Review Article

Immunological and biochemical strategies for the identification of brain tumor-associated antigens

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Various strategies have been used to identify and characterize the antigens associated with human brain tumors. These approaches have included the raising of polyclonal and monoclonal antibodies against tumor antigens and, more recently, efforts toward the direct biochemical identification of such proteins. This review summarizes the progress made in this area, suggests reasons for the broad antigenic cross-reactivity and heterogeneity revealed by these studies, and proposes additional methods for deciphering the complex antigenic composition of human brain tumors.

KEY WORDS - brain neoplasm □ tumor marker □ monoclonal antibody □ brain-neoplasm antigen

Because of potential diagnostic and therapeutic applications, the quest for brain-tumor markers that are specific for central nervous system (CNS) malignancy has received considerable attention over the past 15 years. A tumor marker may be defined as a cellular or viral product that is either unique to, or present in higher concentrations in, transformed cells as compared to normal cells of the same lineage. Truly specific tumor markers that are present only on neoplastic cells are neoantigens, of which the T-antigens encoded by DNA (deoxyribonucleic acid) tumor viruses serve as some of the best examples. Such a marker may be a polypeptide, a carbohydrate, a glycolipid, or a glycoprotein. Certain tumor markers have been found to be related to fetal or differentiation antigens. Such antigens are expressed in greater abundance in cells of common embryological ancestry, yet can vary depending upon the stage of differentiation.

Almost 15,000 cases of primary CNS tumors occur each year in the United States. Despite advances in radiotherapy and the development of chemotherapeutic agents, the survival rate for most malignant brain tumors remains poor. The 2-year survival rate for glioblastoma multiforme is less than 15% to 20%. Thus, there is still an urgent need to develop alternative approaches for the treatment of these malignancies. One avenue of great promise is to 1) identify markers or a panel of markers reliably associated with the neoplasm, and 2) employ conjugated polyclonal or monoclonal antibodies directed against these antigens to deliver radioisotopes, drugs, or cytotoxic agents selectively to the tumor cells. This approach may yield a more specific delivery system for drugs, as well as immunological marker assays permitting the earlier diagnosis of suspected primary or recurrent neoplasms, at which time existing conventional treatments would have a greater likelihood of success because of a smaller tumor burden. Moreover, the specific immunological reagents developed against tumor-associated markers could lead to a more detailed biochemical and molecular characterization of tumor antigens, and possibly provide further insight into their relationship to CNS oncogenesis, growth, and progression. The study of membrane surface antigens may provide clues regarding the influence...
The earliest attempts to identify glioblastoma antigens were those of Siris'104 and Hass,15 who prepared antisera against an alcohol extract of glioblastoma tissue and a solubilized glioblastoma homogenate, respectively. In each case, however, substantial reactivity was present against normal brain antigens. The use of autogenous glioma implants to stimulate patients' immunological response against their own tumors was also unsuccessful in generating reactive sera.42 However, the pioneering clinical studies of Mahaley and colleagues31,67,68 demonstrated that radiiodinated rabbit antihuman glioma antibodies infused in the internal carotid artery could localize preferentially to recurrent glioma cells as measured in tissue fractions, autoradiography, and external brain scans. This provided considerable impetus to develop other polyclonal reagents that could potentially identify brain-tumor markers and thus aid in diagnosis, screening for recurrence, and hopefully immunotherapy, particularly at a time when other laboratories were reporting altered cell-mediated immunity in CNS tumor patients and the possible existence of blocking factors.14,60

An early and novel approach involved autoimmune immunization of patients with saline extracts of their own gliomas to produce human anti-glioblastoma sera that detected an alpha-lipoproteinacious carcino-fetal glial antigen (CFGA) which, however, was subsequently found to be also present in fetal brain (Table 1).176 The existence of blocking factors.14,60

The preparation of rabbit heteroantisera against lyophilized glioblastoma tissue and the astrocytoma line 301, which after extensive absorptions possessed little or no cross-reactivities against normal cells yet reacted with a very high percentage of glioma or astrocytoma lines, lent support to the possibility of tumor-specific markers.25,111 The existence of glioma-specific membrane antigens and a human astrocytoma-associated antigen (HAAA) was thus proposed (Table 1).25,28,111

However, rabbit antisera made in other laboratories against glioma extracts or homogenates cross-reacted with normal fetal brain or adult liver.36,32,74 Rabbit heteroantisera specific for medulloblastoma tumor-associated surface antigens (TSA) were also reported, but these sera were not adequately tested against fetal brain or against other fetal tissues.91 Nevertheless, later preparations of rabbit antisera generated against membranes of glioma tissue and a glioma line still reacted with glioma cells after absorptions with normal tissues including brain,8,93 and identified strong candidates for glioma-related polypeptides at 70, 55, 30, and 10 kD.8

of these antigens upon cell-cell recognition, interaction, and immunosurveillance. Similarly, the characterization of nuclear antigens may help elucidate the underlying processes of genetic regulation and organization.

This review addresses the advances achieved in identifying and characterizing brain tumor-associated antigens. In the interests of brevity, the polyclonal and monoclonal reagents generated so far by various laboratories working in the field have been concisely presented in tabular form, and some of their distinctive and potentially useful reactivities have been summarized graphically. The strategies of immunological as well as direct biochemical identification are discussed, and additional novel genetic approaches are proposed.

### Definitions of Abbreviations

- A = astrocytoma
- AB = adult brain
- AJ-aut = AJ-autologous antibody
- AJ-nat = AJ-natural antibody
- ALL = acute lymphoblastic leukemia
- C3 = 3rd component of complement
- CALLA = common acute lymphoblastic leukemia antigen
- CFGA = carcino-fetal glial antigen
- CGA = common glioma antigen
- CGSA = complex glia-specific antigen
- 2DE = two-dimensional electrophoresis
- DIB = double immunodiffusion
- E = ependymoma
- EBV = Epstein-Barr virus
- EM = electron microscopy
- ENU = ethyl nitrosourea
- FB = fetal brain
- FONA = fetal onconeural antigen
- G = glioma/glioblastoma
- GEA = gliomembryonic antigen
- GFAP = glial fibrillary acidic protein
- GMEM = glioma-mesenchymal extracellular matrix antigen
- HAAA = human astrocytoma-associated antigen
- HLA-DR = human leukocyte antigen-DR
- IA = immune adherence assay
- IEP = immunoelectrophoresis
- IgG = immunoglobulin
- IIF = indirect immunofluorescence
- INMA = interspecies neural membrane antigens
- LAI = leukocyte adherence inhibition
- M = melanoma
- MB = medulloblastoma
- MBA-2 = mouse brain antigens-2
- MHA = mixed hemadsorption assay
- MN = meningioma
- N = neuroblastoma
- OL = oligodendroglioma
- ONA = onconeural antigen
- PBL = peripheral blood lymphocytes
- PEG = polyethylene glycol
- R = retinoblastoma
- RBC = red blood cells
- RIA = radioimmunoassay
- Sch = schwannoma
- SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis
- SPA = staphylococcal protein A assay
- SR = sarcoma
- TSA = tumor-associated surface antigens

