Specific Cellular Immune Responses in Patients with Malignant Gliomas

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ABSTRACT

The leukocyte adherence inhibition assay was used to measure cell-mediated immunity in 26 patients with malignant glial neoplasms and 41 control subjects. A significant inhibition of leukocyte adherence was observed in 21 of 26 (80%) glioma patients in the presence of a 3 M KCl extract of glioma tissue, as compared to that of normal brain extract. Among the control group, no significant difference in the percentage of nonadherent leukocytes was noted in the presence of either antigen. To study the specificity of the reaction, a 3 M KCl extract of meningioma, pituitary tumor, carcinomas of breast, and lung, melanoma, brain, and heart tissues were used as nonspecific antigens. Such studies revealed significantly lower values of nonadherent leukocytes.

These data indicate that patients with malignant glial neoplasms manifest a cellular immune response to glioma-associated antigens which can be measured by the tube leukocyte adherence inhibition assay and that leukocyte adherence inhibition assay may render additional useful information in diagnostic and prognostic evaluation of malignant glial neoplasms.

INTRODUCTION

Recently, several studies have demonstrated the presence of immunological response to glioma-associated antigens in patients with malignant glial neoplasms (1–3, 5, 12, 13, 16, 21, 22). Cellular immune responses are generally believed to be more important in the rejection of solid tumors than are humoral antibodies. Several methods have been utilized to monitor or detect CMI responses to cancer antigens, i.e., lymphocyte stimulation tests (19), leukocyte migration inhibition assays (4, 11), delayed cutaneous hypersensitivity responses to membranes and solubilized extracts of autologous and allogeneic tumor cells (10, 19), and lymphocyte microcytotoxicity assays using tissue culture target cells (9, 15, 23). Lymphocyte cytotoxicity against tumor cells is the most notable and extensively used assay for monitoring the course of disease in cancer patients or for immunodiagnosis. The specificity of the observed reactions and the relative lack of normal reactivity in this assay have been increasingly questioned. In addition, this assay inherits certain technical problems such as types of target cells used, optimal maintenance of cultures, and contamination of tumor cell cultures by normal fibroblasts.

As in most of the biological systems, there is always a need for refinement of the most commonly used assays, and there is even a place for new methodologies. Moreover, because of the heterogeneity of human tumor tissues (in a given kind of tumor), an assay to monitor CMI responses in cancer patients should be relatively simple and should consist of reactions specifically with native, relatively unchanged autologous or allogeneic tumor antigens. Recently, Halliday et al. (7, 8) described a simple and rapid assay for CMI, called LAI assay. This assay has been used in a variety of experimental and clinical tumor systems to study CMI to tumor antigens (6, 7).

We report here that a large number of patients with malignant glial tumors demonstrated CMI responses to autologous as well as allogeneic tumor antigens as measured by LAI assays. We also include evidence that the antigens were absent from a considerable range of normal as well as other types of malignant tissues.

MATERIALS AND METHODS

Patients. All 26 patients providing material for these studies were recruited from a consecutive series of patients with malignant glial tumors treated at the Los Angeles County-University of Southern California Medical Center. All patients had relatively brief histories (less than 6 months) and had been on methylprednisolone therapy for a period of 2 to 5 days, with a mean of 3 days. To assure uniformity in diagnostic appraisal, all pathological materials were reviewed by the director of the Cajal Neuropathological Laboratories at the Los Angeles County-University of Southern California Medical Center. The degree of malignancy observed was considered to be at the level of either a malignant astrocytoma or glioblastoma multiforme in all cases.

A group of 41 control subjects comprised 5 healthy individuals (with no apparent disease), 4 patients with spontaneous subarachnoid hemorrhages secondary to berry aneurysms, 3 patients with cerebral gunshot wounds, 3 patients with simple skull fractures, 4 patients with meningiomas, 5 patients with pituitary tumors, 6 patients with ocular melanomas, 6 patients with carcinomas of the breast, and 5 orthopedic patients, exclusive of CNS trauma.

Tissue Extract (Antigens). Tissue specimens were obtained at the time of surgery, when subtotal excision of the tumors was accomplished. After removal of tissue for routine pathological evaluation, the specimen was transferred in Hanks’ balanced salt solution. The tissue was either used immediately or stored at −70°. Normal brain and normal human heart tissues were obtained from autopsied material within 6 hr after death. KCl extracts (3 M) (Table 1) of various tumor tissues, including 5 gliomas (from Patients F. M., I. J., A. M., H. C., and K. L.), one meningioma (F. T.), one pituitary tumor (A. W.), one carcinoma of the breast (S. J.), one carcinoma of the lung (J. L.), one melanoma (J. B.), and normal human brain and heart tissues were prepared by the modification of the methods of

