Local Administration of Autologous Lymphokine-activated Killer Cells and Recombinant Interleukin 2 to Patients with Malignant Brain Tumors

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ABSTRACT

Lymphokine-activated killer cells (LAK cells) were induced from lymphocytes from patients with malignant glioma by using interleukin 2 (IL-2), and their killing activity was examined. Their LAK activity against Daudi cells was 66.2 ± 13.1% and 48.7 ± 12.7% against self glioma cells, 54.4 ± 10.1% against K562 cells, 43.1 ± 7.9% against Raji cells, and 33.5 ± 16.2% against allogeneic glioma cells. The phenotype of these LAK cells was Leu 1 (++), 2a (±), 3a (++), 7 (+), and 11 (++). The phenotype of precursor LAK cells, on the other hand, was Leu 1 (−), 2a (−), 3a (+), 7 (−), and 11 (+++). Other activated killer cells, including LAK cells, phytohemagglutinin-activated killer cells, autoactivated killer cells, and their precursor LAK cells, were studied serologically in order to identify their phenotypic characteristics. From these data, the LAK cell populations were considered to be polyclonal. Using these LAK cells plus IL-2, local adoptive immunotherapy was undertaken in 23 patients with recurrent malignant glioma. We injected, that is, autologous LAK cells plus IL-2 directly into the cavities of the brain tumors; 1.2 to 324 × 10^6^ LAK cells per ml and 0.8 to 5.4 × 10^1^ units of IL-2 were directly injected into the brain tumor by using an Ommaya reservoir. Definite tumor regression, improvement of some clinical symptoms, and continuous remission over 6 mo or more were observed in six, nine, and three patients, respectively. There were no marked side effects, except for slight fever and chill, in eight and three patients, respectively. These results suggested the possibility of induction of a sufficient number of LAK cells from the lymphocytes of the patients with recurrent malignant glioma, indicating that local adoptive immunotherapy by direct injections of LAK cells and IL-2 into the brain tumor will prove to be an effective means of immunotherapy. Additional follow-up of the patients will be required before its therapeutic value can be established.

INTRODUCTION

Passive transfer of immune lymphocytes is capable of mediating the regression of established tumors in a variety of animal tumor models (1). This approach to cancer therapy has several advantages compared with more commonly used methods of immunotherapy and may provide a cancer-bearing host with components needed to mediate effective antitumor responses.

The presence of a soluble substance which selectively accelerates proliferation of T-lymphocytes, on the other hand, has been reported and termed T-cell growth factor (2). In the Second International Workshop of Lymphokine, this substance was called IL-2 (3), and mouse- and rat-derived IL-2 was purified. It later became possible to produce human rIL-2. The biological significance of IL-2 involves an antitumor action as a result of proliferation and activation of various killer cells. In particular, the incubation of human PBL with IL-2 induces a group of killer cells with low tumor specificitiy that are called LAK cells (4). Recently, it was shown that PBL from cancer patients could be also activated in culture with rIL-2, resulting in the development of effector cells cytotoxic for autologous fresh solid tumor cells. This rIL-2 mediated activation of PBL leads to a unique cytotoxic effector system and has occasionally been used clinically for cancer therapy (5).

We induced LAK cells from PBL of the patients with recurrent malignant glioma by incubation with rIL-2, studied their biological properties, and carried out local AIT in which LAK cells and rIL-2 were injected directly into the brain tumor. In these patients the tumors were all recurrent progressively, which was assessed by clinical examination, CT scan following surgical resection, and radiochemotherapy.

MATERIALS AND METHODS

Patients. We undertook local AIT to 23 patients with recurrent malignant glioma, who had received surgical resection and/or radiochemotherapy as initial treatment. All tumors had been histologically diagnosed as anaplastic astrocytoma by the specimens surgically removed. In the patients whose tumors were located in the superficial cerebral hemisphere and cerebellum, a direct approach was performed, and in the patients whose tumors were located in the deep cerebral hemisphere and brain stem, stereotactic biopsy was done. In these surgical treatments, Ommaya reservoirs were put into the tumor cavities. The patient data and extent of disease are shown in Table 1. Their ages ranged between 5 and 62 yr, and their tumor recurrence was seen between 3 and 14 mo after the initial treatments.

Procedures. In 5 patients, Nos. 1, 2, 3, 4, and 8 in Table 1, PBL were separated on a Ficoll-Hypaque gradient (6) from venous blood obtained by venipuncture. In the other patients, PBL were separated by the same way from venous blood obtained by leukapheresis. PBL were resuspended in RPMI 1640 containing 5% heat-inactivated AB serum (North American Biological, Miami, FL).

