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Uniform Lineage of Oligodendrogliomas

Suzanne M. de la Monte

From the Charles S. Kubik Laboratory of Neuropathology in the Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts

Experimental observations provide evidence that galactocerebrocide-containing (GC^+) oligodendrocytes and glial fibrillary acidic protein-containing ($GFAP^+$) type 2 astrocytes are derived from $A2B5^+$ progenitor cells. Because the cytomorphologic features of oligodendrogliomas resemble those of non-neoplastic oligodendrocytes, it was speculated that neoplastic oligodendroglial cells also have $A2B5^+$ lineage. This hypothesis was investigated by immunostaining histopathologic sections with monoclonal antibodies to GC, A2B5, and GFAP. In 28 tumors, ubiquitous immunolabeling of neoplastic cells with anti-GC and anti-A2B5 was observed. In addition, $GFAP^+/A2B5^+$ astrocytes were present in most mixed glial tumors. The findings suggest that oligodendrogliomas, whether or not they contain foci of astrocytoma, are uniformly derived from $A2B5^+$ progenitor cells. (Am J Pathol 1989, 135:529–540)

The histopathologic features of oligodendrogliomas suggest that these tumors originate from cells carrying the propensity to differentiate into mature oligodendrocytes. As a group, oligodendrogliomas manifest relatively uniform histopathologic and biological behavior, and few histopathologic features have been correlated with long-term prognosis.[1–4] This profile contrasts with the strikingly disparate clinical course of astrocytomas in which prognosis is largely predicted by the histopathologic grade.[5–7] One possible explanation for these intrinsic differences between oligodendrogliomas and astrocytomas is that their histogeneses differ.

Experimental observations imply a dual lineage for macroglial cells. In one of the lineages the progenitor cells contain rat neural antigen-2 ($Ran\text{-}2^+$)[8–10] and lack A2B5 antigen ($A2B5^-$),[10–16] whereas in the other lineage the progenitor cells have the converse immuno-phenotype in that they are $Ran\text{-}2^-$, $A2B5^+$, and designated O-2A.[9–16] $Ran\text{-}2^+$, $A2B5^-$ progenitor cells differentiate into type 1 astrocytes, whereas $Ran\text{-}2^-$, $A2B5^+$ progenitor cells are bipotential with respect to the generation of macroglia and they differentiate into type 2 astrocytes and galactocerebroside-containing (GC^+) oligodendrocytes.[17] *In vitro* immunoreactivity with monoclonal antibodies to tetanus toxin further distinguishes glial cells with $A2B5^+$ as opposed to $A2B5^-$ lineage.[10,15] As $A2B5^+$ progenitor cells differentiate and mature into normal oligodendrocytes they become $A2B5^-$[15] and acquire galactocerebroside (GC) followed by myelin basic protein,[18–21] whereas cells destined to become type 2 astrocytes begin to synthesize glial fibrillary acidic protein (GFAP).[22–24] Type 2 astrocytes assume an intimate relationship with axons of the CNS and do not specifically react to form scar tissue after injury.[25] Their precise role in CNS physiology and development has not been completely defined. In contrast, type 1 astrocytes proliferate and generate scar tissue in response to injury in the central nervous system (CNS).[25]

Because the cytologic features of oligodendrogliomas recapitulate those of non-neoplastic oligodendrocytes, it was speculated that oligodendrogliomas might have $A2B5^+$ lineage. If they do, then the comparatively similar biological behavior of different grades of oligodendroglioma might be attributable to their uniform histogenesis. To examine this hypothesis, 28 biopsy specimens of oligodendroglioma were immunostained to detect A2B5, GFAP, and GC antigens. The findings suggest that oligodendrogliomas do have uniform lineage and derive from $A2B5^+$ (O-2A) progenitor cells.

Experimental Design

All available tissue sections from 28 biopsies of oligodendroglioma diagnosed at the Massachusetts General Hospital between 1976 and 1985 were reviewed to assign histopathologic grades on a scale of 1 to 3 based on cellular density, nuclear pleomorphism, nuclear mitoses, necrosis, and vascularization (Table 1). The oligodendroglioma and astrocytoma components of mixed tumors were graded independently. To be considered an astrocytoma

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Address reprint requests to Dr. Suzanne de la Monte, Division of Neuropathology, Warren 3, Department of Pathology, Massachusetts General Hospital, Fruit Street, Boston, MA 02114.

Table 1. *Histopathologic Criteria Used to Grade Oligodendrogliomas*

Histologic feature	Grade (0–3)	Criteria
Nuclear pleomorphism	1	Small, round, uniform
	2	Larger, varied in size and shape
	3	Large, bizarre
Cellular density	1	Abundant intercellular neuropil
	2	Cytoplasmic borders touching
	3	Cells overlapping
Vascularization	1	Fine branching capillaries
	2	Thick-walled vessels
	3	Glomeruloid proliferation of endothelial cells
Necrosis	0	Absent
	1	Individual pyknotic nuclei
	2	Small clusters of necrotic cells
	3	Sheets of necrotic cells
Mitoses (per 20 400 × fields)	0	Absent
	1	1
	2	2–5
	3	>5

Overall tumor grade: 1, total score less than 5; 2, total score 5 to 8; 3, total score greater than 8.

component, the cells had to be neoplastic and clearly manifest characteristic cytomorphologic features of astrocytic cells in routine histologic sections. Cells having the appearance of oligodendroglioma but immunolabeled with antibodies to GFAP were graded as oligodendroglioma rather than astrocytoma.

