Characterization of lymphoid cells isolated from human gliomas

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To analyze the phenotypic profile of lymphoid cells freshly isolated from surgically resected human gliomas, a double-immunostaining technique was developed which permitted the investigators simultaneously to distinguish between hematogenous and tumor cell populations and to detect expression of lymphocyte-monocyte subset-specific antigens on hematogenous cells. With this technique, the profiles of tumor-infiltrating lymphocytes (TIL's) derived from high- and low-grade gliomas were compared with phenotypes of lymphocytes concurrently isolated from peripheral blood. The total leukocyte cell yield from high-grade glioma cases exceeded that of low-grade cases. In nine high-grade glioma cases the proportion of CD8-positive cells was increased within the TIL population (41.2% ± 1.9%, mean ± standard error of the mean) as compared to the corresponding peripheral blood lymphocyte (PBL) population (30.8% ± 4.1%, p < 0.05). The proportion of natural killer HNK-positive cells, some of which bear the CD8 antigen (although not necessarily the pan T cell antigens CD2 and CD3), was also increased in the TIL's (41.9% ± 4.2%) compared to that found in PBL's (32.1 ± 5.6%, p < 0.05) of high-grade glioma cases. The observed phenotypic pattern of high-grade glioma TIL's is similar to that reported based on immunohistochemical analysis of tumor tissue sections, suggesting that the techniques described here resulted in isolation of lymphoid cells representative of TIL's.

KEY WORDS • brain neoplasm • glioma • tumor-infiltrating lymphocyte • lymphocyte subsets • natural killer cell

LYMPHOCYTIC infiltrates in primary brain tumors were originally described by Bertrand and Man- nen 1 in 1960. Several other studies have since confirmed this finding. 2,4,15,16,19,22 Furthermore, the establishment of a correlation between degree of lymphocytic infiltration and prognosis, as judged by period of survival, 1,4,15,19,22 has prompted several investigators to try to define the properties of these cells in an effort to better understand the intricacies of glioma-immune interactions. This approach has been greatly facilitated by the availability of monoclonal antibodies (MAb's) that can distinguish subsets of lymphoid cells mediating specific immune functions. Immunohistochemical analysis of lymphocyte phenotypes within tissue sections of gliomas 9,14,17,25 has the limitation of not permitting concurrent functional in vitro studies of the cell populations. Protocols reported up to the present have been designed to extract lymphocytes from freshly resected glioma tissue but have resulted in insufficient cell yields to permit immunocytochemical analysis of the cells without prior in vitro expansion of the cell population. 12,13,26

The purpose of the present study was to phenotypically characterize the lymphoid population freshly isolated from surgically resected gliomas of different histological grades and to determine whether the isolated tumor-infiltrating lymphocyte (TIL) population was representative of the TIL population previously characterized by immunohistochemical studies. This communication describes the study and presents the technique for isolation of lymphoid cells from gliomas, the method for distinguishing lymphoid and nonlymphoid cells within the cell isolates, and the results of comparing lymphocyte-monocyte phenotypes of TIL's from high- and low-grade glioma with each other and with corresponding peripheral blood lymphocytes (PBL's).

Materials and Methods

Origin of Cells

The patient group from which the cells were derived consisted of individuals undergoing craniotomy for resection of suspected primary brain tumors. Our study involved 12 patients with a mean age of 52 years. Eight were male (mean age 51 years) and four were female (mean age 54 years). All tumors were histopathologi-
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Methods of Procedure

Isolation of PBL's. Heparinized blood (60 cc) was obtained from the arterial line at the time of operation or by venipuncture in the preoperative pretreatment therapy group. Aliquots (15 ml) of peripheral blood were mixed with 20 cc of phosphate-buffered saline (PBS) in a 50-ml plastic centrifuge tube. A Ficoll-Hypaque gradient was then laid beneath the blood and the tubes were centrifuged at 1800 rpm for 30 minutes at 20°C. The lymphocytes located at the interface between the PBS, and Ficoll-Hypaque layers were collected and washed three times in PBS at 4°C, then centrifuged at 1500 rpm for 15 minutes. Cells were then resuspended in RPMI 1640 medium and counted by means of a hemocytometer.