Purified recombinant IL-2 was provided by Shionogi Chemical Industries, Ltd. PBL was activated to generate LAK cells by in vitro incubation for 4 to 6 days in CM containing 200 units of rIL-2/ml. The PBL were cultured in 25-ml flasks in 10 ml of CM at the concentration of 2 to 5 × 10^6^/ml. Flasks were incubated in 5% CO2 and 95% air at 37°C. After these procedures, LAK activity was measured by 51Cr release assay (Table 2).

Preparation of Target Cells. K562 is the NK-selective myeloid leukemia cell, Daudi cell is the NK-resistant lymphoma line, G-361 is the established melanoma cell, Raji cell is the human lymphoma cell, and NP-1 is the established human glioma cell line. These tumor cells were cultured in CM and used as target cells.

Fresh glioma target cells were produced by preparation of single-cell suspensions from fresh tumor specimens as described by Shapiro et al. (7). The resulting cell suspension was decanted into a tube containing heat-inactivated AB serum, pelleted, and resuspended in CM.

Primary in vitro sensitization was performed by coculture of responder PBL with stimulator incubated vertically in CM in Falcon 3013 flasks (8). All targets were labeled with 50 μCi of Na^24^Cr (American Corp., Arlington Heights, IL) for 90 to 120 min in 1 ml of CM. After washing 4 times, these were added to various numbers of effector lymphocytes in Titiertek microculture plates. The plates were incubated for 4 h at 37°C in 5% CO2. After incubation, supernatants were harvested and counted in a γ-counter. Maximum release was produced by incubation of the targets with 1% Triton-X. Spontaneous release

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1 To whom requests for reprints should be addressed.

2 The abbreviations used are: IL-2, interleukin 2; rIL-2, recombinant interleukin 2; PBL, peripheral blood lymphocytes; LAK, lymphokine-activated killer; AIT, adoptive immunotherapy; CT, computerized tomography; CM, complete medium; NK, natural killer.

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Table 1 Patients' profile treated with AIT using LAK cells and interleukin 2

