In vitro cytolysis of primitive neuroectodermal tumors of the posterior fossa (medulloblastoma) by lymphokine-activated killer cells

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Short-term stimulation of nonantigen-primed peripheral blood mononuclear leukocytes with interleukin-2 generates a population of oncolytic effectors designated "lymphokine-activated killer" (LAK) cells. These LAK cells express potent lytic activity against a wide spectrum of fresh or cultured autochthonous (patient's own) and allogeneic (unrelated) tumors, yet specifically spare normal tissues. In this study, cells derived from primitive neuroectodermal tumors of the posterior fossa (PNET-PF) were examined for their sensitivity to LAK cytolyis utilizing an in vitro 4-hour chromium-51-release assay. Five early-passage cell lines, derived from primary PNET-PF, demonstrated significant sensitivity to LAK cell cytolysis. Lysis was equally effective in culture medium and cerebrospinal fluid. Three freshly excised PNET-PF exhibited similar susceptibility to lysis by autochthonous LAK cells. Greatly increased expansion of LAK cell cultures could be achieved by short-term stimulation with monoclonal anti-CD3 antibodies in addition to interleukin-2 activation. These findings constitute the preliminary in vitro foundations for potential intrathecal adoptive immunotherapy of PNET-PF with LAK cells.

KEY WORDS - primitive neuroectodermal tumor - medulloblastoma - interleukin-2 - lymphokine-activated killer cells - immunotherapy - brain neoplasm

Primary malignant brain tumors are among the most difficult tumors to manage. Despite aggressive surgical resection and radiation therapy, the outcome for patients with these tumors remains quite poor. The 5-year survival rate for patients with primitive neuroectodermal tumors of the posterior fossa (PNET-PF) — one of the most common central nervous system (CNS) malignancies in children — is 40% to 60% when treated with surgery and radiation therapy. Irradiation is associated with significant morbidity in young children, including marked impairment of intellectual capacity in long-term survivors. For this reason, radiation therapy has been abandoned by many workers in children under 2 years of age. Despite extensive trials with various chemotherapeutic agents, alone or in combination, the beneficial effects of this modality have proved marginal. The need for alternative therapies that are more efficacious and less detrimental is obvious. Recent advances in molecular biology and cellular immunology have generated renewed interest in the activation of lymphocytes capable of mediating tumor destruction. Lymphokine-activated killer (LAK) cells, generated by incubating peripheral blood mononuclear leukocytes (PB M) in the presence of interleukin-2 (IL-2), express potent cytotoxicity against a variety of tumors. Lymphokine-activated killer cells are efficacious in the treatment of several different tumor types in animal models, and recent reports on human trials have shown clinical responses in certain tumors treated with LAK cells plus IL-2. Although many questions remain concerning LAK cells, further clinical investigation of this agent in adoptive immunotherapeutic protocols appears justified.

The current study was undertaken to determine the potential sensitivity to LAK cells of tumor cells derived from PNET-PF. Cultured and fresh PNET-PF-derived tumor cells were tested for their sensitivity to both the patient's own (autochthonous) and unrelated (allogeneic) IL-2-activated PBM (that is, LAK cells). The expression of LAK cell activity in an environment similar to the CNS was also studied by performing
Definitions and Abbreviations

AIM-V = defined serum-free lymphocyte medium
anti-CD3 = monoclonal anti-CD3 (OKT3) antibody
CM = complete human feeding medium
$^{51}$Cr = sodium chromate-51
HBSS = Hanks’ balanced salt solution
IL-1 = human recombinant interleukin-1
IL-2 = human recombinant interleukin-2
LAK = lymphokine-activated killer
LU = lytic units
PBM = peripheral blood mononuclear leukocytes
PNET-PF = primitive neuroectodermal tumors, posterior fossa
%SL = percentage specific lysis

Parallel cytotoxicity assays in cerebrospinal fluid (CSF). Finally, the problem of obtaining sufficient numbers of PBM to generate LAK cells in younger patients harboring PNET-PF was addressed. An alternative method for the more rapid expansion of LAK effectors utilizing a monoclonal antibody (OKT3) to the T-cell receptor-associated CD3 epitope was evaluated.

