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Immunology

Cell surface antigens of human astrocytoma defined by mouse monoclonal antibodies: Identification of astrocytoma subsets

(hybridoma/differentiation antigens/glial fibrillary acidic protein)

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ABSTRACT **The surface antigens of cultured human malignant astrocytomas were analyzed by using mouse monoclonal antibodies. BALB/c mice were immunized repeatedly with either SK-MG-1 [a glial fibrillary acidic protein (GFA)-negative astrocytoma line] or SK-AO2 (a GFA-positive astrocytoma line). After fusion with NS/1 mouse myeloma cells, 12 antibody-producing clones were selected for detailed study. Serological analysis permitted the identification of nine distinct antigenic systems. Four monoclonal antibodies (Ab AJ225, Ab AO10, Ab AJ8, and Ab AO122) identified cell surface antigens preferentially expressed on tumors of neuroectodermal origin, and these antibodies subdivided the astrocytoma panel into distinguishable subsets. The determinants detected by Ab AO10 and Ab AJ8 showed mutually exclusive expression on the astrocytoma lines. The AO10 and AJ8 phenotypes appeared to reflect the differentiation state of the cultured cells; 4/7 AO10-positive astrocytomas expressed GFA, an intracellular astrocyte differentiation antigen, whereas all AJ8-positive astrocytomas (9/9) were GFA-negative. Five antibodies (Ab AJ10, Ab AJ9, Ab AJ17, Ab AJ425, and Ab AJ2) recognized determinants widely distributed on normal and malignant cells. Four antibodies defined in this study precipitated proteins from reduced preparations of radioisotope-labeled SK-MG-1 and SK-AO2 cells: Ab AJ225 (M_r 145,000); Ab AO122 (M_r 265,000); Ab AJ10 (M_rs 195,000 and 165,000); and Ab AJ2 (M_rs 170,000, 140,000, 140,000, and 28,000).**

Our initial analyses of cell surface antigens of human malignant melanoma and human renal cancer identified by mouse monoclonal antibodies have been described (1, 2). We now report a comparable analysis of human malignant astrocytoma.

MATERIALS AND METHODS

Tissue Culture. Astrocytoma and other human tumor cell lines and short-term cultures of normal human skin fibroblasts and kidney epithelial cells have been described (3, 4, 5). Cultured melanocytes from skin were provided by A. Houghton.

Serological Procedures. Direct serological tests were performed with an anti-mouse Ig mixed-hemadsorption assay (1). Direct test conditions and absorption procedures were identical to those previously described for the staphylococcal protein A mixed-hemadsorption assay (4). Heat stability of the antigenic determinants was assessed by heating the cell suspension to 100°C for 5 min and then testing for residual antigenic activity in absorption tests. Glial fibrillary acidic protein (GFA) was demonstrated by an indirect immunofluorescence test using monospecific rabbit antiserum provided by L. F. Eng.

Immunizations. BALB/c mice were immunized with either the GFA-negative astrocytoma line SK-MG-1 [designated AJ in a previous publication (3)] or the GFA-positive astrocytoma line SK-AO2 (established by J. R. Shapiro and W. R. Shapiro, Laboratory of Neuro-Oncology, Sloan-Kettering Institute). For the initial immunization, 1×10^7 astrocytoma cells were injected subcutaneously with Freund's complete adjuvant. Five to 10 subsequent immunizations were carried out at 2-week intervals by intraperitoneal inoculation of 1×10^7 tumor cells in the absence of adjuvant. Immunized mice were sacrificed 3 days after the last injection.

Production of Mouse Monoclonal Antibodies. The fusion of immune spleen cells with mouse myeloma MOPC-21 NS/1 cells (ratio 5:1) was performed as described (1, 2). Fused cells were grown in selective medium and subcloned by limiting dilution as previously described (1, 2). For initial screening, supernatants were tested for antibody activity on a panel of cultured cells consisting of three astrocytomas (including the immunizing line), two melanomas, five carcinomas, and adult and fetal skin fibroblasts. Antibody subclass was determined by double diffusion in agar with anti-Ig heavy-chain-specific reagents (Bionetics, Kensington, MD). Cultures of cloned hybridomas were injected subcutaneously into *nu/nu* (athymic) mice (NIH Swiss background). Sera from mice with progressively growing tumors were used for serological and biochemical characterization.

