 19–27) were compared with a paired t test. On average the AUC levels were lower in the second half, but the difference was not significant.

Peritoneal Cells. The generation of peritoneal LAK activity is detailed in Table 4. LAK assays were done with cells obtained just prior to rIL-2 infusions. Some patients could not be evaluated on particular days because either there was no peritoneal fluid or the Tenckhoff catheter would not drain. Usual MNC separation methods using a Ficoll-Hypaque discontinuous gradient were not successful in removing peritoneal eosinophils. When peritoneal cells after separation methods contained <30% MNC the absence of lytic activity was considered not evaluable. Occasionally, on later days in the protocol schedule, peritoneal fluid could be obtained but was essentially acellular. LAK activity was not detected in peritoneal MNC obtained prior to or immediately after the first i.p. rIL-2 infusion on day 0 (5 evaluable patients, Table 4). Four days after the first

---

Table 2  Toxicity

<table>
<thead>
<tr>
<th>Patient</th>
<th>Weight gain†</th>
<th>Eastern Cooperative Oncology Group (days at PS ≥ 3)</th>
<th>Nausea‡</th>
<th>Vomiting‡</th>
<th>Diarrhea‡</th>
<th>rIL-2-related fever</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>17</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>14</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>11</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>24</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>13</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

* By National Cancer Institute Common Toxicity Criteria.  † Weight gain: 0, <5% ; 1, 5–9.9% ; 2, 10–19.9% ; 3, ≥20%.
‡ Ascites thought to be tumor associated, rather than treatment related.  ‡ Abbreviated course (6 doses) because of peritonitis, treatment related.

---

Table 3  Peripheral WBC changes

<table>
<thead>
<tr>
<th>Lymphocytes (µl)</th>
<th>Eosinophils (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Patient</td>
<td>High</td>
</tr>
<tr>
<td>1</td>
<td>1,653</td>
</tr>
<tr>
<td>2</td>
<td>1,584</td>
</tr>
<tr>
<td>3</td>
<td>396</td>
</tr>
<tr>
<td>4</td>
<td>824</td>
</tr>
<tr>
<td>5</td>
<td>856</td>
</tr>
<tr>
<td>6</td>
<td>636</td>
</tr>
<tr>
<td>7</td>
<td>1,800</td>
</tr>
<tr>
<td>8</td>
<td>819</td>
</tr>
<tr>
<td>9</td>
<td>1,463</td>
</tr>
<tr>
<td>10</td>
<td>1,180</td>
</tr>
</tbody>
</table>

* On day 0 when no eosinophils were reported in the differential a value of 1% was used in order to calculate a ratio.
infusion, 4 of 5 evaluable patients had peritoneal cells expressing low levels of LAK activity (<5 LU). By day 7 (24 h after the third infusion of IL-2), peritoneal MNC from 5 of 8 evaluable patients exhibited a wide range of LAK activity (<5-288 LU). By day 11 peritoneal MNC from all 6 evaluable patients demonstrated lytic activity (10-375 LU). This activity was persistent even after incubation for 24-48 h in medium without rIL-2. Addition of rIL-2 (100 units/ml) to the assay did not increase the lytic activity. On day 18, 6 days following the last primary infusion, peritoneal cells from 3 of 4 patients continued to manifest the capacity to lyse Daudi targets. Following rIL-2/LAK infusion on days 19 and 21, peritoneal MNC from all evaluable patients had LAK activity on days 25-28.

The initial pretreatment peritoneal cell analysis varied considerably among patients. Only 2 patients had peritoneal fluid obtainable prior to treatment. For other patients the initial analysis was done on cells obtained by lavage immediately after rIL-2 infusion. The majority of the pretreatment peritoneal cells were eosinophils in 4 patients, neutrophils in 2 patients, and MNC in 3 patients. Cellular populations varied considerably during the treatment for each patient and from patient to patient. There did not seem to be a correlation between rIL-2 dose level and cell type or numbers of peritoneal cells obtained. MNC tended to predominate during days 7-14, followed by an influx of eosinophils, often making up >80% of the cells by the end of the treatment. A rise in the percentage of MNC in the peritoneal fluid correlated with an increase in the percentage of CD56-positive MNC and enhanced lytic activity.

