Histol Histopathol (1998) 13: 73-79

Histology and Histopathology

Lectinhistochemistry of mixed gliomas demonstrating an intermediate cell type

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Summary. 18 cases of low-graded mixed gliomas were studied using the two lectins Concanavalin A (Con A) and Peanut lectin (PNA). Con A stained cytoplasm and processes of tumoral astrocytes, whereas PNA stained cell membranes of tumoral oligodendrocytes. Con A and PNA are reliable markers for astrocyte and oligodendrocyte areas of mixed gliomas, respectively. A part of cells were overlappingly positive for both lectins. They expressed an oligosaccharide pattern of both glioma types and represented a third, intermediate cell type of mixed gliomas. The existence of intermediate cells close to astrocytic and oligodendroglial cell types in mixed gliomas could result from transformation processes of neoplastic glial cells or from the malignant transformation of a common glial precursor cell.

Key words: Mixed glioma, Intermediate glial cells, Lectinhistochemistry

Introduction

In mixed gliomas the conventional hematoxylin-eosin stain does not allow one to sufficiently differentiate astrocytes and oligodendrocytes. The glial fibrillary acidic protein (GFAP) used as marker for astroglia does not reliably differentiate between astrocytic and oligodendrocytic tumor parts because in some cases GFAP is also expressed in neoplastic oligodendrocytes (Eng and Rubinstein, 1978; DeArmond et al., 1980). These tumor cells are then called gliofibrillary oligodendrocytes (Herpers and Budka, 1984). There is no specific oligodendroglial marker known so far. Markers such as myelin basic protein (MBP), myelin-associated glycoprotein (MAG) and Anti-Leu 7, GFAP, S-100 protein, and carboanhydrase C are not specific or of diagnostic value for oligodendrogliomas (Schwechheimer, 1990).

Former lectinhistochemical studies of human astrocytomas and oligodendrogliomas showed lectins to be useful in the diagnosis of these tumors (Schwechheimer et al., 1983; Kabori et al., 1988; Wang et al., 1989; Cruz-Sánchez et al., 1991; Figols et al., 1991, 1993). Lectins are glycoproteins characterized by their ability to specifically bind different carbohydrate moieties (Goldstein et al., 1980; Danguy et al., 1988).

In this study the lectins Concanavalin A (Con A) and Peanut Agglutinin (PNA) were used. Con A binds specifically to D-mannose and D-glucose and, PNA shows a specific binding to D-galactose and N-acetyl-galactosamine (Goldstein and Poretz, 1986). The lectin-histochemistry of mixed gliomas has yet to be studied. We therefore correlated lectinhistochemical and immunohistochemical staining of mixed glioma and furthermore evaluated lectinhistochemical methods for the histological characterization of mixed gliomas.

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Materials and methods

Formalin-fixed tissues of 18 cases of low-grade mixed gliomas (grade II) obtained from the files of the Institute of Neuropathology of the Free University of Berlin were used in this study. There were 11 males and 7 females. The mean age was 43 years with a range between 22 and 75 years. All tumors were intracranial and located predominantly in the frontal and temporal lobes.

Paraffin-embedded tissues were cut at 4 μm and were stained with hematoxylin-eosin (HE). Immunostaining with glial fibrillary acid protein (GFAP) was carried out by the avidin-biotin method (ABC-Kit of Vecta-Stain). Four μm-thin slices were deparaffinized in two baths of xylene for 5 min each followed by two 5 min baths of acetone. The sections were transferred to distilled water and then to two consecutive baths (5 min each) in Tris-saline-buffer (TPS), pH 7.4, containing 1 mM of $MgCl_2$, $MnCl_2$ and $CaCl_2$ (Schulte and Spicer, 1983). The sections were immersed in a bath of TBS containing 0.1% trypsin for 10 min. To block the endogenous peroxidase activity the sections were immersed in absolute ethanol with 30% H_2O_2 in relation 100:1 for 20 min. After two 5 min baths in TBS the sections were covered with the lectins for 90 minutes in a moist chamber at room temperature. The lectins (Sigma

Chemical Co.) were conjugated with horseradish-peroxidase and were applied diluted in TBS (Con A 20 μg/ml, PNA 15 μg/ml). Per 1 ml diluted lectins, 5 ml Triton X-100 was added to improve lectin reactivity. Two 5 min baths in TBS followed. Then the sections were covered with diaminobenzidine (DAB) solution freshly prepared from one DAB tablet (Sigma Chemical Co.) dissolved in 15 ml 0.1 M Tris-HCL-buffer to which 12 μl H_2O_2 was added.

