In Vivo and In Vitro Effect of Adoptive Immunotherapy of Experimental Murine Brain Tumors Using Lymphokine-activated Killer Cells

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ABSTRACT
Adoptive immunotherapy for the experimental murine brain tumor was investigated by using lymphokine-activated killer (LAK) cells both in vivo and in vitro. Supernatants of 48-h culture medium of spleen cells from Fischer rats with IL-2 or a monoclonal antibody to interleukin 2 (IL-2). LAK cells were generated by cocultivation of spleen cells from Fischer rats with IL-2 with the peak reactivity on Day 2 or 3 of culture. Lytic activity was observed against not only syngeneic tumor cells but also allogeneic and xenogeneic tumor cells, while no lytic activity was observed against normal brain cells. The cell death test, dye exclusion test, and immunofluorescence method using monoclonal antibodies revealed that LAK cells partially belonged to the population of the activated T-cell group, but the precursor cells did not react with any monoclonal antibodies used. On the basis of these results in vivo study was performed. LAK cells and immune spleen cells were adoptively transferred to the rats i.v. or intratumorally (i.t.) on the seventh day after the inoculation of T9, a gliosarcoma induced by methylcholangthrene from Fischer rats, into the right basal ganglia. Then the survival rate and necrotic foci were compared between the two groups treated with spleen cells and the control. The survival rate of the groups treated with LAK cells was significantly higher than that of the control (administered i.v.; P < 0.01, administered i.t.; P < 0.05). But the treatment with immune spleen cells was not effective. The incidence and area of necrotic foci in the tumors treated with LAK cells were greater than those of the others. Microautoradiography was also performed using [3H]thymidine-labeled LAK cells, which were administered i.v. to the mice on the 14th day after the inoculation of T9. It was revealed that LAK cells accumulated in the lung shortly after the administration and then in the liver and spleen, especially in the white pulp. IL-2 inhibitor activity of the sera from the tumor-bearing rats was greater than that of normal rats (P < 0.001), but it was depressed markedly by cyclophosphamide (P < 0.01). The adoptive transfer of LAK cells may be one of the effective treatments of malignant brain tumor. The nature of IL-2 inhibitors is necessary to be clarified for more effective immunotherapy.

INTRODUCTION
Various immunological treatments of malignant brain tumors have been done in combination with surgical removal, irradiation, anticancer agents, or biological response modifiers. But they have been proved unsatisfactory.

With the advancement of molecular biology, characteristics of lymphokines have been defined. Especially, IL-2 is worth notice, because it can induce LAK cells from normal lymphocytes with ease and in large quantity, which have a high cytotoxic effect on tumor cells without MHC restriction, even on natural killer-resistant tumor cells. It is certain that LAK cells play a major role in the further adoptive immunotherapy of malignant tumors.

Some experiments about the effects of LAK cells were performed using models of lung metastasis (1-3). As far as we know, however, there has been no report concerning the effects on brain tumors. In this paper, we describe biological characteristics of LAK cells generated in vitro from normal spleen cells of rats and in vivo effects on the experimental brain tumors. Examined intensively are the survival rate in comparison with the effect of immune spleen cells, organ distribution of LAK cells by microautoradiography, and its histological findings. We also report IL-2 inhibition in the serum from the tumor bearers, the activity of which increased more than that of normal serum with the progression of the tumors. There are some reports of IL-2 inhibitors (4-7). Our IL-2 inhibitor inhibited IL-2-dependent cells to incorporate [3H]TdR in response to IL-2 and was suppressed markedly by cyclophosphamide as Hardt reported (5). However, it is a new finding that IL-2 inhibition is increasing with the progression of the brain tumor, which suggests a dominant suppressor mechanism in the brain tumor as well as other malignant tumors, though the cerebrum has been considered to be an immunologically privileged site.

MATERIALS AND METHODS
Animals. Inbred male Fischer and Wistar rats, 7 to 8 wk old, were obtained from the Animal Center of our Institute.

