Phenotype and Functional Activity of Tumor-infiltrating Lymphocytes Isolated from Immunogenic and Nonimmunogenic Rat Brain Tumors

Jone-Jun Tzeng, Rolf F. Barth, Charles G. Orosz, and Steven M. James

Departments of Pathology [J.-J. T., R. F. B.] and Surgery [C. G. O., S. M. J.J, The Ohio State University, Columbus, Ohio 43210

ABSTRACT

The purpose of the present study was to define the immunogenicity of two transplantable rat gliomas, designated F98 and D74, and to relate this to the phenotype and functional activity of tumor-infiltrating lymphocytes (TIL). Fischer rats, immunized with irradiated F98 tumor cells and challenged with intracerebral implants of ten F98 cells, had a median survival time of 49 days compared to 36 days for nonimmunized controls. In contrast, no statistically significant increase in survival times were noted in animals similarly immunized and challenged with the D74 tumors. No in vivo protection could be demonstrated in animals immunized and cross-challenged with either F98 or D74 glioma cells. Lymph node lymphocytes and TIL, isolated from animals immunized and challenged with F98 cells, were more cytolytically active than effector cells obtained from D74-immunized animals. Phenotypes of TIL isolated from intracerebral F98 gliomas of immunized rats were 52% OX-8+ and 21% W3/25+ compared to 31% OX-8+ and 19% W3/25+ for D74-immunized gliomas. Cytolytic activity against glioma targets was mediated by OX-8+ TIL, as determined by cell depletion experiments. Limiting dilution analysis showed that cytolytic T-lymphocyte precursors were present in TIL of F98 gliomas of immunized rats at a frequency of 1/3547 and were specific for F98 targets, while natural killer cell-like activity was low. Our data indicate that the F98 glioma was more immunogenic than the D74 glioma, as evidenced by increased numbers and activity of cytolytic effector cells and their precursors among TIL. This may explain in part the longer survival times observed in immunized animals challenged intracerebrally with the F98 gliomas compared to D74-immunized and -challenged hosts.

INTRODUCTION

Recently, there has been increasing interest in using adoptive immunotherapy with LAK cells3 and rIL-2 to treat patients with glioblastoma multiforme (1-3). The clinical results, however, were disappointing even with direct i.t. administration of LAK cells and rIL-2 (4). This has led us to investigate the potential of adoptive immunotherapy under the most optimized experimental conditions using a well-characterized rat glioma model. In this study (5), we utilized highly purified adherent LAK cells and rIL-2 in an in vitro Winn neutralization assay. More than 99% of the glioma cells were killed, and yet this was still insufficient to achieve a cure (5). Histopathological examination of tumors from treated animals revealed significant amounts of infiltrating lymphocytes in the gliomas. These observations prompted us to study the immunogenicity of the F98 and D74 gliomas and to correlate this with their ability to evoke a cellular immune response.

Although the brain has been considered to be an immunologically “privileged” site (6, 7), considerable evidence has accumulated indicating that mononuclear cells can infiltrate the parenchyma of brain tumors (8-12). Bertrand and Mannen (13) reported that there were lymphocytic infiltrates surrounding gliomas, but the idea that these were a manifestation of host resistance was first proposed by Ridley and Cavanagh (14) in 1971. Subsequent studies have attempted to correlate the degree of lymphocytic infiltration with survival time in brain tumor patients (15-17). Nevertheless, there have been no experimental studies relating the intensities of the mononuclear cell infiltrations of brain tumors to host survival time. Human gliomas are composed of phenotypically and genetically heterogeneous cell populations (18), and these may evoke cellular immune responses of differing intensities against tumor cells with distinct antigens. With the increasing interest in TIL and their potential use in cancer therapy (19, 20), we thought that our tumor model would lend itself to a careful analytical study of the functional properties of TIL in brain tumors. In the present study, we have related the immunogenic status of two rat glioma clones with host survival and with the phenotype and functional activity of TIL. A microculture method has been used to quantify the frequency of CTL-P among TIL isolated from F98 and D74 gliomas, to delineate the specificity of TIL against tumor targets, and to compare the frequencies of CTL-P in TIL isolated from i.c. sites with those from s.c. sites of gliomas.