Adjacent sections (8- to 10-μm thick) from one to three paraffin blocks containing the most representative sample of tumor were immunostained with mouse monoclonal antibodies to GC, GFAP (Dako Products), and A2B5 (antibodies to GC and A2B5 are available from the American Type Culture Collection) antigens using the avidin-biotin-horseradish peroxidase complex (Vector Laboratories, Burlingame, CA) method, according to the manufacturer's protocol, with 3-3'diaminobenzidine (Sigma Chemical Co., St. Louis, MO) as the chromogen. They were then counterstained with hematoxylin, dehydrated in graded alcohols, and cleared in xylenes. The tissue sections were incubated with primary antisera for 1 hour, and the entire immunostaining protocol was carried out at room temperature in humidified chambers. Nonspecific binding with anti-A2B5 was eliminated by initially digesting the sections with trypsin (0.25% in 10 mM phosphate buffered saline, pH 7.4, for 30 minutes at 37 C). All specimens had been fixed in 10% formaldehyde solution for several hours and processed for paraffin embedding in a routine fashion. The quality of staining was uniform and reproducible under these circumstances. Tissues fixed and processed using other methods were not studied.

The densities of GC^+, $GFAP^+$, and $A2B5^+$ neoplastic cells were estimated as follows: 0%, less than 5%, less than 10%, less than 25%, less than 50%, less than 95%, and 95% to 100%. In addition, the proportion of unlabeled neoplastic cells was estimated by subtracting from 100% the total percentage of nonoverlapping areas of GC^+, $GFAP^+$, and $A2B5^+$ cells within the tumors. In general, this process was facilitated by the tendency of cells of a given type to form discrete aggregates that were readily identifiable in adjacent sections derived from the same block and paraffin ribbon. Because the results of double-labeling immunocytochemistry were technically unsatisfactory, the presence of tumor cells bearing two or all three antigens was assessed by comparing the patterns and distributions of immunolabeling in adjacent sections. This process was abetted in part by the distinctive cytomorphology of immunolabeled neoplastic cells. Correlation matrix analysis (Systat programs, Evanston, Ill) was used to determine whether the patterns of immunostaining were associated with the histopathologic grade of the tumors.

Results

Histopathological Features

Based on cytomorphology alone, 21 of the 28 tumors (75%) were classified as oligodendroglioma-predominant, ie, as containing more than 60% oligodendroglioma cells; six (21%) had approximately equal mixtures (40% to 60%) of oligodendroglioma and astrocytoma; and one (4%) was astrocytoma predominant. Grade 1 oligodendroglioma was present in 13 (46%) tumors, grade 2 in 11 (39%), and grade 3 in four (14%). Of the 20 mixed glial tumors, eight (40%) had grade 1 astrocytoma present, eight (40%) had grade 2 present, and four (20%) had grade 3 present.

Cytologic Features of Immunolabeled Neoplastic Cells

Galactocerebroside was detected in 27 (96%) tumors, 21 (78%) of which had cytomorphologic features of oligodendroglioma, ie, they had small round dark nuclei and scant eosinophilic or transparent cytoplasm giving the appearance of a perinuclear halo. In the remaining six, GC^+ cells were identified in both the oligodendroglioma and astrocytoma components of the tumors. The intensity of immunolabeling varied in that cells with large amounts of

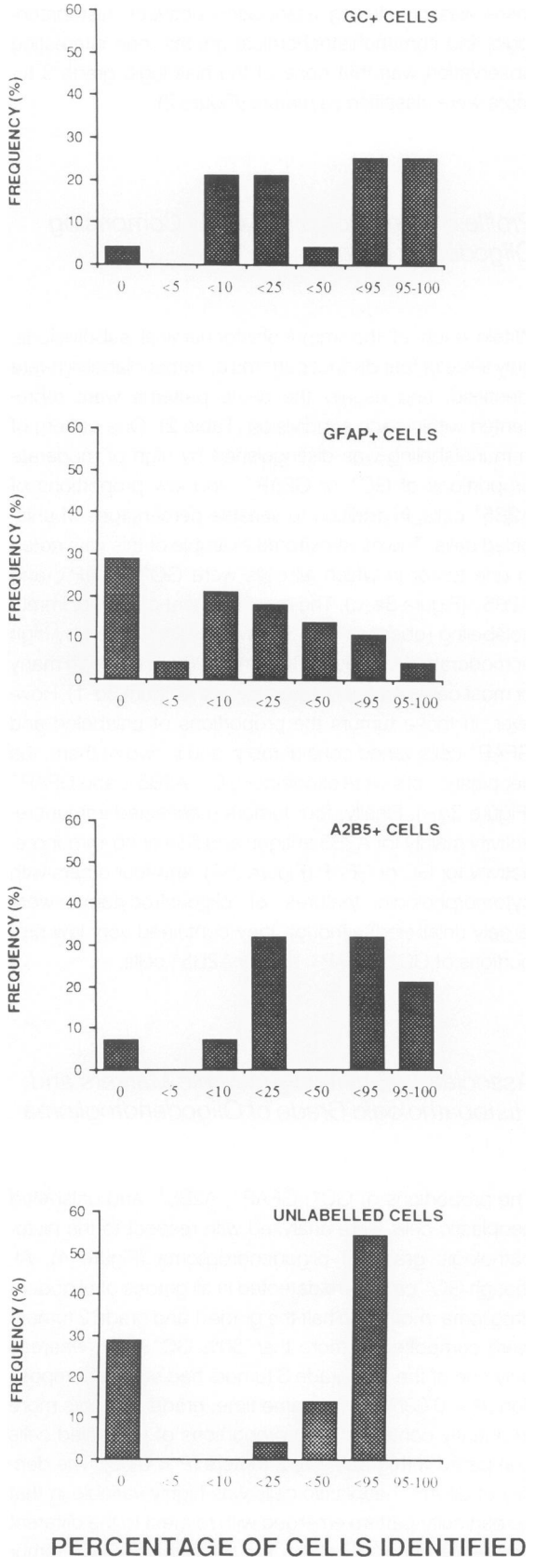


Figure 1. *Percentages of the 28 oligodendrogliomas (all grades) containing different densities of GC$^+$, GFAP$^+$, A2B5$^+$, and unlabeled cells. Moderate (25% to 50%) or high (>50%) densities of GC$^+$ and A2B5$^+$ cells were observed in half the tumors. Only one tumor lacked GC$^+$ cells and two others lacked A2B5$^+$ cells. However, all tumors were GC$^+$, A2B5$^+$, or both. Although GFAP$^+$ cells were observed in 68% of the tumors, usually the densities were low and GFAP$^+$ tumor cells were usually also A2B5$^+$. Moderate or high densities of unlabeled cells were observed in 62% of the cases, whereas in 35% none of the cells were unlabeled; ie, all neoplastic cells were immunolabeled with at least one of the three primary antibodies used.*

visible cytoplasm tended to be more heavily immunolabeled than those with small amounts of transparent cytoplasm. The selectivity of immunolabeling for GC in oligodendroglial cells was similar to that previously described in the CNS of experimental animals using monoclonal antibodies.[26]

