ABSTRACT

In a Phase I study, recombinant interleukin-2 (IL-2) or autotoxhous
lymphokine-activated killer (LAK) cells were used to treat nine patients
with malignant glioma. One patient received the combination of IL-2 and
LAK cells. LAK cells were generated by culturing IL-2 with peripheral
blood lymphocytes obtained from brain tumor patients. Escalating doses
of LAK cells (10^8-10^9) or recombinant IL-2 (10^6-10^8 units) were admin-
istered by direct injection into the brain tissue surrounding the cavity left
following operative tumor removal. There have been no signs of systemic
or neurotoxicity following treatment. The tumor selective killing of the
LAK cells used for these treatments was demonstrated by their ability to
lyse glioma cells but not normal cells in vitro using a chromium release
microcytotoxicity assay.

INTRODUCTION

The poor prognosis of patients with malignant glioma despite
combined treatment with surgery, radiation, and chemotherapy
(1, 2) has generated interest in immunotherapy as an adjunctive
mode of treatment. In 1982, it was first reported that cancer
patients' lymphocytes could be activated in vitro to a tumoricid-
al state merely by incubation with IL-2 (2) alone for a minimum of
2 days (3). The ability of these LAK to lyse autologous
natural killer resistant fresh uncultured melanoma, sarcoma, and
adenocarcinoma was profound.

We have demonstrated recently (4) that brain tumor patient
lymphocytes cultured with recombinant interleukin-2 consis-
tently lysed fresh and/or cultured autologous malignant glioma
cells in a short term (4-h) chromium release microcytotoxicity
assay. These results prompted initiation of the present clinical
trial. We now report the results of the part of our Phase I NIH
study in which nine patients were treated either with LAK cells
or IL-2. The lack of toxicity in these patients has allowed us to
continue with the potentially therapeutic part of our Phase I
study in which LAK and IL-2 will be administered simultane-
ously. To date, one patient has received this combined therapy,
and the results are included in this report.

MATERIALS AND METHODS

Patient Selection. Criteria for patient entry into the study included
the following: (a) age over 18; (b) histological evidence of malignant
glioma; (c) life expectancy of at least 6 weeks; (d) recovery from any
apparent toxic side effects of all prior chemotherapy or radiotherapy;
(e) Kattrovsky rating over 50; (f) adequate hematological status (WBC
greater than or equal to 4,000/mm^3 and platelet count greater than or
equal to 100,000/mm^3); and (g) tumor of sufficient size as determined
by CAT scan to warrant surgical intervention. Furthermore, patients
who had received radiation or chemotherapy in the preceding 4 weeks
or surgery in the previous 6 months were excluded from the study. All
patients had progressive disease documented by clinical examination
and computed tomography scan, and all gave informed consent.

Prior to the initiation of our Phase I trial, a preliminary study was
performed (4) in which we tested the comparative ability of brain tumor
patient PBL cultured with IL-2 to lyse autologous fresh glioma in a
chromium release microcytotoxicity assay. Representative results from
this study are presented in this report. These in vitro studies were
carried out in the same manner, as described below, as was the testing of
the LAK cells used for injections of patients treated as part of the
Phase I trial.

Trial Design. Escalating doses of recombinant interleukin-2 or au-
tologous LAK cells were injected intracerebrally at the time of crani-
otomy for tumor removal. Four patients received IL-2 (10^6-10^8 units
total), and five patients were administered LAK cells (10^6-10^8 cells).
Patient profiles including the dose and type of therapy administered
are shown in Table 1.

Interleukin-2 and Generation of LAK Cells. IL-2 was generously
provided by Cetus Corporation (Emeryville, CA). Purification and
titration of this IL-2 has been described previously (5). This IL-2 had
specific activities of approximately 10^6 units per ml, and IL-2 from Lot
LP 232 and Lot LP 260 were used for these studies. LAK cells used
for injection were generated from PBL of the glioma patients as follows.
Two to 3 days prior to surgery for tumor removal, blood was withdrawn
from a peripheral vein. PBL were then separated from other leukocytes
by ficoll-Hypaque gradient centrifugation according to methods de-
scribed previously (6). PBL were washed three times with HBSS and
then resuspended in complete medium (RPMI 1640 containing 5%
human AB serum (heat inactivated) (Advanced Biotechnologies, Inc.,
Silver Spring, MD), glutamine (300 mg/ml), penicillin (100 mg/ml),
and streptomycin (100 mg/ml) (Biofluids, Inc., Rockville, MD)). The
PBL were activated to generate LAK by in vitro incubation for 2 days
in media containing 100 Cetus units of IL-2 per ml. PBL from patients
C, D, G, and H were cultured in 75-cm^2 flasks in 50 cm^3 of complete
medium at a concentration of 1 x 10^6 cells/ml. Due to the large number
of cells involved, PBL from patient I were cultured in 175 cm^2 flasks
in 175 ml of complete medium at a concentration of 2.5 x 10^6 cells/
ml. The flasks were incubated upright in 5% CO_2 at 37°C.