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Location of glioma</th>
<th>Previous therapy</th>
<th>Period of recurrence after initial treatment (mo)</th>
<th>Neurological status at AIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>F</td>
<td>Pons</td>
<td>Biopsy, 6000 R, ACNU&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4</td>
<td>Remarkable gait disturbance, dysphagia</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>M</td>
<td>Pons</td>
<td>Biopsy, 8500 R, ACNU</td>
<td>6</td>
<td>Vegetative state</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>F</td>
<td>Thalamus</td>
<td>Partial removal, 6000 R, ACNU, PEP</td>
<td>9</td>
<td>Vegetative state</td>
</tr>
<tr>
<td>4</td>
<td>13</td>
<td>F</td>
<td>Frontal</td>
<td>Removal, 6500 R, ACNU, PEP</td>
<td>17</td>
<td>Drowsy state</td>
</tr>
<tr>
<td>5</td>
<td>18</td>
<td>F</td>
<td>Temporal</td>
<td>Removal (3 times), 8000 R, ACNU, PEP</td>
<td>40</td>
<td>Slight dysarthria</td>
</tr>
<tr>
<td>6</td>
<td>34</td>
<td>M</td>
<td>Temporal</td>
<td>Removal, 6000 R, ACNU, PEP</td>
<td>11</td>
<td>Hemiplegia, dysarthria</td>
</tr>
<tr>
<td>7</td>
<td>18</td>
<td>M</td>
<td>Thalamus</td>
<td>Biopsy, 6800 R, ACNU, BLM</td>
<td>3</td>
<td>Headache only</td>
</tr>
<tr>
<td>8</td>
<td>42</td>
<td>F</td>
<td>Thalamus</td>
<td>Removal (2 times), 6200 R, ACNU, BLM</td>
<td>8</td>
<td>Vegetative state</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>M</td>
<td>Pons</td>
<td>Biopsy, 7800 R, ACNU, BLM</td>
<td>7</td>
<td>Akinesis mutism</td>
</tr>
<tr>
<td>10</td>
<td>29</td>
<td>M</td>
<td>Parietal</td>
<td>Removal (3 times), 4200 R, ACNU, PEP</td>
<td>9</td>
<td>Hemiplegia</td>
</tr>
<tr>
<td>11</td>
<td>54</td>
<td>M</td>
<td>Occipital</td>
<td>Removal, 5500 R, ACNU</td>
<td>4</td>
<td>Mental dullness</td>
</tr>
<tr>
<td>12</td>
<td>43</td>
<td>M</td>
<td>Temporal</td>
<td>Removal, 6000 R, BLM, ACNU</td>
<td>8</td>
<td>Dysarthria</td>
</tr>
<tr>
<td>13</td>
<td>34</td>
<td>M</td>
<td>Parietal</td>
<td>Removal, 6000 R, ACNU</td>
<td>8</td>
<td>Dysarthria, appetite loss</td>
</tr>
<tr>
<td>14</td>
<td>30</td>
<td>M</td>
<td>Thalamus</td>
<td>Biopsy, 6000 R, BLM</td>
<td>9</td>
<td>Gait disturbance</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>F</td>
<td>Mildbrain</td>
<td>Biopsy, 4500 R</td>
<td>2</td>
<td>Diploia only</td>
</tr>
<tr>
<td>16</td>
<td>8</td>
<td>M</td>
<td>Pons</td>
<td>Biopsy, 4500 R</td>
<td>3</td>
<td>Gait disturbance</td>
</tr>
<tr>
<td>17</td>
<td>32</td>
<td>F</td>
<td>Frontal</td>
<td>Removal, 6000 R, ACNU</td>
<td>9</td>
<td>Mental retardation</td>
</tr>
<tr>
<td>18</td>
<td>45</td>
<td>M</td>
<td>Temporal</td>
<td>Removal, 5500 R</td>
<td>20</td>
<td>Headache only</td>
</tr>
<tr>
<td>19</td>
<td>62</td>
<td>M</td>
<td>Temporal</td>
<td>Removal (2 times), 6000 R, ACNU</td>
<td>11</td>
<td>Hemiplegia</td>
</tr>
<tr>
<td>20</td>
<td>20</td>
<td>M</td>
<td>Pons</td>
<td>Biopsy, 6000 R, ACNU</td>
<td>6</td>
<td>Vegetative state</td>
</tr>
<tr>
<td>21</td>
<td>48</td>
<td>M</td>
<td>Parietal</td>
<td>Removal, 6000 R, ACNU</td>
<td>11</td>
<td>Hemiplegia</td>
</tr>
<tr>
<td>22</td>
<td>37</td>
<td>M</td>
<td>Parietal</td>
<td>Removal, 6000 R</td>
<td>13</td>
<td>Convulsion</td>
</tr>
<tr>
<td>23</td>
<td>32</td>
<td>F</td>
<td>Parietal</td>
<td>Biopsy, 6000 R, ACNU</td>
<td>12</td>
<td>Hemiplegia</td>
</tr>
</tbody>
</table>

<sup>a</sup> All patients were histologically diagnosed as having anaplastic astrocytoma.

<sup>b</sup> ACNU, 1-(4-amino-2-methyl-5-pyrimidinyl)methyl-3-(2-chloroethyl)-3-nitrosourea hydrochloride; PEP, pepleomycin; BLM, bleomycin.

Table 2 Schedule for the local adoptive immunotherapy to the patients with malignant glioma

1. Separation of peripheral blood lymphocytes
   Ficoll-Conray gradient

2. CM for LAK cells
   RPMI 1640 containing 5% heat-inactivated AB serum

3. Activation of LAK cells
   Incubation for 4 to 5 days in CM containing rIL-2; 2 x 10<sup>4</sup> cells/ml; rIL-2, 200 units/ml

4. Measurement of cytotoxicity
   Target cells: K562; Daudi; Raji; glioma cells; etc.
   E.T<sup>+</sup> ratio: 1:20; 1:50; 1:100
   4-h <sup>51</sup>Cr release assay

5. LAK cell infusion
   Suspended in CM
   Local administration of LAK and rIL-2 via Ommaya's reservoir

was produced by incubation of targets with CM alone. The percentage of lysis was calculated by the formula

\[
\% \text{ of lysis} = \frac{\text{experimental cpm} - \text{spontaneous cpm}}{\text{maximum cpm} - \text{spontaneous cpm}} \times 100\%
\]

Data are reported as the mean ± SE.

Serologic Depletion Studies. PBL were resuspended at 1 x 10<sup>7</sup>/ml of RPMI 1640 at 4°C. The monoclonal antibody was added directly to the cells at a 1:20 dilution. The Leu reagents were purchased from Becton, Dickinson Co., Sunnyvale, CA. Antibody incubation was performed for 1 h at 4°C with occasional mixing. The cells were pelleted, and complement was added in RPMI 1640. Newborn rabbit serum was used as a source of complement. Complement lysis was performed at 37°C for 1 h at a 1:10 dilution. After washing twice with phosphate-buffered saline, PBL were resuspended in CM.

