Immunohistochemical Analysis of Tumor-infiltrating Lymphocytes and Adhesion Molecules (ICAM-1, NCAM) in Human Gliomas

Ryuya YAMANAKA, Ryuichi TANAKA, and Takafumi SAIITO

Department of Neurosurgery, Brain Research Institute, Niigata University, Niigata

Abstract

The presence of tumor-infiltrating lymphocytes (TILs) and the expression of adhesion molecules were examined in 12 glioma tissues. Most TILs were T lymphocytes, and both cytotoxic/suppressor and helper/inducer T lymphocyte phenotypes were found. Neural cell adhesion molecule was intensely expressed in 11 gliomas, and intercellular adhesion molecule-1 (ICAM-1) in seven. Many TILs were found only in gliomas expressing ICAM-1, but there was no relationship with clinical course in the patients.

Key words: immunohistochemistry, intercellular adhesion molecule-1, neural cell adhesion molecule, T lymphocyte subset, tumor-infiltrating lymphocyte

Introduction

Cell adhesion molecules are important in many physiological processes involving cell-to-cell and viral-cellular interactions. Particularly in immunological reactions, many cell adhesion receptors contribute to the formation of intercellular contacts.

The lymphocyte function-associated antigen-1 (LFA-1) is a member of the integrin family of cell surface receptors and is found on a number of immunological cell types, including B and T lymphocytes, natural killer (NK) cells, granulocytes, monocytes, and macrophages. The intercellular adhesion molecule-1 (ICAM-1) is a ligand for the LFA-1 adhesion receptor, and is present in both hematopoietic and other cell types, including vascular endothelium, fibroblasts, squamous cell carcinoma, colon carcinoma, hepatoma, and melanoma. The interaction of LFA-1 and ICAM-1 is an important component of leukocyte adhesion in various immunological and inflammatory reactions, and may contribute to the localization of leukocytes in tumors. In addition, ICAM-1 is involved in specific viral-cellular interactions, since it is the major cell surface receptor for rhinoviruses.

Neural cell adhesion molecules (NCAMs) are a family of closely related cell surface glycoproteins believed to mediate cell-to-cell interactions via a homophilic binding site in a Ca²⁺-independent manner. NCAM is a ligand of NK cells and is important in tumor cell-NK cell interactions. NCAM is also expressed in some human neuroectodermal tumors.

ICAM-1 and NCAM are expressed with high frequency in many malignant tumors of non-lymphoid origin. Expression is modulated on both normal and malignant cells by a number of cytokines, including interleukin-1, interferon-γ, and tumor necrosis factor-α. The role of ICAM-1 and NCAM in the biology of tumor cells is not presently known. However, the involvement of ICAM-1 and NCAM in cell-to-cell interactions, the ability of anti-ICAM-1 monoclonal antibodies (MAbs) to inhibit the lysis of tumor cells by cytotoxic T cells and likewise anti-NCAM MAbs inhibiting lysis by NK cells, and the significantly higher expression of ICAM-1 in metastases than in primary melanoma lesions suggest that these molecules may regulate the interaction of malignant cells with the host immune system and possible involvement in the metastatic spreading of tumor cells.

The present study examined biopsy specimens of glioma tissues by the avidin-biotin-peroxidase complex (ABC) method using MAbs to lymphocyte...
differentiation antigens and to NCAM and ICAM-1 to investigate the phenotypes of tumor-infiltrating lymphocytes (TILs) and expression of adhesion molecules in gliomas.

**Materials and Methods**

Surgical specimens of 12 gliomas were used. Table 1 shows the histological diagnoses of these tumors. Tissue from the normal frontal lobe of a 19-year-old male who died of cardiopulmonary insufficiency after a traffic accident removed soon after death was used as the control. All samples were snap frozen in OCT compound (Miles Scientific, Division of Miles Laboratory, Naperville, Ill., U.S.A.) and stored at −70°C until use.