### Brain Tumor-Associated Antigens Detected by Conventional Polyclonal Sera

The earliest attempts to identify glioblastoma antigens were those of Siris'104 and Hass,15 who prepared antisera against an alcohol extract of glioblastoma tissue and a solubilized glioblastoma homogenate, respectively. In each case, however, substantial reactivity was present against normal brain antigens. The use of autogenous glioma implants to stimulate patients' immunological response against their own tumors was also unsuccessful in generating reactive sera.42 However, the pioneering clinical studies of Mahaley and colleagues31,67,68 demonstrated that radiiodinated rabbit antihuman glioma antibodies infused in the internal carotid artery could localize preferentially to recurrent glioma cells as measured in tissue fractions, autoradiography, and external brain scans. This provided considerable impetus to develop other polyclonal reagents that could potentially identify brain-tumor markers and thus aid in diagnosis, screening for recurrence, and hopefully immunotherapy, particularly at a time when other laboratories were reporting altered cell-mediated immunity in CNS tumor patients and the possible existence of blocking factors.14,60

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- TSA = tumor-associated surface antigens
Brain tumor-associated antigens

Moreover, two nonhuman primate antisera made against a glioma line still reacted with 7/12 and 3/11 glioma cell lines, respectively, following adult and fetal brain absorptions, thereby suggesting the possible existence of glioma-associated specificities distinct from normal brain determinants.116

The study of the reactivities of patients' sera and peripheral blood lymphocytes against their own tumor cells and tumor lines revealed immunological responses against glioma and/or astrocytoma cells by a wide variety of assays including indirect immunofluorescence,70,105 mixed hemadsorption, immune adherence, and protein A assays.85 lymphocyte-mediated cytoxicity,64 leukocyte-adherence inhibition,5,98 and microcytotoxicity.27,58 Some of these assays also demonstrated cross-reactivities with autologous fibroblasts, adult brain, or fetal brain and tissues (Table 1). Part of the difficulty lay in the considerable number and quantity of antigens that normal and malignant brain tissue have in common. Thus, for example, when fetal brain was used as the immunogen, the rabbit antisera readily detected antigens in a high percentage of neuroblastoma cells (Table 1).34,59

Nevertheless, the analysis of autologous reactivities permitted an elegant classification of cultured astrocytoma cell surface antigens into at least three classes using the sera of 30 astrocytoma patients.85 Class I antigens, such as those identified by the sera of patients A.C. and B.C. in that study, could be detected only on autologous astrocytoma cells, whereas the far more common Class III antigens were found to be broadly distributed on normal and neoplastic cells by the sera of patients with astrocytomas. Class II astrocytoma cell surface antigens such as AJ-autologous antibody (AJ-aut) were present on astrocytomas as well as shared by some neuroblastoma and melanoma cells. The AJ-aut antigen was subsequently shown to be closely related serologically to the human melanoma antigen AH, a probable GD2 ganglioside,114 and has a counterpart antigen, AJ-natural antibody (AJ-nat), detected by certain healthy donor sera.45 These cell surface determinants shared by cells of neuroectodermal origin, first detected with polyclonal reagents, would soon be confirmed with monoclonal antibodies (Table 2) on a broad variety of neural crest-derived tumors.

In part due to complex cross-reactivities, possible heterogeneity in antigenic expression, and the large number of related antigens identified by xenogeneic immunization, polyclonal serological methodologies have not been able to clearly define a single glioma-specific antigen to date. However, the difference between an "absolute" specificity and a "therapeutically functional" specificity (which can be exploited clinically in a particular patient) may be most pertinent to the above serological analysis. Perhaps one can disregard an immunological reagent's cross-reactivities with fetal brain and with other tumor cells if in the clinical setting it is functionally specific and does not bind to adult brain or to other normal tissues.

Attempts to Identify Brain-Tumor Antigens Using Monoclonal Antibodies

With the advent of hybridoma technology57 yielding potentially unlimited quantities of monoclonal antibodies secreted by indefinitely proliferating hybrid lines, a new era was envisioned in the investigation of tumor antigens. It was hoped that carefully screened monoclonal antibodies, synthesized by a clonal population originating from one antibody-producing cell (and thus directed against a single antigenic determinant), would be able to overcome the complexities of antigenic cross-reactivities previously detected by polyclonal serological analysis. Furthermore, it was hoped that monoclonal reagents would aid in deciphering interspecies brain antigens like interspecies neural membrane antigens (INMA)1 and mouse brain antigen-2 (MBA-2),70 and would shed light on the possible transformation-associated changes in the expression of various nervous system-specific proteins such as S-100,105,75,76 neuron-specific enolase 14-3-2,109,87 the astrogial-marker glial fibrillary acidic protein (GFAP),2,13,32,35 alpha-2-glycoprotein (NSA3 or hyaluronectin),34,113 and the neuron-marker neurofilament polypeptides.62,92 Moreover, it was hoped that analysis with monoclonal antibodies would help unravel the potential immunological role of lymphocyte subpopulations that infiltrate gliomas in effecting an immunosuppression from within an environment of partial immunological privilege.4 Using OKT and Leu monoclonal antibodies as reagents in flow cytometric immunofluorescence and immunoperoxidase methods have already revealed a relative suppressor-cytotoxic T lymphocyte predominance in peripheral blood as well as in tumor parenchyma of glioma patients.59,110 This may help to explain, in part, the depression of cell-mediated immunity and the possible presence of blocking factors reported earlier in patients with intracranial tumors.14,60