Clinical Trial Design. In the 23 recurrent glioma patients in whom standard initial treatment had failed, about 2 to 17 x 10<sup>7</sup> of autologous LAK cells and 50 to 400 units of rIL-2 were injected directly into the recurrent tumor cavities in one puncture through an Ommaya reservoir. This treatment was repeated 2 to 3 times a wk and continued for 5 to 7 wk. After this concentrated therapy, local AIT was continued once or twice a month in several effective cases. Total dosages of LAK cells and rIL-2 are shown in Table 6. A rather wide range of the dosage was due to the patient nutritional status and/or clinical responses. When the patients' condition was poor, massive lymphocytes were not obtained, and when the tumor regrowth was progressive in spite of the treatment, local AIT was given up. We continued local AIT as maintenance therapy in the patients whose clinical responses were obtained in the first therapeutic period.

RESULTS

Cytotoxic Activity of LAK Cells of Patients with Malignant Glioma. PBL from the volunteers and the patients were tested for lysis of the known NK-sensitive target cell, K562, and other target cells. These PBL were, then, activated with rIL-2 and tested for lysis against these cells (Table 3). Fresh PBL from all the patients demonstrated slight NK-mediated lysis of K562 (18.4 ± 5.2%), but no significant lysis of the autologous tumor cells was found by the same fresh PBL. However, when these PBL were cultured with rIL-2, LAK activity was generated in all the patients.

The use of Daudi cells as target cells allowed induction of cytotoxicity of more than 60% from the patients' lymphocytes, showing its killing activity of 54% and 43% against K562 cells and Raji cells, respectively. Furthermore, the killing activity of more than 33% against autologous and allogeneic glioma cells was found by the same fresh PBL. However, when these PBL were cultured with rIL-2, LAK activity was generated in all the patients.

was produced by incubation of targets with CM alone. The percentage of lysis was calculated by the formula

\[
\% \text{ of lysis} = \frac{\text{experimental cpm} - \text{spontaneous cpm}}{\text{maximum cpm} - \text{spontaneous cpm}} \times 100\%
\]

Data are reported as the mean ± SE.
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Table 3 Killing of target cells from patients' PBL cultured with or without rIL-2

<table>
<thead>
<tr>
<th>Effector cells</th>
<th>Autologous PBL</th>
<th>Autologous BTC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Allogeneic BTC</th>
<th>K562</th>
<th>Daudi</th>
<th>Raji</th>
<th>G361</th>
<th>NP-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBL (34)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.8 ± 2.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.2 ± 3.1</td>
<td>18.4 ± 5.2</td>
<td>5.8 ± 3.1</td>
<td>6.2 ± 2.5</td>
<td>5.0 ± 2.7</td>
<td>6.5 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>LAK (23)</td>
<td>1.8 ± 1.1</td>
<td>48.7 ± 12.7</td>
<td>33.5 ± 16.2</td>
<td>54.4 ± 10.1</td>
<td>66.2 ± 13.1</td>
<td>43.1 ± 7.9</td>
<td>52.1 ± 18.1</td>
<td>49.3 ± 12.3</td>
</tr>
</tbody>
</table>

<sup>a</sup> BTC, brain tumor cell.
<sup>b</sup> Numbers in parentheses, number of times.
<sup>c</sup> Mean ± SD.

was induced. It was thus suggested that sufficient cytotoxicity might be induced from the patients' lymphocytes by using rIL-2, although the cytotoxic level is less than that from healthy controls.

As shown in Table 3, allogeneic normal LAK cells were capable of lysing fresh tumor cells. This LAK killing activity was not restricted to autologous tumor cells, but was also expressed against allogeneic fresh and cultured lines, such as Daudi, Raji, G-361, and NP-1. By contrast, LAK cells from the patients did not kill normal autologous PBL. From these findings we suspected that the target specificity might be very broad beyond tumor specificity.

As we reported previously (9), determination of the cytotoxicity induced for various tumor cells with time revealed that the level reached a peak after about 4 or 5 days of incubation with rIL-2. It was accordingly decided that the lymphocytes after incubation with IL-2 for about 4 days should be used as LAK cells for injection.

Serological Phenotype of LAK Cells and LAK Precursor Cells.