1 This work is supported in part by the Robert E. and May R. Wright Foundation Award 41.
2 To whom requests for reprints should be addressed.
3 The abbreviations used are: CMI, cell-mediated immunity; LAI, leukocyte adherence inhibition; CNS, central nervous system; MEM, Eagle’s minimum essential medium; PBL, peripheral blood leukocytes; NAL, nonadherent leukocytes; PBS, phosphate-buffered saline.
between tumor extracts and normal brain extracts was determined by another laboratory individual who does not have access to the code or to the knowledge of the diagnosis of the patient. All cell counts are made by at least 2 researchers. In order to eliminate any possibility of bias in the results, all assay values were run in sextuplicate. Percentage of nonadherent cells was obtained by comparing with those tubes containing buffer alone. Mean percentage of nonadherent cells and S.E.’s were calculated for each sample, and statistical significance of comparison between tumor extracts and normal brain extracts was determined by Student t test. \( p <0.05 \) was considered significant.

In order to eliminate any possibility of bias in the results, all blood samples and antigen extracts are routinely coded by a laboratory individual who is not involved in the actual performance of the assays. Detailed execution of the experiment is carried out by another laboratory individual who does not have access to the code or to the knowledge of the diagnosis of the patient. All cell counts are made by at least 2 researchers.

**RESULTS**

The nonadherence of the PBL from 5 consecutive patients with gliomas admitted to the Neurological Surgery Unit of the Los Angeles County-University of Southern California Medical Center was measured in glass tubes in the presence of 3 m KCl extracts of tumor tissues obtained from the same patients (autologous reaction), as well as in the presence of normal brain extracts. Each sample was run in 6 replicates. The mean percentage of NAL in a given sample was calculated as a difference of mean percentage of NAL in the presence of antigen and PBS alone. The results of such a study are shown in Table 2. The percentage of NAL was consistently significantly high in the presence of tumor extracts as opposed to normal brain extracts. No significant difference in the values of NAL was noted in autologous or allogeneic reactions.

Our preliminary study did not reveal any significant difference in the value of LAI in the presence of autologous or allogeneic tumor extracts. Therefore, nonadherence of PBL from 26 glioma patients and 41 control subjects was measured in the presence of 3 m KCl extracts of either glioma (A. M.) or normal brain or heart tissue.

No significant difference in the range of percentage of NAL was seen when leukocytes were incubated with heart antigens or PBS alone. However, in both of these cases, the percentage of NAL was markedly lower than in the tubes containing either normal brain antigens or glioma antigens (Tables 3 and 4).

When PBL from a consecutive series of 26 glioma patients were incubated in a medium containing 400 mg of protein per ml of either glioma or normal brain tissue, a significant LAI \( p <0.05 \) was obtained in 21 of 26 glioma patients (80.7%) as compared to that of normal brain antigens (Table 3; Chart 1). The mean percentage of NAL in the presence of glioma antigen (specific antigen) among glioma patients varied between 2 and 61%, with a mean value of 32.8%. In the presence of normal brain antigens, the percentage of NAL ranged between 2 and 35, with a mean of 15.3%.

Among a group of 20 control subjects, no significant difference in the percentage of NAL was noted with glioma or normal brain antigen, except Patients H. P. and G. C. Both of these patients had severe head trauma. As in the glioma patients, the values of NAL were markedly lower with heart antigens and PBS alone when compared with those of glioma or normal brain antigens (Table 4; Chart 2). In this group, the percentage of NAL varied between 2 and 43 (mean value, 13.4%) and 8 and 45 (mean value, 18.0%) with normal brain and glioma antigens, respectively. Within the control population, the patients with brain injury or brain trauma appeared to display a...
of such reactivity among other CNS or non-CNS tumor patients. Therefore, LAI assays on PBL from 4 patients with meningiomas, 5 patients with pituitary tumors, 6 patients with ocular melanomas, and 6 patients with carcinomas of the breast, in the presence of glioma antigens (A. M.), were performed. The results are summarized in Table 5. Values of LAI were significantly high in all 4 meningioma patients in the presence of normal brain and glioma extracts. However, no significant difference in the percentage of NAL

Table 5

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Heart antigen</th>
<th>Normal brain antigen</th>
<th>Glioma antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS trauma</td>
<td>1. M. G.</td>
<td>10 ± 4*</td>
<td>24 ± 15</td>
</tr>
<tr>
<td>2. H. P.</td>
<td>10 ± 5</td>
<td>13 ± 10</td>
<td>34 ± 16</td>
</tr>
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<td>3. G. M.</td>
<td>7 ± 4</td>
<td>12 ± 5</td>
<td>16 ± 6</td>
</tr>
<tr>
<td>4. S. P.</td>
<td>7 ± 4</td>
<td>6 ± 3</td>
<td>15 ± 9</td>
</tr>
<tr>
<td>5. G. C.</td>
<td>8 ± 0</td>
<td>36 ± 18</td>
<td>54 ± 18</td>
</tr>
<tr>
<td>6. F. A.</td>
<td>5 ± 2</td>
<td>23 ± 12</td>
<td>23 ± 14</td>
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Aneurysm