The results presented in this report support an adoptive immunotherapeutic approach, consisting of intrathecal administration of IL-2 and LAK cells as an adjuvant to the treatment of PNET-PF.

Materials and Methods

Source of Tumor Cells

A summary of the pertinent patient information is presented in Table 1. Ten patients were studied, all of whom underwent suboccipital craniectomy for debulking of their tumors. Sterile samples were obtained after adequate tissue was allocated for pathological examination.

Lymphocyte Culture Medium

Complete human feeding medium (CM) or a defined serum-free lymphocyte medium (AIM-V)* was used for the culture of human lymphocytes. The CM was prepared from RPMI 1640, 5% heat-inactivated human AB serum,† 300 μg/ml glutamine, 100 U/ml penicillin, 50 μg/ml gentamicin, and 10 mM HEPES buffer. To the AIM-V, which is specifically defined for ex vivo activation of lymphocytes, 20 mM HEPES buffer was added.

Cerebrospinal Fluid

The CSF used in the study was obtained from an external ventriculostomy placed in a patient for control of intracranial pressure. The CSF was separated into aliquots and stored at 4°C.

Interleukin-2

Human recombinant IL-2‡ was separated into aliquots of 10⁶ Cetus units/ml and stored at 4°C until used. The gene for IL-2 was isolated from a high-producer human cell line, Jurkat. Interleukin-2 is expressed at high levels in recombinant Escherichia coli from which it is purified to apparent homogeneity.§ The endotoxin level in the purified IL-2 was less than 0.1 ng/10⁶ units, as measured in the standard Limulus assay.

Generation of LAK Cells

The PBM were obtained from healthy allogeneic donors or from the tumor-bearing patients listed in Table 1. Heparinized peripheral blood was diluted 1:1 with Hanks’ balanced salt solution (HBSS) and centrifuged over a lymphocyte separation medium‖ at 450 G for 20 minutes. The cells collected at the gradient interface, designated PBM, were washed three times in HBSS and finally resuspended at the appropriate concentration in culture medium. The PBM were either used fresh or were cryopreserved at 10⁷ cells/ml in 90% human AB serum and 10% dimethyl sulfoxide. Cryopreservation was performed by first cooling vials containing the tumor cells in racks filled with 100% ethanol, transferring the racks to −70°C for 4 hours, and then moving the vials to vapor-phase liquid nitrogen freezers. Lymphokine-activated killer cells were generated by preparing cultures at 10⁶ PBM/ml of culture medium with IL-2 added to a final concentration of 1000 U/ml. The incubations were carried out at 37°C in humid 5% CO₂ for the times indicated.

Anti-CD3/IL-2-stimulated PBM were generated by adding 10 ng/ml of anti-CD3 (OKT3),‖ an immunoglobulin G₂, monoclonal antibody directed against the T-cell receptor-associated CD3 complex, directly to IL-2 containing cultures of PBM. The PBM were incubated in the presence of anti-CD3 for 2 days, at which time the cultures were washed three times in HBSS and resuspended in IL-2 containing CM as indicated above.

Tumor Target Cells

Sterile tumor samples were transported from surgery on saline-moistened sponges. Necrotic tissue was removed and the tumor was minced into 1-sq mm pieces in culture medium. Tumor targets designated CW, EE, NQ, RB, and SN were cultured on 100-sq cm Petri dishes in Dulbecco’s modified essential medium/Ham’s

* AIM-V provided by GIBCO Laboratories, Grand Island, New York.
† AB serum provided by Hazelton Corp., Denver, Pennsylvania.
‡ Human recombinant IL-2 provided by the Cetus Corp., Emeryville, California.
§ Lymphocyte separation medium provided by Litton Biometrics, Kensington, Maryland.
‖ OKT3 provided by Ortho Diagnostics Inc., Raritan, New Jersey.
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F-12 with 10% fetal calf serum in humid 5% CO₂ at 37°C. Culture medium was changed biweekly. Cell lines were passaged at confluence with 0.25% trypsin in phosphate-buffered saline with 0.02% ethylenediaminetetra-acetic acid without calcium or magnesium as the cells reached a confluent monolayer. Cultured targets were used at the passage number indicated in Table 2.