Assuming that LAK cells play important roles in killing glioma cells in these patients, LAK cells were studied serologically to identify their phenotypic characteristics. To detect LAK activity, Daudi cells were used as target cells. Various studies have been performed to characterize the LAK phenomenon in humans (10, 11). As shown in Table 4, the LAK effectors were highly sensitive to Leu 1 (85% reduction), Leu 3a (78% reduction), and partially sensitive to Leu 7 (56% loss of activity). These LAK cells were, furthermore, found to be minimally sensitive to Leu 2a (20% reduction) and extremely sensitive to Leu 11 (86% loss of activity).

Table 4 Monoclonal antibody depletion of cells required to generate LAK effectors and LAK precursors

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% of lysis (target, Daudi; E:T,&lt;sup&gt;a&lt;/sup&gt; 50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>52.3 ± 8.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C&lt;sup&gt;c&lt;/sup&gt; only</td>
<td>47.6 ± 7.8&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Leu 1 + C&lt;sup&gt;c&lt;/sup&gt;</td>
<td>82.2 ± 7.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Leu 2 + C&lt;sup&gt;c&lt;/sup&gt;</td>
<td>39.1 ± 8.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Leu 3 + C&lt;sup&gt;c&lt;/sup&gt;</td>
<td>113.3 ± 7.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Leu 7 + C&lt;sup&gt;c&lt;/sup&gt;</td>
<td>23.3 ± 7.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Leu 11 + C&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.8 ± 4.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> E:T, effector:target; C, complement.
<sup>b</sup> In Experiment A, PBL from glioma patients were incubated for 5 days with IL-2. Then, the cell depletion assay was done using monoclonal antibodies, and their cytotoxicities to Daudi cells were examined.
<sup>c</sup> In Experiment B, PBL from the patients were treated with monoclonal antibodies. After incubation with monoclonal antibodies, PBL from the patients were incubated for 5 days with IL-2. Then, their cytotoxicities to Daudi cells were examined.
<sup>d</sup> Mean ± SD.

Table 5 Characteristics of activated killer cells

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>PAK&lt;sup&gt;a&lt;/sup&gt;</th>
<th>AAK</th>
<th>LAK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development kinetics</td>
<td>Days 2, 3</td>
<td>Days 3, 4</td>
<td>Days 4, 5</td>
</tr>
<tr>
<td>Stimulus</td>
<td>PHA</td>
<td>Autologous tumor cells treated with MMC</td>
<td>rIL-2</td>
</tr>
<tr>
<td>Serological phenotype of effector</td>
<td>Leu 1 (+)</td>
<td>Leu 1 (+)</td>
<td>Leu 1 (++)</td>
</tr>
<tr>
<td>Serological phenotype of precursor</td>
<td>Leu 3 (+)</td>
<td>Leu 3 (++)</td>
<td>Leu 7 (-)</td>
</tr>
</tbody>
</table>

<sup>a</sup> PAK, phytohemagglutinin-activated killer cells; AAK, autoactivated killer cells; PHA, phytohemagglutinin; MMC, mitomycin C.

Then we tested whether LAK processor cells expressed any serological phenotypes. After treatment with Leu 1, 2a, and 7, generation of LAK activity was hardly interrupted. LAK precursors were also found to be minimally sensitive to Leu 3a (65% reduction) and extremely sensitive to Leu 11 (87% reduction). It seemed that the phenotype of LAK effectors was different from that of LAK precursors, because Leu 1 and 7 depressed LAK activity remarkably in spite of no influence on induction of the LAK activity from LAK precursors. It was also revealed that the cells required to generate LAK appear to be distinct from NK cells, since the LAK cell precursors did not express Leu 7. From this study, LAK cells induced by rIL-2 were considered to possess membrane antigens to Leu 1, 3a, 7, and 11 monoclonal antibodies. In addition, killing activity was not induced satisfactorily even by the addition of rIL-2 after treatment with Leu 3a and 11, indicating that the LAK precursors were Leu 3a- and 11-positive cells. Lymphokine activation with IL-2 is only one technique for producing activated killer cells in humans (12). In other studies, we have shown that activated killer cells can be generated by phytohemagglutinin (phytohemagglutinin-activated killer cells) and by pool allosensitization (allo-activated killer cells).<sup>3</sup> These data were compared with those obtained by a similar assessment using killer cells activated with phytohemagglutinin or autoactivated killer cells. Three phenotypes were considered to represent different polyclonal cell populations (Table 5).