Three patients (patients H, I, and J) were treated with more than 1
x 10^6 LAK cells. In order to obtain this large number of PBL, these
patients underwent lymphocytopheresis at the NIH Apheresis Unit
utilizing the IBM 2997 continuous flow separator (7). After the 2-day
incubation period, the cells were washed three times in HBSS and
resuspended in a final volume of 5 cm^3 of HBSS for intracerebral
injection. An aliquot of these LAK cells was cryopreserved and subse-
quently thawed for use as effector cells in the microcytotoxicity assay
as described below. In addition, PBL that were not cultured with IL-2
were also cryopreserved and used as effector cells.

Injection of IL-2 or LAK Cells. IL-2 or LAK cells were injected
directly into the brain tissue surrounding the cavity left following
operative tumor removal. IL-2 or LAK were suspended in 5 cm^3 of
HBSS and injected through a 20-gauge blunt brain cannula. LAK and
IL-2 were administered via multiple intracerebral injections (5-8, de-
pending on the size of the tumor), distributed evenly around the
resection cavity. LAK or IL-2 were injected at a rate of approximately
1 cm^3/min. Prior to injection, an aliquot of the LAK cell suspension
was sent for gram stain analysis and culture, and another aliquot was
cryopreserved for later use in immunological testing.

Immunological Testing of LAK Cells. The results from three separate
in vitro studies are reported. The first involved a preliminary study (4)
which led to the initiation of the present Phase I trial. Specifically, we
tested the comparative ability of brain tumor patient PBL cultured with
IL-2 to lyse autologous fresh glioma. In the second, the ability of the
LAK cells administered to patients in the Phase I trial to lyse glioma
and computed tomography scan, and all gave informed consent.

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1 To whom requests for reprints should be addressed.

2 The abbreviations used are: IL-2, interleukin 2; LAK, lymphokine-activated
killer cells; CAT, computed axial tomography; PBL, peripheral blood lympho-
cytes; HBSS, Hanks' balanced salt solution; CBC, complete blood count.

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was tested. The results of these two studies are reported in Table 2. In the third study, the effect of various culture conditions on the generation of LAK from patients involved in the Phase I trial was examined, with the results given in Table 3.

At the time of in vitro testing, the LAK cells and PBL which had been previously cryopreserved were thawed and used as effector cells. Target cells included autologous fresh glioma (if available), autologous glioma grown in tissue culture (if available), allogeneic tissue culture glioma, allogeneic PBL, and Daudi lymphoma cells. Daudi is a natural killer cell resistant tumor cell line grown in tissue culture in complete media. The malignant and glial properties of tissue culture tumor lines established from fresh brain tumor specimens in our laboratory have been confirmed by ultrastructural (8, 9), biochemical (10), and biophysical (11) techniques. The tissue culture lines were maintained (4, 12) and the chromium release microcytotoxicity assay was performed (3) as described previously. Single cell suspensions of fresh glioma were prepared according to the modification of Shapiro’s (13) methods as reported previously (4). Briefly, tumor tissue was minced into 1-mm³ pieces and then passed through a 20-gauge needle multiple times. Single cells were separated from debris by Ficoll-Hypaque gradient centrifugation.

The percentage of specific lysis in the chromium release assay was calculated by the formula:

\[
\text{% killing} = \frac{\text{Experimental cpm} - \text{spontaneous cpm}}{\text{Maximal cpm} - \text{spontaneous cpm}} \times 100
\]

All determinations were made in triplicate, and data are reported as the mean ± SE.

