Expression and modulation of major histocompatibility antigens on murine primary brain tumor in vitro

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Lysis of tumor cells by activated cytotoxic lymphocytes requires their recognition of antigens associated with major histocompatibility complex molecules. The authors studied the constitutive expression of Class I and Class II major histocompatibility complex antigens on mouse brain-tumor cells and the capacity of different cytokines and cytokine combinations to alter this expression in vitro. Cells from the murine glioma 26 (GL26), glioma 261 (GL261), and ependymoblastoma A (EpA) cell lines were established in monolayer culture and treated for 48 hours with either alpha interferon, gamma interferon, tumor necrosis factor alpha, tumor necrosis factor alpha plus gamma interferon, or interleukin-2. They were then analyzed by flow cytometry for baseline and cytokine-altered major histocompatibility complex expression.

All cell lines had a similar constitutive major histocompatibility complex pattern with low Class I antigen expression and no detectable Class II antigen expression. Alpha interferon substantially induced and up-regulated Class I antigen expression, but had no effect on Class II antigen expression. Gamma interferon also stimulated up-regulation of Class I antigen expression, generally doubling the anti-Class I antigen fluorescence of treated cells. Its effect on Class II antigen expression was more extensive. In the GL26 and GL261 cell lines, the expression of Class II antigen determinants increased up to 12 x and 14 x control values and as many as 75% of cells that had no detectable constitutive expression of Class II antigen expressed this antigen after priming with gamma interferon. The addition of tumor necrosis factor alpha to gamma interferon further increased Class II antigen expression on EpA tumor cells only. Interleukin-2 and tumor necrosis factor alpha alone had no effect on Class I or Class II antigen expression of any cell lines.

It is concluded that Class I and Class II antigen expression in mouse glioma cell lines is induced and enhanced after treatment with certain cytokines in vitro. Use of these cell lines to create in situ primary brain tumors in C57BL/6 mice should provide an excellent animal system to study major histocompatibility complex modulation in brain tumor cells and to examine the potential impact of major histocompatibility complex up-regulation on the response of brain tumors to immunotherapy.

KEY WORDS: brain neoplasm · histocompatibility · antigen · immunotherapy · interferon · mouse

Despite advances in the understanding of tumor biology at the cellular and genetic level, there has been no significant recent increase in the survival period of patients with glioblastomas. Less than one-half of patients survive at 1 year, most die within 2 years, and the limitations of surgery and radiation therapy are well known. Since an important advantage of immunotherapy is its potential for high tumor specificity, it has been investigated in various forms to examine antitumor activity in patients with brain tumors. However, numerous trials using intravenous, intratumoral, and intrathecal immunotherapy have had limited or no success.6,7,11,15 Recently, new methods of adoptive immunotherapy have been successful in treating certain advanced cancers outside the central nervous system.10,13,16,20-22 In animal models and in patients, disseminated tumors in the lungs, peritoneal, liver, and other locations have been reduced or eliminated by the administration of activated lymphocytes and cytokines. Nonetheless, immune responses against brain tumors remain elusive. Animal brain tumors are unresponsive to interleukin-2 and lymphokine-activated killer cells5 and to tumor-infiltrating lymphocytes (TIL's) (SC Saris, unpublished data). Furthermore, in clinical
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trials, many patients with stable or regressing tumors in extracranial sites have rapidly progressive metastatic disease in the brain.9

One explanation for the apparent immune privilege of tumors in the brain is poor expression of major histocompatibility antigens. A cell-mediated immune response against tumors can only occur in the presence of appropriate major histocompatibility complex antigens on the surface of tumor cells.2 T-helper cells recognize foreign antigens when they are presented in association with Class II major histocompatibility complex antigens, and cytotoxic T cells recognize targets carrying Class I antigen molecules on their cell surface. In vivo studies by Weber, et al.,12 have documented the importance of major histocompatibility complex expression for recognition of tumors by therapeutic TIL's. They conclude that proper major histocompatibility complex antigen expression is probably necessary, but not sufficient, for an effective immune response to a given tumor target.22

Little is known about expression of major histocompatibility complex antigens by animal brain tumors. For this reason, we analyzed expression of these cell-surface determinants on three murine gliomas and examined their potential for up-regulation after exposure to one of five cytokines or cytokine combinations in vitro. If this expression was found to be similar to that observed in patients with gliomas, an animal model would then be available in which to conduct correlative in vivo studies to determine whether major histocompatibility complex antigen up-regulation can bring about increased effectiveness of immunotherapy against brain tumors.