This study used mouse MAbs to Leu-1 (pan T lymphocyte), Leu-2a (cytotoxic/suppressor T lymphocyte), Leu-3a (helper/inducer T lymphocyte), Leu-12 (pan B lymphocyte), ICAM-1, and NCAM (Becton Dickinson & Co., Sunnyvale, Cal., U.S.A.).

The frozen sections were immunostained by the ABC method. Frozen sections, 5 μm thick, were air-dried, fixed in acetone for 10 minutes, and incubated with normal horse serum at 4°C overnight. Sections were then incubated with MAbs diluted appropriately in the range of 1:20 to 1:160 for 60 minutes, with 1:50 diluted biotinylated horse anti-mouse immunoglobulin G (IgG) (Vector Laboratories, Inc., Burlingame, Cal., U.S.A.) for 30 minutes, and with 1:300 diluted ABC (Vector Laboratories, Inc.) for 30 minutes. The peroxidase reaction was performed by incubating sections for 10 minutes in 100 ml of 0.05 M Tris-HCl buffer, pH 7.5, containing 20 mg of 3,3'-diaminobenzidine tetrahydrochloride and 0.005% hydrogen peroxide. Sections were then counterstained lightly with hematoxylin. Normal mouse IgG1 or IgG2a was used instead of the primary antibodies in the control specimen.

The reactivity of the antibodies was evaluated by the ABC method using frozen sections of human tonsil. Quantitative analysis of TILs counted positive cells on micrographs of the serial sections stained for the lymphocyte differentiation antigens.

**Results**

Examination of normal brain tissue showed no positive reaction in the neurons and glial cells for any of the lymphocyte differentiation antigens. The majority of glial cells were weakly stained by NCAM antibody but not by ICAM-1 antibody. The blood vessel walls were intensely stained by ICAM-1 antibody (data not shown).

A variable number of TILs was found in seven of the 12 glioma specimens, mainly in the perivascular area. There were no or only a few TILs in the tumor tissues in the other five. Most TILs were stained with Leu-1 antibody (Fig. 1A), but no or only a few cells were stained with Leu-12 antibody (Fig. 1D). Both Leu-2a-positive and Leu-3a-positive cells were found in the T lymphocytes (Fig. 1B, C). Table 2 shows the counts of positive cells in gliomas. The Leu-3a/2a ratios estimated in the seven specimens ranged from 0.3 to 1.3. Leu-2a-positive cells were predominant in four specimens, and Leu-3a-positive cells in three.

The great majority of tumor cells were distinctly stained with NCAM antibody in 11 of the 12 glioma specimens (Table 2, Fig. 2). Various staining patterns with ICAM-1 antibody were observed (Table 2). Most tumor cells were stained in four specimens (Fig. 3A), stained and unstained neoplastic areas were observed in three, and the entire neoplastic area was unstained in the other five (Fig. 3B).

Table 2 shows that among the seven specimens expressing ICAM-1 antigens, all had many TILs with Leu-3a/2a ratios ranging from 0.3 to 1.3. The five specimens negative for ICAM-1 included only a few TILs.

**Discussion**

The presence of TILs has been examined immunohistochemically in a variety of human malignant tumors including malignant melanoma, ovarian neoplasms, and breast carcinomas. Most TILs are T lymphocytes and both cytotoxic/suppressor and helper/inducer T lymphocyte phenotypes are present. The correlation between the presence of TILs and the expression of major histocompatibility an-
Fig. 1  Micrographs showing immunostaining for lymphocyte differentiation antigens on TILs in a glioma (Case 5). Serial sections were stained with anti-Leu-1 (A), anti-Leu-2a (B), anti-Leu-3a (C), and anti-Leu-12 (D). Light counterstaining with hematoxylin, ABC method, ×250.

Fig. 2  Micrograph showing immunostaining for NCAM on tumor cells. Positive glioma (Case 1): most tumor cells are stained. Faint nuclear staining is due to hematoxylin counterstaining. ABC method, ×250.