GFAP$^+$ cells were detected in 18 (64%) tumors. Two tumors with cytomorphologic foci of astrocytoma had GFAP$^+$ stroma but not cells. Otherwise, the cells comprising the foci of astrocytoma were uniformly and intensely GFAP$^+$. In 13 (72%) tumors, many GFAP$^+$ neoplastic cells had features of oligodendroglioma as well as immunoreactivity for GC, A2B5, or both. Thus, true oligoastrocytoma cells (GC$^+$/GFAP$^+$) were detected in a large percentage of the tumors.

A2B5 antigen was nearly as prevalent as GC antigen (93% *versus* 96% of the tumors). In nine (32%) cases it was detected in both the oligodendroglioma and astrocytoma components, in 12 (43%), in only the oligodendroglioma portion, and in four (14%) in only the astrocytoma portion. For the most part, A2B5$^+$ cells were also GC$^+$, GFAP$^+$, or both, but A2B5$^+$, GC$^-$, and GFAP$^-$ neoplastic glial cells were detected as well. In addition, two tumors contained A2B5$^+$, GC$^-$, and GFAP$^-$ neoplastic ganglion cells within foci of ganglioglioma. Only two tumors lacked A2B5$^+$ neoplastic cells: in one, 20% of the cells were GC$^+$ and 80% were not immunolabeled with any of the antibodies, and in the other, all cells were GC$^+$ and GFAP$^-$.

Aggregates of neoplastic glial cells not labeled by any of the three antibodies were observed in 20 (71%) tumors. Such cells had histopathologic features indistinguishable from GC$^+$ or A2B5$^+$ oligodendroglioma cells.

Relative Proportions of Different Immunolabeled Neoplastic Cells in the Tumors

Although GC$^+$ neoplastic cells were detected in 97% of the tumors, their densities varied. High densities (estimated as <95% or 95% to 100%) of GC$^+$ cells were detected in 14 (50%) tumors, moderate densities (<25% or

<50%) in seven (25%), and low densities (0, <5%, or <10%) in six (21%). In seven cases (25%) virtually all (99% to 100%) neoplastic cells were GC^+ (Figure 1).

Although $GFAP^+$ neoplastic cells were detected in a high percentage of the tumors, their densities were generally low. In more than half the cases, $GFAP^+$ neoplastic cells were either absent (N = 8; 29%) or were less than 10% of the tumor population (N = 7; 25%). In the few examples (N = 4; 15%) in which $GFAP^+$ cells made up high proportions of the tumor population, those same cells were also GC^+, $A2B5^+$, or both. In tumors with moderate densities of $GFAP^+$ cells (N = 9; 39%), it was mainly the astrocytoma components that were immunolabeled, but the cytomorphologically oligodendroglioma components were often $GFAP^+$ as well.

The pattern of immunostaining for A2B5 paralleled that of GC in that GC^+ cells were usually also $A2B5^+$. A2B5 antigen was widespread and detectable in a large proportion of the cells in each tumor, although the intensity of immunostaining varied. In six tumors (21%), more than 95% of the cells showed intense immunolabeling with anti-A2B5.

High densities of unlabeled cells were present in 15 tumors (54%), but in all cases at least 10% of the tumor cells were labeled by one or more antibodies. On the other hand, in eight (29%) tumors, none of the cells were unlabeled, ie, all neoplastic cells were immunolabeled with at least one of the antibodies.

Immunohistochemical Reclassification of Tumors and Correlation with Histopathologic Grade

The patterns of immunolabeling initially appeared complex because the high degree of variation in the proportions of unlabeled and $GFAP^+$ cells afforded a multitude of unique profiles. To simplify the presentation of data and to correlate patterns of immunohistochemical staining with histopathologic grade, the tumors were reclassified as primitive, intermediate, or mature based on the percentages of unlabeled cells. The assumptions made were 1) the most mature tumors should have high densities of GC^+ or $GFAP^+$ cells and ought to lack or have very low densities (<5%) of unlabeled cells; 2) less mature tumors should be largely composed of $A2B5^+$ cells with low or moderate densities of GC^+ or $GFAP^+$ cells; and 3) the least mature tumors should mainly have unlabeled cells. Using this scheme, 12 (43%) tumors were reclassified as primitive because they contained 50% or more unlabeled cells, six (22%) were reclassified as intermediate, and ten (37%) were reclassified as mature because at least 90% of the tumor cells were either GC^+ or $GFAP^+$. Although there was no striking association between histopathologic and immunohistochemical grades, one interesting observation was that none of the histologic grade 3 tumors were classified as mature (Figure 2).

Profile of Immunostained Cells Comprising Oligodendrogliomas

Within each of the immunohistochemical subdivisions, only three or four distinct patterns of immunolabeling were identified, and usually the same patterns were represented within each subdivision (Table 2). One pattern of immunolabeling was distinguished by high or moderate proportions of GC^+ or $GFAP^+$, and low proportions of $A2B5^+$ cells, in addition to variable percentages of unlabeled cells. The most extreme example of this was noted in one tumor in which all cells were GC^+, $GFAP^-$, and $A2B5^-$ (Figure 3a–c). The most frequent pattern of immunolabeling (observed in 47%) was characterized by high or moderate densities of GC^+ and $A2B5^+$ cells, with many or most cells immunoreactive for both (Figure 3d–f). However, in those tumors the proportions of unlabeled and $GFAP^+$ cells varied considerably, and in two of them, the neoplastic cells were altogether GC^+, $A2B5^+$, and $GFAP^+$ (Figure 3g–i). Finally, four tumors manifested immunoreactivity mainly for A2B5 antigen and little or no immunoreactivity for GC or GFAP (Figure 3j–l), and four others with cytomorphologic features of oligoastrocytoma were largely unlabeled, although they contained very low proportions of GC^+, $GFAP^+$, and/or $A2B5^+$ cells.