Tumor Cells. T9, a gliosarcoma induced by methylcholangthrene from Fischer rats, was kindly given by Dr. J. Yoshida, Department of Neurosurgery, Nagoya University. KMT-17, a fibrosarcoma induced by methylcholangthrene from Fischer rats, was a gift from Dr. M. Hosokawa, Department of Pathology, Hokkaido University. Brain tumor cells of Fischer rats were induced by Rous sarcoma virus. YAC-1 (mouse T-cell leukemia), K562 (human, chronic myeloid leukemia), Raji cell (Burkitt's lymphoma), G361 (human malignant melanoma), and cells from newborn rats' brain as normal cells were used.

Spleen Cells. Spleens were removed aseptically and crushed with a hub of a syringe in CM consisting of RPMI-1640 (GIBCO, Grand Island, NY) supplemented with 0.1 mM nonessential amino acids and 10 mM 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid, 5 x 10^-3 M 2-mercaptoethanol, 100 µg/ml of streptomycin, 100 units/ml of penicillin, 0.03% glutamine, and 10% heat-inactivated fetal bovine serum (GIBCO, Grand Island, NY). The cell suspension was passed through a single layer of a gauze, and the erythrocytes were lysed osmotically with buffered ammonium chloride solution at room temperature for 8 min. The cells were then centrifuged and washed 3 times with CM. Fresh spleen cells prepared in this way were used for LAK cell generation, as control effector cells, or as responder cells in MLTC. In the experiments of adoptive immunotherapy, spleen cells from immunized rats given injections of MMC-treated T9 (1 x 10^7 cells) several weeks before were used as immune spleen cells.

IL-2 Preparation. Spleen cells of Wistar rats (1 x 10^6 cells/ml in CM) were incubated for 48 h with 5 µg/ml of Con A (Miles Laboratories, Elkhart, IN). The supernatants were collected by centrifugation, passed through 0.45-µm filters (Millipore Corp., Bedford, MA), and stored at -20°C before use.

IL-2-dependent Cells. Normal spleen cells (1 x 10^6 cells/ml in CM) from Wistar rats were incubated with Con A (10 µg/ml) for 48 h at 37°C, 5% CO2. Then they were harvested and washed 3 times with CM. These cells were cultured again with IL-2 for 7 to 10 days at 37°C, 5% CO2.
Induction of LAK Cells. Fresh Fischer spleen cells (4 × 10^7/ml in CM) were placed into 75-cm^2 flasks (Falcon Labware; Falcon, Oxnard, CA) with IL-2 in the same volume as the cell suspension for in vivo use, and for the in vitro test they were placed into 25-cm^2 flasks (Falcon Labware). No IL-2 was added to control flasks. The flasks were incubated at 37°C, 5% CO_2. The cells were harvested and washed 3 times with PBS and finally resuspended in PBS for in vivo study and in CM for in vitro study. Cells of 1 to 5-day incubation were tested for the maximum cytotoxic activity, and the cells with the maximum activity were used for in vitro study.

MLTC. Tc (3 to 5 × 10^7/ml in CM) suspended with MMC (50 μg/ml) was incubated for 45 min at 37°C, 5% CO_2, with intermittent gentle shaking and then washed 3 times with CM. Fresh spleen cells from Fischer rats and MMC-treated Tc were placed into 6-well culture plates (Becton Dickinson, Oxnard, CA) at the appropriate responder/stimulator ratio at 37°C, 5% CO_2, for 5 days. Thus generated effector cells were tested for cytotoxicity by the 4-h 51Cr release assay.

In Vitro Cytotoxicity Assay. The 4-h 51Cr release assay was used. Briefly, target cells were labeled with 50 μCi of Na^251CrO_4 (NEN Research Products, Boston, MA) for 2 h in 0.5 ml of CM. They were then washed 3 times with CM and added at 1 × 10^6 cells/well to various numbers of the effector cells in round-bottomed microplates (Flow Laboratories, McLean, VA) in triplicate. The plates were incubated for 4 h at 37°C, 5% CO_2, and the culture supernatants were harvested with the Skatron Titertek system (Skatron A. S., Lierbyen, Norway) and counted in a gamma counter. Maximum release was measured by incubation of the targets in 0.1% Triton X. Spontaneous release was measured by incubation of the targets in CM alone. The percentage of lysis was calculated by

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

All data are reported as the mean value in triplicate.