Peripheral MNC Priming from i.p. rIL-2. The number of WBCs obtained from the 4 days of leukapheresis, the cell yield following ex vivo IL-2 incubation, and the total i.p. infused lytic units of LAK generally correlated with rIL-2 dose (Table 5). Patients 2 and 8 were exceptions. Patient 2 had large numbers of adherent cells in the ex vivo activated cultures. Because patient 8 was not administered the sixth infusion of IL-2 (day 12), 5 days instead of 3 elapsed between the last i.p. rIL-2 infusion and leukapheresis. This delay may explain the slightly lower leukapheresis and LAK yields obtained and the considerably decreased number of lytic units generated for this patient.
Fig. 4. AUC (units·h/ml) of serum IL-2 levels after each IL-2 infusion for each of three dose levels given: A, 5 × 10^8 units/m^2; □, 2 × 10^9 units/m^2; △, 8 × 10^9 units/m^2. Points, means; bars, ±SE.

Table 4 Peritoneal lytic activity (LU/10^6 peritoneal MNC)*

<table>
<thead>
<tr>
<th>Study day</th>
<th>0</th>
<th>4</th>
<th>7</th>
<th>11</th>
<th>14</th>
<th>18</th>
<th>21*</th>
<th>25</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

% Evaluable patients with lytic activity

- 0 LU, <10% specific lysis at effector:target ratio of 40:1; <5 LU, >10-<30% specific lysis at effector:target ratio of 40:1.
- Cells obtained before LAK/IL-2 infusion.
- NO, not obtained.
- Cells cultured 24-48 h in media without IL-2 before assayed for lytic activity.
- Due to eosinophilia; NE, not evaluable (ascites collected but not assayed because of insufficient numbers of MNC).
- Acellular ascites; NE, not evaluable (ascites collected but not assayed because of insufficient numbers of MNC).

Table 5 IL-2, i.p., priming of peripheral lymphocytes for ex vivo LAK activation

<table>
<thead>
<tr>
<th>Dose: 5 × 10^8 units/m^2</th>
<th>Total leukopak cell yield (4 days) × 10^8</th>
<th>Total LAK yield (4 days) × 10^8</th>
<th>Mean LU/LAK (4-day range)</th>
<th>Total LU infused × 10^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>24</td>
<td>16</td>
<td>(21–82)</td>
<td>826</td>
</tr>
<tr>
<td>Patient 2</td>
<td>28</td>
<td>12</td>
<td>&lt;5</td>
<td>46</td>
</tr>
<tr>
<td>Patient 3</td>
<td>23</td>
<td>10</td>
<td>116 (18–355)</td>
<td>304</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dose: 2 × 10^9 units/m^2</th>
<th>Total leukopak cell yield (4 days) × 10^8</th>
<th>Total LAK yield (4 days) × 10^8</th>
<th>Mean LU/LAK (4-day range)</th>
<th>Total LU infused × 10^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 4</td>
<td>35</td>
<td>20</td>
<td>158 (99–152)</td>
<td>1322</td>
</tr>
<tr>
<td>Patient 5</td>
<td>47</td>
<td>18</td>
<td>155 (80–189)</td>
<td>3,348</td>
</tr>
<tr>
<td>Patient 6</td>
<td>40</td>
<td>22</td>
<td>426 (355–470)</td>
<td>7,167</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dose: 8 × 10^9 units/m^2</th>
<th>Total leukopak cell yield (4 days) × 10^8</th>
<th>Total LAK yield (4 days) × 10^8</th>
<th>Mean LU/LAK (4-day range)</th>
<th>Total LU infused × 10^3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 7</td>
<td>69</td>
<td>48</td>
<td>381 (373–389)</td>
<td>18,000</td>
</tr>
<tr>
<td>Patient 8</td>
<td>54</td>
<td>36</td>
<td>107 (97–117)</td>
<td>3,758</td>
</tr>
<tr>
<td>Patient 10</td>
<td>70</td>
<td>63</td>
<td>396 (301–494)</td>
<td>25,448</td>
</tr>
</tbody>
</table>