Association Between Antigenic Markers and Histopathologic Grade of Oligodendroglioma

The proportions of GC^+, $GFAP^+$, $A2B5^+$, and unlabeled neoplastic cells were analyzed with respect to the histopathologic grade of oligodendroglioma (Figure 4). Although GC^+ cells were detected in all grades of oligodendroglioma, more than half the grade 1 and grade 2 tumors were composed of more than 50% GC^+ cells, whereas only one of the four grade 3 tumors had such a composition ($P < 0.005$). At the same time, grade 3 tumors more frequently contained high proportions of unlabeled cells compared with grade 1 or 2 tumors ($P < 0.05$). The density of $GFAP^+$ neoplastic cells was highly variable in that no particular pattern emerged with respect to the different grades of tumor. Similarly, the density of $A2B5^+$ tumor cells did not correlate with tumor grade.

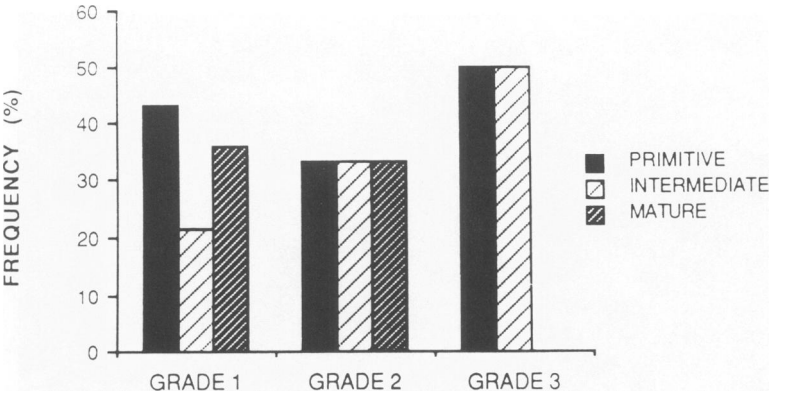


Figure 2. *Immunohistochemical reclassification of tumors and correlation with histopathologic grade of oligodendroglioma. The proportions of unlabeled and A2B5$^+$/GC$^-$, GFAP$^-$ cells were used to reclassify the tumors as primitive (N = 12, 43%), intermediate (N = 8, 29%), or mature (N = 8, 29%) (also see Table 2). There were no significant correlations with respect to histopathologic and immunohistochemical grades, but an interesting finding was that none of the histologic grade 3 tumors were classified as mature.*

Discussion

All 28 oligodendrogliomas were GC$^+$, A2B5$^+$, or both, and in general substantial proportions of the tumor cell populations expressed both. This implies 1) that oligodendroglioma cells bear the same antigenic markers of differentiation as non-neoplastic immature oligodendrocytes; and 2) that oligodendrogliomas have A2B5$^+$ lineage. Experimental paradigms of oligodendrocyte differentiation have demonstrated a gradual disappearance of A2B5 antigen[17] coupled with cessation of the mitotic cycle and sequential antigenic expression of galactocerebrocide followed by myelin basic protein,[21] reflecting a transition to mature myelin-producing cells. Thus, the nearly universal expression of GC and A2B5 in oligodendrogliomas is reminiscent of what occurs during maturation of non-neoplastic oligodendrocytes. Assuming that A2B5 and GC antigens are also expressed sequentially in neoplastic cells, it seems likely that oligodendroglioma cells on the whole are halted in their differentiation at a stage at which the lineage marker (A2B5) is still present and the cells are identifiable as committed to differentiate and mature primarily along the lines of oligodendroglia.

GFAP$^+$ neoplastic cells with cytomorphologic features of oligodendroglioma were frequently immunoreactive with antibodies to GC, A2B5, or both GC$^+$/GFAP$^+$ cells are *bona fide* oligoastrocytomas. Although similar GFAP$^+$ neoplastic cells were described by others,[4,27,28] they were not previously shown to be GC$^+$ as well. Oligoastrocytoma cells appear antigenically similar to the oligoastrocyte transitional cells observed experimentally.[29] The existence of such transitional cells is explained by the finding that during differentiation of normal oligodendroglia there is a window in time when both GC and GFAP antigens are expressed, but with further differentiation the GFAP disappears. It has been suggested that in this situation the immunoreactive GFAP may be soluble because intermediate filaments are lacking by ultrastructural examination.[29] The same may be true of oligoastrocytoma cells.

In addition to the labeling of the astrocytoma component with anti-A2B5, foci of ganglioglioma present in two tumors were also A2B5$^+$. This is of interest because, experimentally, immature developing neuronal cells are often A2B5$^+$.[15,30] This observation, together with the finding of GC$^+$/GFAP$^+$ and GFAP$^+$/A2B5$^+$ neoplastic cells within the tumors, suggests that both pure and mixed oligodendrogliomas share the same histogenesis as normal oligodendrocytes, type 2 astrocytes, and a subset of neurons, and thus are all derived from A2B5$^+$ progenitor cells. This

Table 2. *Subcategorization of 28 Oligodendrogliomas According to the Proportions of GC$^+$, GFAP$^+$, A2B5$^+$, and Unlabeled Cells*

Category	GC$^+$	GFAP$^+$	A2B5$^+$	Number (%)
Mature (<10% unlabeled)	High	Variable	High	8 (29)
	High	Low	Low	1 (4)
	Moderate	Low	High	1 (4)
Intermediate (10% to 50% unlabeled)	Moderate	Variable	Moderate	3 (11)
	Moderate*	Moderate*	Low	2 (7)
	Low	Moderate	Moderate	1 (4)
Primitive (>50% unlabeled)	Moderate*	Moderate*	Low	4 (14)
	Low	Low	Low	4 (14)
	Moderate	Variable	Moderate	2 (7)
	Low	Variable	Moderate	2 (7)

* Indicate either one or both.