Cell Surface Phenotype Determination. The surface phenotype was determined by the cell depletion test, dye exclusion test, and immunofluorescent method, using mAbs: W3/13 (pan T-cell marker); W3/25 (helper T-cell marker); OX4 (Ia marker); OX8 (killer-suppressor T-cell marker) (Sera-Lab, England); rabbit C' (Cedarlane, Canada), and FITC-labeled anti-mouse-goat IgG (Fab') (TAGO; Burlingame, CA).

Cell Depletion Test. Fresh spleen cells from Fischer rats and LAK cells (1 × 10^6 cells/0.5 ml in RPMI-1640 medium) in tubes (Becton Dickinson) were incubated with mAbs (0.5 μl, 1:1 dilution) for 60 min at 4°C. Supernatants were removed after centrifugation (1300 rpm, 10 min), and then 1 ml of C' (1:10 dilution) was added. The tubes were incubated for 40 to 50 min at 37°C, 5% CO_2, and then washed 3 times with RPMI-1640 medium. Thus treated spleen cells were further incubated for 2 to 3 days with IL-2 to obtain LAK cells. The cells were harvested and tested for cytotoxic activity to determine the surface phenotype of LAK cells and their precursor.

Dye Exclusion Test. LAK cells treated with mAb were offered to a 0.5% trypan blue dye exclusion test. The cytotoxic index was calculated by

CI = 100 × \frac{\%\text{ of cyt (mAb + C')} - \%\text{ of cyt (C' alone)}}{\%\text{ of cyt (C' alone)}}

Immunofluorescence. LAK cells and fresh spleen cells from Fischer rats were treated with mAbs as mentioned above. One hundred μl of FITC-labeled anti-mouse-goat serum (1:50 dilution) were added and incubated for 30 min at 37°C, 5% CO_2. The cells were washed 3 times with N_2Na solution. After removal of supernatants by centrifugation, the cells were enclosed on nonfluorescent glass slides with 50% glycerine buffer. The percentage of antigen-positive cells in three different fields was calculated under a fluorescence microscope.

In Vivo Effects of LAK Cells and Immune Spleen Cells. Tc (1 × 10^5 cells/3 μl in PBS) was inoculated into the right basal ganglia of Fischer rats by a microsyringe. On the seventh day, LAK cells were injected via lateral tail veins (5 × 10^6 cells) and i.t. (1 × 10^6 cells), and immune spleen cells were injected i.v. (1 × 10^6 cells) and i.t. (1 × 10^6 cells). Then the survival rate of each group was compared statistically by the Cox-Mantel test, where P < 0.05 was considered significant.

Histological Study. Brains treated with LAK cells or immune spleen cells were fixed with 10% formalin, and then necrotic foci and infiltrating cells were estimated in hematoxylin-eosin stain.

Microautoradiography. LAK cells (2 × 10^7/ml in CM) were labeled with [3H]TdR by 4-h incubation at 37°C, 5% CO_2. The cells were washed 3 times with PBS, and then 1 × 10^4 cells were administered to the rats bearing brain tumors via lateral tail veins 14 days after the inoculation. Organ distribution of LAK cells was studied by autoradiography using a 6-μm slice of brains, spleens, livers, lungs, and thymuses fixed with 10% formalin.

IL-2 Inhibitor in the Serum from Brain Tumor-Bearing Rats. IL-2-dependent cells (5 × 10^6 cells/50 μl in CM) and sera from normal and tumor-bearing rats were dispensed into flat-bottomed microplates (Becton Dickinson). IL-2 (50 μl) was added to each well of the plate and incubated for 24 h, and then [3H]TdR (1 μCi) was added to incubate for 4 h. The cells were harvested to count the incorporated [3H]TdR by a fluid scintillation counter. The percentage of inhibition was calculated by

\[
\%\text{ of inhibition} = \left(1 - \frac{\text{cpm when serum was added}}{\text{cpm when medium was added}} \right) \times 100
\]

The results were analyzed statistically by the t test.