**Pretreatment and Followup Investigations.** Prior to treatment, all patients underwent history and physical examination, Karnovsky performance status, CAT scan with contrast, CBC with differential WBC count, and a biochemical profile, including electrolytes, serum creatinine, calcium, magnesium, uric acid, chest radiograph, and electrocar-

### Table 2 Killing of glioblastoma by brain tumor patient lymphocytes cultured without IL-2 (PBL) and with IL-2 (LAK)

<table>
<thead>
<tr>
<th>Patient study group</th>
<th>Effector cells</th>
<th>Autologous fresh glioma</th>
<th>Autologous tissue culture glioma</th>
<th>Allogeneic tissue culture glioma</th>
<th>Autologous PBL</th>
<th>Allogeneic PBL</th>
</tr>
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<tbody>
<tr>
<td>Preliminary study</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>PBL</td>
<td>6</td>
<td>6</td>
<td>NT*</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>2</td>
<td>PBL</td>
<td>0</td>
<td>-8</td>
<td>-1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>PBL</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>PBL</td>
<td>-6</td>
<td>1</td>
<td>-5</td>
<td>-2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>PBL</td>
<td>36</td>
<td>36</td>
<td>NT*</td>
<td>NT</td>
<td>NT</td>
</tr>
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<td>Phase I study</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>PBL</td>
<td>3</td>
<td>6</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>PBL</td>
<td>2</td>
<td>8</td>
<td>-3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>PBL</td>
<td>46</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>PBL</td>
<td>6</td>
<td>12</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>PBL</td>
<td>38</td>
<td>38</td>
<td>NT*</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>J</td>
<td>PBL</td>
<td>9</td>
<td>10</td>
<td>NT*</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

* Effector:target = 80:1.
* The “Preliminary Study” group represents a separate group of brain tumor patients whose lymphocytes were studied prior to the initiation of our Phase I study.
* NT, not tested due to inaccessibility of tissue.
IL-2 AND LAK IN THE TREATMENT OF GLIOMA

Table 3 Comparative lysis of tumor targets by patient I lymphocytes cultured with IL-2 for varying time periods and at different cell concentrations

<table>
<thead>
<tr>
<th>Culture contents</th>
<th>% killing of target cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responder cell</td>
<td>No. of days of culture</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal volunteer PBL</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
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<tr>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Patient I PBL</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
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<tr>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
</tr>
</tbody>
</table>

* NT, not tested.

RESULTS

None of the 10 patients treated with IL-2 or LAK exhibited signs or symptoms of toxicity. One patient, patient F, died 3 months post surgery and injection of 10^6 units IL-2. The cause of death was progressive tumor growth, as demonstrated by CAT scan. There was no evidence of skin reactions associated with allergic responses, and the CBC and electrolyte patterns remained normal pre- and post-treatment. CAT scans with contrast obtained on patients 7 to 10 days post-treatment were not different from scans obtained following uncomplicated craniotomy for tumor removal, suggesting that intracerebral injection of IL-2 or LAK cells is not associated with radiographic changes.

Compared to pretreatment status, the neurological status of 8 of 9 patients has either remained the same or improved slightly. Our first patient (patient A) developed a left sided hemisensory neglect which was evident on the first post-operative day. CAT scan at that time demonstrated that this focal deficit was secondary to a stroke involving the right posterior thalamus. This stroke was most likely due to the surgical sacrifice of a small feeding arteriole in the course of removing a deep temporal lobe lesion. At 6 months followup, this patient's deficit had not significantly improved. Patient D became moderately lethargic the first post-operative day, and a CAT scan at that time demonstrated pneumocephalus. The pneumocephaly resolved and the patient became fully alert 24 h following placement of 100% O2.

In our preliminary study, patient lymphocytes cultured without IL-2 did not lyse autologous or allogeneic glioma cells (Table 2). However, when these cells were cultured with IL-2, LAK cells were generated which produced significant lysis of autologous fresh glioma, as well as both autologous and allogeneic tissue culture glioma. LAK cells did not lyse autologous normal PBL. When the lymphocytes of patients involved in our Phase I study were tested, the generation of LAK cells which significantly lysed allogeneic tissue culture glioma was seen in 4 of 6 cases (Table 2).

The inability to detect significant LAK cell killing by lymphocytes from patients G or I (Table 2) prompted subsequent retesting. Two weeks following craniotomy and injection of lymphocytes, blood was redrawn from patient I (we were unable to obtain blood from patient G), and the PBL were cultured with 100 Cetus units of IL-2 per ml in 25-cm² flasks in 10 cm³ of complete media. PBL obtained from normal volunteers were similarly treated. As can be seen (Table 3), when PBL from normal volunteers or patient I were cultured with IL-2, LAK cells were generated which produced marked and comparable degrees of lysis of both Daudi tumor and glioma. LAK cells could be produced following culture of PBL with IL-2 for 2, 3, or 4 days at concentrations of either 1 or 2.5 × 10^6 cells/ml. Normal volunteers' PBL cultured without IL-2 did not lyse Daudi or glioma. The glioma cells used in these experiments were obtained from the same tissue culture line as used in the experiments described in Table 2. Furthermore, at the time of retesting, patient I was receiving steroid therapy at the same dosage as when initially tested.