MATERIALS AND METHODS

Tumor Cells. Glioma clones, designated F98 and D74, were derived from an undifferentiated neoplasm induced by N-ethyl-N-nitosourea in an inbred CD Fischer rat (21). The in vitro and in vivo morphology and growth of these tumors have been described in detail (22, 23). Their in vivo biological behavior closely simulates that of human glioblastoma multiforme in that they cannot be cured by either chemotherapy or radiotherapy and kill by virtue of the fact that they form expanding intracranial masses (24, 25). The Moloney virus-induced YAC-1 T cell lymphoma was used as the indicator of NK activity. All tumor cell lines were grown in Dulbecco’s modified Eagle’s medium (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (HyClone, Logan, UT), 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mm L-glutamine. They were propagated in vitro for fewer than 10 passages.

Animals and Rat Brain Tumor Model. Six-week-old male Fischer rats weighing 150–170 g were purchased from Charles River (Wilmington, MA). The implantation procedure of Kobayashi et al. (26) has been utilized. Briefly, rats were sedated with ketamine/xylazine (1:2/1), a plastic screw was embedded in the skull, and tumor cells were implanted through a central hole of the plastic screw into the right caudate nucleus via a Hamilton syringe fitted with a 5-mm-long 26-gauge needle. Twenty thousand F98 glioma cells were injected in 10 μl of Dulbecco’s modified Eagle’s medium containing 1% agarose at a gelling temperature of <30°C. Animals were fed ad libitum and were sacrificed when they became ataxic or had periorbital bleeding. Survival times were determined by adding 1 day to the date of euthanasia.

Immunization of Animals. Fischer rats were immunized with F98 or D74 tumor cells using a procedure similar to that described by Ibayashi

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2 To whom requests for reprints should be addressed, at 165 Hamilton Hall, 1645 Neil Avenue, Columbus, OH 43210.

3 The abbreviations used are: LAK cells, lymphokine-activated killer cells; i.t., intratamural; TIL, tumor-infiltrating lymphocytes; rIL-2, recombinant interleu-
kin 2; MST, mean survival time; CTL-P, cytolytic T-lymphocyte precursors; I//, minimal frequency estimate; 1/CL, 95% confidence limit of the frequency esti-
mate; i.e., intracierbral; PBMC, peripheral blood mononuclear cells; NK cells, natural killer cells; HBSS, Hank’s balanced salt solution; aAGs, polyclonal rabbit anti-asialo GM1.

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et al. (27). For immunization, 10⁷ glioma cells were injected s.c. into the dorsal flank. One week following implantation, the tumor mass was surgically excised, and 10⁶ tumor cells that had been X-irradiated (9000 cGy) were injected s.c. weekly for 6 weeks. One week thereafter, viable tumor cells were injected s.c. or i.c. for further studies.

Isolation of Tumor-infiltrating Lymphocytes. Rat gliomas were excised aseptically, minced into 1- to 2-mm fragments, pressed through a wire mesh, and stirred in phosphate-buffered saline solutions containing 0.025% collagenase (Sigma, St. Louis, MO) and 0.002% DNase (Sigma) for 2-4 h at ambient temperature. Cell suspensions were subjected to discontinuous density gradient centrifugation, as described by Whiteside et al. (28). The mononuclear cell-enriched fractions were collected at the interface between 75 and 100% Ficoll-Hypaque (density, 1.077; Sigma). Medium for growing TIL consisted of RPMI 1640 (GIBCO) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 5 x 10⁻³⁵ mCi/ml Na²⁵°CrO₄, 1 μM sodium pyruvate, and 2 mM L-glutamine. TIL were pooled from 3–5 individual gliomas. The average yield of TIL from F98 and D74 gliomas varied from 10⁴ to 6 x 10⁶/g of tumors.