However, the results of numerous monoclonal antibody studies (Table 2) have largely supported the earlier findings with polyclonal antibodies of antigens common to cells of neuroectodermal origin. Many of the anti-glioma or anti-astrocytoma monoclonal antibodies such as CG12, AO10, and G13-C6 possess broad activities against glioma, melanoma, and neuroblastoma cells, as well as fetal brain (Table 2).20,23,34,94 For example, the reactivity profiles of two of four different anti-astrocytoma murine monoclonal antibodies (AO10 and AJ8) are very similar in their binding to astrocytoma, melanoma, and epithelial cancer cell lines (Fig. 1).20 The AO10 and AO122 monoclonal antibodies also cross-reacted with fetal and adult brain (Table 2). Nevertheless, astrocytoma determinants were clearly present since several of the murine monoclonal antibodies were capable of immunoprecipitating proteins from labeled astrocytoma cell extracts; AJ225 and AO122 precipitated 145- and 265-kD polypeptides, respectively.20

The nature of the immunogen appeared immaterial.
<table>
<thead>
<tr>
<th>Antigen or Polyclonal Antibody</th>
<th>Immunogen</th>
<th>Type of Polyclonal Reagent</th>
<th>Assays</th>
<th>Molecular Identification</th>
<th>Neoplastic Cells Present in:†</th>
<th>Normal Cells Cross-Reactive With:†</th>
<th>Authors &amp; Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFGA</td>
<td>autoimmunization of patients with saline extracts of own tumor</td>
<td>human antisera</td>
<td>DID, absorptions</td>
<td>lipoprotein (alpha-1-mobility)</td>
<td>7/7 G, 4/4 A, 0/2 MN, 0/2 E tumor extracts</td>
<td>FB</td>
<td>Trouillas, 1971; Trouillas, 1972</td>
</tr>
<tr>
<td>HAAA</td>
<td>cells of astrocytoma line 301</td>
<td>rabbit heteroantiserum</td>
<td>dye exclusion cytotoxicity, absorptions</td>
<td>ND</td>
<td>7/7 A, 0/3 MN lines; 2/2 A tissue homogenates</td>
<td>none detected after absorptions</td>
<td>Coakham, 1974; Coakham &amp; Lakshmi, 1975</td>
</tr>
<tr>
<td>glioma-specific membrane antigens</td>
<td>lyophilized glioblastoma tissue (1 tumor)</td>
<td>rabbit heteroantiserum</td>
<td>IIF, DID, absorptions</td>
<td>ND</td>
<td>15/15 G lines (3 IIF patterns)</td>
<td>none detected after absorptions</td>
<td>Wahlström, et al., 1974</td>
</tr>
<tr>
<td>glioblastoma-associated antigen</td>
<td>saline extracts of glioblastoma</td>
<td>rabbit heteroantiserum</td>
<td>DID, IEP, absorptions</td>
<td>beta-mobility</td>
<td>13/16 G, 2/2 A, 0/3 MN, 0/2 neuroinoma saline tissue extracts</td>
<td>embryonic brain (8 to 10 wks)</td>
<td>Kehayov, 1976</td>
</tr>
<tr>
<td>meningeoma-associated embryonic antigen</td>
<td>saline extract of 8- to 10-wk embryonic brain</td>
<td>rabbit heteroantiserum</td>
<td>DID, IEP, absorptions</td>
<td>ND</td>
<td>5/6 MN, 0/16 G, 0/2 A, 0/3 MN, 0/2 embryonic brain saline tissue extracts</td>
<td>embryonic brain (8 to 10 wks)</td>
<td>Kehayov, et al., 1976</td>
</tr>
<tr>
<td>glioblastoma/reactive glia-associated antigen &quot;G&quot;; astrocytoma-associated antigen &quot;A&quot;</td>
<td>sera from patients</td>
<td>IIF, absorptions</td>
<td>ND</td>
<td>7/7 sera on G (3 lines), 6/7 sera on A (1 line) cultures for &quot;G&quot; antigen</td>
<td>6/7 sera on a reactive glial line; 3/3 healthy donor sera also reactive with G cultures</td>
<td>Solheid, et al., 1976</td>
<td></td>
</tr>
<tr>
<td>malignant gial tumor-associated antigen(s)</td>
<td>sera from glioma patients</td>
<td>IIF, absorptions</td>
<td>ND</td>
<td>2/17 sera for autologous surface antigens, 5/21 sera for autologous cytoplasmic antigens, &amp; 10/21 sera for allogeneic cytoplasmic antigens of G cells</td>
<td>none with normal brain tissue</td>
<td>Sheikh, et al., 1977</td>
<td></td>
</tr>
<tr>
<td>human brain-tumor cell surface antigens</td>
<td>glioma tissue homogenate &amp; sediment</td>
<td>rabbit heteroantiserum</td>
<td>MHA, Ouchterlony, absorptions</td>
<td>ND</td>
<td>5/5 G, 3/3 MN, 2/2 A, 2/2 E, 2/2 neuroinomas, 1/1 OL cultures</td>
<td>FB, HeLa cells</td>
<td>Miyake, et al., 1977</td>
</tr>
<tr>
<td>lack of glioblastoma- or meningeoma-associated antigens</td>
<td>Triton X-100/sodium barbital extracts of glioblastoma &amp; meningeoma tissue</td>
<td>rabbit heteroantiserum</td>
<td>absorptions, quantitative IEP</td>
<td>ND</td>
<td>glioblastoma &amp; meningeoma tissue extracts</td>
<td>adult liver (yet not in AB or FB)</td>
<td>Dittmann, et al., 1977</td>
</tr>
<tr>
<td>HB (human brain) antigen</td>
<td>homogenate of fetal brain (14 wks gestation)</td>
<td>rabbit heteroantiserum</td>
<td>IIF, indirect RIA, absorptions</td>
<td>ND</td>
<td>2/2 N lines, 6/6 N tissues, 0/22 leukemic blasts, 1 embryonal rhabdomyosarcoma line</td>
<td>5% to 10% of normal PBL, 0/7 thymocytes</td>
<td>Casper, et al., 1977</td>
</tr>
<tr>
<td>CGSA</td>
<td>adult rat brain homogenate</td>
<td>rabbit heteroantiserum</td>
<td>IIF, absorptions</td>
<td>ND</td>
<td>10/10 G, 5/5 A, 8/8 OL, 2/2 anaplastic G, 1/1 Sch, 0/6 MN tissues</td>
<td>AB (especially astrocytes)</td>
<td>Lach &amp; Weinträuber, 1978</td>
</tr>
<tr>
<td>brain-tumor surface antigens</td>
<td>autoantigous patients' sera</td>
<td>IIF</td>
<td>ND</td>
<td>9/14 G, 2/9 A, 1/1 E, 2/2 SR, 0/6 MN tissues or cultures</td>
<td>ND</td>
<td>Böker, 1978</td>
<td></td>
</tr>
</tbody>
</table>