Immunoprecipitation Procedures. Antibodies were tested for precipitating activity by using radiolabeled antigen from detergent-solubilized extracts of the immunizing cell line. Three different labeling procedures were used. Labeling with [^{3}H]glucosamine (New England Nuclear, 30–60 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) or with [^{35}S]methionine (New England Nuclear, 1,000 Ci/mmol) and extraction with Nonidet P-40 (NP-40) buffer were carried out as described (1, 2). In some experiments the [^{35}S]methionine-labeled extract was fractionated on a 1-ml concanavalin A-Sepharose (Pharmacia) column, using 0.15 M NaCl/0.01 M Tris·HCl, pH 7.3/0.1% NP-40 as column buffer, and eluting with 0.2 M methyl α-D-mannoside. ^{125}I labeling of solubilized membrane preparations followed Brown *et al.* (6), except that the gel filtration step before iodination was omitted. Membrane preparation was carried out according to Natori *et al.* (7), except that the buffer during disruption was supplemented with 10 mM $MgCl_2$ and 2 mM phenylmethylsulfonyl fluoride. Protein-conjugated ^{125}I was estimated by measuring radioactivity in samples precipitated with cold 10% (w/v) trichloroacetic acid and then washed with ethanol and acetone.

Radioimmunoprecipitation procedures with [^{3}H]glucosamine- and [^{35}S]methionine-labeled samples were carried out as described (1, 2). For ^{125}I-labeled samples, aliquots (200 μl) were first cleared of nonspecific binding material by treating with *nu/*

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Abbreviations: GFA, glial fibrillary acidic protein; EBV, Epstein–Barr virus; NP-40, Nonidet P-40.

nu mouse serum (1 μl), rabbit anti-mouse IgG (15 μl) (Cappel Laboratories, Cochranville, PA), and *Staphylococcus aureus* (15 μl) (Bethesda Research Laboratories, Bethesda, MD). To aliquots (200 μl) of the precleared supernatant solution (5×10^5 cpm of ^{125}I-labeled protein), 0.1 or 1 μl of antibody and 15 μl of rabbit anti-mouse IgG were added. Immune complexes were isolated with *S. aureus* as described (6), except that 0.1 ml of normal rabbit serum was added to the second wash buffer to reduce background binding. Labeled antigen was eluted from the pellet and analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis and two-dimensional electrophoresis as described previously (8, 9) except that iodoacetamide (14 mg/ml) was added to the sample buffer when nonreduced samples were analyzed.

RESULTS

From four fusions of NS/1 myeloma with spleen cells from mice immunized with SK-MG-1 (three fusions) or SK-AO2 (one fusion), 12 antibody-producing clones were selected for detailed analysis (Table 1). The serological specificity of these antibodies was tested on a panel of 49 established human cell lines (Table 2). The antibodies were also tested on short-term cultures of human adult and fetal skin fibroblasts, kidney epithelial cells, and melanocytes. In most cases, serological analysis consisted of both direct and absorption tests. Melanocytes were studied only by direct test, and lymphoblastoid lines, erythrocytes, adult brain, and fetal brain were analyzed only by absorption tests.

Monoclonal antibodies Ab AJ225, Ab AO10, Ab AJ8, Ab AO122, Ab AJ10, Ab AJ9, Ab AJ17, Ab AJ425, and Ab AJ2 defined nine distinct cell surface antigenic systems. Eight have been selected for detailed presentation (Table 2). Ab AJ2 will be discussed in a subsequent publication. (Of the remaining three monoclonal antibodies listed in Table 1, Ab AO50 and Ab AO92 were serologically related to Ab AO122, and Ab AJ60 was similar to Ab AJ10.)

AJ225 Antigenic System. Direct tests and absorption analysis with Ab AJ225 indicated that the determinant detected by this antibody was largely restricted to astrocytoma cell lines (Table 2). Although all astrocytoma lines absorbed Ab AJ225, the titers in direct serological tests permitted a division of cultured astrocytomas into two groups on the basis of quantitative differences in antigen expression; 12/16 tumors were high expressors and 4/16 were low expressors. The only other tumor lines expressing high levels of AJ225 were 1/10 melanomas and 1/17 epithelial cancers; 2/4 renal carcinomas expressed low levels of the antigen demonstrable by absorption tests. In addition, T-cell leukemia cells (MOLT-4) absorbed Ab AJ225 reactivity. EBV-transformed B cells, adult and fetal skin fibroblasts, kidney epithelial cells, and homogenates of adult and fetal brain did not absorb. The results of direct serological tests on melanocytes suggested low levels of AJ225 expression on this normal cell type.