Effect of Cyclophosphamide on IL-2 Inhibitor Activity. Tc (1 × 10^7 cells) was injected s.c. into the hind legs of normal rats. On the 27th day, cyclophosphamide (60 mg/kg) was injected i.p. Changes of IL-2 inhibitor activity in the serum within 24 h were compared between cyclophosphamide-treated and nontreated rats statistically by r test.

RESULTS

LAK Activity

Cytocidal effects of LAK cells reached the maximum on 2 to 3 days of culture and then decreased relatively rapidly (Table 1).

Target Specificity of LAK Cells. Because of the result mentioned above, LAK cells of 2-day culture were used to study cytocidal effects on various target cells. The result shows that not only syngenic tumor cells but also allogenic and xenogenic tumor cells were susceptible to LAK cells. But normal brain cells remained intact (Table 2).

Cytocidal Effect of the Effector Cells from MLTC

Specific effector cells against Tc were not generated in MLTC. When MLTC was performed with IL-2, effector cells

<table>
<thead>
<tr>
<th>Day</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tc</td>
<td>Control</td>
<td>11.4³</td>
<td>30.8</td>
<td>27</td>
<td>6.6</td>
<td>6.1</td>
</tr>
<tr>
<td>K562</td>
<td>2.1</td>
<td>3.7</td>
<td>-2.5</td>
<td>-8.3</td>
<td>-4.1</td>
<td>3.5</td>
</tr>
</tbody>
</table>

* Mean of triplicate determinations; E/T = 100; target cell = Tc.

Table 2 Target specificity of LAK cells

<table>
<thead>
<tr>
<th>Target cells</th>
<th>E/T</th>
<th>LAK cell</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tc</td>
<td>100</td>
<td>30</td>
<td>-1.3</td>
</tr>
<tr>
<td>K562</td>
<td>50</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Raji cell</td>
<td>50</td>
<td>20</td>
<td>1.5</td>
</tr>
<tr>
<td>G361</td>
<td>50</td>
<td>48.2</td>
<td>12.1</td>
</tr>
<tr>
<td>YAC-1</td>
<td>50</td>
<td>23.4</td>
<td>-10</td>
</tr>
<tr>
<td>KMT-17</td>
<td>40</td>
<td>13.7</td>
<td>-0.5</td>
</tr>
<tr>
<td>RSV tumor</td>
<td>50</td>
<td>17.2</td>
<td>8</td>
</tr>
<tr>
<td>Normal brain cells⁴</td>
<td>100</td>
<td>6.5</td>
<td>5.2</td>
</tr>
</tbody>
</table>

* Mean of triplicate determinations.

² Normal brain cells obtained from newborn Fischer rats were not lyed.
killed not only T₆, but also K562. This indicates that an antigen-specific killer was not induced (Table 3).

Serological Phenotype of LAK Cells

LAK cells generated from mAb+C⁺ treated spleen cells and those from fresh spleen cells had almost the same activity, while the lytic activity was suppressed when LAK cells were treated with the mAb, W₃/₁₃ or OX₄. The same phenomenon was observed in the dye exclusion test and immunofluorescent method. The results indicate that the phenotype of the LAK cells is W₃/₁₃, OX₄-positive, or activated T-cells, and precursor LAK cells have none of the antigen response to the mAbs used (Tables 4 to 6).

In Vivo Effect of Adoptive Immunotherapy

Survival Rate in the Groups Treated with LAK Cells. The mean survival time (mean ± SD, days) was 21.1 ± 3.0 (n = 14) in controls, 23.8 ± 4.2 (n = 18) in the group with administration i.t., and 25.1 ± 2.9 (n = 14) in the group with administration i.v. The survival rate was compared statistically between the groups by the Cox-Mantel test, where P < 0.05 in the group with administration i.t. and P < 0.01 in the group with administration i.v. The survival rate was compared statistically between the treated groups and the control by the Cox-Mantel test. The treated groups both with i.v. administration of immune spleen cells and the control. No significant difference was found between them.