* Table 1: Brain tumor-associated antigens detected by polyclonal reagents.
<table>
<thead>
<tr>
<th>Medulloblastoma</th>
<th>Medulloblastoma (BC) tissue cells</th>
<th>Rabbit heteroantiserum</th>
<th>Immunocytoadhesion (rosette formation), absorptions</th>
<th>ND</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.C. &amp; B.C. (Class I) astrocytoma antigens</td>
<td>NA</td>
<td>Autologous human sera</td>
<td>MHA, IA, anti-C3-MHA, protein A assay, absorptions</td>
<td>ND</td>
</tr>
<tr>
<td>AJ-aut (Class II) astrocytoma antigen</td>
<td>NA</td>
<td>Autologous human sera</td>
<td>MHA, IA, anti-C3-MHA, protein A assay, absorptions</td>
<td>ND, yet serologically related to AH, a GD2 ganglioside</td>
</tr>
<tr>
<td>CGA; GEA</td>
<td>NA</td>
<td>Patients' PBL</td>
<td>Lymphocyte-mediated microcytotoxicity, monolayer absorptions</td>
<td>ND</td>
</tr>
<tr>
<td>Glioma-associated specificities M5-30, M5-2</td>
<td>Glioma line U-251 MG</td>
<td>Primate (Macaca fascicularis) antisera</td>
<td>14C-nicotinamide release microcytotoxicity, absorptions</td>
<td>ND</td>
</tr>
<tr>
<td>ONA cells from neuroblastoma line LA-N-1</td>
<td>Rabbit heteroantiserum</td>
<td>125I-SPA, absorptions</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Native glioma antigens</td>
<td>Patients' sera &amp; peripheral blood leukocytes</td>
<td>IIF, LA assay</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>FONA-1, FONA-2 1st-trimester FB tissue</td>
<td>Rabbit heteroantiserum</td>
<td>125I-SPA, absorptions</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Glioma-associated membrane antigens</td>
<td>Triton X-100 extract of plasma membranes from glioma tissue (for rabbit immunizations)</td>
<td>Rabbit heteroantiserum, Ig fraction of glioma patients' sera</td>
<td>EM, SDS-PAGE, DED, countercurrent electrophoresis, absorptions microcytotoxicity, IA, absorptions</td>
<td>70 kD, 55 kD, 30 kD, 10 kD</td>
</tr>
<tr>
<td>Glioma-associated antigen N.A.</td>
<td>NA</td>
<td>Autologous patients' sera</td>
<td>Rabbit heteroantiserum</td>
<td>ND</td>
</tr>
<tr>
<td>Restricted glioma-associated antigen</td>
<td>Membrane-enriched fractions of glioma line LN-18</td>
<td>Serum from healthy, non-transfused male donor 337</td>
<td>IA, anti-C3-MHA, protein A assay, absorptions</td>
<td>ND</td>
</tr>
<tr>
<td>AJ-nat astrocytoma-related antigen</td>
<td>NA</td>
<td>Autologous patients' sera</td>
<td>Microcytotoxicity</td>
<td>ND</td>
</tr>
</tbody>
</table>

* The polyclonal reagents are summarized in chronological order based on the year that the study was reported. Their reactivities in neoplastic as well as in normal cells are given. All lines and tissues are of human origin unless indicated otherwise. See Definitions of Abbreviations table; NA = not applicable; ND = not determined.

† The designation "x/y" indicates the number (x) of lines or tissues positive out of the number (y) tested.
to the outcome of the reactivity profile. When fetal brain at 20 weeks gestation (for 4D2cl 6 and 1H8cl 3) or membranes from melanoma lines (for Me1-5, Me1-14, Me3-TB7, Me4-F8, and Me5-D5) served as the sensitizing immunogen in murine hybridoma production, the resulting monoclonal antibodies also recognized a broad range of neural crest-derived cells, including glioma, melanoma, and neuroblastoma lines (Table 2). Shared antigens were also detected in hematopoietic and lymphoid cells (Table 2), including common acute lymphoblastic leukemia antigen (CALLA), human leukocyte antigen (HLA-DR), and platelet glycoprotein IIb-IIIa. In retrospect, this brain-hematopoietic cell cross-reactivity was somewhat more understandable since the Thy-1 antigen had previously been shown to be shared by thymocytes, certain leukemias, and brain. The extensive sharing of antigenic determinants by CNS tumor tissue and hematopoietic cells has recently been once again confirmed by indirect immunofluorescence using a panel of monoclonal antibodies raised against human hematopoietic cells.

Nevertheless, the anti-astrocytoma monoclonal antibody analysis did yield particularly rewarding dividends concerning astrocyte differentiation. At least nine distinct astrocytoma cell surface antigenic systems were defined, of which two (the determinants AO10 and A8) were expressed in a mutually exclusive fashion on defined, of which two (the determinants AO10 and A8) astrocytoma cell lines were GFAP-negative, whereas the astrocytoma lines examined. All nine A8-positive astrocytoma cell lines were GFAP-negative, whereas 57% of AO10-positive lines also expressed GFAP. Since prior evidence suggested that the expression of GFAP was not only have helped to classify the antigen systems of cells in the astrocyte lineage, it was proposed that A8, AO10, and GFAP typing may help to determine whether astrocytic elements were precursor, immature, or mature astrocytes. An A8+ AO10- GFAP expression would characterize undifferentiated precursors, whereas an A8+ AO10+ GFAP antigen typing would identify differentiated mature astrocytes. Intermediate stages would represent in part the approximately 40% of A8+ AO10+ lines that were GFAP-. Thus, monoclonal antibodies against astrocytoma cell surface antigens not only have helped to classify the antigen systems of these tumor lines but have also suggested their state of differentiation. A typing scheme such as this to determine differentiation status, if found reliable, could play an important role in neuropathology for the diagnosis and assessment of patient prognosis.