Ab AJ225 identified a heat-labile determinant and immunoprecipitated a protein with M_r 145,000 from ^{125}I-labeled SK-MG-1 (Fig. 1). This band was not detected in immunoprecipitates from cells labeled with [^{3}H]glucosamine or [^{35}S]methionine. In some experiments, this antigen appeared as a closely spaced doublet. The pI of the 145,000 M_r component was 4.8. Without reduction a single band with M_r 150,000 was identified relative to reduced standards. The inability to immunoprecipitate the AJ225 antigen after metabolic labeling raised the possibility that this determinant was a fetal calf serum component adsorbed to the cell surface. However, the highly restricted distribution of this antigen, the failure of fetal calf serum to inhibit Ab AJ225, and the ability of fresh astrocytoma tissue to absorb Ab AJ225 speak strongly against this possibility.

AO10 and AJ8 Antigenic Systems. The AO10 and AJ8 antigenic systems are described together because, with two exceptions, they subdivided 16 cultured astrocytomas into mutually exclusive AO10-positive and AJ8-positive subsets. The exceptions were SK-MG-10, which expressed neither antigen, and SK-MG-12, which expressed both.

The AO10 antigen was identified on 7/16 astrocytoma lines. As shown in Table 2, 4/7 AO10-positive astrocytomas were GFA-positive. The antigen was also demonstrated on cells from 3/10 melanomas, 2/2 neuroblastomas, and a T-cell leukemia. Direct serological tests failed to demonstrate the AO10 determinant on epithelial cancer cells; however, absorption of Ab AO10 indicated low levels of antigen expression on 2/17 of these lines. EBV-transformed B cells did not absorb Ab AO10. The AO10 determinant was detected in adult and fetal brain but not identified on adult and fetal skin fibroblasts, kidney epithelial cells, or melanocytes.

The AJ8 antigen was detected on 9/16 astrocytoma lines. All AJ8-positive astrocytomas were GFA-negative (Table 2). The AJ8 antigen was also demonstrated on 4/10 melanoma cell lines. Direct serological tests failed to demonstrate the AJ8 determinant on epithelial cancer cells; however, absorption analysis detected low levels of antigen expression on 4/17 cell lines. Neuroblastoma cells (0/2), EBV-transformed B cells, and T-cell leukemia cells did not absorb Ab AJ8. AJ8 was detected on adult and fetal skin fibroblasts and melanocytes, but not on cultured kidney epithelial cells or in adult or fetal brain.

The AO10 determinant was heat labile, suggesting that it resided on a protein, but Ab AO10 did not immunoprecipitate a detectable component from [^{3}H]glucosamine-, [^{35}S]methionine-, or ^{125}I-labeled SK-AO2 cells. The AJ8 determinant was also heat labile and could not be immunoprecipitated from

Table 1. Derivation of mouse hybridomas producing monoclonal antibodies reacting with surface antigens of human malignant astrocytoma cells

Fusion no.	Astrocytoma cell line used for immunizations	No. of immunizations	No. of positive wells/total no. of wells	No. of clones isolated and analyzed	Antibodies characterized
1	SK-MG-1	6	17/480	4	AJ2 (γ1)*, AJ8 (γ1)*, AJ9 (γ1)*, AJ17 (γ1)*
2	SK-MG-1	6	10/480	3	AJ60 (γ1), AJ225 (μ)* AJ425 (γ1)*
3	SK-MG-1	8	59/360	1	AJ10 (γ1)*
4	SK-AO2	10	20/360	4	AO10 (γ1)*, AO50 (γ1), AO92 (γ1), AO122 (γ1)*

* Prototype antibodies (see Table 2).