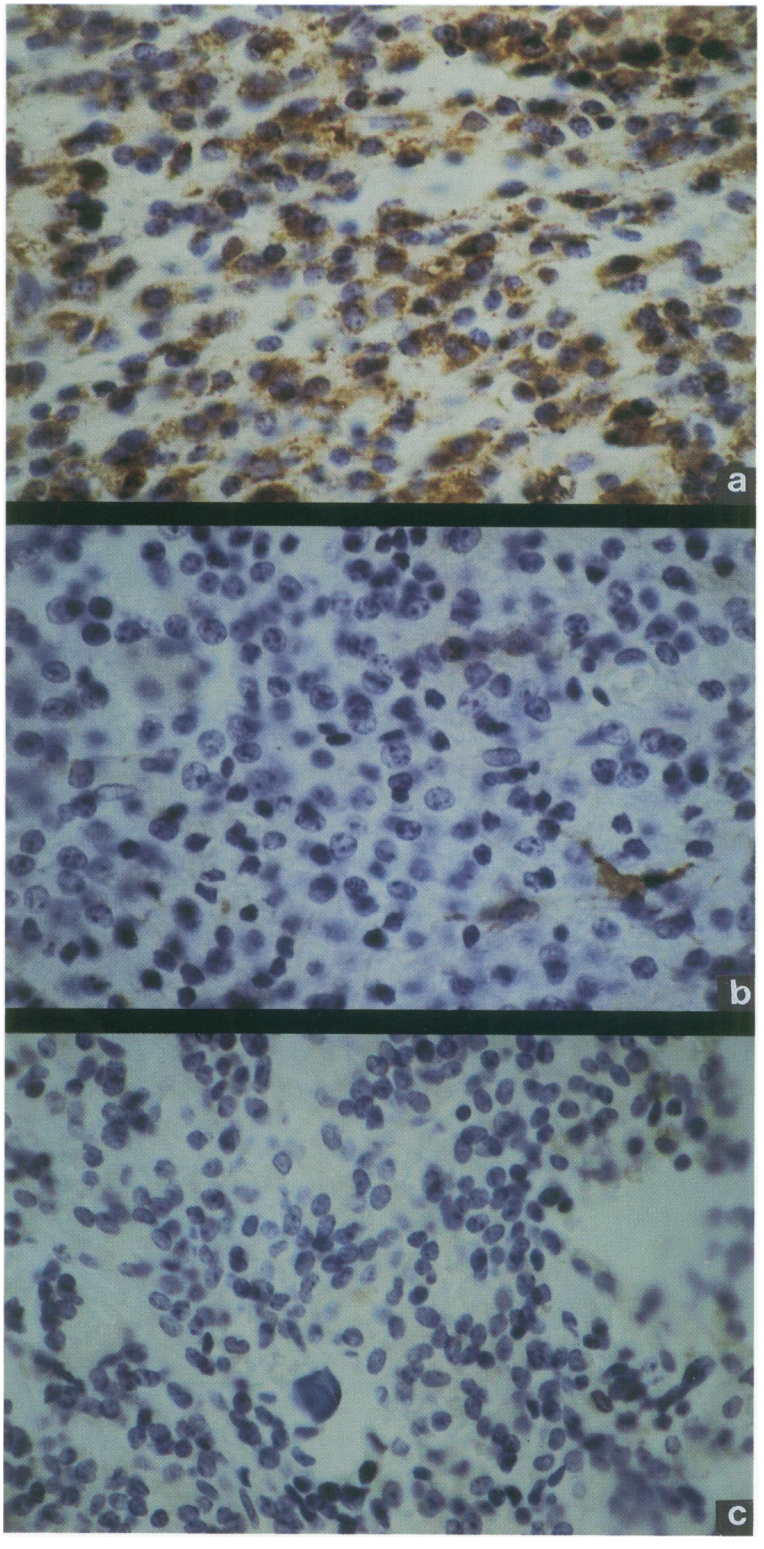


Figure 3. *Patterns of immunohistochemical staining of oligodendrogliomas. Each set of three figures depicts adjacent sections from a single tumor immunostained with anti-GC (top panel), anti-GFAP (middle panel), or anti-A2B5 (bottom panel). One pattern of immunolabeling (N = 2, 7%) was distinguished by high or moderate proportions of GC$^+$ and low proportions of GFAP$^+$ and A2B5$^+$ cells* **(a–c)**. *The most frequent pattern of immunolabeling (47%) was characterized by high of GC$^+$ and A2B5$^+$ cells, with many or most cells immunoreactive for both, and few or no GFAP$^+$ cells* **(d–f)**. *In two tumors (7%), the neoplastic cells were altogether GC$^+$, A2B5$^+$, and GFAP$^+$* **(g–i)**. *Four tumors (14%) manifested immunoreactivity mainly for A2B5 antigen, and little or no immunoreactivity for GC or GFAP* **(j–l)** *(all ×825).*

counters the observations made with astrocytomas in which only 24% were found to have A2B5$^+$ lineage.[31]

Understanding the origin of unlabeled neoplastic cells is germane to the interpretation of cell lineage analysis of glial tumors. Because A2B5 antigen is expressed quite early in glial differentiation,[9,12,16] its absence in immature (unlabeled) cells programmed to become oligodendroglia indicates a very primitive and perhaps stem cell stage. By