Table 2  Immunostaining for lymphocyte differentiation antigens on TILs and adhesion molecules on tumor cells in gliomas

<table>
<thead>
<tr>
<th>Case No.</th>
<th>No. of immunostained TILs*</th>
<th>Ratio of Leu-3a/2a</th>
<th>Stained tumor cells**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leu-1 Leu-12 Leu-2a Leu-3a</td>
<td></td>
<td>ICAM-1 NCAM</td>
</tr>
<tr>
<td>1</td>
<td>254 1 160 56</td>
<td>0.4</td>
<td>+ +</td>
</tr>
<tr>
<td>2</td>
<td>36   5 18 6</td>
<td>0.3</td>
<td>+ +</td>
</tr>
<tr>
<td>3</td>
<td>61   2 23 18</td>
<td>0.8</td>
<td>± +</td>
</tr>
<tr>
<td>4</td>
<td>&lt;10</td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>476  2 190 220</td>
<td>1.2</td>
<td>+ +</td>
</tr>
<tr>
<td>6</td>
<td>&lt;10</td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2504 1 678 859</td>
<td>1.3</td>
<td>+ +</td>
</tr>
<tr>
<td>8</td>
<td>&lt;10</td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>151 0 78 46</td>
<td>0.6</td>
<td>± +</td>
</tr>
<tr>
<td>10</td>
<td>&lt;10</td>
<td>- +</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>189 0 58 73</td>
<td>1.3</td>
<td>± +</td>
</tr>
<tr>
<td>12</td>
<td>&lt;10</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*<10: no or only a few TILs were found. **+: positive staining, ±: positive and negative staining, -: negative staining.

Adhesion Molecules on the Glioma Cell

Table 2  Immunostaining for lymphocyte differentiation antigens on TILs and adhesion molecules on tumor cells in gliomas

=tions on tumor cells has also been investigated. However, only a few reports have investigated the immunological nature of TILs in brain tumors. Von Hanwehr et al. observed TILs in four of six gliomas, finding some lymphocytes showed Leu-2a predominance whereas others did not. Saito et al. showed a correlation between the number of TILs

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and major histocompatibility class II antigen expression on tumor cells. Gliomas were evenly divided into human leukocyte common antigen (HLA)-DR-positive cases with many TILs and HLA-DR-negative cases with few TILs.

Most TILs in our glioma specimens were T lymphocytes, and both cytotoxic/suppressor and helper/inducer T lymphocyte phenotypes were observed, supporting the previous studies. B lymphocytes formed only minor or negligible components in the TIL population. Leu-3a/2a ratios ranged from 0.3 to 1.3, and were usually low compared to that of 1.8 ± 0.6 in peripheral blood lymphocytes. This suggests that TIL infiltration had some immunological mechanism rather than simple extravasation from the blood flow.

Expression of adhesion molecules may be connected with TIL infiltration into tumors. Tomita et al. reported that renal cell carcinoma with a higher degree of mononuclear cell infiltration expressed ICAM-1 more frequently and intensely. Our study showed that adhesion molecules were frequently demonstrated in our glioma specimens. Eleven of the 12 gliomas showed intense positive staining for NCAM, and seven of the 12 showed ICAM-1-positive staining as well.

ICAM-1 is clearly of major importance in interactions between the target cell and cytotoxic T lymphocyte. The TILs observed in our study may have been immunologically related to the tumor cells through immunoochemical alteration of the ICAM-1 antigen. We therefore analyzed the relationship between TILs and expression of ICAM-1 antigens on the tumor cells. Seven glioma specimens were ICAM-1 positive with many TILs and five were ICAM-1 negative with few TILs. There is clearly a correlation between the number of TILs and ICAM-1 antigen expression on tumor cells. In glioma patients, ICAM-1-positive tumors were always accompanied with many TILs. However, there was no apparent relationship between the histological diagnosis, clinical course, and these results (Table 2). Further studies including polymorphic determinations of ICAM-1 and the relationship between T lymphocyte function and surface antigens would be of value in proving or disproving the presence of a local immune response against brain tumors.

Acknowledgments

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Address reprint requests to: R. Yamanaka, M.D., Department of Neurosurgery, Brain Research Institute, Niigata University, 1–757 Asahimachi-dori, Niigata 951, Japan.