Table 2. Characterization of eight prototype mouse monoclonal antibodies detecting cell surface antigens on human malignant astrocytoma cells

Cells	Ab AJ225		Ab AO10		Ab AJ8		Ab AO122		Ab AJ10		Ab AJ9		Ab AJ17		Ab AJ425	
	Titer $\times 10^{-3}$	Abs.	Titer $\times 10^{-3}$	Abs.	Titer $\times 10^{-3}$	Abs.	Titer $\times 10^{-3}$	Abs.	Titer $\times 10^{-3}$	Abs.	Titer $\times 10^{-3}$	Abs.	Titer $\times 10^{-3}$	Abs.	Titer $\times 10^{-3}$	Abs.
Astrocytoma (GFA)*																
SK-MG-1 (−)	100	+	−	−	10	+	−	−	100	+	100	+	100	+	100	+
SK-MG-2 (+)	−	+	10	+	−	−	100	+	100	+	1,000	+	10	+	−	+
SK-MG-3 (−)	10	+	−	−	−	+	1,000	+	100	+	1,000	+	100	+	100	+
SK-MG-4 (−)	100	+	100	+	−	−	1,000	+	10	+	1,000	+	10	+	10	+
SK-MG-7 (−)	100	+	−	−	−	+	100	+	10	+	1,000	+	10	+	1	+
SK-MG-9 (−)	−	+	100	+	−	−	1	+	1	+	100	+	1	+	1	+
SK-MG-10 (−)	10	+	−	−	−	−	−	−	100	+	100	+	1	+	−	+
SK-MG-11 (−)	10	+	−	−	10	+	1,000	+	1	+	1	+	1	+	1	+
SK-MG-12 (−)	10	+	10	+	100	+	−	−	10	+	1,000	+	100	+	100	+
SK-MG-13 (−)	10	+	−	−	100	+	10	+	−	+	100	+	1	+	100	+
SK-MS (−)	−	+	−	−	100	+	1	+	100	+	100	+	10	+	10	+
SK-AO2 (+)	−	+	1,000	+	−	−	1,000	+	−	+	1	+	1	+	10	+
T98 (−)	1	+	−	−	−	+	−	−	10	+	10	+	10	+	100	+
U178MG (−)	100	+	−	−	10	+	−	−	100	+	1,000	+	1	+	1	+
U251MG (+)	1	+	1,000	+	−	−	−	−	100	+	1,000	+	10	+	100	+
U373MG (+)	100	+	1,000	+	−	−	−	−	100	+	100	+	100	+	100	+
Neuroblastoma																
SK NMC	−	+	1,000	+	−	−	−	−	−	−	1,000	+	100	+	100	+
SK NSH	−	−	100	+	−	−	−	−	−	−	1,000	+	−	−	−	+
Melanoma																
SK-MEL-13	−	−	−	−	1,000	+	100	+	100	+	100	+	−	+	100	+
SK-MEL-28	−	−	−	−	1,000	+	100	+	10	+	100	+	100	+	100	+
SK-MEL-29	−	−	−	−	−	−	−	−	1,000	+	1,000	+	100	+	100	+
SK-MEL-31	−	−	−	+	−	−	1,000	+	100	+	100	+	1	+	10	+
SK-MEL-33	−	−	−	+	−	−	−	−	100	+	100	+	100	+	100	+
SK-MEL-37	−	−	10	+	−	−	100	+	10	+	100	+	10	+	10	+
SK-MEL-44	100	+	−	−	−	−	100	+	100	+	10	+	−	+	1	+
SK-MEL-93	−	−	−	−	1,000	+	100	+	1,000	+	100	+	1	+	100	+
SK-MEL-124	−	−	−	−	−	−	100	+	100	+	100	+	100	+	100	+
MeWo	−	−	−	−	10,000	+	100	+	−	−	1,000	+	10	+	10	+
Epithelial cancers																
Lung																
SK-LL-LC	−	−	−	−	−	−	−	−	−	−	1	+	1	+	−	+
SK-LC-6	10	+	−	−	−	−	−	−	100	+	10	+	10	+	100	+
Breast																
AlAb	−	−	−	−	−	−	−	−	−	−	1,000	+	10	+	100	+
BT-20	−	−	−	−	−	−	−	−	−	−	1,000	+	10	+	1	+
CAMA	−	−	−	−	−	−	−	−	−	−	−	−	100	+	100	+
MCF-7	−	−	−	−	−	−	−	−	−	−	100	+	10	+	100	+
SK-BR-3	−	−	−	−	−	−	−	−	−	−	100	+	10	+	100	+
Colon																
HT-29	−	−	−	−	−	−	−	−	−	−	100	+	1	+	−	+
SW-1116	−	−	−	−	−	+	−	−	−	−	100	+	1	+	−	+
SW-1222	−	−	−	−	−	+	−	−	−	−	100	+	1	+	−	+
Renal																
SK-RC-1	−	+	−	−	−	+	−	−	100	+	100	+	10	+	100	+
SK-RC-6	−	−	−	+	−	−	−	−	100	+	100	+	10	+	100	+
SK-RC-7	−	−	−	−	−	−	−	−	100	+	100	+	10	+	100	+
SK-RC-9	−	+	−	−	−	+	−	−	1,000	+	100	+	10	+	100	+
Bladder																
RT-4	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	+
T-24	−	−	−	−	−	−	−	−	100	+	1,000	+	1,000	+	1,000	+
Cervix																
ME-180	−	−	−	−	−	−	−	−	−	−	100	+	−	−	−	+
Lymphoblastoid cells																
EBV B cells																
AH		−				−				−		−		−		+
BT		−				−				−		−		−		+
BD				−				−								
T cells																
MOLT-4		+		+		−		−		−		−		−		+
Normal human cells																
Brain																
Adult		−		+		−		+		+		+		+		+
Fetal		−		+		−		+		+		+		+		+
Adult skin fibroblasts	−	−	−	−	−	+	100	+	−	+	−	+	1	+	−	+
Fetal skin fibroblasts	−	−	−	−	−	+	10	+	−	+	−	+	100	+	1	+
Adult kidney epithelium	−	−	−	−	−	−	−	−	100	+	100	+	10	+	100	+
Melanocytes	1		−		10		100		1,000		100		10		100	
Erythrocytes		−		−		−		−		−		−		−		−