Materials and Methods

Animals and Tumor Cells

Twelve-week-old female C57BL/6 mice were used in all experiments.* Cryopreserved tumor fragments of the murine glioma 26 (GL26), glioma 261 (GL261), and ependymoblastoma A (EpA) cell lines were obtained;† these are primary mouse brain-tumor cells that were induced in the brains of C57BL/6 mice after intracerebral implantation of methylcholanthrene pellets.4 Although these tumor lines do not express glial fibrillary acidic protein positivity (unpublished observations), they grow well after intracerebral inoculation in mice, demonstrate a moderate degree of invasiveness and infiltration, and result in death of the animal from mass effect in approximately 3 weeks. For these experiments, the tumors were grown in the flanks of C57BL/6 mice and maintained by serial subcutaneous transplantation approximately once every 3 weeks.

Preparation of Monolayer Cultures

After the mice were sacrificed by cervical dislocation, the flank tumors were removed and minced using sterile technique. This slurry was stirred in a flask for 2 hours at room temperature in 28 cc of Hanks' balanced salt solution and 5 cc each of deoxyribonuclease, hyaluronidase, and collagenase. The resultant single-cell suspension was filtered through a nylon mesh and counted by trypan blue dye exclusion. One million viable tumor cells were placed in flasks containing 15 ml of complete medium consisting of Dulbecco's minimum essential medium with 10% fetal calf serum, 0.05 mM 2-mercaptoethanol, 0.1 mM nonessential amino acids, 0.1 mM sodium pyruvate, 300 mg/ml L-glutamine, 100 U/ml penicillin, 100 mg/ml streptomycin, and 50 mg/ml gentamicin sulfate. Cells were incubated at 37°C in an atmosphere of 5% CO2. Cells at confluence were subcultured and used in either the second or third passage for experiments.

Experimental Design

One million tumor cells were placed in each of six flasks containing complete medium. No cytokines were added to the first flask. The following cytokines were added to the five remaining flasks: tumor necrosis factor alpha (100 U/ml); gamma interferon (100 U/ml); alpha interferon (1000 U/ml); interleukin-2 (1000 U/ml); and gamma interferon (100 U/ml) plus tumor necrosis factor alpha (100 U/ml).‡ After 48 hours, cells were harvested using standard trypsinization techniques. Each experiment was repeated at least twice.

To determine the duration of up-regulation after cytokine priming, five flasks were prepared with one million GL621 cells. Each was incubated with gamma interferon plus tumor necrosis factor alpha (100 U/ml each). After 48 hours, the cells in one flask were harvested and analyzed for Class I and Class II antigen expression. The media in the other five flasks were removed and replaced with complete media. The cells in these flasks were harvested at 2, 4, 5, 6, and 7 days and analyzed for Class I and Class II antigen expression. This experiment was repeated twice.