⁵¹Cr Release Assay. Tumor cells (3 x 10⁶) were labeled with 200 μCi of Na²⁵¹CrO₄ (specific activity, =10–35 mCi/ml; Amersham, Arlington Heights, IL) for 90 min at 37°C. Five thousand target cells were added to each well of 96-well round-bottomed microtiter plates (Corning, Corning, NY). Following this, effector cells were added, the volumes were adjusted to 0.2 ml, and the plates were incubated at 37°C in a humidified incubator containing 5% CO₂ for 18 h. At the end of this time, the plates were centrifuged at 300 x g for 30 s, and 100-μl aliquots of the supernatant were removed from each well for counting in a gamma scintillation counter. The percentage of specific lysis was determined by subtracting the cpm in the absence of tumor cells from the cpm in the presence of tumor cells and dividing by the cpm in the absence of tumor cells. The average percentage of specific lysis was 60 ± 10% for F98 glioma cells and 40 ± 10% for D74 glioma cells.

 RESULTS

In Vivo Survival following Tumor Immunization. A Kaplan-Meier plot of the survival experiment is shown in Fig. 1. MSTs were 22 days for F98-immunized rats challenged with 2 x 10⁶ F98 glioma cells compared to 18 days for nonimmunized animals implanted with F98 (Table 1). Differences in survival times were significant at P = 0.03, as determined by means of the Wilcoxon rank sum and Kruskal-Wallis tests (30, 31).

<table>
<thead>
<tr>
<th>Immunizing cells</th>
<th>Challenge cells</th>
<th>MST ± SD</th>
<th>Median</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>F98</td>
<td>18 ± 1</td>
<td>18</td>
<td>0.03</td>
</tr>
<tr>
<td>F98</td>
<td>F98</td>
<td>20 ± 2</td>
<td>22</td>
<td>NS</td>
</tr>
<tr>
<td>D74</td>
<td>F98</td>
<td>16 ± 1.5</td>
<td>16</td>
<td>NS</td>
</tr>
<tr>
<td>None</td>
<td>D74</td>
<td>15 ± 3</td>
<td>16</td>
<td>NS</td>
</tr>
<tr>
<td>F98</td>
<td>F98</td>
<td>15 ± 1.7</td>
<td>15</td>
<td>NS</td>
</tr>
</tbody>
</table>

a Rats were immunized by inoculating 1 x 10⁶ F98 or D74 cells over the dorsal area of animals. One week after the inoculation, tumor mass was surgically removed, and 1 x 10⁶ irradiated tumor cells were injected s.c. once a week for 6 weeks.

b One week following the immunization, 2 x 10⁶ F98 or D74 cells were implanted stereotactically into the right caudate nucleus.

Table 1 Effects of tumor immunization on the survival of glioma-bearing rats

Fig. 1. Effects of tumor immunization on the survival of glioma-bearing rats. F98 glioma-immunized animals were challenged with either F98 (□) or D74 (○) tumor cells at a dose of 2 x 10⁶. D74-immunized rats were challenged with the same number of D74 (●) or F98 (●) cells. Nonimmunized rats were implanted with F98 (○) or D74 (○) cells as controls. Rats were euthanized, and the percentages of survival times were shown in the Kaplan-Meier plot. Enhanced survival was seen in F98-immunized animals challenged with F98 gliomas.
MSTs (16 days) were similar for nonimmunized and D74-immunized animals challenged with 20,000 D74 cells. In order to determine the in vivo reactivity of immunization, F98- and D74-immunized animals were cross-challenged with either D74 or F98 tumor cells, respectively. There were no significant differences in the survival times of cross-challenged animals as compared to nonimmunized controls with either the F98 or D74 tumors. In order to determine whether immunized rats were capable of eliminating small tumor burdens, graded numbers of glioma cells ranging from 10 to 10,000 were implanted into the right carotid artery. The mean survival times of cross-challenged animals were calculated from survival times of seven animals except as noted.