It was also readily apparent that certain monoclonal antibodies possessed distinctive “discordant” reactivities that could offer potential diagnostic benefits as part of an antibody panel. For example, AJ225 was far more “astrocytoma-specific” because of its lower reactivities with melanoma and epithelial cancer lines than were the other three anti-astrocytoma monoclonal antibodies (Fig. 1). Conversely, AO122 reacted with 80% of the melanoma lines but with none of the 17 epithelial cancer lines (Fig. 1). These data immediately suggested that monoclonal reagents, particularly as part of an immunological panel, could specifically differentiate between tumor types as well as between tumor lines and thus provide an initial basis for comparison and subsequent characterization.

Examples of such discordance, which could serve both diagnostic and neuropathological investigations, were searched out among the other reported monoclonal reagents (Table 2 and Fig. 2). The data reported in the primary literature have been summarized in bar graph format by the authors, as in Fig. 1. Murine monoclonal antibodies UJ127:11 and UJ13A against fetal brain homogenate were quite disparate in their reactivity profiles to glioma, ependymoma, and meningioma tissues (Fig. 2 left). Whereas UJ13A was pan-neuroectodermal (pan-NE) in its reactivity, UJ127:11 was far more restricted and reacted principally with neural tumors such as medulloblastoma and neuroblastoma, and very infrequently with glioma, ependymoma, or meningioma tissues. The UJ13A monoclonal antibody will most probably have limited clinical application because of its lack of specificity.

Discordance in melanoma reactivity was noted in the anti-glioma line monoclonal antibodies G13-C6 and 81C6 (Fig. 2 center); G13-C6 reacted with a very high percentage (89%) of melanoma lines by radioimmunoassay, while glial-associated 81C6, which identifies the glial-mesenchymal extracellular matrix antigen (GMEM), reacted with a low percentage (14%) of such melanoma lines by radioimmunoassay. Both monoclonal reagents identified shared neuroectodermal antigens common to glia and neuroblastoma lines (Fig. 2 center). Yet, the cross-reactivity of 81C6 with hepatic tissues (Table 2) suggests that liver localization may restrict its clinical usefulness.

Discordance in glial versus melanoma reactivity was also apparent in the anti-glioma line monoclonal antibodies BF7, GE2, and CG12 (Fig. 2 right). Only CG12 identified antigens in a large fraction of melanoma lines; the reactivity profiles of GE2 and BF7 were more
### TABLE 2

<table>
<thead>
<tr>
<th>Antigen or Monoclonal Antibody</th>
<th>Immunogen</th>
<th>Type of Monoclonal Reagent</th>
<th>Assays</th>
<th>Molecular Identification</th>
<th>Neoplastic Cells Present in:†</th>
<th>Normal Cells Cross-Reactive With:‡</th>
<th>Authors &amp; Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>PI 153/3 cells of neuroblastoma line IMR6</td>
<td>murine hybridoma sup &amp; ascites</td>
<td>microcytotoxicity, indirect RIA, absorptions</td>
<td>ND</td>
<td>6/6 N, 1/2 R, 1/1 G cell lines</td>
<td>large amounts in FB; small amounts in AB, fetal spleen, null ALL, some B-ALL &amp; normal B cells</td>
<td>none detected</td>
<td>Kennett &amp; Gilbert, 1979; Kennett, et al., 1980</td>
</tr>
<tr>
<td>PI 125/10 cells of neuroblastoma line IMR6</td>
<td>murine hybridoma sup &amp; ascites</td>
<td>microcytotoxicity, indirect RIA, absorptions</td>
<td>ND</td>
<td>6/6 N, 1/2 R, 1/1 G cell lines; hepatoma SKHep-1, fibrosarcoma HT1080, lymphoblastoid SCBM cell lines</td>
<td>fetal fibroblast line IMR90</td>
<td>none detected</td>
<td>Kennett &amp; Gilbert, 1979</td>
</tr>
<tr>
<td>R2 antisera cells of melanoma line SK-MEL-28</td>
<td>murine hybridoma sup &amp; sera from tumor-bearing nu/nu mice</td>
<td>microcytotoxicity, indirect RIA, absorptions</td>
<td>glycolipid determinants</td>
<td>16/16 M, 2/5 A lines</td>
<td>weak reactivity with adult brain &amp; melanocytes</td>
<td>Dippold, et al., 1980</td>
<td></td>
</tr>
<tr>
<td>19-19 cells of melanoma line SW 691</td>
<td>murine hybridoma sup</td>
<td>indirect RIA, MHA, absorptions</td>
<td>ND</td>
<td>31/44 M cell lines &amp; early cultures; 4/7 A (RIA), 1/2 A (MHA) lines</td>
<td>none detected</td>
<td>Herlyn, et al., 1980</td>
<td></td>
</tr>
<tr>
<td>Nu4B cells from SW 691 × mouse fibroblast IT 22 somatic cell hybrid (691-I-5-Nu)</td>
<td>murine hybridoma sup</td>
<td>indirect RIA, MHA, absorptions</td>
<td>ND</td>
<td>45/46 M cell lines &amp; early cultures; 6/7 A (RIA), 1/2 A (MHA) lines</td>
<td>1/4 normal fibroblast lines by RIA &amp; MHA</td>
<td>none detected</td>
<td>Herlyn, et al., 1980</td>
</tr>
<tr>
<td>7.51 &amp; 7.60 cells of melanoma line CaCL 78-1</td>
<td>murine hybridoma sup</td>
<td>indirect RIA, MHA, absorptions</td>
<td>ND</td>
<td>8/8 M, 3/3 N, 4-5/5 G, 4-5/5 R lines; 3/5 M &amp; 2/2 R tissues</td>
<td>FB</td>
<td>Schnegg, Diserens, Carrel, et al., 1981; de Tribolet, et al., 1982</td>
<td></td>
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<tr>
<td>165 cultured melanoma cells</td>
<td>murine hybridoma sup</td>
<td>indirect 125I-SPA, absorptions</td>
<td>ND</td>
<td>3-4 M, 2/3 G, 1/4 SR, 0/10 N cell lines</td>
<td>fetal lung, kidney, colon, muscle</td>
<td>Seeger, et al., 1981</td>
<td></td>
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<tr>
<td>4D2cl 6 homogenate of FB (20 wks gestation)</td>
<td>murine hybridoma sup &amp; ascites</td>
<td>cell surface indirect RIA, 14C-nicotinamide release cytotoxicity, PAP, IIF, absorptions</td>
<td>ND</td>
<td>5/14 G, 1/2 M, 1/3 N, 0/1 MB lines by RIA: 13/13 G, 1/1 N tissues by PAP</td>
<td>1/5 fetal fibroblast lines by RIA; adult spleen &amp; FB, liver, spleen by PAP</td>
<td>Wikstrand &amp; Bigner, 1982</td>
<td></td>
</tr>
</tbody>
</table>

* The monoclonal reagents are summarized in chronological order based on the year that the study was reported. Their reactivities in neoplastic as well as in normal cells are given. All lines and tissues are of human origin unless indicated otherwise. See Definitions of Abbreviations table; ND = not determined; sup = supernatant.