Titer: − indicates no reaction in direct tests at a dilution of 1:1,000 (i.e., the entry in the column would be <1.0). Abs., absorption tests. Sera (diluted to end point) were absorbed with the indicated cell type and tested for residual activity for SK-MG-1 (Ab AJ225, Ab AJ10, Ab AJ9, Ab AJ17, Ab AJ425), U25IMG (Ab AO10), or SK-MEL-28 (Ab AJ8, Ab AO122); +, complete absorption; −, no absorption. EBV indicates cells transformed by Epstein–Barr virus.

* GFA expression was determined by reactivity of cultured astrocytoma cells (formaldehyde/acetone fixed) with rabbit anti-GFA antiserum (dilution 1:500) in indirect immunofluorescence tests.

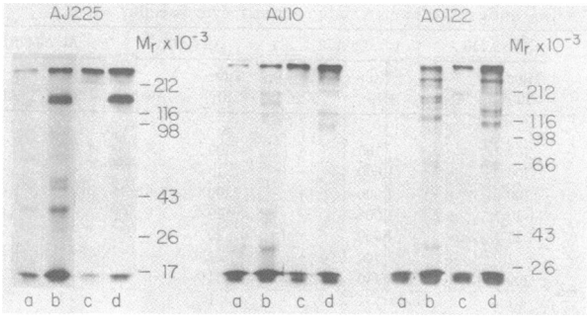

FIG. 1. Autoradiograms of immunoprecipitates obtained with Ab AJ225, Ab AJ10, and Ab AO122 from ^{125}I-labeled membrane extracts of SK-MG-1 and SK-AO2 analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis. Labeled SK-MG-1 cells were used for the analysis of Ab AJ225 and Ab AJ10, and labeled SK-AO2 cells for the analysis of Ab AO122. Lanes a and c are control immunoprecipitates obtained with *nu/nu* mouse serum. Lanes b and d are immunoprecipitates obtained with the designated monoclonal antibody. Lanes a and b are reduced samples; lanes c and d are nonreduced samples. Ab AJ225 immunoprecipitates were analyzed with 9% acrylamide gels; Ab AJ10 and Ab AO122 immunoprecipitates, with 7.5% acrylamide gels. Optimal immunoprecipitation was obtained with 1.0 μl of Ab AJ225 and 0.5 μl of Ab AO122 and Ab AJ10. The molecular weight standards were myosin (212,000), β-galactosidase (116,000), phosphorylase (97,500), bovine serum albumin (66,000), ovalbumin (43,000), concanavalin A (26,000), and myoglobin (17,000).

[^{3}H]glucosamine-, [^{35}S]methionine-, or ^{125}I-labeled SK-MG-1 cells.