Cytotoxic Activity of Effector Cells following Immunization. Table 3 summarizes the cytolytic activity of effector cells isolated from F98- or D74-immunized animals. For rats immunized with the F98 tumor and challenged with the same tumor, the mean percentage of specific lysis against F98 targets, at an effector/target cell ratio of 40/1, was 49.3% for TIL, 48.4% for lymphocytes isolated from ipsilateral popliteal lymph node, −2.0% from contralateral popliteal lymph node, and −3.8% for PBMC. In contrast, the cytolytic activities of ipsilateral popliteal lymph node and TIL isolated from D74-immunized animals were only 12.4 and 13.2%, respectively. In order to test for cross-reactivity, either immunized rats were challenged with a different tumor cell clone or their effector cells were tested against a different target cell clone (i.e., F98 versus D74 or D74 versus F98). The cytolytic activity of effector cells was markedly decreased under both conditions (i.e., F98 versus F98, 48.4%, compared to F98 versus D74, 8.1%).

Phenotype of TIL. TIL were isolated from intracerebral tumors of immunized and nonimmunized rats. The phenotype of TIL isolated from i.c. F98 gliomas of F98-immunized rats was 52% OX-8+ and 21% W3/25+ compared to 31% OX-8+ and 19% W3/25+ for D74-immunized animals (Table 4). AGM1+ cells were <15% and ED1+ were <7% of the cells present in the TIL population. In an attempt to determine which subpopulation of TIL was responsible for their cytolytic activity, cell depletion experiments were performed. Treatment with anti-OX-8 antibody plus complement reduced the cytolytic activity of F98 TIL isolated from s.c. tumors from 43.7 to −2.5% and D74 TIL from 11.5 to −1.8% (Table 5), indicating that OX-8+ cells were the effector population. Similar changes also were observed for TIL isolated from i.c. sites of F98 and D74 gliomas. There were no significant reductions in the cytolytic activity of TIL treated with either anti-W3/25 or anti-AGM1 antibodies.

Microcultures for CTL-P. Microcultures were tested for cytotoxic activity against 51Cr-labeled tumor targets, and the frequencies of CTL-P in TIL fractions were determined by limiting dilution analysis. In preliminary experiments (data not shown), optimum culture conditions were determined by incubating graded numbers of TIL with 105 irradiated splenocytes, 106 irradiated tumor cells, and 10 units/well of rIL-2 for 8 days in individual experiments. Since microcultures were set up with rIL-2 as a growth factor, it was necessary to exclude the possibility that some of the cytolytic activity was attributable to activated NK cells. This was done by depleting the subpopulation of TIL with various antibodies plus complement, then determining the frequencies of CTL-P in the remaining TIL against F98 tumor cells and a NK-sensitive YAC-1 cell line. Prior to depletion, the frequency of CTL-P in F98 TIL was 1/3,547 against F98 targets compared to 1/12,770 against YAC-1 NK-sensitive targets (Table 6). Following treatment with anti-OX-8 antibody and complement, the frequency of CTL-P decreased to 1/131,300 against F98 targets but was essentially unchanged against YAC-1 targets, indicating that their activity was not attributable to NK cells. Following treatment with anti-AGM1 antibody and complement, the frequency of CTL-P was reduced to 1/193,092 against YAC-1 targets compared to 1/3,820 against F98 targets, thereby proving that the F98 TIL were specific for F98 targets, and little of their activity could be attributed to NK cells.

Frequencies of CTL-P in TIL. In order to determine whether
controls.

One week later. TIL were isolated from tumors and incubated under the same culture conditions as described in Table 6. Nonimmunized animals were used as controls.

The percentage of positive cells was expressed as the mean ± SD for three independent experiments as determined by an indirect immunofluorescent assay.