† The designation "x/y" indicates the number (x) of lines or tissues positive out of the number (y) tested.

‡ The authors and year of publication are listed for each antigen.

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**TABLE 2 (continued ↓)**
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<th>Antigen or Monoclonal Antibody</th>
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</tr>
</thead>
<tbody>
<tr>
<td>7H10cl 4</td>
<td>homogenate of FB (20 wks gestation)</td>
<td>murine hybridoma sup &amp; ascites</td>
<td>cell surface indirect RIA, 14C- misc-oxinate release cytotoxicity, PAP, IIF, absorptions</td>
<td>ND</td>
<td>13/14 G, 0/2 M, 1/3 N, 1/1 MB lines by RIA; 13/13 G, 1/1 N tissues by PAP</td>
<td>adult spleen &amp; FB, liver, spleen, thymus by PAP</td>
<td>Wikstrand &amp; Big-ner, 1982</td>
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<td>1H8cl 2</td>
<td>homogenate of FB (20 wks gestation)</td>
<td>murine hybridoma sup &amp; ascites</td>
<td>cell surface indirect RIA, 14C-misc-oxinate release cytotoxicity, PAP, IIF, absorptions</td>
<td>ND</td>
<td>7/14 G, 2/3 N, 0/2 M, 1/1 MB lines by RIA; 9/15 G tissues by PAP</td>
<td>2/3 fetal fibroblast lines by RIA; FB &amp; spleen by PAP</td>
<td>Wikstrand, et al., 1982</td>
</tr>
<tr>
<td>1H8cl 3</td>
<td>homogenate of FB (20 wks gestation)</td>
<td>murine hybridoma sup &amp; ascites</td>
<td>cell surface indirect RIA, 14C-misc-oxinate release cytotoxicity, PAP, IIF, absorptions</td>
<td>ND</td>
<td>9/14 G, 2/3 N, 1/2 M, 1/1 MB lines by RIA; 12/15 G tissues by PAP</td>
<td>adult spleen &amp; FB, liver, spleen by PAP</td>
<td>Wikstrand, et al., 1982</td>
</tr>
<tr>
<td>AJ225</td>
<td>cells from astrocytoma line SK-MG-1</td>
<td>murine hybridoma sup &amp; sera from tumor-bearing nu/ nu mice</td>
<td>MHA, absorptions, immunoprecipitation</td>
<td>145 kD</td>
<td>16/16 A, 1/10 M, 2/4 renal carcinoma, 1/17 epithelial cancer, T-cell leukemia MOLT-4 cell lines</td>
<td>melanocytes</td>
<td>Cairncross, et al., 1982</td>
</tr>
<tr>
<td>AO10</td>
<td>cells from astrocytoma line SK-AO2</td>
<td>murine hybridoma sup &amp; sera from tumor-bearing nu/ nu mice</td>
<td>MHA, absorptions</td>
<td>ND</td>
<td>7/16 A, 3/10 M, 2/2 N, 2/17 epithelial cancer, T-cell leukemia MOLT-4 cell lines</td>
<td>AB &amp; FB</td>
<td>Cairncross, et al., 1982</td>
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<tr>
<td>AJ8</td>
<td>cells from astrocytoma line SK-MG-1</td>
<td>murine hybridoma sup &amp; sera from tumor-bearing nu/ nu mice</td>
<td>MHA, absorptions</td>
<td>ND</td>
<td>9/16 A, 4/10 M, 0/2 N, 4/17 epithelial cancer cell lines</td>
<td>melanocytes; adult &amp; fetal skin fibroblasts</td>
<td>Cairncross, et al., 1982</td>
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<tr>
<td>AO122</td>
<td>cells from astrocytoma line SK-AO2</td>
<td>murine hybridoma sup &amp; sera from tumor-bearing nu/ nu mice</td>
<td>MHA, absorptions, immunoprecipitation</td>
<td>265 kD</td>
<td>9/16 A, 8/10 M, 0/2 N cell lines</td>
<td>AB &amp; FB; melanocytes; adult &amp; fetal skin fibroblasts</td>
<td>Cairncross, et al., 1982</td>
</tr>
<tr>
<td>Me1-5, Me1-14, Me3-TB7, Me4-F8, Me5-D5</td>
<td>membrane-enriched fractions from melanoma lines Me-43 or IGR-3</td>
<td>murine hybridoma sup &amp; ascites</td>
<td>indirect RIA, absorptions</td>
<td>protein determinants abolished by trypsin, unaffected by tunica-myecin or neuraminidase</td>
<td>ranging from 10/12-12/12 (11/ 11) M, 12/30-23/36 (18/28) A, &amp; 1/3-3/3 N cell lines</td>
<td>none detected (Me5-D5 not tested with FB)</td>
<td>Carrel, et al., 1982; de Trbolet, et al., 1984</td>
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<tr>
<td>G13-F6</td>
<td>whole cells from glioma line LN-18</td>
<td>murine hybridoma sup &amp; ascites</td>
<td>indirect RIA, absorptions</td>
<td>trypsin-sensitive protein determinant as above</td>
<td>8/9 M, 9/13 G, 1/3 N cell lines</td>
<td>FB</td>
<td>Carrel, et al. 1982</td>
</tr>
<tr>
<td>Antigen</td>
<td>Description</td>
<td>Reactivity</td>
<td>Notes</td>
<td></td>
<td></td>
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<tr>
<td>P-64 peptide, 217c</td>
<td>rat C6 glioma cells</td>
<td>murine hybridoma sup</td>
<td>direct $^{131}$I- &amp; $^{125}$I-SPA, immunoprecipitation</td>
<td>64 kD</td>
<td>1/1 human glial tumor line; C6 rat glioma cells; ENU-induced transformed rat oligodendrocytes; spontaneously transformed rat astrocytes; two rodent hepatoma lines (weak binding)</td>
<td></td>
<td></td>
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<tr>
<td>LGL1-P64</td>
<td>PEG fusion of intratumoral (glioma) lymphocytes from patients with human myeloma line LICR-LON-HMy2</td>
<td>human hybridoma sup</td>
<td>indirect RIA</td>
<td>ND</td>
<td>1/1 G, 1/1 colon carcinoma, &amp; 1–2/2 lung carcinoma lines; positive radiolocalization with ID6 of recurrent G in patient</td>
<td></td>
<td></td>
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<tr>
<td>UJ127:11</td>
<td>homogenate of FB (16 wks gestation)</td>
<td>murine hybridoma sup &amp; ascites</td>
<td>IIF, indirect RIA, immunoprecipitation, absorptions</td>
<td>220–240 kD glycoprotein</td>
<td>10/10 Sch, 8/10 MB, 8/8 N, 1/40 G, 0/18 A, 0/8 M, 0/14 E, 0/6 OL, 0/16 MN, 0/3 PNET tissues</td>
<td></td>
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<tr>
<td>UJ13A</td>
<td>homogenate of FB (16 wks gestation)</td>
<td>murine hybridoma ascites</td>
<td>indirect RIA, IIF, absorptions</td>
<td>ND</td>
<td>40/40 G, 18/18 A, 14/14 E, 6/6 OL, 10/10 Sch, 14/16 MN, 10/10 MB, 8/8 N, 3/3 PNET, 0/8 M tissues; positive radiolocalization of tumors in 3 of 5 patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GMEM 81C6</td>
<td>cell suspensions of glioma line U-251 MG</td>
<td>murine hybridoma sup &amp; ascites</td>
<td>cell surface indirect RIA, IIF, PAP, absorptions, immunoprecipitation</td>
<td>230 kD (210-kD minor band) glycoprotein</td>
<td>14/16 G, 1/7 M, 1/3 N, 2/6 SR lines by RIA; 10/11 G, 1/6 A, 4/4 M, 2/2 fibrosarcoma, 1/1 Wilms' tumor, 1/1 ovarian carcinoma tissues by PAP; positive radiolocalization of G line in athymic mice &amp; rats</td>
<td></td>
<td></td>
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<tr>
<td>M148</td>
<td>homogenate of medulloblastoma tissue</td>
<td>murine hybridoma ascites</td>
<td>IIF, immunoprecipitation</td>
<td>130 kD &amp; 110 kD glycoproteins Ib/IIa in platelet membranes</td>
<td>2/3 N lines; 4/6 MB, 1/2 N, 4/4 Ewing's SR, 3/3 rhabdomyosarcoma, 1/1 hepatoblastoma, 1/1 teratocarcinoma tissues</td>
<td></td>
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</tr>
<tr>
<td>GMEM-related antigen 2A6</td>
<td>medulloblastoma-derived line TE-671</td>
<td>murine hybridoma sup &amp; ascites</td>
<td>avidin-biotin-peroxidase</td>
<td>ND</td>
<td>9/10 G, 1/4 MB tissues</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The monoclonal reagents are summarized in chronological order based on the year that the study was reported. Their reactivities in neoplastic as well as in normal cells are given. All lines and tissues are of human origin unless indicated otherwise. See Definitions of Abbreviations table; ND = not determined; sup = supernatant.