Fluorescence-Activated Cell Sorter Analysis

Tumor-cell surface-antigen phenotyping was performed with a fluorescence cell sorter.§ Briefly, 1 × 10⁶ cells were aliquoted per tube and incubated for 30 minutes at 4°C with 10 to 15 μl of the appropriate primary antibody. These cells were then incubated with 10 μl fluorescein isothiocyanate-labeled goat antibodies

* C57BL/6 mice supplied by Small Animal Section, National Institutes of Health, Bethesda, Maryland.
† Cryopreserved tumor fragments supplied by the Division of Cancer Treatment Repository, Frederick, Maryland.
‡ The recombinant cytokines were gifts of the following corporations: interleukin-2, Cetus, Emeryville, California; alpha interferon, Hoffman-LaRoche, Nutley, New Jersey; murine gamma interferon, Genzyme, Boston, Massachusetts; and tumor necrosis factor alpha, Cellular Products, Inc., Buffalo, New York.
§ FACScan fluorescence cell sorter manufactured by Becton Dickinson, Mountain View, California.
to mouse immunoglobulin G (IgG) heavy and light chains and analyzed by flow cytometry. For each flask of experimental cells, four analyses were performed. In the first, no primary antibody was added in order to determine background fluorescence. In the second, an isotype-specific antibody against antigen Ia was added as an irrelevant control to determine the amount of nonspecific staining. In the third, the antibody against Class I antigen was added. In the fourth, the antibody against Class II antigen was added.

**Antibodies**

The antibody against Class I antigens K and D was designated 28-8-6; the antibody against Class II antigen Ia was designated 25-9-17. Both were murine IgG1. Isotype-specific irrelevant antibody was directed against Ia.

**Data Analysis**

To determine the level of major histocompatibility complex expression, the flow cytometry profile of the

**TABLE 1**

Expression of major histocompatibility complex Class I antigens on mouse brain tumor cells before and after treatment with cytokines

<table>
<thead>
<tr>
<th>Cell Line &amp; Cytokine</th>
<th>% Expression</th>
<th>Fluorescence Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Treated</td>
</tr>
<tr>
<td>cell line EpA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-IFN</td>
<td>87 ± 19</td>
<td>20 ± 10</td>
</tr>
<tr>
<td>γ-IFN</td>
<td>87 ± 12</td>
<td>52 ± 6</td>
</tr>
<tr>
<td>TNF-α</td>
<td>82 ± 19</td>
<td>41 ± 17</td>
</tr>
<tr>
<td>TNF-α + γ-IFN</td>
<td>69 ± 42</td>
<td>13 ± 13</td>
</tr>
<tr>
<td>IL-2</td>
<td>82 ± 27</td>
<td>43 ± 12</td>
</tr>
<tr>
<td>cell line GL26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-IFN</td>
<td>68 ± 28</td>
<td>46 ± 15</td>
</tr>
<tr>
<td>γ-IFN</td>
<td>77 ± 32</td>
<td>79 ± 52</td>
</tr>
<tr>
<td>TNF-α</td>
<td>79 ± 33</td>
<td>46 ± 22</td>
</tr>
<tr>
<td>TNF-α + γ-IFN</td>
<td>77 ± 33</td>
<td>97 ± 61</td>
</tr>
<tr>
<td>IL-2</td>
<td>84 ± 22</td>
<td>41 ± 22</td>
</tr>
</tbody>
</table>

* All numbers were rounded off to the nearest integer and are expressed as means ± standard deviations. EpA = murine epidermal-blasteroma A cell line; GL26 = murine glioma 26 cell line; GL26 = murine glioma 26 cell line; α-IFN = alpha interferon; γ-IFN = gamma interferon; TNF-α = tumor necrosis factor alpha; IL-2 = interleukin-2.

† Percentage of cells that constitutively express Class I antigens at baseline and after cytokine treatment.

‡ Mean channel fluorescence before cytokine priming (baseline) and after modulation with cytokines. The cytokine effect was calculated as the mean channel fluorescence after cytokine modulation divided by the mean channel fluorescence before cytokine modulation.

untreated cells was compared to that of unstained control cells. The percentage of cells to the right of the curve for unstained control cells was considered positive for the determinant being examined. A fluorescence of 10 to 100 was considered a low degree of antigen expression, 100 to 200 was considered a medium degree, and 200 to 300 was considered a high degree. 