Time of reaction depended on promptness of reaction but it was not longer than 5 minutes. Then, the sections were rinsed in distilled water and counterstained with hematoxylin. After dehydration in ethanol and xylol, slices were occluded with Vitro-Clud (Langenbrinck).

Two different types of negative controls were performed: replacing lectins by incubation with TBS alone; and replacing lectins by a mixture of 1:1 of each lectin with its corresponding inhibitory sugar (final concentration of the mixture 0.2M). By both methods staining was completely neutralized.

Results

Two types of mixed glioma were differentiated: the type AAOO, i.e. separated areas of astrocytoma and oligodendroglioma, and the type AOAO, i.e. intermingled astrocytoma- and oligodendroglioma-cells. From a total of 18 cases 9 tumors were classified as mixed glioma type AAOO, and 9 tumors were classified as mixed glioma type AOAO. Con A marked cytoplasm and processes of astrocytes (Fig. 1). PNA marked cell membranes of oligodendrocytes (Fig. 3).

The results of the semiquantitative analysis of lectin and GFAP staining is presented in Tables 1 to 3. The histological type of mixed glioma found (AAOO or AOAO) is noted, as well as the percentage of positive

Table 1. GFAP reactivity in mixed gliomas.

TYPE	REACTIVITY
AAOO	
AA:	75-100% of cells +++
	2 cases: 25% of cells +++
OO:	4 cases: 5-25% of cells +++
	4 cases: nearly no positive cells
	1 case: 50% of cells +++ (mostly oligodendrocytes)
AOAO	4 cases: 50% of cells +++
	5 cases: 5-25% of cells ++

+++: intense reactivity; ++: moderate reactivity; +: weak reactivity.

Table 2. Con A reactivity in mixed gliomas.

TYPE	REACTIVITY
AAOO	
AA:	75-100% of cells + to +++
OO:	5-25% of cells ++
	1 case: nearly no positive cells
AOAO	5 cases: 75-90%% of cells ++
	4 cases: 25-50% of cells ++

+++: intense reactivity; ++: moderate reactivity; +: weak reactivity.