Clinical Application of Local AIT. Lymphocytes from the patients were separated and incubated according to the method described above, and killing activity was induced by addition of rIL-2. Then the lymphocytes were again injected along with rIL-2 into the brain tumor tissues. This procedure, i.e., local AIT, was performed in 23 patients (Table 1). The total dosage...
of local AIT and clinical responses are shown in Table 6. CT scans were obtained from all patients before and after treatment. Definite tumor regression on CT scans, improvement of some clinical symptoms like mental signs, and continuous remission over 6 mo or more were observed in 6, 9, and 3 patients, respectively. There were no marked side effects except slight fever and chill in 8 and 3 patients, respectively.

Patient 1, N. M., an 8-yr-old female (Case 1), had suffered from headache and disturbance in gait since July 1984, from which a diagnosis of pontine glioma was made. In spite of radiotherapy at 6000 rads and chemotherapy, it became impossible for her to walk without support, resulting in readmission. Since the CT scintigram revealed a distinctly enlarged tumor, 3.2 × 10^6 rIL-2-activated lymphocytes and 1400 units of rIL-2 were locally injected through an Ommaya reservoir. Distinct tumor regression was observed on the CT scintigram, the symptoms were markedly improved, and the patient was discharged walking without support (Fig. 1).

Patient 2, H. K., a 43-yr-old male (Case 12), noticed headache and alophasia in May 1985 and visited a nearby hospital. A left temporal tumor was indicated by CT scanning. Tumor resection was followed by radiotherapy at 6000 rads and chemotherapy. Four mo later, a decrease in headache and disturbance in gait appeared. Since a CT scintigram revealed relapse of the tumor, 32.4 × 10^6 rIL-2-activated lymphocytes and 2100 units of rIL-2 were locally injected. A CT scintigram taken about 2 mo after AIT revealed disappearance of the tumor, and there has been no relapse of the tumor since then (Fig. 2).

Patient 3, H. S., a 54-yr-old male (Case 11), underwent walking without support (Fig. 1).

The above three patients are the examples of cases in which AIT was markedly effective, but transient fever and chill have been observed as side effects in 8 and 3 patients, respectively. No other severe side effects have been observed, and biochemical examinations revealed no abnormal findings. These results suggest that adoptive immunotherapy is effective for relapsed malignant glioma, and that it is possible to induce a sufficient number of LAK cells from the lymphocytes of the patients with recurrent malignant glioma.

Patients with recurrent malignant glioma have been generally thought to have recurrence in a few months. In our series, however, we experienced 14 patients who showed no direct response to local AIT but are still alive free from recurrence. This fact makes us assume that there might be a mediating effect of local AIT. Additional follow-up of the patients will be required before its therapeutic value can be established.

### DISCUSSION

There have been many reports on various types of immune deficiency states accompanied by malignant brain tumors (13, 14), and the mechanism of immunosuppression has become an

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**Table 6 Clinical responses to the AIT in 23 patients with recurrent malignant glioma**