Extraction of TIL's. The surgical specimen was extensively washed of its contaminating blood using PBS. Vascular and meningeal elements as well as non-tumor brain tissue were removed with sterile scissors and forceps. The tumor tissue was then cut into 1- to 2-sq mm pieces, suspended in RPMI medium, and passed through a 210-μm nylon mesh bag with the help of a 10-cc syringe plunger. No enzymatic treatment of the tissue was used in the procedure. To isolate the cellular elements from the tissue suspension, 10-cc aliquots of the tissue-RPMI suspension were laid over 10 cc of a 90% Percoll density gradient and then centrifuged at 15,000 rpm for 30 minutes at 4°C. After centrifugation, the thick layer of myelin found at the interface of the RPMI-Percoll gradient was discarded.

Phenotypic Analysis of Lymphoid Cells

Aliquots of the cells isolated from the blood and from the tumor tissue were immunostained with MAb's (minimum of 25,000 cells per type of MAb used) immediately following the cell isolation. The immunostaining procedure involved a double-labeling technique designed to distinguish lymphoid from nonlymphoid cells and to determine the phenotypes of cells within the lymphoid population. Aliquots (50 μl) of the cells were pelleted in Eppendorf® tubes using a microcentrifuge, and then incubated for 30 minutes at 4°C with 50 μl of saturating dilutions of one of the panel of mouse anti-human lymphocyte or monocyte MAb's listed in Table 1. The cells were then washed three times, again pelleted, and incubated with 50 μl of 1:100 diluted biotinylated polyclonal goat anti-mouse immunoglobulins* for 30 minutes at 4°C. After a further three washes, the cells were pelleted and exposed to 10 μl of a 1:100 dilution of avidin phycoerythrin. In order to distinguish leukocytes from tumor cells, all cell aliquots were again pelleted and incubated with 20 μl of undiluted fluorescein-conjugated monoclonal goat anti-human common leukocyte antibody Hle-1† for 30 min-

* Biocan manufactured by Jackson ImmunoResearch Laboratories, Inc., West Grove, Pennsylvania.
† Hle-1 monoclonal antibody obtained from Becton-Dickinson Labware, Lincoln Park, New Jersey.

The cellular elements, including lymphoid and gliala cells, were then recovered from within the gradient and washed three times in RPMI medium. The red blood cell-enriched fraction at the bottom of the gradient was discarded. After the cells were washed, their numbers and viability were determined using a hemocytometer and trypan blue exclusion. Since many of the tumor cells lose their processes and become rounded during the isolation procedures, lymphoid and tumor cells could not be clearly distinguished morphologically at this stage.

<table>
<thead>
<tr>
<th>Monoclonal Antibody</th>
<th>Cell Surface Antigen-Cell Subset</th>
<th>Low-Grade Gliomas</th>
<th>High-Grade Gliomas</th>
<th>Total Glioma Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKT-11</td>
<td>CD2 pan T cell</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
<tr>
<td>Leu 2a</td>
<td>CD8 suppressor cytotoxic</td>
<td>65.2 ± 2.7</td>
<td>43.2 ± 2.7</td>
<td>63.7 ± 3.4</td>
</tr>
<tr>
<td>Leu 3</td>
<td>CD4 helper/inducer</td>
<td>45.8 ± 5.5</td>
<td>31.4 ± 5.0</td>
<td>42.1 ± 3.3</td>
</tr>
<tr>
<td>HNK-1</td>
<td>natural killer + CD8-positive cells</td>
<td>21.8 ± 12.6</td>
<td>32.1 ± 5.6</td>
<td>41.9 ± 4.2</td>
</tr>
<tr>
<td>OKM 1 or Leu M5</td>
<td>CD11b or CD11c monocyte-macrophage</td>
<td>10.6 ± 4.7</td>
<td>27.3 ± 6.7</td>
<td>27.9 ± 3.0</td>
</tr>
<tr>
<td>Leu 2 + 9.3</td>
<td>CD8:CD28, cytotoxic</td>
<td>75.3 ± 3.5</td>
<td>75.3 ± 3.5</td>
<td>75.3 ± 3.5</td>
</tr>
</tbody>
</table>

* Cells derived from glioma tissue (tumor-infiltrating lymphocytes, TIL's) and peripheral blood (peripheral blood lymphocytes, PBL's) of patients with high-grade (nine cases) and low-grade (three cases) gliomas. Except for Leu 2 + 9.3 immunostaining studies, data indicate the mean percentage of hematogenous cells (± standard error of the mean) expressing a specific lymphocyte-monocyte antigen as detected by monoclonal immunostaining antibody and flow cytometry analysis. Leu 2 + 9.3 results are expressed as the percentage of CD8-positive cells expressing CD28. Normal data for peripheral blood: 30.2% ± 1.5% CD8-positive cells; 47.3% ± 1.9% CD4-positive cells. Significance of difference between results with same symbols †, ‡, and §: p < 0.05 (paired t-test).
utes at 4°C. The cells were washed a final three times and then analyzed using flow cytometry. Controls included samples stained with each primary and secondary antibody alone.