† The designation "x/y" indicates the number (x) of lines or tissues positive out of the number (y) tested.
"glial-specific" and revealed reactivities with higher percentages of glioma and schwannoma lines. Clearly, panels of monoclonal antibodies are needed, since "discordant" reactivities may represent the limit of specificity of monoclonal antibodies when it comes to highly conserved and shared neuroectodermal and differentiation antigens.

The difference between "absolute" and "therapeutically functional" specificities, as mentioned before, is also very pertinent to the use of monoclonal reagents in the clinical setting. Monoclonal antibodies that are "cross-reactive" with fetal brain and various tumor cells may nevertheless be therapeutically efficacious in a particular patient with a single tumor type, provided that they do not react extensively with normal adult tissues.

Probably the best evidence to date for possible glioma-specific tumor markers has come not from human but rather from animal model systems. Monoclonal antibodies have detected antigens apparently specific for chemically induced rat glioma cells. Recently, the putatively tumor-specific murine monoclonal antibody 217c raised against C6 rat glioma cells has been shown to immunoprecipitate a 64-kD polypeptide only from the immunizing C6 rat glioma line and chemically induced oligodendrocyte or spontaneously transformed astrocyte rat lines, yet not from control cell lines. However, the applicability and relevance of data derived from animal cell lines to the clinical setting still needs to be clearly demonstrated.

**Limitations to an Immunological Approach**

There are several underlying premises basic to the immunological approach which must be met in order for it to succeed. First, the tumor marker must be consistently associated with the brain neoplasm, either because the marker is unique to the transformed cells or is present in greater abundance in them than in normal brain. Second, the marker must be sufficiently antigenic for the current methods of developing polyclonal and monoclonal antibodies to be successful. This may be a problem when dealing with low-abundance, sequestered, cell-cycle-specific, or lipid-associated antigens. These factors, coupled with the likely immunocompromised state of patients with a malignancy and the potential difficulty in antigen presentation due to both the blood-brain barrier and the absence of a CNS lymphatic system, may account for only a small minority of glioma patients developing a measurable humoral response against their neoplasm. Immunosurveillance and effector-arm function may also be less efficient in "immunologically privileged sites" such as the brain.

Third, there are technical difficulties such as the separation of blood elements and endothelium from the tumor tissue preparation. This problem can be circumvented in part by the use of tumor-cell tissue culture lines that are composed of relatively homogeneous cell populations. The advantage of using a tumor-cell line must be weighed against the observation that cells in culture may express antigens differently, both qualitatively and quantitatively, than cells *in vivo*. This is especially problematic with tissue culture lines of high passage number. Fourth, the developed immunological reagents must be monospecific, have a high titer, and possess high-affinity association constants. Monoclonality does not rule out cross-reactivity. A monoclonal antibody, which for example exquisitely binds to an epitope within a domain common to many cellular proteins, would be extensively cross-reactive and thus not useful because of its lack of specificity. Moreover, a particular antibody species may bind to several dissimilar antigenic determinants, a property termed "linked specificities." Fifth, the above approach, although promising, depends upon a reliable and clinically convenient delivery system.