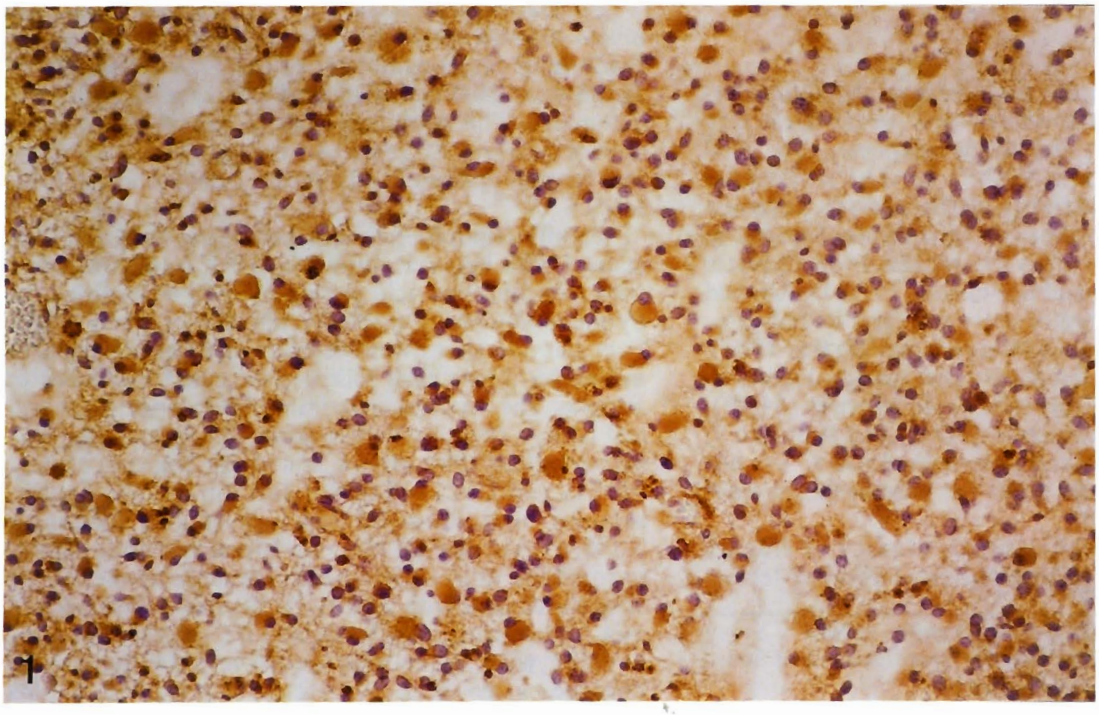

Fig. 1. Con A-positive astrocyte area of a mixed glioma type AAOO. The same case as Fig. 3. x 200

glial cells of the tumor or the astrocytoma-(AA) or oligodendroglioma-area (OO) respectively, and the intensity of staining.

In the astrocytoma areas of the mixed gliomas of AAOO type (Figs. 1, 5) GFAP marked the astrocytes in 7 cases nearly completely positively, in 2 cases only one quarter of astrocytes were GFAP-positive. Con A stained astrocytes in all cases nearly completely positively. In the same cases PNA marked a small part of cells, which represented morphologically partially oligodendrocytes

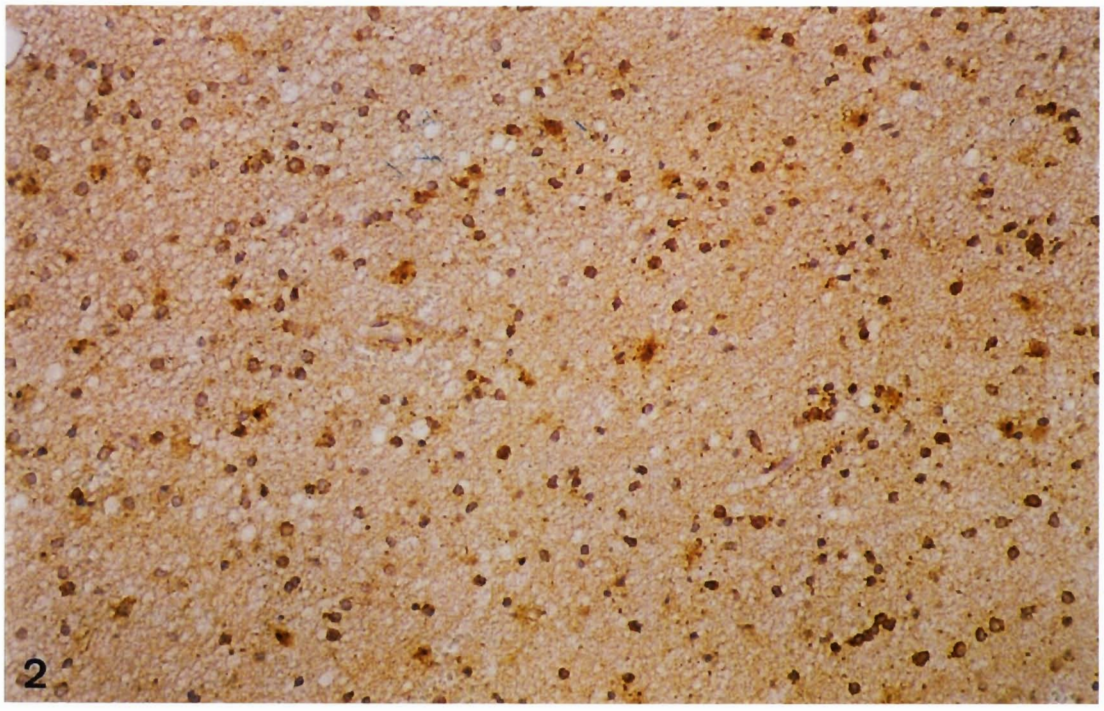

Fig. 2. Con A-positive astrocyte area of a mixed glioma type AOAO. Con A reactivity of astrocytes in a cytoplasm pattern. x 200