Table 3 Specific CTL induction by MLTC

<table>
<thead>
<tr>
<th>% of lysis* in cell depletion test</th>
<th>10⁻⁴</th>
<th>20</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLTC only</td>
<td>11.5</td>
<td>0.14</td>
<td>2.9</td>
<td>6.6</td>
</tr>
<tr>
<td>MLTC + IL-2</td>
<td>14.5</td>
<td>(13.2)</td>
<td>14.3</td>
<td>(23.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18.5</td>
<td>(20.9)</td>
</tr>
</tbody>
</table>

* R/S in MLTC.

Table 4 Serological phenotypes of precursor LAK and LAK cells by cell depletion test using mAb

<table>
<thead>
<tr>
<th>% of lysis* in cell depletion test</th>
<th>10⁻⁴</th>
<th>20</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen cell†</td>
<td>33.9</td>
<td>30.5</td>
<td>29</td>
<td>30.1</td>
</tr>
<tr>
<td>LAK cell</td>
<td>30.6</td>
<td>30.6</td>
<td>21</td>
<td>38</td>
</tr>
</tbody>
</table>

* Mean of triplicates; E/T = 100, target = T₆.
† Spleen cells were treated with mAb and C⁺ or C⁻ alone. No remarkable changes in the lytic activity were found.
‡ When LAK cells were treated with W₃/₁₃ + C⁺ and OX₄ + C⁺, the lytic activity was depleted.

Survival Rate in the Groups Treated with Immune Spleen Cells. The mean survival time was 22.3 ± 3.9 (n = 19) in the group with administration i.v. and 19.4 ± 1.4 (n = 8) in the group with administration i.t. But the survival rate was not significant between the group with administration i.v. and the control. In the group with administration i.t., statistical estimation was not performed because of its smaller number (Fig. 2).

Histological Examination

Necrosis in the brain tumors was found in 8 of 13 cases (61.5%) in the group with administration of LAK cells i.v., 11 of 14 cases (78.6%) in the group with administration of LAK cells i.t., 2 of 8 cases (25%) in the group with administration of immune spleen cells i.v., and 0 of 7 cases (0%) in the group with administration of immune spleen cells i.t. The incidence of spontaneous necrosis seemed to be about 30% considering that of the control, 8 of 27 cases (29.6%), which suggested the incidence of necrosis was made higher by the administration of LAK cells regardless of its administration route. When the necrotic area is compared in a longer diameter shorter diameter crossing at right angles, the necrotic area is wider in the group treated with LAK cells than in the groups with immune spleen cells, where the necrotic foci were 4.9 mm² of LAK cells i.v., 4.2 mm² i.t., 1.3 mm² of immune spleen cells i.v., and 2.4 mm² in the control. The necrotic foci were mainly localized in the central portion of the tumors. It was very interesting, however, that micronecrotic foci were scattered in the tumors in some cases to which LAK cells were administered i.v. No significant difference was found between them.

Table 5 Serological phenotypes of LAK cells by dye exclusion test

<table>
<thead>
<tr>
<th>% of specific mAb positive cells</th>
<th>10⁻⁴</th>
<th>20</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td>W₃/₁₃</td>
<td>25.5</td>
<td>5.9</td>
<td>15.4</td>
<td>6.8</td>
</tr>
<tr>
<td>W₃/₂₅</td>
<td>39.8</td>
<td>8.4</td>
<td>24.5</td>
<td>6.4</td>
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</table>

* Mean of triplicates; E/T = 100, target = T₆.
† There were more W₃/₁₃ and OX₄ positive cells in LAK cells. Cells in three different fields were counted.

Fig. 1. Effects of adoptive immunotherapy with immune spleen cells. Immune spleen cells from Fischer rats immunized against T₆ were administered to the rats bearing brain tumor 7 days after inoculation of T₉ into the right basal ganglia: 5 x 10⁴ LAK cells for i.v. injection and 1 x 10⁴ for i.t. injection. Their survival rates were compared between the treated groups and the control by the Cox-Mantel test. The treated groups both with i.v. administration of LAK cells and i.t. administration showed significantly higher rates than the control (P < 0.01 and P < 0.05, respectively).