**TABLE 2**

Expression of major histocompatibility complex Class II antigens on mouse brain tumor cells before and after treatment with cytokines

<table>
<thead>
<tr>
<th>Cell Line &amp; Cytokine</th>
<th>% Expression</th>
<th>Fluorescence Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Treated</td>
</tr>
<tr>
<td>cell line EpA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-IFN</td>
<td>1 ± 1</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>γ-IFN</td>
<td>1 ± 2</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0</td>
<td>7 ± 4</td>
</tr>
<tr>
<td>TNF-α + γ-IFN</td>
<td>1 ± 1</td>
<td>8 ± 6</td>
</tr>
<tr>
<td>IL-2</td>
<td>1 ± 1</td>
<td>5 ± 6</td>
</tr>
<tr>
<td>cell line GL26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-IFN</td>
<td>5 ± 9</td>
<td>7 ± 12</td>
</tr>
<tr>
<td>γ-IFN</td>
<td>4 ± 6</td>
<td>35 ± 25</td>
</tr>
<tr>
<td>TNF-α</td>
<td>4 ± 7</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>TNF-α + γ-IFN</td>
<td>3 ± 2</td>
<td>51 ± 19</td>
</tr>
<tr>
<td>IL-2</td>
<td>6 ± 10</td>
<td>3 ± 2</td>
</tr>
</tbody>
</table>

* All numbers were rounded off to the nearest integer and are expressed as means ± standard deviations. EpA = murine epidermal-blasteroma A cell line; GL26 = murine glioma 26 cell line; α-IFN = alpha interferon; γ-IFN = gamma interferon; TNF-α = tumor necrosis factor alpha; IL-2 = interleukin-2.

† Percentage of cells that constitutively express Class I antigens at baseline and after cytokine treatment.

‡ Mean channel fluorescence before cytokine priming (baseline) and after modulation with cytokines. The cytokine effect was calculated as the mean channel fluorescence after cytokine modulation divided by the mean channel fluorescence before cytokine modulation.

**Constitutive Expression of Major Histocompatibility Complex Antigens**

Expression of Class I and Class II antigens was similar among the three glioma lines we examined (Tables 1 and 2). The percentages (means ± standard deviations)
Major histocompatibility antigen expression on brain tumors

Fig. 1. *In vitro* effects of cytokines on tumor cell major histocompatibility complex Class I antigen expression (left) and Class II antigen expression (right). Tumor cells were prepared as described. Flow cytometry profiles are shown with fluorescent channel number measured logarithmically on the x axis and cell number units on the y axis. Profiles are shown for untreated cells (solid line) and cells treated with alpha interferon (α-IFN, dotted line), gamma interferon (γ-IFN, dotted/dashed line), or tumor necrosis factor alpha (TNF) plus γ-IFN (dashed line). Mean channel fluorescence values are shown for untreated cells and for cells treated with α-IFN, γ-IFN, or TNF + γ-IFN.

Effects of Cytokines on Class I Antigen Expression

Tumor necrosis factor alpha and interleukin-2 had no effect on Class I antigen expression in any cell line, although in occasional experiments there was a minimal effect (Table 1 and Fig. 1 left). Alpha interferon caused substantial up-regulation in all cell lines. In experiments where untreated cells did not express Class I antigens, alpha interferon induced expression in most of the cells. In one experiment with GL26 cells, 62% of untreated cells expressed Class I antigens; after treatment with alpha interferon, 96% of cells expressed this molecule. In cell lines that already expressed Class I antigen, incubation with alpha interferon doubled or tripled the MCF. In EpA, GL261, and GL26 cells, treatment with alpha interferon increased MCF from 20 ± 10 to 52 ± 34, 14 ± 6 to 46 ± 15, and 26 ± 11 to 50 ± 17, respectively. Gamma interferon induced a modest, but significant, mean increase of Class I antigen expression of 1.7 ×, 1.5 ×, and 2.1 × control values for the EpA, GL26, and GL261 cell lines, respectively. The addition of tumor necrosis factor alpha to gamma interferon augmented the up-regulating effect of gamma interferon on EpA cells.