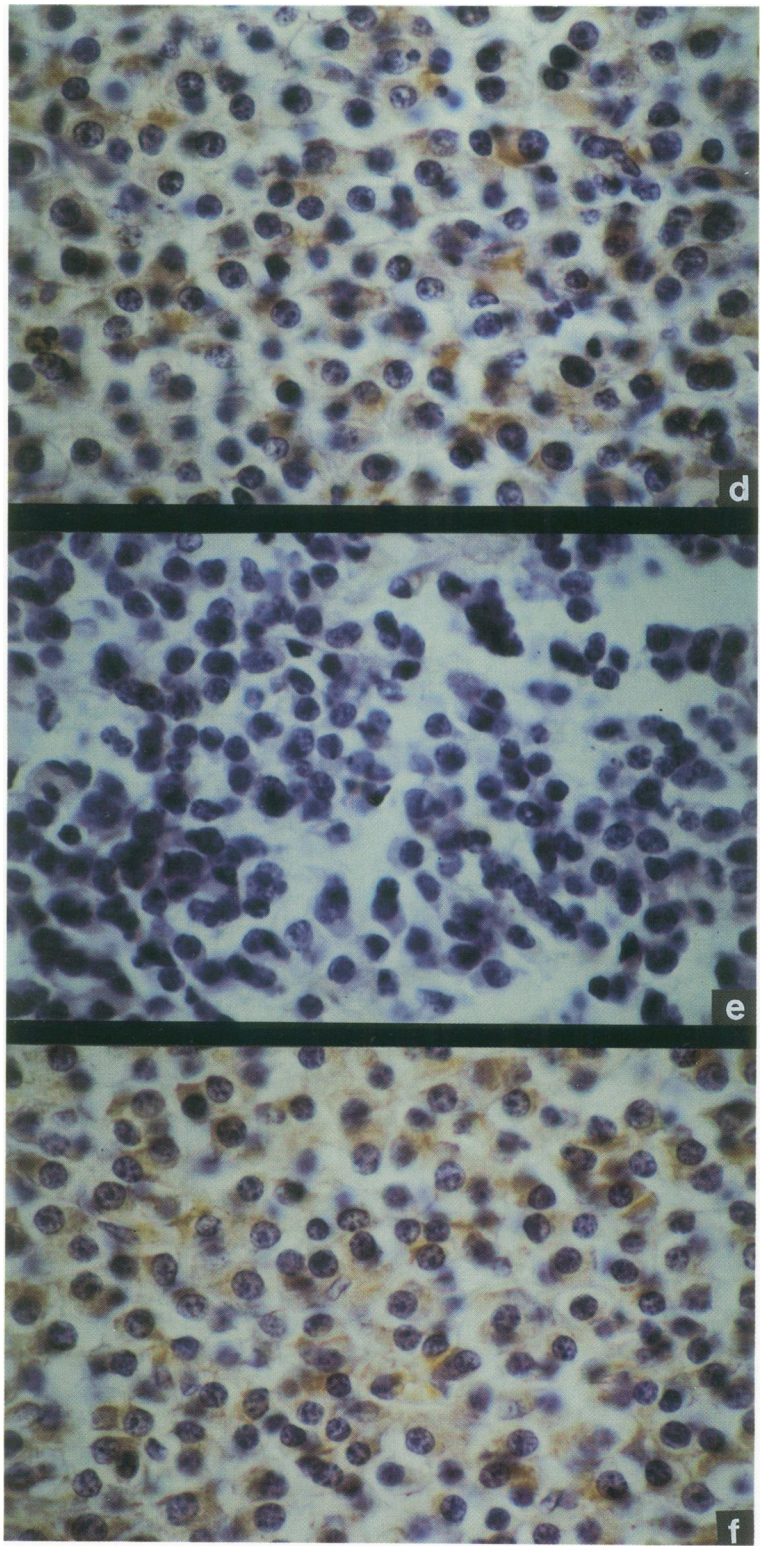
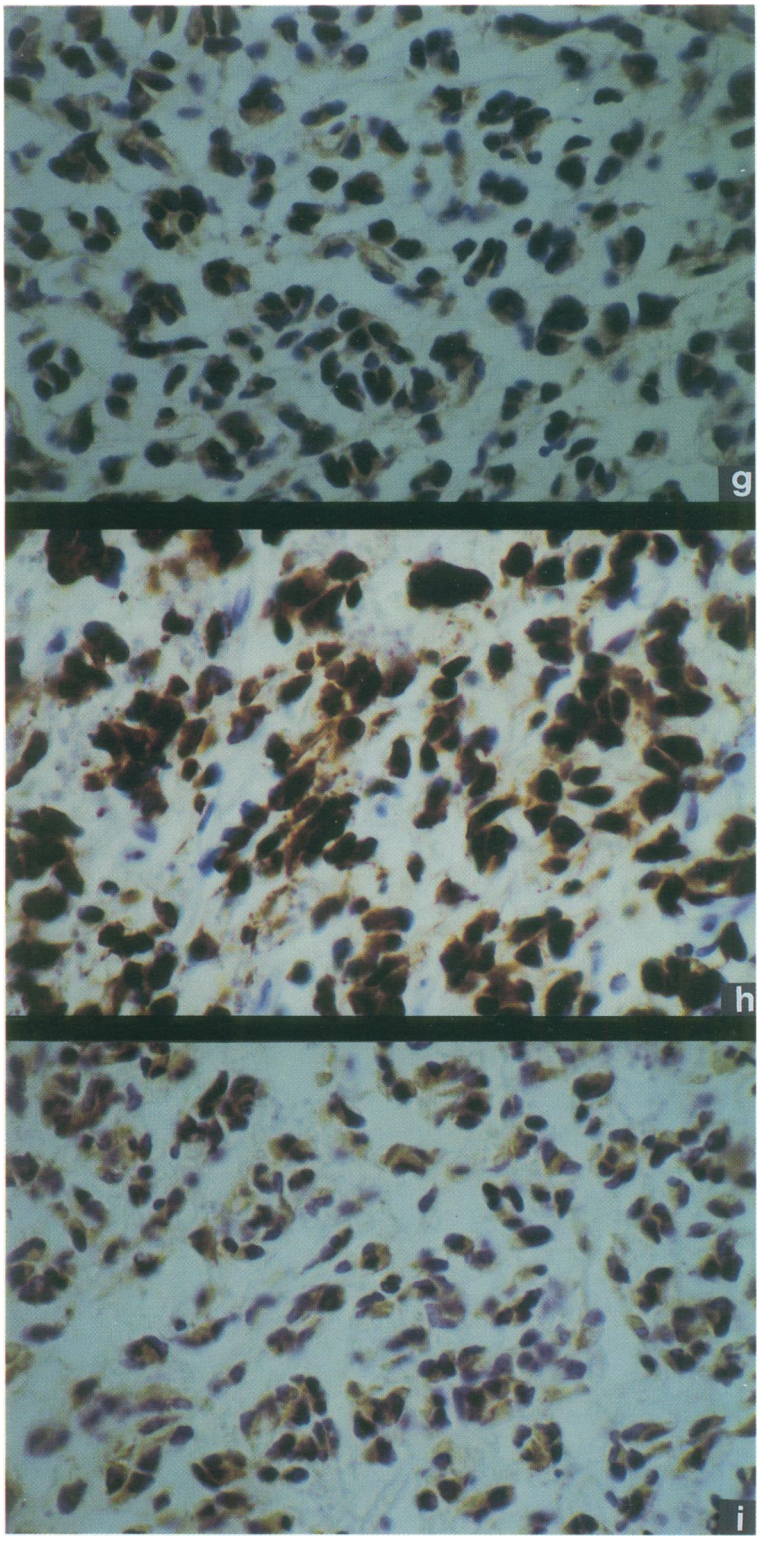