Peripheral MNC LAK Activity. Fig. 5 illustrates the percentage of specific lysis of Daudi targets by peripheral MNC from all 10 patients after i.p. infusions of rIL-2. There was a progressive increase in lytic activity (mean percentage of specific lysis), reaching a peak on day 18 and persisting until day 28. The inducibility of peripheral LAK activity did not appear to correlate with i.p. rIL-2 dose, since patients from each of the 3 dose levels had >20% specific lysis on days 25 and 27. One patient (patient 3) who was receiving the lowest dose of rIL-2 (5 × 10^8 units/m^2) had high levels of peripheral LAK activity after the first i.p. infusion of IL-2 and maintained this throughout the study. The large standard error of the mean percentage of specific lysis of all patients (Fig. 5) reflects this patient's high peripheral LAK activity. Lytic activity of peripheral MNCs could be greatly enhanced after several i.p. IL-2 treatments (from 8 to 60% specific lysis) when rIL-2 (100 units/ml) was added during the 4-h assay. Peripheral MNC from 10 normal individuals exhibited a similar enhancement of lytic activity if IL-2 was present during the assay (from <5% specific lysis to 20 ± 11%), however, not as dramatic as the patients' peripheral MNC after i.p. IL-2 priming. The increase in these primed peripheral lymphocytes coincided with an increase in the per-

6307
that these studies used Cetus rIL-2 (Emeryville, CA) which has been shown, on a unit for unit basis, to be more potent than Roche rIL-2. Clearly, ascites will limit dose and frequency of treatment particularly when the peritoneum is already abnormal as in patients with advanced ovarian cancer or other malignancies metastatic to the peritoneum.

Other authors have raised concerns about the potential development of peritoneal fibrosis following i.p. biological therapy (30). Such fibrosis could lead to either bowel obstruction requiring surgical intervention or significant occlusion of the peritoneal cavity precluding continued i.p. therapy. D’Acquisto et al. (11) noted evidence of peritoneal inflammation in a trial of i.p. recombinant γ-interferon and postulated a direct effect of the interferon as a cause. Our trial experience suggests that, although rIL-2-related fibrosis may be a problem, it should be evaluated in the context of the clinical setting. Ovarian cancer is a disease that naturally produces bowel obstruction, and fibrosis at second- or third-look laparotomies is not uncommon in this population. Evaluation of the fibrogenic pathogenesis and development of preventive measures are needed.

A potential kinetic advantage of i.p. over i.v. therapy includes a high i.p. to peripheral ratio of drug with resulting reduction in systemic toxicity (31). Indeed, in this trial the dose-limiting toxicity of hypotension seen with high-dose i.v. bolus rIL-2 was avoided. The 2-log advantage of i.p. over serum IL-2 levels seen in this trial is similar to that of other studies (3, 30). Thus, the toxicity profile is altered and high IL-2 levels in the peritoneum and maintenance of modest but prolonged serum levels are achieved. In fact, peripheral levels of IL-2 approximated that necessary for LAK activation in vitro (32).

We noted an initial lymphopenia, a rebound lymphocytosis, and marked eosinophilia similar to that reported for i.v. IL-2 (33). Based on this trial leukapheresis might be more productive if started 48 rather than 72 h after the last rIL-2 primary infusion. Leukapheresis was scheduled for day 15 which for some patients was 1–2 days after peak lymphocytosis. MNC yields from apheresis at our highest i.p. rIL-2 dose level approximated those obtained after high-dose i.v. bolus rIL-2 by Rosenberg et al. (19). The latter investigators reported a 66.5 × 10⁹ median total cell yield from 5 days of leukapheresis after an i.v. dose of 10⁴ units/kg, 3 times/day, for 5 days, compared with a cell yield of 69, 54, and 70 × 10⁹ from our three patients receiving high-dose rIL-2 i.p. (Table 5). In a previously reported i.p. rIL-2/LAK trial (30) patients were initially primed i.v. for 3 days in their first cycle and then administered rIL-2 with LAK i.p. followed by a second cycle of leukapheresis and i.p. rIL-2/LAK. Thus, that protocol differed from ours in rIL-2 dose, timing, and source. They obtained a mean LAK yield from each day of leukapheresis of 10.8 × 10⁹ (cycle 1 i.v. priming) and 7.7 × 10⁹ (cycle 2 i.p. priming). Our mean LAK yield for each day of leukapheresis was 3.2, 5, or 12.3 × 10⁹ for low-, medium-, and high-dose levels of rIL-2 in our protocol. The range of total number of LAK generated in our patients receiving the highest dose was 36–63 × 10⁹, while those administering high-dose rIL-2 i.v. report a range of 20–152 × 10⁹ (mean 76 × 10⁹) (33). We conclude that i.p. rIL-2 can adequately generate sufficient peripheral cells for ex vivo generation of LAK.