Alternatively, fragments of tumor were dissociated using a modification of the overnight enzymatic digestion technique described by Rayner, et al. In brief, fragments were incubated with constant stirring in RPMI 1640 without serum containing 2 mg/ml collagenase (Type IV), 5 U/ml hyaluronidase (Type V), and 0.2 mg/ml deoxyribonuclease (Type I) for 2 hours at room temperature.* Dissociated tumor cells were passed through a 100-gauge nylon mesh and then washed twice in HBSS. Viable tumor cells were isolated from debris by centrifugation over lymphocyte separation medium at 450 G for 20 minutes. Tumor cells were collected from the interface, washed twice with HBSS, and resuspended in CM. The cells were used immediately or were cryopreserved, as described above.

**Cytotoxicity Assay**

Tumor cytolysis by LAK cells was determined in a standard 4-hour chromium-51 (⁵¹Cr)-release assay. Cultured tumor cells were harvested from Petri dishes with 0.25% trypsin, or alternatively, cryopreserved fresh tumor cells were rapidly thawed and washed twice more in 10 times the volume of medium. Cultured cells were labeled by exposure to 250 µCi ⁵¹Cr in 1 ml of medium at 37°C for 1 hour and fresh tumor cells were labeled with 400 µCi ⁵¹Cr in 0.5 ml of medium at 37°C for 2 hours. Cells were washed in medium twice and incubated for an additional 30 minutes prior to three final washes. Then, 0.1 ml of medium containing 2.5 × 10⁷ tumor cells was added to round-bottom microtiter plates in triplicate or quadruplicate patterns. Effector cells were added to the wells at various effector:target ratios determined with the following formula:

\[
\text{Percentage specific lysis (\%SL)} = \frac{\text{measured cpm} - \text{spontaneous cpm}}{\text{total cpm} - \text{spontaneous cpm}} \times 100.
\]

Spontaneous and maximal releases were determined from the mean results in six wells, and all experimental determinations were made in triplicate or quadruplicate and are reported as %SL ± standard error of the mean. The data presented in the tables have been rounded off to one significant digit.

Lytic units (LU) were calculated from the lysis data in order to describe the cytotoxic potential of the cells within the cultures of PBM. The use of multiple effector:target ratios allows an approximation of the linear rise portion of the sigmoidal killing curve. Based on a line of best fit calculated by linear regression for the linear rise portion of the data, the number of killer cells required to effect a 30% specific lysis of the target population (%SL = 30) was determined. Lytic units are calculated by the product of 10⁶ and the inverse of the number of PBM required to yield SL of 30% (that is, 10⁶/number of PBM required for 30% SL). The total lytic potential of a given culture is expressed as the product of the calculated LU and the total cell count of the culture divided by 10⁶ (that is, [LU × total cell number]/10⁶).

**Results**

It was found that early-passage cell lines derived from PNET-PF were efficiently killed by LAK cells and were resistant to lysis by unstimulated PBM. To obtain LAK cells, PBM derived from normal healthy donors were cultured in CM for 7 days with IL-2, as described above. Cultured PBM (LAK cells) were then tested for their oncolytic potential against early-passage cell lines derived from the primary tumors described in Table 1. Fresh unstimulated PBM served as control cultures. The IL-2-stimulated (LAK cells) cultures exhibited potent cytotoxicity against all of the tumor lines tested, regardless of the passage number of the particular cell line (Table 2). In contrast, these same cell lines were unable to lyse the early-passage cell lines derived from PNET-PF, even when tested against 30% specific lysis. Determined with the following formula:

\[
\text{Percentage specific lysis (\%SL)} = \frac{\text{measured cpm} - \text{spontaneous cpm}}{\text{total cpm} - \text{spontaneous cpm}} \times 100.
\]
considerably more resistant to killing by the fresh PBM. Cell line NQ derived from Case 3 was the only target that exhibited significant susceptibility to killing by fresh PBM. However, the specific lysis of NQ mediated by LAK cells was much more potent than PBM. These data indicate that LAK cells are capable of efficiently killing cultured allogeneic PNET-PF at low effector:target ratios in short-term \(^{51}\)Cr-release cytotoxicity assays.