Figure 3 *(continued)*

the same token, the absence or markedly reduced expression of A2B5 antigen in association with moderate or high densities of GC^+ or $GFAP^+$ neoplastic cells indicates an advanced degree of differentiation because this is precisely the antigenic phenotype of non-neoplastic postmitotic oligodendrocytes and type 2 astrocytes. In this



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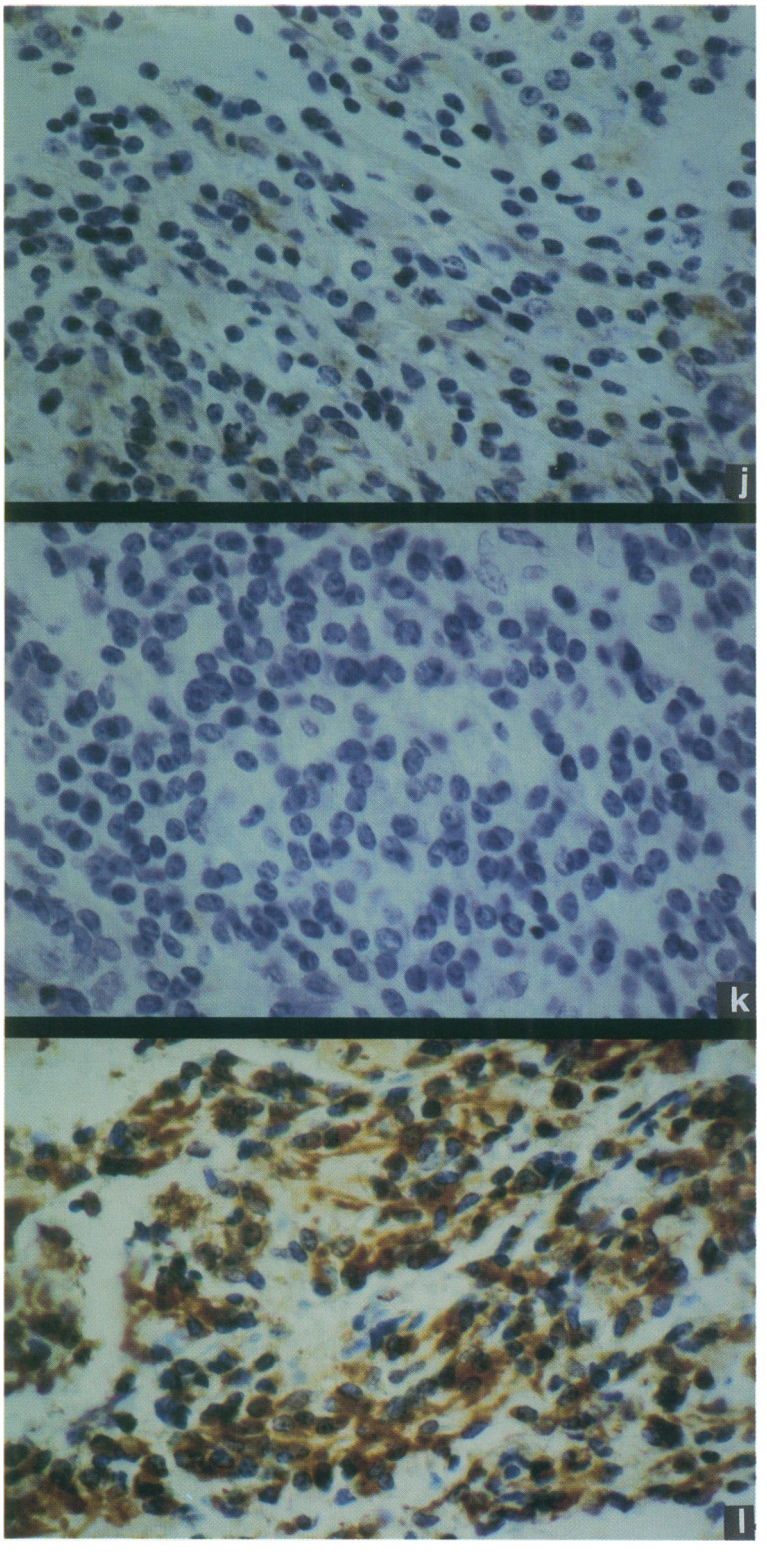
context it is reasonable to hypothesize that the unlabeled neoplastic cells in oligodendrogliomas represent either de-differentiated cells that have lost antigenic expression of A2B5, GC, and/or GFAP, or they are precursors of $A2B5^+$ progenitor cells, ie, are pluripotent (stem) cells capable of differentiating along either $A2B5^+$ or $A2B5^-$ lin-