Although the blood-brain barrier appears to be somewhat permeable in the vicinity of the tumor, as evidenced by contrast enhancement on computerized tomography scans, it may nevertheless prove to be a formidable deterrent to the delivery...
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of antibodies. The data from many clinical studies may be obscured by limitations in the delivery system. Modification of the blood-brain barrier, intra-arterial therapy, and use of conjugated Fab immunoglobulin fragments may help to overcome this obstacle. Difficulty in interpretation of clinical results may be compounded by the host’s generation of anti-bodies as well as by the interaction of immunoglobulins with immune modifiers such as the endogenous interleukins. Finally, it should be pointed out that many normal brain proteins are highly antigenic since they are relatively isolated from the immune system by the blood-brain barrier. Consequently, any effort to raise a high-titered antibody against a CNS-associated tumor is likely to lead to cross-reaction with normal brain tissue, resulting in an autoimmune allergic encephalitis.

A number of factors may have contributed to the lack of tumor type specificity exhibited by the reported monoclonal reagents (Table 2). First, a large number and amount of cellular constituents, particularly structural ones, are commonly shared. Murine hybridoma production against human antigens involves xenogeneic immunization which can emphasize antibody manufacture against certain common cell surface components. In part, this problem, as well as that of patients producing antibodies against murine immunoglobulins, can be solved by developing human hybridomas.

Second, some of the complexities detected in monoclonal as well as polyclonal serological analysis may have arisen from the marked phenotypic heterogeneity of human glioma lines and their cell subpopulations. The development of a stem cell assay system is one potential way to help minimize the effect of cell line heterogeneity in the testing of chemotherapeutic agents. Finally, monoclonality does not rule out cross-reactivity, and the exquisite binding of monoclonal antibodies to a particular epitope is irrelevant if the epitope is part of a common domain shared by many molecules.

Possibilities for Direct Biochemical and Genetic Identification

Due to the complexity and number of proteins in tumors, and the limitations of protein separation techniques, most workers have chosen to raise antibodies to glioma tissues without attempting to identify and isolate the specific proteins against which these antibodies are directed. Other more direct approaches to identifying tumor-associated antigens are now within the realm of possibility. The introduction and refinement of two-dimensional gel electrophoresis (2DE) and silver staining have resulted in renewed interest in the "fingerprinting" of proteins in various biological materials. These two-dimensional protein patterns provide not only the molecular weights and isoelectric points of the constituent polypeptides but also semiquantitative information on their relative amounts. Using these techniques, Heydorn, et al., recently described normal CNS proteins in rat brain. Subsequently, these techniques were applied to normal human brain and demonstrated a fairly constant protein pattern associated with different samples of normal human cerebral cortex. These particular gels displayed proteins with molecular weights ranging from 14 to 100 kD and isoelectric points of 4.7 to 7.0. Initially, almost none of the over 150 "spots" seen on the gels were known, but by means of immunoblotting and co-migration techniques several of the major spots have been identified (Fig. 3 upper). A wide variety of human brain tumors have recently been studied using 2DE, and this investigation has revealed that the different tumor types have fairly characteristic protein patterns which differ substantially from that seen in normal brain. Moreover, application of these techniques has demonstrated large amounts of GFAP in astrocytomas, a prominent vimentin complex in meningiomas, and neuron-specific enolase in medulloblastomas. Of particular significance was the fact that characteristic protein profiles were found for high- and low-grade astrocytomas, juvenile astrocytomas, ependymomas, and medulloblastomas, and that each tumor profile contained certain polypeptide spots that were not detected or were clearly reduced in normal human cortex (Fig. 3 lower). Efforts are now under way in our laboratory to isolate these tumor-associated proteins from the malignant astrocytoma gels. The production of polyclonal and monoclonal antibodies against the proteins eluted from these singly excited spots may yield reagents capable of specifically binding certain CNS tumors.

There are several potential limitations associated with the above approach. First, several polypeptides lie outside the molecular weight and isoelectric point range that must arbitrarily be chosen for such studies, and thus may escape detection. Second, not all proteins lend themselves to separation by 2DE because of their solubility, charge, or staining characteristics. Third, polypeptides initially identified as possibly tumor-associated may later be found to be present in body organs other than the brain. Finally, several of the tumor-associated proteins may be cytosolic or nuclear in location rather than on the tumor-cell surface membrane. While this would obviously limit their usefulness as "target" proteins, they could nevertheless yield important data relating to the metabolism and composition of the tumor.

Another direct approach which could supplement the above study would be the complementary DNA (cDNA) cloning of RNA (ribonucleic acid) from fresh brain-tumor tissue into an efficient expression vector such as lambda-gt 11. Subtraction hybridization or labeled RNA from normal human brain, as well as both seronegative and extensively absorbed anti-tumor sera, can serve as controls in the screening process to identify potentially unique genetic inserts and their products. Monospecific sera, in turn, can be generated against these genetically dissected products before returning the investigation to tumor tissue. The genetic dissection of complex antigenic systems by employing...
FIG. 3. Silver-stained two-dimensional gel of normal human cerebral cortex (upper) including polypeptide spots the identities of which have been established. The positions of potential tumor-associated polypeptides (lower), identified on gels of astrocytomas, ependymomas, and medulloblastomas, have been superscribed onto the normal gel pattern. CTX = cerebral cortex; IFP = intermediate filament protein; GFAP = glial fibrillary acidic protein; sGOT = serum glutamic-oxaloacetic transaminase; NSE = neuron-specific enolase. Data derived from Narayan, et al.176

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recombinant cloning coupled with gene transfer techniques has already proved successful in the unequivocal identification of a transformation-associated nuclear antigen of Epstein-Barr virus. Thus, genetic dissection can contribute significantly to the specificity attained by immunological means alone.

Conclusions

Advances in identifying and characterizing human brain tumor-associated antigens by means of both polyclonal sera and monoclonal antibodies have been reviewed. In neither case were truly brain tumor-specific antigens documented. Instead, broadly shared neuroectodermal and common differentiation antigens were detected in glioma, astrocytoma, neuroblastoma, melanoma, and hematopoietic cells. Nevertheless, discordant reactivity profiles were found in certain monoclonal antibodies that would make these reagents potentially useful in tumor diagnosis and therapy, possibly as part of an antibody panel. Attempts at the direct identification of tumor markers and cDNA cloning are two new approaches to this problem of major biological and clinical significance.

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References

the use of microcytotoxicity and immune adherence assays. JNCI 64:223–233, 1980
67. Mahaley MS Jr: Immunological studies with human
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107. Studer A, de Tribolet N, Diserens AC, et al: Characteri-


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