To further define the lymphoid cell population bearing the CD8 antigen (that is, the suppressor/cytotoxic cells), aliquots of cells were double-immunostained with an MAb (9.3) directed against the CD28 antigen present on the cytotoxic cells of the CD8 population.‡ The anti-9.3 MAb incubation step was followed by incubation with fluorescein-labeled rabbit anti-mouse antibody (50 μl of 1:50 dilution). Phycoerythrin-conjugated MAb directed against the CD8 antigen (10 μl of stock anti-Leu 2a antibody) was then added to the cells.

Method of Flow Cytometry Analysis

In our initial analyses of cells double-stained with anti-pan leukocyte antibody and anti-lymphocyte-monocyte subset antibodies, between 2500 and 5000 total cells were enumerated. This form of analysis provided data on the proportion of total cells which were of lymphoid origin, as well as on the proportion of particular subsets within the lymphoid population. In later studies, only cells immunostained with the anti-pan leukocyte antibody were analyzed, resulting in a greater sampling of cells for the lymphocyte-monocyte phenotype analysis.

Immunological Tumor Histology Correlations

Each tumor studied was graded histologically using the Kernohan scale by reviewing the pathological specimen.

Functional In Vitro Studies

The functional properties of TIL’s and PBL’s were assessed by determining the extent of cell proliferation following 14 days of in vitro culture. For these studies, 5 × 10⁶ of either PBL’s or TIL’s, suspended at 1 × 10⁶ cells/ml in RPMI medium plus 10% fetal bovine serum (FBS) and 0.1 μg/ml gentamicin, were placed in U-bottom microwells which also contained 5 × 10⁶ irradiated autologous mononuclear cells as a source of feeder cells, OKT3 (10 ng/ml final concentration), and interleukin-2 (IL-2, 10 U/ml) prepared from MLA cell line supernatant. The final volume in each microwell was 200 μl. The IL-2 was supplemented twice weekly. Additional feeders were provided on a biweekly basis. The microwells were split into multiple wells, as required. Cell counts of PBL’s and TIL’s were conducted after 14 days.

To assess the functional properties of PBL’s passed through our TIL extraction procedure, 10⁶ of these PBL’s were suspended in flat-bottomed microwells in 200 μl of RPMI medium supplemented with 10% FBS and OKT3 (10 ng/ml final concentration). After 72 hours, the microwells were pulsed with 1 μCi of ³H-thymidine and harvested 5 hours later with an automated cell harvester. Dried filters were analyzed in a liquid scintillation counter.

Statistical Analysis

Phenotypic data derived from TIL’s and PBL’s were compared using both paired and unpaired t-tests. Mean values are expressed ± standard error of the mean.

Results

The yield of TIL’s from individual glioma cases depended on the size of the specimen and the tumor grade. To obtain adequate cell numbers for our studies, specimens estimated by the surgeon to be larger than 1 cu cm were required. The usual specimen size was 1 to 2 cu cm, but exact determinations of size were difficult because of the irregularity of the samples and their frequent fragmentation. No consistent difference was noted in specimen size between high- and low-grade glioma cases. The mean cell yield was 3.5 × 10⁶ cells, with the highest being 9.0 × 10⁶ total cells (that is, combined lymphoid and nonlymphoid cells) and the lowest yield was 1.4 × 10⁵ cells. The lowest cell yields were obtained from those cases subsequently found to have the lowest grade of malignancy.

The proportion of total cells derived from the tumor tissue staining with the anti-pan leukocyte antibody was 45%, 67%, 60%, and 11% for the four high-grade glioma cases; for the two low-grade glioma cases, the proportions were 61% and 60%. An example of the analysis from a low-grade glioma case is presented in Fig. 1. As shown, all cells contained within squares 2 and 4 are positively labeled with anti-HLe-1 and are considered to be cells of lymphoid origin. The proportion of these anti-HLe-1-positive cells expressing a particular lymphocyte or monocyte surface marker can then be determined.