These studies showed that LAK cells effectively kill early-passage PNET-PF targets suspended in CSF. The ability of IL-2-stimulated PBM to express functional LAK cell activity in an environment similar to the CNS was tested by performing \(^{51}\)Cr-release killing assays with the effector and target cell mixtures suspended in CSF. No significant differences were noted when killing assays were performed in CSF versus tissue culture medium. The data in Table 3 indicate that CSF does not inhibit the recognition and killing of PNET-PF-derived cell lines by LAK cells.

It was also shown that LAK cells, but not unstimulated PBM, expressed significant cytotoxicity against fresh autochthonous tumor. The ability of lymphocytes derived from tumor-bearing patients to develop LAK cell function against the patient’s own (autochthonous) tumor was tested. Single-cell suspensions of autochthonous tumor targets were cryopreserved immediately after enzymatic disaggregation. Thawed autochthonous target cells were chromium-labeled immediately before the killing assays were performed. All patient-derived PBM, cultured for 7 days in AIM-V with 1000 U IL-2/ml, exhibited significant lysis against their autochthonous fresh tumor (Table 4). In contrast, autochthonous tumor targets exhibited considerable resistance to killing by unstimulated PBM control cultures. The observed killing (%SL) of fresh tumor by LAK cells and especially by unstimulated PBM, was less than for cultured targets.

Finally, the studies demonstrated that short-term exposure of IL-2-stimulated PBM cultures to the monoclonal anti-CD3 antibody greatly increased the yield of LAK cells. Given the patient population afflicted with PNET-PF, it may prove difficult to acquire the large numbers of PBM required for the conventional short-term in vitro generation of LAK cells currently being cultured.

### Table 2

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Passage</th>
<th>PBM 1:1</th>
<th>PBM 10:1</th>
<th>PBM 100:1</th>
<th>LAK Cells 1:1</th>
<th>LAK Cells 10:1</th>
<th>LAK Cells 100:1</th>
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<tr>
<td>CW</td>
<td>8</td>
<td>34 ± 2</td>
<td>68 ± 1</td>
<td>70 ± 4</td>
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<td>70 ± 4</td>
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<td>CW</td>
<td>5</td>
<td>25 ± 2</td>
<td>65 ± 2</td>
<td>81 ± 1</td>
<td>25 ± 2</td>
<td>65 ± 2</td>
<td>81 ± 1</td>
</tr>
<tr>
<td>CW</td>
<td>5</td>
<td>42 ± 1</td>
<td>81 ± 2</td>
<td>82 ± 2</td>
<td>42 ± 1</td>
<td>81 ± 2</td>
<td>82 ± 2</td>
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<td>2</td>
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<td>21 ± 1</td>
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<td>59 ± 2</td>
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<td>81 ± 2</td>
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<tr>
<td>RB</td>
<td>5</td>
<td>5 ± 6</td>
<td>6 ± 1</td>
<td>15 ± 3</td>
<td>11 ± 4</td>
<td>53 ± 3</td>
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<td>RB</td>
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<td>41 ± 3</td>
<td>57 ± 6</td>
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<td>SN</td>
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<td>11 ± 2</td>
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<td>41 ± 3</td>
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<td>SN</td>
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<td>6 ± 1</td>
<td>31 ± 3</td>
<td>54 ± 2</td>
<td>6 ± 1</td>
<td>31 ± 3</td>
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</tbody>
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* PNET-PF = primitive neuroectodermal tumors of the posterior fossa; LAK cells = lymphokine-activated killer cells; PBM = peripheral blood mononuclear leukocytes. The PBM obtained from normal healthy donors either were used fresh (PBM) or were cultured for 7 days with 1000 U interleukin-2/ml complete human feeding medium (that is, LAK cells) as described in the Materials and Methods section. Cultures were harvested and assayed for oncolytic activity against the indicated cultured PNET-PF targets. Tumor destruction is presented as mean percent specific lysis in four wells ± standard error of the mean. Effector:target ratios are indicated above the appropriate data columns.