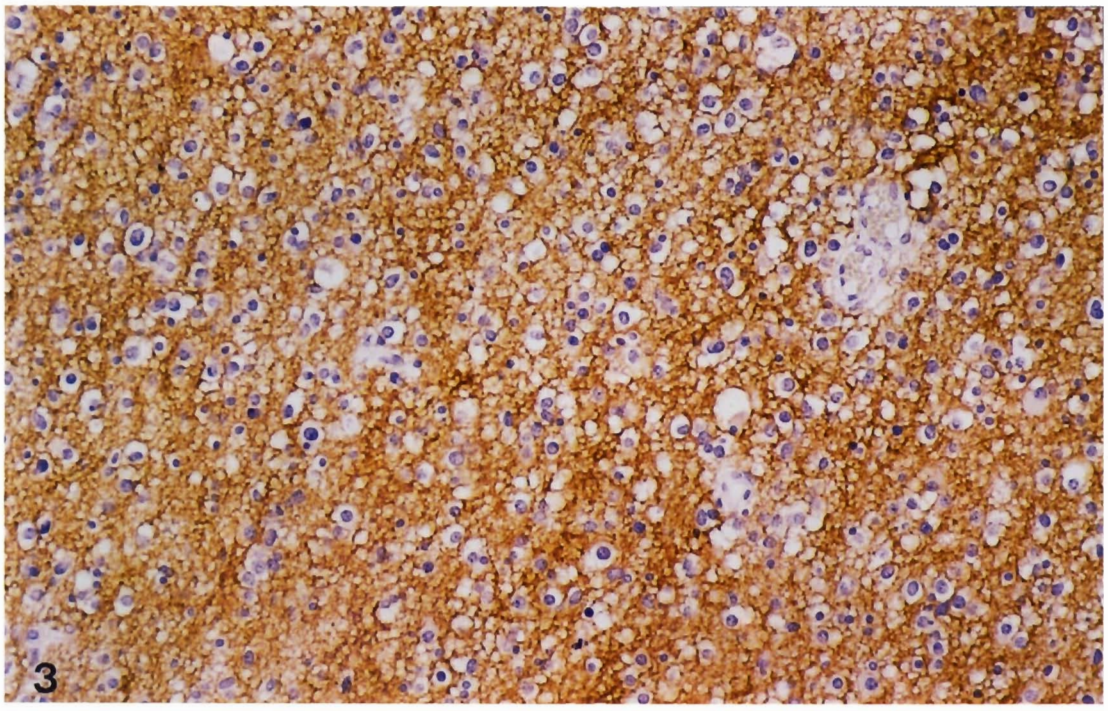

Fig. 3. PNA-positive oligodendrocyte area of a mixed glioma type AAOO. The same case as Fig. 1. x 200

and partially astrocytes, in 7 cases. There were nearly no PNA-positive cells in 2 cases.

In the oligodendroglioma areas of mixed gliomas of type AAOO (Figs. 3, 5) GFAP marked the small astrocyte part in 4 cases. In another 4 cases the existing astrocytes were not all stained by GFAP. In 1 case the oligodendrocytes were 50% positive. Con A stained in all 8 cases the small astrocyte part of the oligodendroglioma area. In 1 case there were nearly no Con A-positive cells. PNA reactivity was in all cases nearly