Comparison of Lymphocyte-Monocyte Subset Profiles between TIL’s and PBL’s

When the glioma group was considered in total, no statistical difference in lymphocyte or monocyte subpopulation phenotypes was found between TIL and PBL populations (Table 1). However, when cases were subdivided on the basis of tumor histology into low-grade and high-grade categories, the proportion of CD8-positive cells in the TIL population of the high-grade tumors was increased (41.2% ± 1.9%) compared to the corresponding PBL preparations (30.8% ± 4.1%, p < 0.05, Table 1). In the low-grade tumor subgroup, the proportion of CD8-positive cells in the TIL’s was, if anything, lower than in the corresponding PBL preparations (Table 1). The proportions of HNK-positive cells in the TIL’s of the high-grade glioma cases (41.9% ± 4.2%) was significantly increased compared to that in peripheral blood samples (32.1% ± 5.6%, p < 0.05). Of the CD8-positive cells present within both the TIL’s

‡ The 9.3 antibody was a gift from Dr. John Hansen, Seattle, Washington.
Lymphoid cells isolated from human gliomas and PBL's of glioma patients, approximately 75% of the cells were immunostained by the 9.3 MAb, a proportion similar to that found in the peripheral blood CD8-positive population.

The proportion of CD4-positive cells in the PBL's of the high-grade glioma cases (34.1% ± 5.0%) was reduced compared to our normal laboratory values (47.3% ± 1.9%, p < 0.05) (Table 1). The CD4-positive proportion in the PBL's of high-grade glioma patients was lower than that found in the TIL's (42.1% ± 3.3%, p < 0.05). The CD4:CD8 ratio in both TIL's and PBL's in the glioma cases was reduced compared to normal values generated in our laboratory.

To determine the effect of the unavoidable use of high-dose corticosteroids on lymphocyte subset proportions in these patients, PBL-monocyte phenotypes were compared from each of two patients before and after institution of corticosteroid therapy (Table 2). In both cases, the corticosteroids lowered the number of CD4-positive cells and increased the proportion of CD8-positive cells.

**Control Experiments**

To address the concern that the cells defined as TIL's are indeed derived from the in vivo tumor infiltrate population and that they are not contaminants from the peripheral blood in which the tumor is soaking when it is received from the operating room, two control experiments were conducted. First, normal brain tissue was obtained in two cases in which a lobectomy was required for decompression. In each instance, both the normal brain specimen and a simultaneously obtained tumor specimen (high-grade glioma) were processed using the TIL isolation protocol described in the Materials and Methods section. The cells derived from both the normal tissue and the tumor specimen were then stained with the common leukocyte MAb anti-Hle-1 and with anti-pan T cell MAb (anti-CD2). Flow cytometry analysis indicated clearly defined T cells only in the cell population derived from the tumor specimen.

The second control experiment consisted in evaluating mitogen-induced proliferative properties of TIL's and PBL's. As shown in Table 3, the cell yield from PBL's in 14-day cultures supplemented with IL-2 significantly exceeded that derived from TIL's. To determine that this reduced proliferative capacity of TIL's was not attributable to the effects of our isolation procedure, an aliquot of PBL's was mixed with a suspension of irradiated normal brain tissue and then subjected to the TIL isolation procedure. The addition of 2 x 10^7 cells to a normal brain tissue specimen resulted in a yield of 1.6 x 10^7 Hle-1-positive cells, suggesting that our procedure could recover 80% of cells within a central nervous system (CNS) tissue specimen. The lymphocyte-monocyte subset phenotype of the cells recovered after passage through brain did not differ from that of PBL's. As shown in Table 4, the PBL's isolated from our TIL procedure retained a high level of proliferative capacity in response of OKT3.

**FIG. 1.** Flow cytometry analysis of 5 x 10^3 cells that have been immunostained with pan-leukocyte antibody anti-Hle-1 (abscissa: green fluorescence, FL1) and anti-CD4 antibody (ordinate: red fluorescence, FL2). Only anti-Hle-stained cells are considered to be of hematogenous origin (Boxes 2 and 4) and are used to calculate the proportion of cells expressing the selected lymphocyte-monocyte antigen (as, for example, CD4-positive cells in Box 2).