† Denotes no significant lysis.

### Table 3

<table>
<thead>
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<th>Cell Line</th>
<th>Passage</th>
<th>Culture Medium</th>
<th>Tumor Destruction</th>
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<tr>
<td></td>
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<td>1:1</td>
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</tr>
<tr>
<td>CW</td>
<td>8</td>
<td>CM</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>EE</td>
<td>2</td>
<td>CM</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>CSF</td>
<td></td>
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<td>13 ± 4</td>
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</table>

* PNET-PF = primitive neuroectodermal tumors of the posterior fossa; CSF = cerebrospinal fluid; LAK = lymphokine-activated killer cells; CM = complete human feeding medium; PBM = peripheral blood mononuclear leukocytes. The PBM, cultured for 7 days in CM containing 1000 U interleukin-2/ml, were assayed for LAK activity against the indicated target. Killing assays were performed with effectors and targets subjected to different ratios in either CM or CSF. Tumor destruction is presented as mean percent specific lysis in four wells ± standard error of the mean.

### Table 4

<table>
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<tr>
<th>Cell Line</th>
<th>IL-2</th>
<th>1:6:1</th>
<th>6:3:1</th>
<th>25:1</th>
<th>100:1</th>
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<tr>
<td>EP</td>
<td>+</td>
<td>3 ± 2</td>
<td>18 ± 1</td>
<td>35 ± 2</td>
<td>41 ± 5</td>
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<tr>
<td>AS</td>
<td>+</td>
<td>12 ± 3</td>
<td>22 ± 7</td>
<td>20 ± 5</td>
<td>30 ± 2</td>
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<tr>
<td>AM</td>
<td>+</td>
<td>5 ± 3</td>
<td>10 ± 2</td>
<td>22 ± 4</td>
<td>37 ± 4</td>
</tr>
</tbody>
</table>

* PNET-PF = primitive neuroectodermal tumors of the posterior fossa; LAK cells = lymphokine-activated killer cells. Peripheral blood mononuclear leukocytes derived from patients with PNET-PF were cultured for 7 days in AIM-V with (+) or without (−) 1000 U interleukin-2/ml. These cultured effectors were then tested for oncolytic potential against fresh autochthonous tumor at the effector:target ratios indicated. Tumor destruction is presented as a mean percent of specific lysis in three wells ± standard error of the mean. 0 = no significant lysis.  R. E. George, et al.  J. Neurosurg. / Volume 69 / September, 1988
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utilized in clinical trials. The data presented in Table 5 show that short-term stimulation with monoclonal anti-CD3 antibody greatly increased the cell number recovered from IL-2 expanded cultures. These anti-CD3/IL-2-stimulated PBM expressed similar or slightly reduced cytolytic activity compared to the population stimulated with IL-2 alone (Table 6). However, an appreciation for the utility of anti-CD3 expansion of IL-2-stimulated cultures is gained by comparing the overall lytic potential of the resultant populations. This oncolytic potential is expressed in Table 6 as LU/culture, which is derived from the product of the lytic potential of the effector cells (that is, the LU) within a given population and the total number of cells within that population (that is, the total cell count). These data indicate that the final oncolytic potential derived from a limited number of starting PBM can be increased four- to sixfold over IL-2 alone in 3-week culture period by simultaneously stimulating IL-2 cultures with anti-CD3.