In the present study we have defined the immunogenicity of two rat glioma clones, F98 and D74, and have characterized the TIL isolated from either s.c. or i.e. implants of these tumors. To our knowledge, this is the first study relating the immunogenicity of gliomas to the phenotype and functional activity of TIL and to the frequencies of CTL-P, which were remarkably similar at the s.c. and i.e. sites. Longer host survival times were correlated with more immunogenic gliomas, greater numbers of OX-8+ TIL, and higher selective cytolytic activity. Low cytotoxic activity and low frequencies of CTL-P were observed in TIL isolated from F98 gliomas of nonimmunized animals. Following immunization the frequency of CTL-P in TIL isolated from either F98 or D74 gliomas of nonimmunized animals increased over 25-fold from 1/77,399 to 1/3,036, while only small increases were found in D74-immunized animals. The frequency of CTL-P was 1/3,036 in F98 gliomas increased over 25-fold from 1/77,399 to 1/3,036, while only small increases were found in D74-immunized animals. This observation might provide the rationale for developing predictive tests for tumor immunogenicity and potential responsiveness to immunotherapy. Since human gliomas presumably are composed of cells of varying antigenicity, quantitative assessment of TIL might promote the development of such tests.

### Table 4 Phenotypes of TIL isolated from gliomas of immunized or nonimmunized animals

<table>
<thead>
<tr>
<th>Antibody</th>
<th>F98 TIL</th>
<th>D74 TIL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonimmunized</td>
<td>Immune</td>
</tr>
<tr>
<td>OX-8</td>
<td>22 ± 5</td>
<td>52 ± 5</td>
</tr>
<tr>
<td>W3/25</td>
<td>19 ± 3</td>
<td>21 ± 6</td>
</tr>
<tr>
<td>AGM1</td>
<td>10 ± 5</td>
<td>14 ± 5</td>
</tr>
<tr>
<td>ED1</td>
<td>7 ± 2</td>
<td>5 ± 1</td>
</tr>
</tbody>
</table>

* Monoclonal anti-rat OX-8 (CD8), W3/25 (CD4), ED1 (macrophage), and AGM1 were used as primary antibodies. F(ab')2 fragment of fluorescein isothiocyanate-conjugated IgG was used as the secondary antibody.

### Table 5 Cell depletion studies of the subset of TIL responsible for their cytolytic activities

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Complement</th>
<th>s.c.</th>
<th>i.c.</th>
<th>s.c.</th>
<th>i.c.</th>
</tr>
</thead>
<tbody>
<tr>
<td>−</td>
<td>−</td>
<td>43.7 ± 5.1</td>
<td>48.5 ± 6.8</td>
<td>11.5 ± 4.0</td>
<td>12.1 ± 1.3</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>51.2 ± 5.7</td>
<td>44.9 ± 6.6</td>
<td>13.1 ± 6.5</td>
<td>14.0 ± 5.6</td>
</tr>
<tr>
<td>OX-8</td>
<td>−</td>
<td>44.4 ± 7.9</td>
<td>46.1 ± 2.3</td>
<td>14.3 ± 6.6</td>
<td>12.4 ± 0.2</td>
</tr>
<tr>
<td>OX-8</td>
<td>+</td>
<td>−2.5 ± 1.8</td>
<td>−1.5 ± 2.7</td>
<td>−1.8 ± 3.1</td>
<td>−1.2 ± 3.7</td>
</tr>
<tr>
<td>W3/25</td>
<td>−</td>
<td>48.1 ± 6.4</td>
<td>47.5 ± 3.5</td>
<td>13.2 ± 3.5</td>
<td>11.8 ± 1.9</td>
</tr>
<tr>
<td>W3/25</td>
<td>+</td>
<td>44.8 ± 6.9</td>
<td>50.8 ± 3.1</td>
<td>11.9 ± 4.3</td>
<td>11.8 ± 1.3</td>
</tr>
<tr>
<td>AGM1</td>
<td>−</td>
<td>55.5 ± 6.1</td>
<td>49.1 ± 3.2</td>
<td>11.1 ± 5.4</td>
<td>12.4 ± 2.2</td>
</tr>
<tr>
<td>AGM1</td>
<td>+</td>
<td>45.9 ± 7.4</td>
<td>47.1 ± 6.9</td>
<td>12.5 ± 4.8</td>
<td>14.2 ± 2.8</td>
</tr>
</tbody>
</table>