AO122 Antigenic System. The AO122 antigen was found on 9/16 astrocytoma lines (Table 2). The pattern of expression on astrocytomas clearly distinguished the AO122 system from the AJ225, AJ8, or AO10 systems. AO122 was strongly represented on 8/10 melanoma lines. Neuroblastoma cells (0/2), epithelial cancer cells (0/17), EBV-transformed B cells, and T-cell leukemia cells did not express AO122. Melanocytes and adult and fetal skin fibroblasts were highly reactive with Ab AO122, and homogenates of adult and fetal brain absorbed Ab AO122. AO122 was not detected on kidney epithelial cells or erythrocytes.

Ab AO122 identified a heat-labile determinant and immunoprecipitated a protein complex from ^{125}I-labeled SK-AO2 cells. Four major proteins with M_rs 265,000, 195,000, 180,000, and 140,000 were identified in reduced preparations (Fig. 1). Without reduction, four components with M_rs 255,000, 150,000, 135,000, and 115,000 were seen. Only the 265,000 M_r band was detected after labeling with [^{35}S]methionine. Ab AO122 did not immunoprecipitate a detectable component from [^{3}H]glucosamine-labeled SK-AO2 cells. Two other monoclonal antibodies, Ab AO50 and Ab AO92, appeared to recognize the AO122 determinant. The pattern of Ab AO50 reactivity was identical to that of Ab AO122, but Ab AO50 did not immunoprecipitate any components from ^{125}I-labeled SK-AO2 cells. Ab AO92 immunoprecipitated the same protein complex as Ab AO122 did, although minor differences were found in the serological reactivity of the two antibodies.

AJ10 Antigenic System. The AJ10 determinant was found to be widely distributed on normal and malignant cells (Table 2). Despite this broad representation, the AJ10 antigen was not detected on any of the breast or colon cancer cell lines. Ab AJ10 identified a heat-labile determinant and immunoprecipitated two proteins with M_rs 195,000 and 165,000 from reduced extracts of [^{35}S]methionine- or ^{125}I-labeled SK-MG-1 cells (Fig. 1). Unreduced samples migrated as two bands corresponding to M_rs 135,000 and 110,000. Ab AJ10 did not immunoprecipitate a detectable component from [^{3}H]glucosamine-labeled cells. A second monoclonal antibody, Ab AJ60, demonstrated serological reactivity identical to that of Ab AJ10 but did not immunoprecipitate the 195,000/165,000 M_r complex.

AJ9, AJ17, AJ425, and AJ2 Antigenic Systems. Ab AJ9, Ab AJ17, Ab AJ425, and Ab AJ2 recognized heat-labile determinants on most normal and malignant cells. Differences in serological reactivity with specific cell lines suggested that they identified different antigenic systems. Ab AJ9, Ab AJ17, and Ab AJ425 did not immunoprecipitate detectable components from [^{3}H]glucosamine-, [^{35}S]methionine-, or ^{125}I-labeled SK-MG-1 cells. Ab AJ2 immunoprecipitated a glycoprotein complex with M_rs 170,000, 140,000, 140,000, and 28,000 from [^{3}H]glucosamine-, [^{35}S]methionine-, and ^{125}I-labeled SK-MG-1 cells and will be discussed in a subsequent publication.

DISCUSSION

This study of human malignant astrocytoma generated a series of mouse monoclonal antibodies that define nine distinct cell surface antigenic systems. These cell surface determinants are characterized as restricted or widely distributed on the basis of their distribution on a large panel of cultured tumor cell types. The restricted antigens (AJ225, AO10, AJ8, AO122) are preferentially expressed on tumors of neuroectodermal origin. The nonrestricted antigens (AJ10, AJ9, AJ17, AJ425, AJ2) are found on virtually all malignant cells. Cell surface determinants present only on tumor cells were not detected in this analysis; the antigens described here are identified as normal cell surface components by their expression on at least one normal cell type. The nonrestricted antigenic systems are expressed on most normal cell types and are found in brain. The restricted systems, on the other hand, have a restricted distribution on normal cells as well. AO10 and AO122 are detected in brain; AJ225 and AJ8 are not. The failure to identify the AJ225 and AJ8 determinants in brain suggests that within the context of normal neural cells AJ225 and AJ8 represent "tumor" antigens. However, endpoint absorptions with homogenates of brain may not be sufficiently sensitive to detect antigens restricted to a small subpopulation of neural cells. Extending the serological analysis to sections of normal brain and astrocytomas will be important in assessing the significance of antigens detected on tumor cells in culture but not demonstrable in normal brain by absorption techniques or binding assays.