<table>
<thead>
<tr>
<th>Case</th>
<th>IL-2 units</th>
<th>LAK cells (× 10^6)</th>
<th>Period of treatment</th>
<th>Clinical response</th>
<th>Reduction on CT scan</th>
<th>Status as of 1/23/88</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1400</td>
<td>3.2</td>
<td>12/21/85-2/30/86</td>
<td>Became able to walk and eat for herself</td>
<td>++</td>
<td>Dead of pneumonia; no recurrence for 6 mo</td>
</tr>
<tr>
<td>2</td>
<td>1600</td>
<td>4.2</td>
<td>3/5/86-5/6/86</td>
<td>No response</td>
<td>-</td>
<td>Dead of disease</td>
</tr>
<tr>
<td>3</td>
<td>1300</td>
<td>2.3</td>
<td>2/12/86-4/1/86</td>
<td>No response</td>
<td>-</td>
<td>Dead of disease</td>
</tr>
<tr>
<td>4</td>
<td>1100</td>
<td>2.7</td>
<td>3/30/86-4/20/86</td>
<td>No response</td>
<td>-</td>
<td>Dead of disease</td>
</tr>
<tr>
<td>5</td>
<td>1800</td>
<td>221</td>
<td>8/20/86</td>
<td>Speak smoothly</td>
<td>+</td>
<td>Alive usefully; no recurrence over 19 mo</td>
</tr>
<tr>
<td>6</td>
<td>800</td>
<td>1.8</td>
<td>4/8/86</td>
<td>No response</td>
<td>-</td>
<td>Alive usefully; being retreated with AIT</td>
</tr>
<tr>
<td>7</td>
<td>1200</td>
<td>11</td>
<td>4/25/86-6/25/86</td>
<td>Headache disappeared</td>
<td>±</td>
<td>Alive with no deficits; follow-up only</td>
</tr>
<tr>
<td>8</td>
<td>1100</td>
<td>1.2</td>
<td>5/3/86-6/2/86</td>
<td>No response</td>
<td>-</td>
<td>Dead of disease</td>
</tr>
<tr>
<td>9</td>
<td>800</td>
<td>4.5</td>
<td>7/3/86-7/30/86</td>
<td>Unchanged</td>
<td>-</td>
<td>Dead of disease</td>
</tr>
<tr>
<td>10</td>
<td>1300</td>
<td>8.6</td>
<td>9/3/86</td>
<td>Unchanged</td>
<td>-</td>
<td>Alive usefully; being retreated with AIT</td>
</tr>
<tr>
<td>11</td>
<td>2100</td>
<td>324</td>
<td>6/18/86-9/25/86</td>
<td>More vivid</td>
<td>+</td>
<td>Dead of disease; recurrence after 8 mo</td>
</tr>
<tr>
<td>12</td>
<td>1900</td>
<td>228</td>
<td>9/10/86</td>
<td>More active</td>
<td>++</td>
<td>Alive usefully; no recurrence over 14 mo</td>
</tr>
<tr>
<td>13</td>
<td>2500</td>
<td>38</td>
<td>10/3/86</td>
<td>Some relief of complaints</td>
<td>-</td>
<td>Alive; being retreated with AIT</td>
</tr>
<tr>
<td>14</td>
<td>3400</td>
<td>45</td>
<td>5/10/87-6/30/87</td>
<td>No response</td>
<td>-</td>
<td>Dead of disease</td>
</tr>
<tr>
<td>15</td>
<td>3200</td>
<td>32</td>
<td>4/30/87</td>
<td>Diplopia disappeared</td>
<td>+</td>
<td>Alive; being retreated with AIT</td>
</tr>
<tr>
<td>16</td>
<td>1600</td>
<td>62</td>
<td>9/3/87-1/2/88</td>
<td>No response</td>
<td>-</td>
<td>Dead of disease; recurrence after 1 mo</td>
</tr>
<tr>
<td>17</td>
<td>3400</td>
<td>72</td>
<td>11/4/87</td>
<td>Mental signs decreased</td>
<td>+</td>
<td>Alive usefully; being retreated with AIT</td>
</tr>
<tr>
<td>18</td>
<td>4800</td>
<td>83</td>
<td>11/2/87-12/25/87</td>
<td>Unchanged</td>
<td>-</td>
<td>Alive usefully; follow-up only</td>
</tr>
<tr>
<td>19</td>
<td>5400</td>
<td>48</td>
<td>10/25/87</td>
<td>Some relief of complaints</td>
<td>-</td>
<td>Alive with disease; being retreated with AIT</td>
</tr>
<tr>
<td>20</td>
<td>4200</td>
<td>85</td>
<td>11/4/87-1/5/88</td>
<td>No response</td>
<td>-</td>
<td>Vegetative state with disease</td>
</tr>
<tr>
<td>21</td>
<td>4300</td>
<td>45</td>
<td>12/6/87</td>
<td>More vivid appearance</td>
<td>±</td>
<td>Alive usefully; being retreated with AIT</td>
</tr>
<tr>
<td>22</td>
<td>4100</td>
<td>48</td>
<td>12/3/87</td>
<td>Convulsion disappeared</td>
<td>-</td>
<td>Alive usefully; being retreated with AIT</td>
</tr>
<tr>
<td>23</td>
<td>4300</td>
<td>48</td>
<td>12/25/87</td>
<td>Unchanged</td>
<td>-</td>
<td>Alive usefully; being retreated with AIT</td>
</tr>
</tbody>
</table>
ADOPTIVE IMMUNOTHERAPY TO GLIOMA PATIENTS