<table>
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<th>Glioma antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. M. G.</td>
<td>8 ± 4</td>
<td>7 ± 4</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>2. H. C.</td>
<td>12 ± 7</td>
<td>43 ± 17 &amp; 45 ± 21</td>
<td></td>
</tr>
<tr>
<td>3. W. U.</td>
<td>6 ± 3</td>
<td>2 ± 1</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>4. G. B.</td>
<td>5 ± 4</td>
<td>8 ± 6</td>
<td>8 ± 5</td>
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Non-CNS trauma

<table>
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<th>Patient</th>
<th>Heart antigen</th>
<th>Normal brain antigen</th>
<th>Glioma antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. G. R.</td>
<td>7 ± 3</td>
<td>2 ± 1</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>2. J. B.</td>
<td>5 ± 2</td>
<td>6 ± 3</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>3. S. R.</td>
<td>7 ± 3</td>
<td>5 ± 3</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>4. M. E.</td>
<td>6 ± 3</td>
<td>3 ± 4</td>
<td>6 ± 3</td>
</tr>
<tr>
<td>5. C. M.</td>
<td>7 ± 3</td>
<td>2 ± 1</td>
<td>11 ± 3</td>
</tr>
</tbody>
</table>

Normal

<table>
<thead>
<tr>
<th>Patient</th>
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<th>Normal brain antigen</th>
<th>Glioma antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. K. S.</td>
<td>7 ± 2</td>
<td>16 ± 7</td>
<td>22 ± 12</td>
</tr>
<tr>
<td>2. H. H.</td>
<td>6 ± 4</td>
<td>18 ± 8</td>
<td>15 ± 8</td>
</tr>
<tr>
<td>3. T. S.</td>
<td>7 ± 3</td>
<td>15 ± 8</td>
<td>17 ± 7</td>
</tr>
<tr>
<td>4. J. H.</td>
<td>5 ± 4</td>
<td>12 ± 6</td>
<td>10 ± 5</td>
</tr>
<tr>
<td>5. C. C.</td>
<td>9 ± 3</td>
<td>11 ± 4</td>
<td>16 ± 6</td>
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* Mean ± S.D.

relatively higher percentage of NAL with normal brain and glioma antigens than did normal healthy volunteers and others. Table 4 shows the percentage of LAI among various control populations measured in the presence of glioma and normal brain tissue antigens, respectively. Four patients with severe CNS trauma (M. G., H. P., G. C., and F. A.) and one patient with an aneurysm (H. C., 45%) consistently showed significantly higher values of LAI with both glioma and normal brain antigens.

The indication of the specificity of LAI in the presence of glioma antigens among glioma patients would be the absence
was apparent when normal brain and glioma antigens were compared. Among patients who only had pituitary tumors, one patient (P. E.) showed significant value of LAI with normal brain and glioma extracts with no significant value of LAI when both normal brain and glioma extracts were compared. None of the 6 ocular melanoma patients indicated specific LAI in the presence of all 3 extracts. Of the 6 patients with carcinomas of the breast, 3 revealed significant LAI with both normal brain and glioma extracts. Again, none of the patients indicated specificity of the reaction with either normal brain or glioma extracts.

Further specificity of the LAI reaction for glioma antigens in glioma patients was sought by studying the LAI of glioma patients in the presence of tumor extracts of a battery of various tumor tissues. In such a study, LAI of PBL from 5 glioma patients was studied in the presence of 3 M KCl extracts of the following tumor tissues: (a) meningioma; (b) pituitary tumor; (c) carcinomas of the breast; (d) carcinomas of the lung; and (e) melanoma. Results are summarized in Table 6. The percentage of LAI was significantly lower in the presence of meningioma, pituitary tumor, carcinomas of the breast, carcinomas of the lung, and melanoma extracts as compared to that of glioma extracts. However, the values for NAL were comparatively higher in the presence of meningioma and pituitary tumors (CNS tumors) as compared to those of non-CNS tumor extracts, i.e., carcinoma of the breast, carcinomas of the lung, and melanoma.

A study of the effect of various concentrations of antigen extracts showed that maximum LAI was observed at a concentration of 400 mg of protein per ml. Increased protein concentrations resulted in inconsistent values among various experiments.