**TABLE 2**

<table>
<thead>
<tr>
<th>Monoclonal Antibody</th>
<th>Cell Surface Antigen</th>
<th>Case 1 Pre-steroids</th>
<th>Post-steroids</th>
<th>Case 2 Pre-steroids</th>
<th>Post-steroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKT 11</td>
<td>CD2</td>
<td>70</td>
<td>80</td>
<td>69</td>
<td>49</td>
</tr>
<tr>
<td>Leu 2a</td>
<td>CD8</td>
<td>36</td>
<td>49</td>
<td>17</td>
<td>22</td>
</tr>
<tr>
<td>Leu 3</td>
<td>CD4</td>
<td>23</td>
<td>8</td>
<td>43</td>
<td>26</td>
</tr>
<tr>
<td>HNK-1</td>
<td></td>
<td>15</td>
<td>20</td>
<td>26</td>
<td>16</td>
</tr>
<tr>
<td>Leu M5</td>
<td>CD11c</td>
<td>3</td>
<td>27</td>
<td>22</td>
<td>—</td>
</tr>
<tr>
<td>Leu 2 + 9.3</td>
<td>CD8:CD28</td>
<td>65</td>
<td>47</td>
<td>73</td>
<td>45</td>
</tr>
</tbody>
</table>

* Data show the percentage of cells expressing the specific antigen. Patients received dexamethasone (Decadron), 16 mg daily.

**TABLE 3**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>PBL's</th>
<th>TIL's</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14 x 10^6</td>
<td>7.9 x 10^6</td>
</tr>
<tr>
<td>2</td>
<td>1.5 x 10^6</td>
<td>1.0 x 10^6</td>
</tr>
<tr>
<td>3</td>
<td>2.8 x 10^6</td>
<td>2.0 x 10^6</td>
</tr>
<tr>
<td>4</td>
<td>2.5 x 10^6</td>
<td>1.0 x 10^6</td>
</tr>
</tbody>
</table>

* Data indicate number of cells recovered after 14 days of in vitro OKT3-stimulated cultures supplemented with interleukin-2 (IL-2). 5 x 10^9 cells were initially cultured with 5 x 10^3 irradiated autologous feeder mononuclear cells in 200 μl medium and OKT3, and IL-2 (10 U/μl) was added twice weekly. TIL = tumor-infiltrating lymphocyte; PBL = peripheral blood lymphocyte.
These results indicate the different functional properties exhibited by TIL's compared to PBL's.

**Discussion**

This study has attempted to characterize the lymphocyte-monocyte infiltrate within glioma tissue using a technique whereby the TIL's are isolated from bulk in vitro proliferative capacity of mononuclear cells (MNC's)*

<table>
<thead>
<tr>
<th>Mononuclear Cells</th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>freshly isolated</td>
<td>18,271 cpm</td>
<td>4153 cpm</td>
</tr>
<tr>
<td>reisolated from normal brain</td>
<td>49,639 cpm</td>
<td>18,044 cpm</td>
</tr>
</tbody>
</table>

* Data indicate results from two separate cases in which MNC's were utilized immediately after isolation from peripheral blood or after being mixed with irradiated normal brain homogenate and then reisolated using the Percoll gradient isolation procedure. 200 µl of 10^8 MNC's suspended in RPMI medium plus 10% fetal bovine serum were cultured with 20 µl of OKT3 (1:10 dilution). Results indicate 

**TABLE 4**

3H-thymidine uptake (counts per minute (cpm)) by cells pulsed for 5 hours after 72 hours in culture. TIL = tumor-infiltrating lymphocyte.

In order to identify the hematogenous cells contained within the TIL bulk-isolated cell population, the common leukocyte antibody anti-Hle-1 (which recognizes CD45 antigen cluster) was utilized. This step is required prior to analysis of lymphocyte or monocyte phenotypes since intrinsic CNS cells lose their morphological features during the isolation procedure and cannot be distinguished from lymphoid cells by morphological criteria alone. As indicated, although usually 60% to 70% of cells isolated from the gliomas were lymphoid in origin, our analysis indicated that, at least in one case, only a minority of the cells were lymphoid. The common leukocyte antibody anti-Hle-1 does not bind to human astrocytes maintained in in vitro culture (data not shown) in contrast to other MAB's such as anti-HNK-1 which do recognize epitopes shared between lymphoid and neural cells.