Effects of Cytokines on Class II Antigen Expression

Tumor necrosis factor alpha, alpha interferon, and interleukin-2 had no effect on the expression of the Class II antigen determinant in any cell line (Table 2 and Fig. 1 right). For EpA cells, gamma interferon caused the fluorescence intensity to increase to 1.9 × control. The addition of tumor necrosis factor alpha to gamma interferon increased this up-regulation to a mean of 4.6 × control. In numerous experiments, there was no Class II antigen expression in control cells, but treatment with tumor necrosis factor alpha plus gamma interferon augmented Class II antigen expression in up to 25% of cells. For GL26 cells, the anti-Class II antigen fluorescence intensity of cells treated with gamma interferon or tumor necrosis factor alpha plus gamma interferon increased 14- to 17-fold. In two of three experiments, there was no pretreatment expression of the Class II antigen; however, after treatment with gamma interferon, 73% of cells expressed this determinant to a low degree (MCF 38 to 58). The addition of tumor necrosis factor alpha to gamma interferon caused 85% of the cells to express major histocompatibility complex Class II antigen, suggesting a minimal additive effect. The results for GL261 cells were similar to those for the GL26 cells, although not as extreme.

*J. Neurosurg.* / *Volume 75* / *December, 1991* 925
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FKJ. 2. Glioma GL261 cell-line kinetics of induction of Class I antigen expression (left) and Class II antigen expression (right) by tumor necrosis factor alpha plus gamma interferon. Flow cytometry analysis was performed using monoclonal antibody 28-8-6 to measure Class I expression and monoclonal antibody 25-9-17 to measure Class II expression. Mean channel fluorescence from the flow cytometry profile of each time point is plotted on the y axis and time is plotted on the x axis.

Duration of Effects

After 48 hours of GL261 cell treatment with tumor necrosis factor alpha plus gamma interferon, the MCF of Class I antigen increased to 62. After the cytokines had been removed, this MCF continued to increase to 100 at 4 days, but had diminished to 75 at 7 days (Fig. 2 left). After 48 hours of treatment, the MCF of Class II antigen increased from less than 5 to 56. After cytokine removal, this decreased rapidly to an MCF of 20 at 4 days and returned to baseline at 6 days (Fig. 2 right). This experiment was repeated twice with similar results (data not presented).

Discussion

Terminology

The majority of cells in the body have antigens on their surfaces that are unique to each person. These are called “major histocompatibility antigens” and were discovered when research was conducted to determine why the immune system of one individual will recognize and attempt to destroy tissue transplanted from another individual. The two main groups of major histocompatibility antigens are the Class I and Class II antigens. It has been discovered recently that the recognition and destruction of tumor cells by the immune system require expression of these antigens on their surfaces. Additional work has determined that, when tumor cells do not have these antigens on their cell membranes, one can “up-regulate” expression with naturally occurring proteins (cytokines) such as gamma interferon, alpha interferon, and tumor necrosis factor alpha. The objective of this study was to define the normal (or constitutive) expression of these antigens on the surface of murine glioma cells and to determine if this expression could be increased with cytokines.

Major Histocompatibility Antigen Expression on Glioma Cells

We observed that the majority of mouse glioma cells expressed low levels of major histocompatibility complex Class I antigens and no detectable Class II antigens. We could find no reports on major histocompatibility complex expression in rat, mouse, or other animal brain tumors with which to compare our findings. Piguet, et al., established 10 human malignant glioma cell lines in vitro and found that all strongly expressed human leukocyte antigen (HLA)-A, HLA-B, and HLA-C (Class I), but only three were HLA-DR (Class II)-positive. In another study by Piguet, et al., nine of 22 established human glioma cell lines were HLA-DR-positive. Saito, et al., performed immunohistochemical analyses on tissues from 14 patients with malignant gliomas: all had intense staining for b-2 microglobulin (Class I); six of 14 specimens were positive for HLA-DR; four were weakly positive, and four were negative. Therefore, our findings in mice correspond with those in humans. Class I antigen expression is usually present, while Class II antigen expression is weak or absent.