**DISCUSSION**

Our data indicate that PBL of a large percentage (80.7%) of glioma patients exhibited a significant (p < 0.05) LAI in the presence of glioma antigens as opposed to normal brain antigens (Tables 2 and 3). PBL of a majority of control donors did not show a significant difference in LAI in the presence of glioma or normal brain antigens (Table 4). This consistently observed difference in reaction frequency of leukocytes to glioma antigens within the
Cellular Immune Response in Malignant Gliomas

Cellular immune response in malignant gliomas has been a subject of extensive research. Distinct cellular sensitization to specific antigens has been observed in glioma patient populations. The LAI assay, among other techniques, has proven useful in detecting cellular sensitization to a specific anti-glioma population and of that between control donors of glioma-associated antigens. It also reveals that the LAI assay can discriminate between glioma patients and those of control subjects, based on the reactions of glioma-associated antigens.

Our studies indicate comparatively high values of NAL in the presence of both glioma and normal brain antigens as compared to heart antigens (Tables 3 and 4), which may be attributed to organ-specific rather than tumor-associated antigens. The facts (a) that a large number of glioma patients showed significant increase ($p < 0.05$) in NAL over normal brain antigens and (b) that leukocytes from patients with other CNS cancers, exclusive of gliomas (i.e., meningioma and pituitary tumors), did not show a difference strengthen the contention that LAI reactivity depicted in glioma patients may be glioma antigen associated.

While it is not absolutely certain that the LAI assay measures exclusively lymphocyte functions, there is considerable evidence that this is the case. According to Grosser et al. (6), the reactive cell was a circulating monocyte that recognized and reacted with tumor antigens by cytophilic antitumor antibody bound to the surface of the monocyte.

In our study, we did not find any significant difference in LAI results in autologous and allogeneic combinations of PBL and tumor extracts (Table 2). This similarity of results strongly suggests that transplantation antigens may not be the cause of the reported reactions. These findings further confirm our previously reported data that gliomas may share antigenic characteristics (22). Using indirect immunofluorescent antibody techniques, we demonstrated that, of 21 sera obtained from patients with histologically proven malignant gliomas, 47% possessed antibodies reactive with cytoplasmic components of allogeneic tumor cells. In vitro, antigenic cross-reactivity between tumors of the same histological types or origin has been reported by other researchers in a variety of clinical tumors, utilizing a variety of immunological methodologies, i.e., lymphocyte cytotoxicity assays (14, 16), serum cytotoxicity assays (12, 13), and immunofluorescent test for antibodies to tumor-associated antigens (22).

Further proof of glioma antigen specificity was sought by reactions of leukocytes from glioma patients with a battery of antigen extracts obtained from normal brain, gliomas, meningiomas, pituitary tumors, carcinomas of the breast, carcinomas of the lung, and melanomas (Table 6). In this test, LAI responsiveness to glioma antigens was significantly higher than all other antigens tested. However, a comparatively higher value of LAI in the presence of meningioma and pituitary tumor extracts (CNS tumor) may be attributed to organ-specific common antigens.

An analysis of LAI responsiveness to normal brain extracts, as well as glioma extracts among various control donors revealed that patients with brain trauma or brain injury consistently showed higher values of NAL than did other donors (Table 4; Chart 2). Among 4 of 10 patients with brain trauma, values for NAL ranged between 23 and 36%. These values were significantly higher than those of other control populations (Table 4). Moreover, a comparison of LAI reactivity of brain trauma patients to normal brain and glioma antigens revealed an overlap in the degree of responsiveness. It is hard to explain this cross-reactivity in LAI results between malignant gliomas and brain trauma patients. However, there are some suggestions that gliomas may share certain antigenic components with normal brain (25). Recently, Thomas (24) used radial immunodiffusion assays and demonstrated circulating brain antigens in 37% of 76 patients with blunt head injury. In light of these studies, it is tempting to suggest that after severe brain injury there is a breakdown of the blood-brain barrier. Therefore, some of the damaged brain cells may release brain components into the circulation; under normal conditions this would not occur. These antigenic components may in turn provoke host immune responses.

In conclusion, the results presented in this report establish the fact that the LAI assay depicts specific anti-tumor activity to glioma antigens in patients with gliomas and that this assay is rapid, specific, and reproducible. The fact is that the LAI assay not only discriminates between gliomas, other cancers, and controls but also obviates the necessity of tissue culture cell lines. Because of the possible variation in the results, however, the tests have to be done repeatedly. This information, coupled with the findings that gliomas may possess common antigens, renders the LAI assay as a potential tool for neurosurgeons to diagnose gliomas early in the course of the disease. This provides them with an opportunity to better manage glioma patients.

REFERENCES


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