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eage. However, the latter possibility is doubtful because if the tumors contained such stem cells a broader range of cell types defined both histopathologically and immunohistochemically should have been evident, eg, the GFAP$^+$ astrocytoma components ought not to have been uniformly A2B5$^+$ as they were. Experimental evidence

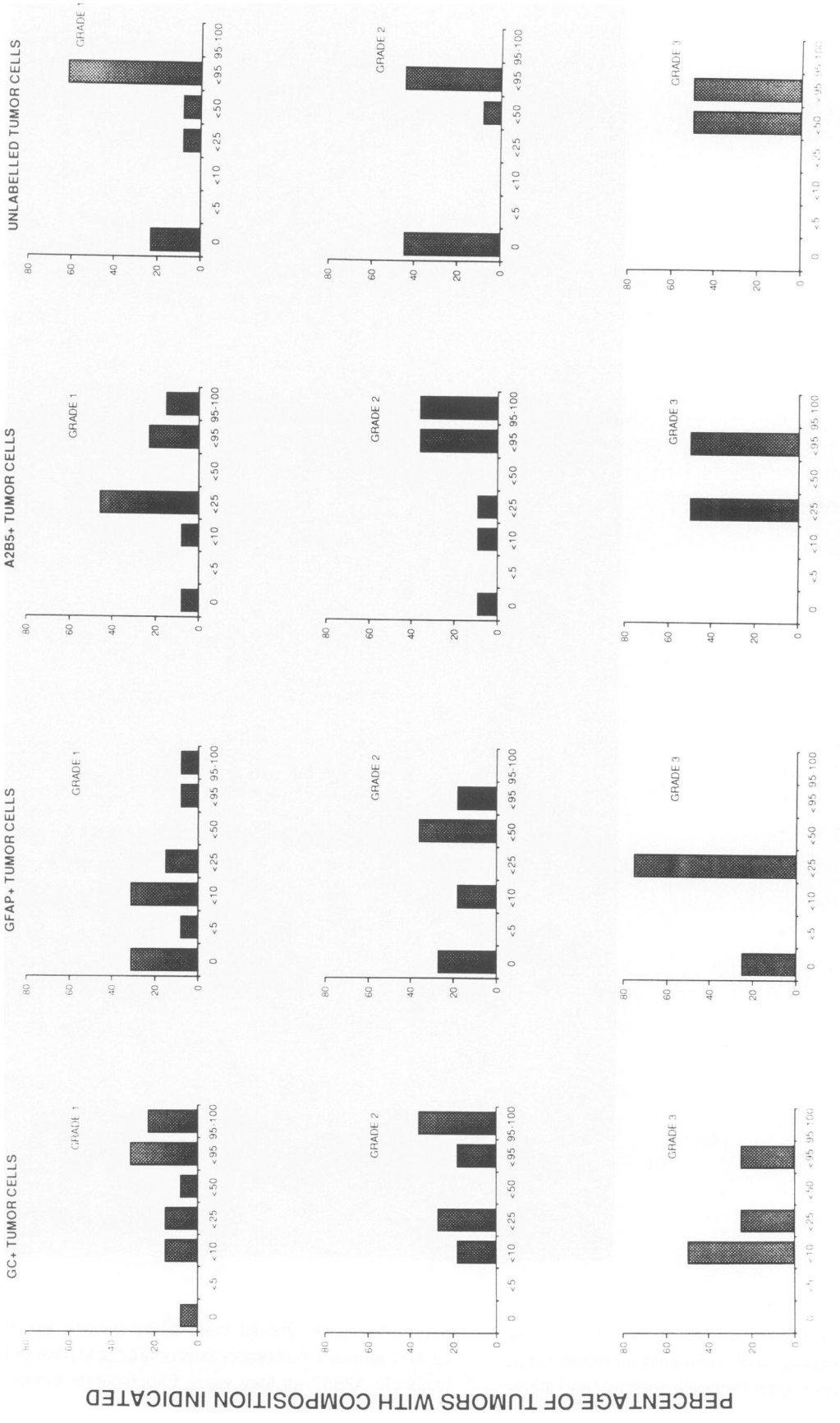

Figure 4. *Density distributions of different cell types with respect to histopathologic grade of oligodendroglioma (grade 1, N = 13; grade 2, N = 11; grade 3, N = 4). The percentage of the indicated cell type is expressed along the abscissa, and the percentage of the tumors with that particular composition is represented along the ordinate. Although GC^+ cells were present in all grades of oligodendroglioma, more than half the grade 1 and grade 2 tumors had greater than 50% GC^+ cells, whereas only one of the four grade 3 tumors had such a high density of GC^+ cells ($P < 0.005$). Moreover, grade 3 tumors more frequently contained high densities of unlabeled cells compared with grade 1 and 2 tumors ($P < 0.05$). The densities of $GFAP^+$ and $A2B5^+$ cells, however, were not correlated with tumor grade.*

suggests that in the adult CNS there is a persistent reservoir of A2B5$^+$ cells with O-2A progenitor-cell-like morphology, and capacity to exhibit an *in vitro* proliferative response to growth factors and differentiate into GC$^+$ oligodendrocytes or type 2 astrocytes.[32] Presumably these progenitor cells could serve as targets for neoplastic transformation and thereby give rise to both pure and mixed oligodendrogliomas. In light of this discussion, the more tenable hypothesis is that the unlabeled neoplastic oligodendroglial cells represent de-differentiated A2B5$^+$ progenitor cells rather than stem cells.

Independent of histopathologic grade, oligodendrogliomas have comparatively invariant and indolent biological behaviors relative to most moderate- and high-grade astrocytomas. This phenomenon may be related to the common histogenesis of oligodendrogliomas from A2B5$^+$ progenitor cells. In support of this argument is the observation that several of the astrocytomas containing moderate or high densities of A2B5$^+$ neoplastic cells had histopathologic features indicative of a good prognosis.[31] One possible interpretation is that A2B5$^+$ glial tumors have a better prognosis than those lacking this particular lineage marker.

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