In this study, subsets of cultured astrocytomas are identified. The biological significance of grouping astrocytomas on the basis of cell surface characteristics is unknown. Observations in other tumor systems suggest that the surface phenotype of transformed cells reflects both the cell of origin and the state of differentiation of the normal counterpart at the time of malignant change (10). Phenotypic differences among astrocytoma lines may be explained in one of three ways. Astrocytoma subsets may point to transformation in one of several developmentally and phenotypically distinct astrocyte lineages. Alternatively, tumor subsets may indicate that transformation has occurred at different points in a single lineage of astrocyte differentiation. Lastly, tumor subsets may reflect the random loss or random expression of astrocyte differentiation antigens.

The division of cultured astrocytomas into mutually exclusive AO10-positive and AJ8-positive subsets and the relationship between the AO10/AJ8 surface phenotype and GFA expression provide an opportunity to speculate on the biological significance of these cell surface markers and of the subsets they define. The intriguing feature of AO10 and AJ8 expression is the essentially nonoverlapping distribution on cultured astrocytomas. It is unlikely that subsetting of this kind is explained by a random expression or loss of the AO10 and AJ8 determinants. However, this reciprocal relationship could be taken as evi-

dence for the existence of two antigenically distinct astrocyte lineages or two antigenically distinct phases in a single lineage. Past studies of GFA have indicated that GFA is absent from pleuripotential neuroglial stem cells and immature astrocyte precursors but is detectable in all mature astrocytes (11). The observation that AO10 is present on GFA-positive tumors and that AJ8-positive tumors are GFA-negative suggests that these cell surface markers reflect the state of differentiation of astrocytomas in culture and lends support to the latter view that transformation has occurred in astrocytes at different points in a single developmental lineage.

Serological typing for AJ8, AO10, and GFA suggests that cultured astrocytomas can be divided into three groups on the basis of differentiation-related phenotypic characteristics. Cultured astrocytomas that are $AJ8^-/AO10^+/GFA^+$ represent more differentiated cell lines; those that are $AJ8^+/AO10^-/GFA^-$ represent less differentiated cell lines; and those that are $AJ8^-/AO10^+/GFA^-$ represent a group at an intermediate stage in differentiation. These relationships are illustrated in Fig. 2. It will now be important to determine whether this grouping of cultured astrocytomas on the basis of differentiation characteristics can be shown with tumors *in vivo*. Antigens AJ225 and AO122 also divide cultured astrocytomas into subsets, but the relationship between their expression and other biological properties of normal and malignant astrocytes is uncertain.

The reciprocal expression of AO10 and AJ8 also extends to normal cells of neuroectodermal origin. Brain, for example, is $AO10^+/AJ8^-$; melanocytes are $AO10^-/AJ8^+$. Preliminary observations with other neuroectodermal tumors, including neuroblastoma and melanoma, indicate a similar pattern of reciprocal antigen expression. Also noteworthy is the detection of AO10 on T-cell leukemia cells (MOLT 4), a pattern reminiscent of that observed with other antigens such as Thy-1 that are shared by T cells and brain (12).

Because of different serological methods, the use of different cell panels, and the limited biochemical characterization of many determinants, especially those that do not precipitate, it is not possible to make direct comparisons between the antigens defined in this report and those described by other investigators in studies of malignant astrocytoma (13). Such comparisons await an exchange of reagents. However, serological differences on a uniform panel of cell lines together with immunochemical differences clearly distinguish the determinants described here from the 12 antigenic systems (gp150, gp95, M_{19}, R_8, O_5, R_{24}, gp160, S_{25}, gp120r, gp120nr, gp115, V_1) previously defined in our laboratory by mouse monoclonal antibodies to human malignant melanoma and human renal cancer cells (1, 2, 14).

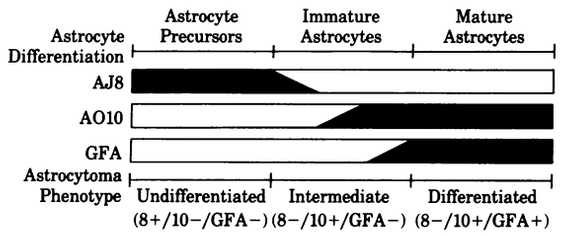

FIG. 2. Proposed relationship between astrocyte differentiation and phenotypic characteristics of cultured astrocytomas, based on serological typing for AJ8, AO10, and GFA (AJ8 AO10 are abbreviated 8 and 10, respectively).

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