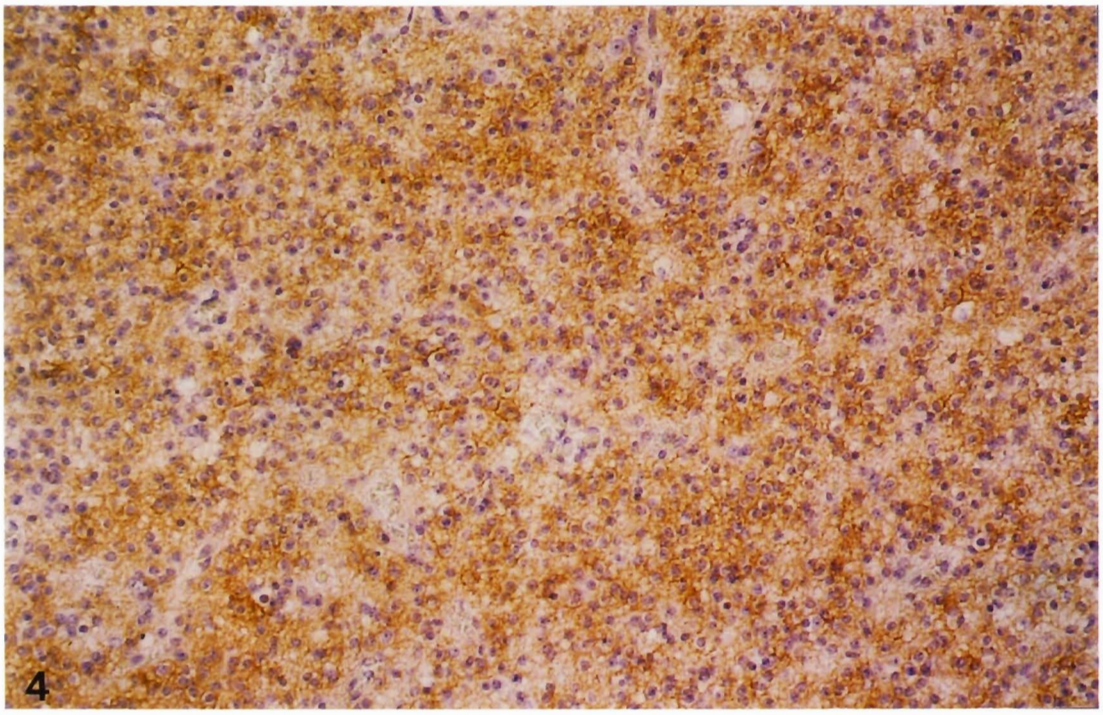

Fig. 4. PNA-positive mixed glioma type AOAO. PNA reactivity of oligodendrocytes in a reticular pattern. x 200

