Immunoprecipitation of a M, 64,000 Glial Tumor-associated Antigen by Monoclonal Antibody 217c

Stephen J. Luner and Jean de Vellis

ABSTRACT

Monoclonal antibody 217c binds to a tumor-associated surface antigen of transformed rat glial cells. Treatment of C6 glioma cells with 2.5% 1-butanol yielded an extract which was active in competitive inhibition of antibody 217c to cell monolayers in an 125I-protein A assay as well as in binding antibody 217c in an enzyme-linked immunodot assay. The antigen, however, was not released in soluble form, but in a particulate fraction which could be pelleted by ultracentrifugation for 2 h at 120,000 × g. Antibody binding activity in the immunodot assay could be destroyed by heating the extract to 100°C for 10 min.

To determine the molecular weight of the antigenic polypeptide, cell monolayer cultures were surface radioiodinated and extracted with Nonidet P-40. Immobilized antibody 217c bound only a single labeled polypeptide with a molecular weight of 64,000 as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. This surface peptide was present in the C6 glioma line as well as in oligodendrocyte and astrocyte cultures transformed either spontaneously or using ethylnitrosourea. It was absent from normal astrocyte and oligodendrocyte cultures of neonatal rat brain. In the glial lines studied the P-64 peptide appears as a surface marker indicating malignant transformation.

INTRODUCTION

While the existence of tumor-associated antigens was first demonstrated by classical serology and cellular immunology, it has been the introduction of the hybridoma technique for monoclonal antibody production that has yielded a wide variety of specific reagents for the detection and characterization of individual molecules associated with malignant transformation. Among tumors of neuroectodermal origin the greatest progress to date has been in the identification of melanoma cell surface peptide antigens by immunoprecipitation of detergent extracts of radiolabeled cells followed by SDS-PAGE of the precipitates (1–3). Other antigens not producing bands by this procedure and not losing activity upon heating have been identified as glycolipids (1, 2).

Glial tumors have been shown to share some of the tumor-associated antigens found on melanoma cells since they bind monoclonal antibodies originating from melanoma-immunized mice (4, 5). Additional human glioma-associated antigens have been defined using monoclonal antibodies derived either from the immunization of mice with human glioma cell lines (6, 7) or from the fusion of intratumoral lymphocytes from glioma patients with a human myeloma line (8).

The 217c monoclonal antibody was developed starting with the immunization of BALB/c mice with the C6 established rat glioma line and has been shown to bind to 3 of 3 rat glial tumor cell lines tested, but not to normal rat tissues or glial cell cultures (9, 10). Three additional monoclonal antibodies have recently been developed against chemically induced rat glioma lines (11).

The present investigation of the antigen against which antibody 217c is directed first tested its extractability from viable cells by 2.5% butanol and then went on to the immunoprecipitation of the peptide antigen from a Nonidet P-40 extract of surface-labeled cells.

MATERIALS AND METHODS

Cell Cultures. Cell lines, all of rat origin, were the C6 established glioma line, a line of oligodendrocytes transformed using ethylnitrosourea (TOP-ET-1), spontaneously transformed astrocytes (BD-ST-1), and nontransformed astrocytes (BD-N-2) (10, 12). Separate primary cultures were prepared essentially as described (13) using shaking to separate the two glial cell populations. Cells were grown in a mixture (1:1) of Dulbecco’s modified essential medium and Ham’s F-12 medium supplemented with 10% fetal bovine serum.

Antigens. The 217c clone (10) was grown either in RPMI 1640 with 10% fetal bovine serum or in Dulbecco’s-Ham’s medium (1:1) supplemented with insulin, transferrin, ethanolamine, and selenium (14), taking culture supernatants as the antibody source. Control immunoglobulin was mouse IgG from Sigma Chemical Co., St. Louis, MO.

Butanol Extraction. Monolayer cultures of C6 glioma cells or primary astrocytes were incubated for 3 h in serum-free medium, then washed 4 times with phosphate-buffered saline, and removed from the flasks using Versene. After an additional saline wash, the cell pellet was extracted (15) with 10 volumes of 2.5% 1-butanol in phosphate-buffered saline for 5 min at 22°C with gentle pipetting. The cells were pelleted with a 5-min low speed centrifugation, and a portion of the low speed supernatant was centrifuged at 120,000 × g for 2 h in the Beckman 40 rotor. Cell viability by trypan blue exclusion remained greater than 85%. Extracts were dialyzed against DPBS and protein content was determined (16).

Immunodot Assay. Following a modification of a described technique (17), 1-µl volumes of extracts were dotted onto strips of Schleicher and Schuell BA85 nitrocellulose membrane which were then blocked for 15 min in 3% bovine serum albumin in TBS, pH 7.4, containing 10% fetal bovine serum. After a 2-h incubation in a 3:1 mixture of 217c supernatant plus blocking solution the strips were washed in TBS on a rotator for 20 min.

Received 8/21/85; accepted 10/29/85.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Operated for the United States Department of Energy by the University of California under Contract DE-AC0376-SF00012. This article was supported by the Director of the Office of Energy Research, Office of Health and Environmental Sciences, Mental Retardation Research Center, Brain Research Institute, Departments of Anatomy and Psychiatry, UCLA School of Medicine, University of California, Los Angeles, California 90024 [J. de V.]

2 To whom requests for reprints should be addressed, at Laboratory of Biomedical and Environmental Sciences, Mental Retardation Research Center, Brain Research Institute, Departments of Anatomy and Psychiatry, UCLA School of Medicine, University of California, Los Angeles, California 90024 [S. J. L.]

3 The abbreviations used are: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; DPBS, Dulbecco’s phosphate-buffered saline; TBS, Tris-buffered saline.
The observation that antibody-binding activity in the 2.5% butanol extract was pelleted by ultracentrifugation at 12,000 x g suggests that the antigen was released from C6 cells as vesicles rather than in soluble form. This would be consistent with the observation that the extract gave a pattern of Coomassie blue staining of the bands characteristic of astrocytes or oligodendrocytes, all of which are negative in the protein A assay.
immunoprecipitation of glial tumor antigen

Fig. 3. Autoradiograph of SDS-PAGE under reducing conditions of an immunoprecipitate with antibody 217c of a Nonidet P-40 extract of surface radioiodinated C6 cells.

Table 1

<table>
<thead>
<tr>
<th>Cells</th>
<th>Antibody</th>
<th>Mouse IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6</td>
<td>356 ± 42</td>
<td></td>
</tr>
<tr>
<td>TOP-ET-1</td>
<td>256 ± 24</td>
<td></td>
</tr>
<tr>
<td>BD-ST-1</td>
<td>332 ± 23</td>
<td></td>
</tr>
<tr>
<td>BD-N-2</td>
<td>350 ± 39</td>
<td></td>
</tr>
<tr>
<td>Primary astrocytes</td>
<td>274 ± 2</td>
<td></td>
</tr>
<tr>
<td>Primary oligodendrocytes</td>
<td>317 ± 83</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± SD of triplicate assays.

ACKNOWLEDGMENTS

We thank Ruth Cole for providing primary glial cultures and Yutaka Kobayashi, Shalini Kumar, Sheila Scully, and Willow Peng for helpful discussions.

REFERENCES

Immunoprecipitation of a $M_r$ 64,000 Glial Tumor-associated Antigen by Monoclonal Antibody 217c

Stephen J. Luner and Jean de Vellis


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/46/2/863

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/46/2/863.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.