We attempted to determine whether our TIL-derived population was contaminated with peripheral blood cells. The fact that our specimens were extensively washed of their blood and debrided of vascular and meningeal elements, and our findings that 1) distinct T cells were recovered only from tumor tissue and not from normal brain (as evaluated by flow cytometry) and 2) marked differences in proliferative ability between TIL's and PBL's existed (even when the PBL's were passed through the same isolation procedures as for the TIL's) led us to believe that the tumor-extracted lymphocytes in this study were indeed TIL's. One cannot rule out that, within the cell population derived from bulk isolation of TIL's, there may be some degree of contamination with peripheral blood cells. Such contamination must be considered when studying the properties of in vitro expanded TIL and PBL populations, particularly in view of the finding that the T cell cloning efficiency of TIL's from gliomas is markedly lower than for PBL's, which is perhaps related to suppressor factor production by glioma cells.

Our phenotypic data, derived from analysis of identified lymphoid cells, suggest that in high-grade tumor cases there is an increase in the proportion of CD8-positive cells within the TIL population compared to corresponding PBL's. This increase is possibly related to the concurrent increase in natural killer cells, some of which are known to express the CD8, although not necessarily the pan T cell antigens CD2 or CD3. Further studies with triple immunolabeling of cells will be needed to document the presence of Hle-1-positive, CD2-negative, CD8-positive cells within the TIL's. Our results regarding overrepresentation of CD8-positive cells in high-grade glioma cases are consistent with those of others using immunohistochemical analysis of tissue sections.

Phenotypic analysis of the PBL's in our patients indicated a reduced proportion of CD4-positive cells and an increased proportion of CD8-positive cells compared to control donors. All of our patients were receiving corticosteroid therapy preoperatively, as likely were the patients whose tumors were used in previously reported immunohistochemical studies. As we have found by studying patients before and during treatment, the dosage of corticosteroids used preoperatively in glioma patients does result in reduced proportions of CD4-positive cells and increased proportions of CD8-positive cells in the peripheral blood. However, Roszman, et al., and Bhondeley, et al., have previously found a reduced proportion of CD4-positive cells in the PBL's of high-grade glioma patients who were not receiving corticosteroid therapy. Elliott, et al., have also demonstrated a functional defect in the CD4-positive helper cell population in patients with high-grade gliomas. We found that the CD4-positive population within the TIL's of high-grade glioma patients was not reduced to the same extent as that of their PBL's. The observation of a lack of overrepresentation of CD8-positive cells in the TIL population derived from gliomas of low-grade malignancy, despite the fact that these patients received the same high dose of corticosteroids preoperatively as the high-grade glioma patients, would suggest that the relative increase in the CD8-positive cells in the high-grade glioma cases is likely not due to the effects of systemic corticosteroid therapy alone.

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The significance of the lymphocyte infiltrate in gliomas remains to be defined, particularly as to whether these cells contain subpopulations capable of affecting tumor growth. The presence of both CD4- and CD8-positive T cell subsets within the TIL's, with some overrepresentation of CD8-positive cells, is similar to the pattern found in chronic non-neoplastic inflammatory diseases of the CNS, including multiple sclerosis and relapsing experimental allergic encephalomyelitis (EAE). 1,2,3,24 With regard to EAE, the bulk of the cells infiltrating the CNS are not antigen-specific, even when the disease is induced with antigen-specific T cells. Analysis of T cell clones derived from TIL's of glioma patients indicates that only a minority of such clones demonstrate glioma-specific cytotoxicity and only a minority of these are restricted to the patient's own glioma. 1,2,13,26 Many of the clones do show natural killer activity. The proportion of glioma-specific cytotoxic clones derived from the TIL population is, however, increased compared to the proportion that can be derived from the corresponding PBL population. 1,2,13,26 As shown in this study and in others, 1,2,13,26 the efficiency of expansion of glioma-derived TIL's, at least in vitro, is markedly reduced compared to that achieved using PBL's. Thus, the overall capacity of TIL's to affect tumor growth must be considered in the context of both the properties of the lymphocyte populations themselves and the capacity of the tumor to inhibit the proliferative, and perhaps the effector, functions of these infiltrating cells.

References


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