Fig. 2. Effects of adoptive immunotherapy with immune spleen cells. Immune spleen cells from Fischer rats immunized against T₆ were administered to the rats bearing brain tumor 7 days after inoculation of T₉ into the right basal ganglia: 5 x 10⁴ LAK cells for i.v. injection and 1 x 10⁴ for i.t. injection. Their survival rates were compared between the treated groups and the control by the Cox-Mantel test. The treated groups both with i.v. administration of LAK cells and i.t. administration showed significantly higher rates than the control (P < 0.01 and P < 0.05, respectively).
specific finding was found with respect to cellular infiltration, like lymphocytes and macrophages, into the tumors. Further investigation is necessary as to when the animals should be sacrificed (Table 7).

Microautoradiographical Findings

3H-labeled LAK cells administered i.v. to the brain tumor-bearing rats accumulated mainly in the lungs after 4 h, dispersed gradually, and accumulated in the livers, spleens much later, mainly in the white pulps. No accumulation in the thymus was found. The tumors were studded with LAK cells, about 10 cells/field (magnification, ×40).

IL-2 Inhibitor Activity in the Serum from Brain Tumor-bearing Rats

IL-2 inhibitor activity in the serum from normal rats was about 50%, while that from the brain tumor-bearing rats was elevated to about 80% through the course ($P < 0.001$) (Fig. 3).

Effect of Cyclophosphamide on IL-2 Inhibitor Activity

IL-2 inhibitor activity of the serum from subcutaneous tumor-bearing rats was depressed by cyclophosphamide injected i.p.

<table>
<thead>
<tr>
<th>Table 7 Relationship between treatment and necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histological findings of the tumor were studied in hematoxylin-eosin stain. Necrotic foci in the tumors treated with LAK cells were more remarkable than those of the others. No other specific findings were observed.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>LAK cells</th>
<th>Immune spleen cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Local†</td>
<td>General‡</td>
</tr>
<tr>
<td></td>
<td>Local†</td>
<td>General‡</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Necrosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>78.5%</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>61.5%</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>−</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>−</td>
<td>21.4%</td>
<td>38.5%</td>
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<tr>
<td></td>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>70.4</td>
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</tr>
<tr>
<td>No. of cases</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td></td>
</tr>
</tbody>
</table>

† Immune spleen cells obtained from immunized rats.
‡ One × 10^7 LAK cells.
§ Five × 10^7 LAK cells.
¶ One × 10^7 immune spleen cells.
# One × 10^8 immune spleen cells.
* Percentage of the cases.
** Necrotic area, mm².

DISCUSSION

Adoptive immunotherapy of malignant neoplasms has been investigated using antigen-specific CTL (9-13), macrophages (14, 15) and LAK cells (1-3), etc. Induction of antigen-specific CTL depends on MHC (16-18) expressed on stimulator cells and antigenicity of tumor cells, which is not easy for practical use. Furthermore, immunosuppressive substances (19) secreted by some tumor cells make it very difficult. Our experiment to induce specific CTL against T6 by MLTC failed. Some macrophages have been said to have an immunosuppressive function (20-22). But compared with CTL and macrophages, LAK cells have the advantage that they are induced from normal spleen cells or peripheral blood lymphocytes easily and have high cytoidal effects on various tumor cells without MHC restriction (23-26), even on natural killer-resistant tumor cells.

We report here biological characteristics of LAK cells generated from rats' spleen cells in vitro and in vivo effects when administered to brain tumor-bearing rats. Cytocidal effects of LAK cells reached a maximum on Days 2 and 3 of culture and decreased relatively rapidly. The kinetics of LAK cells generated from rats' spleen cells seemed more rapid than those from mice and humans (27, 28). LAK cells had nonspecific, MHC-non-restricted cytoidal effects as well as those from mice and humans, but susceptibility to LAK cells was different among tumor cells. Substances expressed on the tumor cells which LAK cells recognize have not been identified. LFA-1, a kind of LFA determined in activated T-cells (29, 30), is thought to conjugate with a TSCB (29, 31). Susceptibility of tumor cells to LAK cells may be dependent on the degree of expression of such Ags as TSCB. The serological phenotype of LAK cells is W3/13 and OX4 positive, while precursor LAK cells have no Ags determined by the mAbs used.