**Discussion**

Primitive neuroectodermal tumors of the posterior fossa, also known as medulloblastomas, comprise 20% of pediatric brain tumors. These highly malignant tumors frequently metastasize via the CSF pathways to the spinal and cranial subarachnoid spaces. At the time of initial presentation, as many as 42% of patients have spinal metastases. Standard therapy for PNET-PF currently consists of gross total tumor resection followed by craniospinal irradiation. Radiation is associated with a high incidence of intellectual impairment, endocrinological disturbances, and growth retardation in young children, and results in 5-year survival rates of only 40% to 60%. Chemotherapy is the sole form of therapy used in children under 2 years of age because of severe side effects of irradiation in this age group. Chemotherapy, however, has not yet proved of benefit in treating PNET-PF. Because surgery, irradiation, and chemotherapy do not adequately treat PNET-PF, additional treatment modalities need to be explored.

Adoptive immunotherapy, a form of treatment in which activated effector cells are introduced into a patient, is currently being investigated as a possible cancer therapy. Lymphokine-activated killer cells possess several attributes that could make them useful in adoptive immunotherapy: 1) they are highly potent against tumors; 2) they require no prior antigen exposure to express their oncolytic effect (that is, they do not require incubation with tumor cells); 3) their recognition mechanism is able to distinguish between normal and malignant cells and thereby spare normal tissue; and 4) they express oncolytic activity against many tumor types. Tumors appear to be unable to develop resistance to LAK cell killing, and large numbers of LAK cells can be generated ex vivo from tumor-bearing patients and appear to be unaffected by the tumor-induced states of immunosuppression. In addition, LAK cells have demonstrated the ability to kill numerous types of human tumors in vitro, and recent adoptive immunotherapeutic trials using concomitant intravenous infusion of ex vivo-generated LAK cells and IL-2 have proved successful in mediating the regression of some metastatic solid tumors in humans. Intracranial gliomas have shown sensitivity to in vitro LAK cell killing, and a Phase I study examining the toxicity of LAK cell and recombinant human IL-2 infusions into glialoma tumor cavities has demonstrated no major side effects. The current study was undertaken to determine whether PNET-PF are sensitive to LAK cell killing. Five early-passage tumor cell lines obtained from PNET-PF were recognized and lysed by LAK cells when

<table>
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<tr>
<th>Source of PBM</th>
<th>Anti-CD3</th>
<th>Culture Time (wks)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>normal donor</td>
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</tr>
<tr>
<td>tumor cells</td>
<td>+</td>
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</tr>
<tr>
<td>CK</td>
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<td></td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>MS</td>
<td>−</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>2</td>
</tr>
</tbody>
</table>

* PBM = peripheral blood mononuclear leukocytes; IL-2 = interleukin 2. The PBM from the indicated donor cell lines were cultured in AIM-V containing 1000 U IL-2/ml with (+) or without (−) 2 days of monoclonal anti-CD3 antibody stimulation (10 ng/ml). Total viable cell counts were determined in each culture at the indicated times with a hemacytometer using 0.2% trypan blue. Cell counts are presented × 10^6.

<table>
<thead>
<tr>
<th>Source of PBM</th>
<th>Anti-CD3</th>
<th>Lysis Activity (Lytic Units/LU)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1.6:1</td>
</tr>
<tr>
<td>CK</td>
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<tr>
<td></td>
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<td>MS</td>
<td>−</td>
<td>1 ± 3</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0</td>
</tr>
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</table>

* LAK = lymphokine-activated killer cells; IL-2 = interleukin-2; PBM = peripheral blood mononuclear leukocytes; LU/Cult = calculated lytic units × total viable cell recovery/10^6. 0 denotes no significant lysis. The PBM cultures described in Table 5 were assayed for oncolytic activity against fresh autochthonous tumor on Day 21. Tumor destruction is presented as mean percent specific lysis in three wells ± standard error of the mean. Lytic units (LU), indicative of cytotoxic potential on a per cell basis, and total lytic units per culture (LU/Cult), describing the oncolytic potential of the total effector population, were calculated as described in the Materials and Methods section. See text for further explanation.
tested in the 4-hour in vitro $^{51}$Cr-release assay. In a similar fashion, three fresh PNET-PF showed sensitivity to LAK cytolysis, although to a lesser degree than cultured targets. Previous studies have demonstrated that glial cells$^{15}$ and other normal tissues$^{13}$ are not lysed by LAK cells. These results suggest that LAK cells are able to recognize and lyse PNET-PF selectively, while specifically sparing normal tissues.