Enriched populations of CD3-negative large granular lymphocytes are efficiently activated by rIL-2 and demonstrate LAK activity within hours. This is in contrast to CD3-positive MNC requiring 2–3 days exposure to IL-2 to generate maximal activity (34). Although peripheral LAK activity was high percentage of CD3-negative, CD56-positive MNC during the treatment course.

DISCUSSION

Optimal treatment with biological response modifiers ultimately requires knowledge of the dose resulting in the greatest desired biological effect. First, however, it is important to have an understanding of the dose range tolerable in humans, the kinds of toxicities encountered, and a description of the kinetic behavior of the agent used. In this trial we evaluated three dose levels of rIL-2, given i.p. as repeated infusions, with the addition of autologous LAK.

The dose-limiting toxicity of rIL-2 given on this schedule was discomfort secondary to ascites accumulation. Patients at the highest dose level gained from 19 to 42% of their baseline weight from fluid retention manifested as ascites and peripheral edema. Because of increasing ascites many rIL-2 infusions were given in volumes of lactated Ringers’ solution smaller than the initially prescribed 1 liter/m². The three patients at the highest dose level who received the full 28-day course of therapy all experienced the need for ascites drainage, dose elimination, or reduction of rIL-2 volume infused. The discomfort from the fluid gain and abdominal distension seen with repeated infusions of 8 × 10⁹ units/m² suggested that at higher dose levels few patients would tolerate even a majority of infusions on this schedule. Therefore, the maximum tolerated dose is considered to be at or near 8 × 10⁹ units/m².

The infusion of LAK did not seem to directly increase toxicity. There were no episodes of wheezing, urticaria, or another anaphylactoid type reactions and no increase in nausea or diarrhea over that seen with i.p. rIL-2 alone. Fluid gain did not completely resolve during days 13–18 prior to the second course of rIL-2, so it is difficult to determine whether LAK influenced ascites accumulation.

Lurie et al. (2) reported a lack of weight gain or ascites accumulation in a trial of i.p. rIL-2 using a daily times five schedule every 2 weeks in which total doses ranged from 4.0 × 10⁴ to 15.6 × 10⁹ units/patient. In contrast, using three days a day dosing and a higher individual dose level Lotze et al. (1) reported that all patients gained weight. It is also worth noting...
in only one patient, we could show that the peripheral MNC from patients receiving i.p. rIL-2 were "primed" for lytic killing and only needed additional exposure to rIL-2 (100 units/ml) during the short assay period to effect lysis. Furthermore, this increase in primed peripheral MNC coincided with an increase in the percentage of CD3-negative, CD56-positive MNC during the treatment course.

This study confirms other clinical reports (5, 7, 30) that following i.p. IL-2 there are large numbers of MNC in the peritoneal cavity which are activated to kill tumor in vitro. Our findings show that this LAK activity corresponded to increases in the percentage of CD3-negative, CD56-positive population of large granular lymphocytes rather than CD3-positive T-cells.

We found that rIL-2 i.p. every 48 h induced peritoneal LAK activity, and this activity persisted for several days after the last i.p. rIL-2 infusion. Although LAK are reportedly dependent on continued exposure to IL-2 for effective killing function (35, 36), these endogenously activated cells retained lytic activity even after being incubated in culture media for 48 h without IL-2. Our findings also indicate that i.p. rIL-2 does not need to be given by continuous infusion (6) or even every 8 h (1, 30) to maintain adequate IL-2 levels to support localized LAK activity. Based on our observations, we suggest that 2 or 3 i.p. rIL-2 infusions/week could maintain cellular lytic activity and perhaps avoid severe toxicities such as ascites, abdominal pain, and intraperitoneal fibrosis.