Effect of Gamma Interferon

In our experiments, gamma interferon had an up-regulating effect that was modest on Class I antigens and profound on Class II antigens. We could find no previous reports on the modulating effect of gamma interferon on rodent brain tumors, although numerous investigators have studied the effect of this cytokine on normal brain cells both in vitro and in vivo. Wong, et al., noted that there were no detectable Class I or II antigen determinants on the astrocytes of newborn mice; however, after incubation with gamma interferon in vitro, all cells expressed a moderate amount of Class I antigen and 10% of cells expressed low levels of Class II antigen. The findings of Fierz, et al., disagree with regard to Class II antigen modulation. They prepared astrocytes from the brains of Lewis rats and demonstrated la antigen expression (Class II) after incubation with 10 U/ml of gamma interferon. Additionally, they demonstrated that only after this modulating effect appeared could these astrocytes present antigen to activated lymphocytes. Pulver, et al., also demonstrated an up-regulating effect on la antigens when human fetal...
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A small, but significant, increase of Class I antigen expression and no effect on Class II antigen expression. Since alpha interferon administered with interleukin-2 causes a synergistic effect in the regression of murine tumors outside the central nervous system, we treated brain-tumor cells with interleukin-2 and measured the effect on Class I and Class II antigen expression. Interleukin-2 had no effect on either.

Biological Relevance

The results of our study demonstrate a variable and often dramatic effect of different cytokines on major histocompatibility complex antigen expression by gliomas in vitro. A similar modulating effect in vivo requires that the cytokine be delivered to the tumor. Chemical characteristics that favor such penetration are a small molecule size, lipophilicity, low molecular binding to serum proteins, and carrier-mediated transport. The cytokines of interest (alpha interferon, gamma interferon, and tumor necrosis factor alpha) are relatively large proteins, and we are not aware of studies investigating their delivery into brain tumors. Penetration of proteins into the cerebrospinal fluid (CSF) may reflect penetration into the brain, and Collins observed that only 1% of plasma alpha interferon (19,000 daltons) penetrated into the CSF of normal rhesus monkeys. The delivery of interleukin-2 (15,000 daltons) was examined in patients receiving treatment for extracranial cancer. At 4 hours, interleukin-2 levels began to rise in the CSF to as high as 9 U/ml and remained so until the end of therapy, at which time the level decreased over about 8 hours until it was undetectable. These reports suggest that the delivery of cytokines into normal brain is low or negligible. Although the permeability of brain-tumor vessels is increased compared to normal blood vessels, it is unclear whether this would increase cytokine delivery to levels necessary to affect major histocompatibility complex antigen expression.

If adequate cytokine delivery to brain-tumor cells occurred, it is unclear if this would cause modulation of major histocompatibility complex antigen expression in the milieu of the brain and, if so, if it would alter clinical responses to immunotherapy. Weber and Rosenberg studied the effect of cytokines on pulmonary metastases in situ in C57BL/6 mice. In one study, immunohistochemical analysis demonstrated that the modulating effects observed in vitro were similar to those that could be demonstrated in vivo. In a second study, Weber et al. showed no efficacy when interleukin-2 and tumor-infiltrating lymphocytes were used to treat pulmonary metastases that expressed low levels of Class I antigens. However, when these metastases were transfected with a gene encoding for Class I antigens, the pulmonary metastases were reduced or eliminated with TLI's and interleukin-2. These data suggest that major histocompatibility complex antigen expression and modulation observed in vitro also occur in vivo, and that expression of major histocompatibility

Effect of Tumor Necrosis Factor Alpha

The addition of tumor necrosis factor alpha to gamma interferon did not add significantly to the up-regulating effect on Class I antigen, and only in one of three glioma lines (EpA) did it add substantially to the up-regulating effect of Class II antigen. We studied the effects of these two cytokines together because of their well-known synergism in the lysis of tumor cells. Fransen et al. observed that tumor necrosis factor alpha plus gamma interferon is far more effective in destroying human breast, cervix, and colon carcinomas in vitro than tumor necrosis factor alpha alone. This was a direct effect rather than mediated by lymphoid effectors, and the modulation of major histocompatibility complex antigens was not studied. Weber and Rosenberg investigated the modulating effect of these cytokines on mouse sarcomas of varying immunogenicity. Their findings concur with ours in regard to Class I antigens, but they observed no induction of Class II antigens with gamma interferon alone or with tumor necrosis factor alpha plus gamma interferon combined.