DISCUSSION

In a previous report we demonstrated that LAK cells generated by culturing brain tumor patient PBL with IL-2 produced marked selective lysis of autologous glioma cells (4). The present Phase I trial demonstrates that IL-2 or autologous LAK cells can be safely administered intracerebrally to patients with malignant glioma. This represents the first report of the use of IL-2 or LAK cells in the treatment of human brain tumors.

Mazumder and Rosenberg (14) and Mule et al. (15) have demonstrated that in experimental mouse models systemic administration of recombinant IL-2 and lymphocytes activated by IL-2 produced regression of established pulmonary metastases. Subsequently, clinical studies utilizing IL-2 alone in patients with solid tumors were initiated by Lotze et al. (16). A dose limiting toxicity was seen following i.v. bolus (10^6 units/kg) or i.v. continuous (300,000 units/h) infusion. Toxic side effects included transient fever and chills, anorexia, malaise, and mild gastrointestinal symptoms. Minimal renal and hepatic toxicity was demonstrated, while hematologic toxicity was limited to mild anemia, thrombocytopenia, and in one case, reversible eosinophilia. Pronounced weight gain occurred and was thought to be secondary to extravascular fluid accumulation. In our study, IL-2 administration was not associated with any toxic side effects. This is almost certainly related to the fact...
that equivalent doses of IL-2 were given by direct intracerebral injection rather than i.v. Probably intracerebral injection of IL-2 does not result in serum levels sufficient to produce systemic toxicity. Interestingly, the systemic infusion of α-interferon has been reported to cause toxic central nervous system effects (17).

Glioblastoma cells have been shown to release a factor inhibiting IL-2 mediated induction of cytotoxic T-cells (18), while our own results have demonstrated that LAK activation is depressed in the presence of glioma cells in vitro (19). Hence, our method of activating LAK in vitro in the absence of glioma followed by administration locally to the brain tumor site appears advantageous. In the patient clinical trial, up to 10^10 LAK cells have been administered intracerebrally without signs of toxicity. To date, there is one other published report (20) of the use of LAK cells activated with IL-2 in the treatment of human cancer. Rosenberg reported that in a Phase I study four patients with tumor involving the lung and liver tolerated i.v. infusion of large numbers of LAK cells. Specific data regarding toxicity was not given.

Young et al. (21) have treated 17 patients with glioblastoma by intratumoral injection of 10^7-10^9 autologous fresh, nonactivated leukocytes. Their finding that 8 of 17 patients sustained clinical improvement and were alive 17 months post-treatment may suggest that an even more marked effect may be attainable following administration of activated tumoricidal lymphocytes. The intracerebral route used in our study and by Young et al. (21) would be most likely to maximize cell-cell contact between killer lymphocytes and residual glioma cells. Furthermore, if injections are made into brain tissue grossly uninvolved with tumor, a relatively uncompromised blood brain barrier may keep LAK cells localized to the injection site. To confirm that LAK cells remain in the local area, we have utilized autoradiography to study the fate of indium-111 labeled LAK cells injected directly into rat brains. We found that at 48 h following injection, 95% of LAK cells remained localized to the injection site.3

In our study, there were two cases (patient G and I) in which we were unable to detect significant killing of tumor in vitro (Table 2). Results from experiments retesting the ability to generate LAK from patient I PBL suggest that this was not related to the concentrations at which PBL were cultured with IL-2 but rather was most likely to be due to complications secondary to handling large volumes of cells. In the future, we hope to eliminate the negative effect of the large culture volumes required when large numbers of PBL are being processed by (a) prewarming the final tissue culture medium to 37°C immediately prior to cell addition, (b) culturing PBL with IL-2 for 3 rather than 2 days, and then (c) demonstrating cytotoxicity to tumor in vitro prior to injection.

In conclusion, results from this Phase I study demonstrate that glioma patients tolerated intracerebral infusion of IL-2 or LAK cells without any evidence of toxic side effects. Animal studies have indicated that the combination of LAK plus IL-2 is often more therapeutic than is LAK alone. Hence, our next series of patients will be treated in the same manner as the tenth patient in this report, i.e., with LAK cells and IL-2 delivered simultaneously.

ACKNOWLEDGMENTS

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3 S. K. Jacobs, D. J. Wilson, P. L. Kornblith, and E. A. Grimm, unpublished data.
Interleukin-2 or Autologous Lymphokine-activated Killer Cell Treatment of Malignant Glioma: Phase I Trial

Steven K. Jacobs, Debra J. Wilson, Paul L. Kornblith, et al.


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