LAK cells from humans are OKT3,8 positive, and those from mice, Thy 1, Lyt2 positive, and both are la positive as well as those from rats (27, 32). LAK cells are thought to partially belong to activated T-cells, but they are also considered polyclonal because the activity was not depleted by mAb W3/13 or OX4. In vivo effects of LAK cells were investigated by using some models of lung-metastasis and skin tumors alone (1-3),
whereas no investigation was performed by using the models of brain tumor.

We neurosurgeons studying immunotherapy of malignant brain tumors think it important to examine the effect of LAK cells on experimental brain tumors. We evaluated the effects of nonspecific adoptive immunotherapy using LAK cells and specific adoptive immunotherapy using immune competent cells from previously immunized rats on the experimental brain tumors. The specific adoptive immunotherapy was studied by using thoracic duct lymphocytes (33), immune spleen cells (34, 35) from immunized animals, and specific CTL (9, 10) generated in vitro. Crum et al. (33) reported that the skin tumor regressed by administration i.v. of TDL from immunized rats. The same effect of immune spleen cells was also reported (34, 35). These cells mentioned above are not cytotoxic in vitro and regarded as mainly consisting of helper T-cells, which were guessed to induce specific killer T-cells in vitro (33, 36, 37).

Other investigators reported that irradiation (35), thymectomy, or pretreatment with cyclophosphamide (38, 39) was necessary for those cells adoptively transferred to exert their antitumor effect in vivo, which means depression of the immune suppressive mechanism dominant in the host-bearing malignant tumors (40, 41). Concerning the idea to expand clones of specific CTL with IL-2 (2), there surely exist some tumor cells which cannot induce CTL as we report. These ideas seem to have many problems for clinical use. Our experiments using immune spleen cells and cyclophosphamide (data not shown) were not effective, indicating a greater participation of the suppressor mechanism.

On the other hand, LAK cells are induced easily and have nonspecific cytoidal effects without MHC restriction in vitro. They were effective in vivo not only for the metastatic or skin tumor models but also for the models of brain tumors as we have reported. Kedar et al. reported that LAK cells were effective in the Winn assay but less in general administration (42). Our experiment revealed that general administration of LAK cells was more effective than local administration contrary to our expectation. Some reasons are considered: (a) the direct intracranial approach might be more stressful for the hosts; (b) the number of LAK cells administered i.t. was less than that administered i.v.; (c) it might be possible for LAK cells to induce a secondary immune response in vivo because they belong to a polyclonal cell group (42), and on microautoradiography the accumulation of LAK cells in the white pulp of spleens may support this; and (d) adoptive immunotherapy was started 7 days after the inoculation of T9, but microautoradiography was performed 14 days after the inoculation. The tumor size on the 14th day was about 12 times as large as that on the seventh day. The larger tumor disturbs cerebral circulation, and it may be difficult for LAK cells to be distributed over the tumor.

Therefore, administration i.v. may be effective only when the tumor is very small.

Furthermore, IL-2 administration in addition to general administration of LAK cells has been said to be able to prolong a life-span of LAK cells and augment the activity (2, 43). Concerning the effect of IL-2 administration alone, the antitumor effect was not observed in some experiments (44), and the shortness of its half-time in vitro (45) and some IL-2 inhibitors in serum pose complex problems. Rosenberg et al. have reported the efficacy of high-dose administration of IL-2 (43, 46), which still has problems of toxicity, the induction of autoimmune diseases (5, 46), generalized capillary permeability leak syndrome (46), etc., to be resolved as soon as possible. Cyclophosphamide inhibited some of the IL-2 inhibitor activities, which meant a removal of one of the factors in the immune suppressive mechanism. Further investigation is necessary.

Adoptive immunotherapy of malignant brain tumors using LAK cells seems to be one of the attractive and promising treatments. But there still exist many problems to be resolved, namely, how to keep hosts' immunity high, what makes tumor cells escape through the immune surveillance, how to augment the expression of tumor antigens, etc.

REFERENCES

EFFECT OF LAK CELLS ON EXPERIMENTAL MURINE BRAIN TUMORS


In Vivo and in Vitro Effect of Adoptive Immunotherapy of Experimental Murine Brain Tumors Using Lymphokine-activated Killer Cells

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