Effect of Alpha Interferon and Interleukin-2

In our glioma cell lines, most cells constitutively expressed Class I antigens, and alpha interferon substantially increased this expression. In contrast, there was no baseline expression of Class II antigens, and alpha interferon did not cause its induction. We could find no previous reports of the effect of alpha interferon on animal brain tumors, but these findings generally agree with studies of other normal and neoplastic tissues. Wong et al. treated the astrocytes of 2-day-old CBA mice with 200 U/ml of interferon-alpha/beta; there was induction of Class I antigen expression in 50% of cells, whereas there was no induction of Class II antigen expression. They suggested that the modulating effect of gamma interferon was 200-fold greater than alpha interferon in induction of Class I antigen. Similarly, Piquet et al. incubated six human glioma cell lines with 5000 U/ml of alpha interferon for 4 days. There was increased expression of antigens HLA-A, HLA-B, and HLA-C in three of the six cell lines, but no effect on HLA-DR. Weber and Rosenberg treated four mouse sarcoma lines with 1000 U/ml and observed

astrocytes were treated with gamma interferon. Piquet et al. treated 10 human glioma cell lines with gamma interferon and observed that Class I antigen expression (already present in all cell lines) was enhanced in seven and Class II antigen expression was induced in three. Thus, our observations are similar to the results of previous investigations of normal astrocytes and human gliomas except that the induction of Class II antigens in the three mouse gliomas was more consistent and extensive.
complex antigens may be a necessary, but not sufficient, condition for mounting an effective immune response against an in situ tumor.

Time Course of Up-Regulation

The kinetic data on the duration of major histocompatibility complex antigen up-regulation in vitro were surprising in that Class I antigen expression rose rapidly, then continued to rise for 3 days after the cytokine was removed. Weber and Rosenberg studied the kinetics of major histocompatibility complex antigen induction in subcutaneous mouse sarcomas after parenteral treatment with alpha interferon. They also observed that the up-regulation of Class I antigen peaked 2 days after the end of treatment, then decayed to baseline over 4 to 7 days. Class II antigens also rose rapidly during treatment, but then rapidly decayed to baseline levels within 4 days. Although in vitro studies are necessary, these results suggest that frequent injections of modulating cytokines would be necessary to maintain increased Class II antigen expression. Less frequent treatments would be necessary for maintenance of increased Class I antigen expression.

Future Research

These findings provide an animal model for the in vitro study of brain-tumor major histocompatibility complex antigen expression and its potential relevance to the treatment of patients with brain tumors. The mouse brain tumors that we studied are similar to humans in regard to Class I and II antigen expression and respond similarly to cytokine treatment. These tumor cells are easily injected into the brains of C57BL/6 mice and result in death of the animal in approximately 3 weeks from an enlarging intracerebral mass. Future investigations using this system should shed light on the effect of cytokines on the lysis of modulated tumor cells by activated lymphocytes in vitro, their effect on brain-tumor major histocompatibility complex antigen expression in situ, and the effect of major histocompatibility complex antigen up-regulation (if present) on the effectiveness of immunotherapy against brain tumors in vivo.

Acknowledgments

The authors gratefully acknowledge the assistance of Jeffrey Weber, M.D., of the Surgical Oncology Branch of the National Cancer Institute, Bethesda, Maryland, for his help in experimental design and review of the manuscript.

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Manuscript received November 9, 1990.
Accepted in final form April 30, 1991.
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