Another approach to i.p. immunotherapy has been to prime with low doses of rIL-2 i.p., expand the intracavitary lymphocytes ex vivo with IL-2, and then reinfuse cells i.p. (2). However, using this approach, total doses of ex vivo activated intracavitary lymphocytes given ranged from only 2 to 20 $\times$ 10^6. It may be that judicious scheduling of i.p. IL-2 can induce endogenously large numbers of LAK and obviate the need for the technically more demanding ex vivo activation of peritoneal cells.

Previous reports of therapy with i.p. or i.v. IL-2/LAK (30, 33) have shown that neither in vitro LAK activity nor numbers of LAK infused were predictive of clinical efficacy or toxicity. We observed one partial response in a patient receiving the highest dose of i.p. rIL-2, but we could not discern a correlation between clinical or laboratory findings (such as LAK activity, MNC numbers and phenotypes) and tumor response. Encouraging clinical responses have been reported with very low doses of rIL-2 (6) but a laboratory correlation to response has not been demonstrated.

We had anticipated that for a given patient and dose level repeated infusions would result in increasing ascites cellularity and because of "vascular leak" increasing serum levels of IL-2. Ununexpectedly, three of four patients at the highest dose level (patients 7, 8, and 9) experienced the accumulation of large volumes of essentially cell-free ascites after receiving several infusions of i.p. rIL-2. This phenomenon was observed prior to the infusion of LAK. This reduction of peripheral IL-2 levels over time is in contrast to that seen by others with three times a day dosing, where during a second cycle of i.p. rIL-2 peripheral levels were actually higher than during the first cycle despite the same treatment dose (30). The therapy-induced ascites we observed was associated with low levels (AUC $<$ 10, peak $<$ 1 unit/ml) or an absence of detectable IL-2 in the serum and high i.p. IL-2 levels (2000$\rightarrow$25,000 units/ml 48 h post-IL-2 infusion). It is possible but not likely that this extreme hypocellularity is due to simple dilution or sampling error from peritoneal pockets of fluid. Another possible explanation for the absence of cells in the fluid is that the activated MNC become adherent to the peritoneal surface. A subpopulation of IL-2-activated MNC (1–4%) are known to become adherent. This enriched population of cells has been shown to be CD3 negative CD56 positive, to have higher proliferative potential, and to be more cytotoxic (37). Another possibility was that ascites fluid was toxic to the cells. To test this, we activated normal lymphocytes with rIL-2 in the presence of this ascites fluid but toxic effects on normal MNC were not detected (data not shown). The hypocellularity of ascites in our patients may be related to the low peripheral IL-2 levels noted, but the mechanism of such an interaction is unclear.

In conclusion, this trial of i.p. rIL-2 with LAK resulted in a dose-limiting toxicity of symptoms related to ascites accumulation. In a subsequent phase II trial using this schedule a dose no greater than 8 $\times$ 10^6 units/m^2 body surface area/dose should be used. Leukapheresis could perhaps be more productive if started a day earlier. Peripheral IL-2 levels generally varied with dose level and i.p. levels early in the treatment period. Later measurements demonstrated decreasing peripheral IL-2 levels despite maintenance of the same i.p. dose. Intraperitoneal IL-2 induced durable regional LAK activity which correlated with an increase in the percentage of MNC as well as the percentage of CD56-positive MNC in the peritoneum. In addition, peripheral blood MNC were primed by i.p. IL-2 for lytic activity which was inducible with brief in vitro exposure to IL-2. This priming correlated with an increase in CD56-positive peripheral MNC and with a rebound lymphocytosis 48–72 h after i.p. rIL-2. A clearer understanding of peritoneal changes after several infusions of i.p. rIL-2 will help improve the design of further clinical trials using i.p. immunotherapy.

ACKNOWLEDGMENTS

We wish to thank Pamela Vacek, Chris Blair, and Greg Goodwin for computer and statistical support, Cheryl Chabot and the Clinical Research Center nurses for the care given the patients, and Cathy Larsson, Franny Matthews, Cassandra Vertone, and Eva Champoux for their assistance in this project.

REFERENCES

8. Einhorn, N., Ling, P., Einhorn, S., and Strander, H. A phase II study on...


Phase I Trial of Intraperitoneal Recombinant Interleukin-2/Lymphokine-activated Killer Cells in Patients with Ovarian Cancer


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/50/19/6302

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.