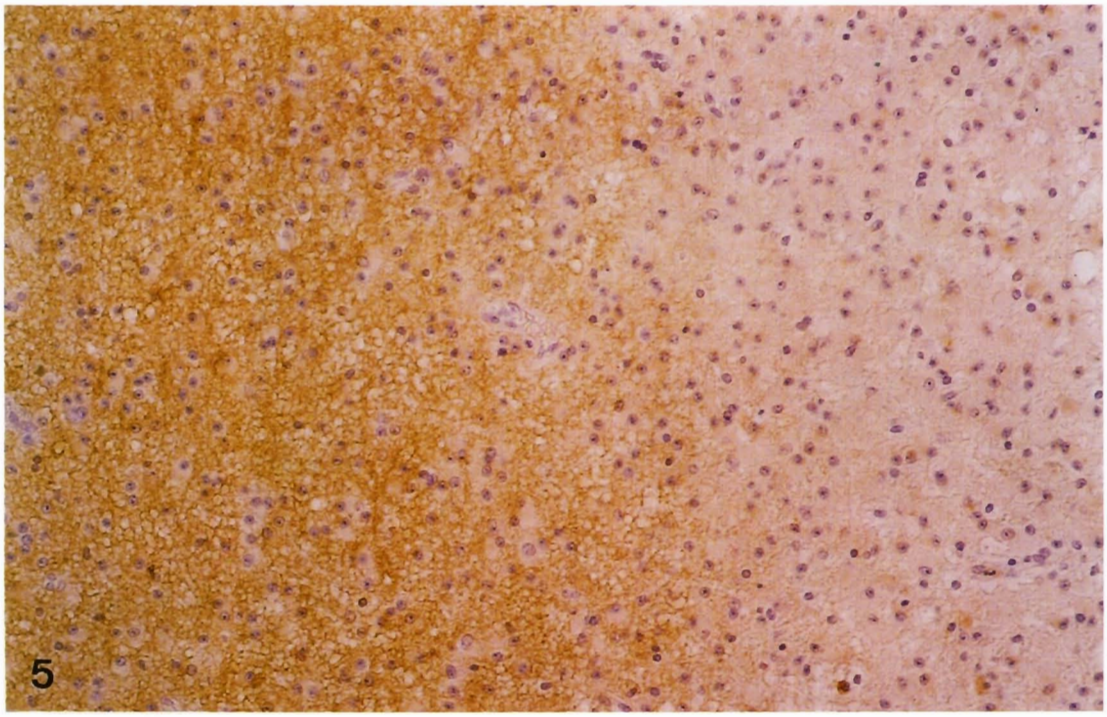

Fig. 5. PNA-staining of the transition zone of an oligodendrocyte to an astrocyte area of a mixed glioma type AAOO. Oligodendrocytes are stained brownish in a reticular pattern. In the astrocyte area some in a cytoplasmatic pattern weakly stained astrocytes are seen. x 200

Table 3. PNA reactivity in mixed gliomas.

TYPE	REACTIVITY
AAOO	
AA:	5-25% of cells ++ 2 cases: nearly no positive cells
OO:	90-100% of cells +++
AOAO	50-90% of cells ++ 1 case: 10% of cells ++

+++: intense reactivity; ++: moderate reactivity; +: weak reactivity.

complete.

In mixed gliomas of type AOAO (Figs. 2, 4) GFAP marked the astrocyte part completely in 4 cases. In 5 cases only a part of the existing astrocytes were GFAP-positive. Con A marked most cells in 5 cases and one third of the cells in 4 cases. PNA marked the majority of cells as oligodendrocytes in 8 cases, in 1 case only 10% of cells were PNA-positive.

Correlating the GFAP-, Con A- and PNA-stainings there were similar results in 13 cases regarding the composition of tumor areas. In comparison to the other stainings, GFAP marked less astrocytes in 7 cases. In 1 case more cells were GFAP-positive than in the other stains; the cells were GFAP-positive oligodendrocytes. Con A marked relatively more astrocytes in 4 cases, and in 3 cases less astrocytes in comparison to the other stains. PNA marked more oligodendrocytes in 8 cases and in 1 case less oligodendrocytes than expected by the other stains. In 3 cases PNA marked cells which were morphologically considered to be astrocytes.

Discussion

In the study of 18 low-grade mixed gliomas the differentiation of astrocyte and oligodendrocyte areas was not sufficiently possible with conventional histological methods. The GFAP-immunohistochemical results did not allow a reliable differentiation between astrocyte and oligodendrocyte tumor areas because of incomplete GFAP-reactivity of astrocytes. This was also reported by Iglesias (1986). Additionally, oligodendrocytes have been found to be GFAP-positive in oligodendrogliomas by Eng and Rubinstein (1978) and DeArmond et al. (1980) and in oligoastrocytomas by Sarkar et al. (1988) and Escalona-Zapata and Nieves (1992). These GFAP-positive oligodendrocytes are phenotypically not different from neoplastic oligodendroglia and were called gliofibrillary oligodendroytes (Herpes and Budka, 1984).

In the mixed gliomas of our study, ConA marked the cytoplasm and processes of tumoral astrocytes, which is in line with the findings of other authors (Schwechheimer et al., 1984; Cruz-Sánchez et al., 1991; Figols et al., 1991, 1993). Con A therefore is a reliable marker for astrocyte areas of mixed gliomas. The oligodendrocytes reacted Con A-negative in the studied mixed gliomas supporting the report by Figols et al. (1991, 1993), although some cases in our study have been found where Con A stained cells that morphologically resembled oligodendrocytes. In contrast to these results other authors found that Con A always marks well-differentiated oligodendroglial cells (Schwechheimer et al., 1984; Wang et al., 1989; Cruz-Sánchez et al., 1991). The reason for these diverging results can only partially be explained by variable existence of gliofibrillary oligodendrocytes. Rather the different methods using might be held responsible. Differences in the kind of lectins, the marking of lectins used and in the fixation and staining procedures might produce different results.