* Antibodies (1:25 dilution for OX-8 and W3/25 and 1:100 for AGM1) were added directly to the TIL and incubated for 1 h at 4°C with intermittent mixing. Cells then were pelleted, and low-toxicity rabbit complement was added at 1:6 dilution at 37°C for 1 h.

### Table 6 Limiting dilution analysis of the specificity of TIL isolated from F98 gliomas against F98 and YAC-1 targets

<table>
<thead>
<tr>
<th>Target cells</th>
<th>F98</th>
<th>YAC-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1/f</td>
<td>1/CL</td>
</tr>
<tr>
<td>None</td>
<td>3,547</td>
<td>2,760–4,936</td>
</tr>
<tr>
<td>OX-8</td>
<td>131,300</td>
<td>76,482–163,710</td>
</tr>
<tr>
<td>OX-8</td>
<td>3,291</td>
<td>2,589–4,514</td>
</tr>
<tr>
<td>AGM1</td>
<td>3,820</td>
<td>3,013–5,218</td>
</tr>
</tbody>
</table>

* Cell depletion studies of F98 TIL were carried out as described in Table 5, following which graded numbers of responder TIL were seeded into round-bottom microtiter plates together with 10° irradiated rat splenocytes, 10° irradiated F98 tumor cells, and 10 units/well of rIL-2 and incubated in a humidified atmosphere of 5% CO₂ at 37°C for 8 days. Different ¹⁸⁶Cr-labeled targets were then added to each well for another 6 h.

### Table 7 Frequencies of CTL-P in TIL isolated from different sites of immunized or nonimmunized animals bearing F98 or D74 gliomas

<table>
<thead>
<tr>
<th>Responder</th>
<th>Site</th>
<th>F98 TIL</th>
<th>1/f</th>
<th>1/CL</th>
<th>P</th>
<th>F98 TIL</th>
<th>1/f</th>
<th>1/CL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>F98 TIL</td>
<td>i.e.</td>
<td>77,397</td>
<td>94,613–175,905</td>
<td>0.4097</td>
<td>3,036</td>
<td>2,406–4,122</td>
<td>0.9988</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F98 TIL</td>
<td>s.c.</td>
<td>72,991</td>
<td>47,459–157,977</td>
<td>0.5531</td>
<td>3,046</td>
<td>2,398–4,176</td>
<td>0.9741</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F98 TIL</td>
<td>PBMC</td>
<td>79,390</td>
<td>50,124–190,874</td>
<td>0.8894</td>
<td>44,104</td>
<td>30,840–77,390</td>
<td>0.8278</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D74 TIL</td>
<td>i.e.</td>
<td>97,790</td>
<td>50,536–168,855</td>
<td>0.2150</td>
<td>14,964</td>
<td>11,705–20,748</td>
<td>0.8070</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D74 TIL</td>
<td>s.c.</td>
<td>93,401</td>
<td>57,650–245,881</td>
<td>0.7515</td>
<td>19,376</td>
<td>14,654–28,588</td>
<td>0.9641</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D74 TIL</td>
<td>PBMC</td>
<td>101,903</td>
<td>62,564–274,560</td>
<td>0.2110</td>
<td>30,840–77,390</td>
<td>0.8278</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Rats were immunized with F98 or D74 cells as in Table 1. One week following the immunization, animals were challenged with glioma cells over i.e. or s.c. sites. One week later, TIL were isolated from tumors and incubated under the same culture conditions as described in Table 6. Nonimmunized animals were used as controls.