It is known that PNET-PF are in direct contact with and spread via the CSF pathways. Treatment of these CSF-borne metastases has proved to be difficult. One method of treatment might be to administer LAK cells and IL-2 directly into the ventricular system so that they disperse along the CSF pathways. If PNET-PF retained their sensitivity to LAK cytolysis in CSF, this form of therapy could eradicate residual tumor without the harmful side effects that radiation or chemotherapy produce.

The next series of experiments examined whether PNET-PF retained their sensitivity to LAK cells in CSF, an environment presumably more analogous to in vivo conditions. Two early-passage PNET-PF-derived cell lines were tested for LAK cell sensitivity in 4-hour $^{51}$Cr-release cytotoxicity assays run simultaneously in CSF and in culture medium. Both cell lines were lysed equally well in CSF and medium, indicating that LAK cells expressed no inhibitory effect on the ability of LAK cells to recognize and lyse tumor cells. In previous studies, tumors sensitive to LAK cell killing in vitro have been sensitive to LAK cytolysis in vivo.$^{22}$

In human adoptive immunotherapy trials carried out to date, LAK cells have been generated by leukapheresis of large numbers of lymphocytes ($10^{10}$ PBM) followed by 2- to 4-day incubations in the presence of IL-2.$^{16,28-30}$ Typically, PNET-PF occur in the pediatric age group in which leukapheresis is not easily accomplished. Sufficient numbers of LAK cells for use in immunotherapeutic protocols could be generated for these children by prolonged in vitro expansion of a smaller number of PBM in culture; however, this adds considerable risk of bacterial or fungal contamination and may delay the initiation of therapy. A more effective means for the rapid generation of sufficient numbers of LAK cells for adoptive immunotherapy would be helpful in this patient population.

A recently reported technique for LAK cell expansion involves the short-term simultaneous stimulation of PBM with IL-2 and the monoclonal antibody directed against the T-cell receptor-associated CD3 (OKT3) epitope.$^{23}$ Likewise, this report indicates that anti-CD3-stimulated expansion of patient-derived LAK cells can, over a 3-week culture period, yield a several-fold increase in cell recovery relative to IL-2 activation alone. On a per-cell comparison (that is, LU value), these anti-CD3/IL-2-generated effector cells expressed similar potency relative to LAK cells generated in IL-2 alone. The total lytic potential recovered from a starting population of PBM (that is, LU/culture), however, was increased four to six times. Ochoa, et al.,$^{23}$ reported further enhancement of the lytic potential for these anti-CD3/IL-2-activated killer cells using beta-IL-1, gamma-interferon, or beta-interferon. Preliminary findings in our laboratory show that LAK cell potency can be significantly boosted above that achieved using IL-2 stimulation alone, given appropriate lymphokine/monokine signaling. Although the optimal culture conditions for the generation of the highest yield of the most potent effector population have not been determined, the described anti-CD3/IL-2-activation protocol allows for the rapid generation of sufficient numbers of LAK cells for the initiation of adoptive immunotherapeutic trials. Future work will undoubtedly provide improvements in all aspects of the immunotherapeutic application of LAK cells.

**Conclusions**

Cultured and fresh PNET-PF were sensitive to cytolysis by LAK cells, and patients afflicted with these tumors retained their ability to generate LAK cells. It was also shown in an in vitro system that CSF did not inhibit the LAK cell-mediated lysis of tumor. Additionally, a technique applicable to clinical trials by which the expansion of LAK cells can be greatly increased without detracting from their lytic abilities was evaluated and applied. These findings constitute preliminary in vitro foundations for potential intrathecal adoptive immunotherapy of PNET-PF with LAK cells as an adjuvant to current treatment.

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**References**

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