In contrast to Con A, PNA stained the cell membranes of oligodendrocytes in the studied mixed gliomas, as found by other authors in oligodendrogliomas (Schwechheimer et al., 1983; Cruz-Sánchez et al., 1991; Figols et al., 1991, 1993). Accordingly, PNA has to be regarded as a reliable marker of the oligodendrocyte areas in mixed gliomas. Interestingly, Wang et al. (1989) found no PNA reactivity in oligodendrogliomas. Astrocytes were rarely stained by PNA in the studied mixed gliomas, in accordance with Figols et al. (1991, 1993) and Wang et al. (1989). On the contrary, Schwechheimer et al. (1983) described a clear PNA-reactivity of astrocytomas.

In comparing the lectin reactivity in mixed gliomas of type AAOO and AOAO it became obvious that in some cases cells were overlappingly positive for Con A and PNA. Morphologically, these cells resembled either oligodendrocytes or astrocytes, or could not be identified as either cell type. Cells stained by both Con A and PNA expressed an oligosaccharide pattern that has both astrocyte and oligodendrocyte characteristics. On the grounds of their mixed or intermediate staining characteristic, these cells besides astrocytes and oligodendrocytes represent a third cell type called intermediate cell type. Large parts of mixed gliomas consist of intermediate cells that are immunologically conform in lectinhistochemistry. These intermediate cells that are potentially unique to mixed gliomas represent a cell type that combines characteristics of astrocytes and oligodendrocytes.

The existence of intermediate cells as a third cell type in mixed gliomas might have implications for the pathogenesis of mixed gliomas. The mechanisms by which different cell populations form mixed gliomas are unknown (Russell and Rubinstein, 1989). The simultaneous malignant transformation of two different cells in the same brain region is considered unlikely (Kleihues et al., 1995). Mixed gliomas could develop from a single neoplastic glial cell type that transforms to other glial cell lines (Hart et al., 1974; Meneses et al., 1982). This would imply the oligodendroglial transformation of an astrocytoma (Kamitani et al., 1987, 1988) or the astrocyte transformation of an oligodendroglioma (Escalona-Zapata and Nieves, 1992). The transformation of cell morphology with phenotypes

intermediate between astrocytes and oligodendroyctes following modification of pericellular millieu has been described (Murabe and Sano, 1981). Dyer and Philibotte (1995) showed that a clone of a glioma cell line derived from transgenic mice is capable of differentiating into an oligodendrocyte-like cell or an astrocyte-like cell depending on culture conditions. Furthermore, this clone has been shown to be capable of transforming directly from the oligodendrocyte-like to the astrocyte-like cell and vice-versa. During this transformation, a mixture of cell phenotypes is seen. It is possible that astroglial, oligodendroglial and transitional glial tumor cells represent phenotypes derived from a single neoplastic cell that is environmentally adaptable. The intermediate cells expressing both astrocyte and oligodendrocyte characteristics in lectinhistochemistry would represent a transitional form between these two phenotypes. Therefore, it is possible that one tumorigenic event could lead to the development of a glial neoplasm, and in relation to environmental signals phenotypic changes are induced.

The neoplastic transformation of a common glial precursor cell, from which astrocytes and oligodendrocytes develop, could be another explanation for the appearance of different cell populations in mixed gliomas. Raff et al. (1983) demonstrated two subsets of glial precursor cells, one of them giving rise to both oligodendrocytes and astrocytes. Recent molecular genetic studies demonstrated a distinct pattern of loss of heterozygosity for oligodendrogliomas and oligoastrocytes on the one hand and for astrocytes, on the other. Oligodendrogliomas and oligoastrocytes show loss of heterozygosity most frequently on 19q while astrocytomas show loss of heterozygosity most frequently on 19p (Ritland et al., 1995). Shared allelic losses on chromosome arms 1p and 19q in both oligodendrogliomas and oligoastrocytomas were found by Kraus et al. (1995). Investigating the oligodendroglial and astrocytic areas of mixed gliomas further showed that losses of heterozygosity in 1p and 19q were present in both tumor portions. These results suggest, in agreement with the lectinhistochemical demonstration of an intermediate cell type in mixed gliomas of our study, that the oligodendroglial and astrocytic areas of mixed gliomas are derived from a common cell of origin. This common cell of origin may be the common precursor cell (Raff et al., 1983). The regional differentiation into either oligodendroglial or astrocytic phenotypes is likely to be mediated by epigenetic factors (von Deimling et al., 1995).

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Accepted June 14, 1997