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vide information on their immunogenicity.

In the past it has been thought the brain was an immunologically privileged site due to the relative impenetrability of the blood-brain barrier and the lack of lymphatic drainage of the central nervous system (6, 7). More recent data clearly indicate that this is indeed not the case and that allografts implanted i.c. can be vigorously rejected (32–34). In the present study, tumor immunization increased the survival times of animals implanted with gliomas compared to those of nonimmunized rats. However, as few as ten F98 or D74 tumor cells were lethal in either immunized or nonimmunized animals, and there is reason to believe that a single cell may be sufficient to kill the host (5).

Clones F98 and D74 were both derived from the same N-ethyl-N-nitrosourea-induced glioma. Although no significant differences in survival times of F98- or D74-immunized animals were observed following cross-challenge, TIL isolated from these tumors demonstrated low levels of cross-reactivity. Chemically induced rodent tumors initially were reported to express distinct tumor-associated antigens that generally were unique to individual tumors and did not cross-react with other tumors in vivo (35). When monoclonal antibodies are used, however, there is increasing evidence that cross-reactive oncofetal antigens are expressed on chemically and virally induced tumors (36, 37), and this is further supported by the low level in vitro cytolytic cross-reactivity that we observed.

Lymphocytes isolated from F98-immunized animals had weak cytotoxic activity against D74 targets and low frequencies of CTL-P against D74 compared to F98 targets, indicating that limiting dilution analysis can selectively detect tumor-reactive cytolytic T-lymphocytes. The low cytotoxic activity and frequency (<0.1%) of CTL-P may have been due to suppressive factors produced by a subset of TIL (38–40) or to inhibitory substances secreted by the tumor cells themselves (12, 41). Fontana et al. (42–44) have reported that human glioblastoma cells secreted a transforming growth factor β-like substance that inhibited the growth of T-cells and the generation of lymphokine-activated killer cells. We have observed similar effects to be mediated by the tissue culture supernatants from F98 glioma cells (5), and these may have had an inhibitory effect on the growth of CTL-P among TIL.

The major histocompatibility complex class I gene products play an important role in antitumor immunity (45). Qualitative and quantitative changes of major histocompatibility complex class I antigens on tumor cells have been associated with differing host immune responses (46, 47). In the present study, we observed higher frequencies of CTL-P among TIL isolated from F98 compared to those of D74 gliomas of immunized animals. However, there was a similar expression of major histocompatibility complex class I antigens on F98 and D74 glioma cells, as defined by a monoclonal antibody OX-18. The biochemical nature of the putative tumor-associated antigen(s) and its relationship to the immunogenicity of these tumors remain to be determined.

Several investigators have attempted to define the immunogenicity of brain tumors by measuring their growth at s.c. sites (27) or to determine the effects of i.t. infusion of LAK cells on s.c. implanted gliomas (48). These studies were based on the premise that s.c. gliomas mimic the biological behavior of i.c. tumors. In order to verify this we determined the cytolytic activities of TIL isolated from i.c. and s.c. glioma sites and the frequencies of CTL-P present in TIL populations. No significant differences were observed between the activities of TIL isolated from the two sites, and there were overlapping ranges in the frequencies of CTL-P. Our data provide the first direct assessment of the functional activities and proliferative potential of CTL-P of TIL isolated from i.c. gliomas. Our results clearly indicate that i.c. tumors are capable of evoking an immune response in peripheral lymph nodes. If the converse of this also is true, then there is a rational basis for the treatment of brain tumors by means of systemic immunotherapy (49).

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REFERENCES

FUNCTIONAL ACTIVITY OF TUMOR-INFILTRATING LYMPHOCYTES


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Jone-Jiun Tzeng, Rolf F. Barth, Charles G. Orosz, et al.


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