issue (15). To make immunological approaches more effective and reliable for the treatment of these patients, it seems important to enhance antitumor immunity by using various lymphokines (16). IL-2 has been used as a lymphokine which makes the immune response to the tumor in a favorable direction by enhancement and activation of various types of killer cells (17). LAK cells induced with IL-2 show low tumor specificity and accordingly possess wide-ranging antitumor activity, unlike natural killer cells. There is thus no damage to normal cells. In addition, LAK cells, unlike cytotoxic T-lymphocytes, show antitumor cell activity even in their nonsensitized state, and it is easily induced. Their application to immunotherapy for tumors is thus increasing. Cheever et al. (18) propagated self lymphocytes in vitro, resensitized them to the tumor, and injected them again into the patients. Immune mouse lymphocytes themselves possess antitumor activity, and in vitro incubation was important only from the aspect of proliferation of effector cells. Thereafter, Mills et al. (19) conducted in vitro experiments to give antitumor capability to lymphocytes from tumor-bearing mice with weak antitumor activity, with the disadvantage that living cells were used as sensitized antigens. Since Rosenberg et al. (1) reported the effectiveness of adoptive immunotherapy with LAK cells induced with rIL-2, LAK cells have been increasingly used. However, there still remain clinical problems, one of which is that a life span of LAK cells is short when they are administered in vitro (20). So lymphocytes from the patients with recurrent malignant glioma were incubated in the presence of rIL-2 and examined for induction of killing activity. Wide-ranging killing activity of more than 60% and of 30 to 50% was induced against autologous tumor cells and other tumor cells, respectively.

Then we have demonstrated in these patients that PBL cultured in rIL-2 have lytic activity for autologous fresh tumor cells. In 17 of the 23 patients, significant lysis of fresh tumor cells was seen. Six patients who did not lyse autologous tumor were immunoincompetent, for PBL from these patients showed depressed lymphocyte blastogenesis and NK activity, although the basic mechanism for the killing of tumor cells by LAK cells is unclear. A variety of nonspecific lytic interactions with tumor cells caused by human lymphoid cells have been described (20–23). These phenomena include anomalous cytotoxicity (24), activated cell killing (25), N-cell activation (26), polyclonal activation by lectins (27), and activation of human cells by in vivo sensitization (28). Then, surface antigens of the induced LAK cells were analyzed. Our studies revealed that these LAK cells belonged to the population of Leu 1 (+/-), 2a (-/-), 3a (+/-), 7 (+), and 11 (+/-)-positive cells, while the precursor LAK cells mainly belonged to the population of Leu 3a- and 11-positive cells. Therefore, the induced LAK cells were considered to be polyclonal killer cell populations with clearly different properties from those of conventional cytotoxic T-lymphocytes and natural killer cells.

After the basic study described above, we carried out local AIT in the 23 patients. All patients with their malignant glioma mainly located in the deep portion of the brain had advanced or unresectable brain tumor and had not responded to previous standard therapy, including surgery and radiochemotherapy. The average period before recurrence was 4 mo. Some clinical improvement such as mental signs, gait disturbance, and motor weakness was observed in 9 cases, and tumor regression was clearly observed on CT scintigrams in 6 cases. In 3 of the 6 cases, remarkable objective tumor regression was observed (Figs. 1 to 3), and in 3 of the 9 effective cases, clinical remission continued over 6 mo. This adoptive immunotherapy seemed to be more effective in the young patients, because their immunity was considered to be maintained comparatively.

Although side effects caused by this therapy including fever, hyperbilirubinemia, and respiratory insufficiency have been reported (29), in our cases no marked side effects were observed.
ADOPTIVE IMMUNOTHERAPY TO GLIOMA PATIENTS

except transient fever and chill, indicating that this procedure might be effect for the patients with malignant glioma in whom any conventional procedure has failed. One of the advantages of this approach is in its broad antitumor specificity. The practicality and safety in administering this therapy to large numbers of patients remain to be fully defined. In our experiences, those patients whose lymphocytes showed strong LAK activity responded to the local AIT. But some cases did not respond clinically to this treatment in spite of strong induction of LAK activity in vitro.

Direct action of rIL-2 and enhancement of the antitumor effect due to an increase in various killer cells accompanying rIL-2 administration may be possible reasons for the induction of the effector system in vivo. In the patient in whom local AIT was ineffective in spite of the favorable data in vitro, there may be some disturbance or inhibitory factors in the mechanism.

Our experience offers hope that this therapy may be effective for patients with malignant brain tumors, but there are problems to be investigated which include elucidation of the interaction between immunologically competent cells of the host and cultured lymphocytes, detection of a population which will provide a true therapeutic effect in populations of injected lymphocytes (30–32), development of a more effective method to administer the ideal population (33), elucidation of the mechanism by which LAK cells exert their activity, and a study to find more effective adoptive immunotherapy.

REFERENCES

Local Administration of Autologous Lymphokine-activated Killer Cells and Recombinant Interleukin 2 to Patients with Malignant Brain Tumors

Seiichi Yoshida, Ryuichi Tanaka, Nobuyuki Takai, et al.


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