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Lectins as differentiation markers of human gliomas

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Summary. The lectins Concanavalin A (Con A), *Ricinus communis* agglutinin (RCA-I), Peanut agglutinin (PNA) and Wheat germ agglutinin (WGA) as well as the immunomarkers for glial fibrillary acidic protein (GFAP) and myelin basic protein (MBP) were used in a series of 21 glial tumors (4 pylocytic astrocytomas, 5 grade II astrocytomas, 3 anaplastic astrocytomas, 4 glioblastomas and 5 oligodendrogliomas).

ConA binds to all tumoral astrocytes in low grade astrocytomas, as well as to well differentiated tumoral astrocytes in anaplastic astrocytomas and glioblastomas. RCA-I has a similar behaviour. PNA, and to a lesser degree WGA, binds selectively to the oligodendroglial plasma membrane in well differentiated oligodendrogliomas. The results suggest that these lectins are markers of differentiation in gliomas rather than of malignancy.

Key words: Lectins, Gliomas, Tumoral differentiation markers

Introduction

In gliomas, an increased number of mitotic figures, cellular density, necrosis, atypical nuclei, endothelial proliferation etc., are considered anaplastic features. Prognosis of tumors exhibiting these characteristics, with very few exceptions (Mork et al., 1986), is poor. In many cases such anaplastic signs correlate poorly with the postoperative survival (Ross and Rubinstein, 1989).

The introduction of immunohistochemistry into histopathological routine supplies some new data which can help to establish a prognostic judgement. Thus, the scarcity of expression of glial fibrillary acidic protein (GFAP) in anaplastic artrocytomas, or the increased expression of vimentine in anaplastic glial cells (Roessma et al., 1986; Cruz-Sánchez et al., 1989), can help in establishing degrees of malignancy.

In the search for new markers for tumor cells, lectins have been used, although more often in tumors outside the CNS (Bramwell and Harris, 1978; Newman et al., 1979). Lectins (from the latin word *legere*, to bind), are glycoproteins derived from different natural sources, mainly plants, and are characterized by their ability to specifically bind different carbohydrate moieties. In malignant cells these receptors were reported to be abnormally crowded in coarse masses over the cellular membrane (Rapin and Burger, 1974; Louis et al., 1981). These characteristics would permit, at least theoretically, the recognition of malignancy.

Recently, lectins have been used in CNS tumors with contradictory results (Schwechheimer et al., 1983, 1984a,b; Wang et al., 1989).

In the present study staining by different lectins has been correlated with histological characteristics of anaplasia in astrocytomas and oligodendrogliomas. The normal and/or reactive neural tissue which generally accompanies tumors in surgical biopsies has also been studied.

Materials and methods

21 glial tumors served for the present study: 4 pilocytic astrocytomas (grade I), 5 grade II astrocytomas, 3 anaplastic astrocytomas (grade III), 4 glioblastomas and 5 oligodendrogliomas (grade I, II and III). The tumors were embedded in paraffin, cut at 7 µm and stained by the haematoxylin-eosin (HE), Nissl, Van-Gieson and PAS techniques. Glial fibrillary acidic protein (GFAP) and myelin basic protein (MBP) immunohistochemical procedures were performed. The GFAP procedure was carried out by the avidin-biotin method, using a polyclonal antibody (Dako, Denmark) at a concentration of 1:500, and then developing the final reaction product with diaminobenzidine (DAB).

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The MBP technique was performed using the alkaline phosphatase-antialkaline phosphatase (APAAP) method, with a monoclonal antibody from SEROTEC (U.K.) at a concentration of 1:100.

Lectins marked with horseradish-peroxidase (HRP) (characteristics and concentrations listed in Table 1) were obtained from Sigma Chemical Co. (USA). 5-7 μm thick paraffin sections were deparaffinized in two baths of xylene of 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-buffered-saline (TBS) containing 1mM of $CaCl_2$, $MgCl_2$ and $MnCl_2$, pH 7.4 (Streit et al., 1985). The sections were immersed in a bath of TBS containing 0.1% trypsin for 10 minutes (Büttner-Ennever et al., 1989). To block the endogenous peroxidase activity, the slides were immersed in a 1.5% H_2O_2 methanol bath for 20 minutes. After two 5 min baths in TBS, the sections were covered by the lectins diluted in TBS (Table 1) and incubated in a moist chamber at room temperature for 90 minutes. After another two 5 minutes baths in TBS, the sections were covered with a freshly prepared DAB solution in Tris-HCl 0.1 M at a final concentration of 1 mg/ml containing 7 μl/ml of H_2O_2. This step was also done in a moist chamber for 5 minutes. After rinsing in distilled water, the slides were counterstained with haematoxylin, dehydrated with alcohols, then xylene and mounted in Permount.

Two different types of negative controls for each tumor were used: 1) replacing lectins by incubation with TBS only; and 2) preincubation of the lectins with their corresponding inhibitory sugar at a concentration of 0.2 M.

Results

Normal structures of the CNS

Both the cytoplasm and the plasma membrane of neurons were stained with ConA and WGA. The cytoplasm and the processes of both cortical and white matter astrocytes were also labelled with ConA. Only reactive astrocytes were stained by RCA-I. Resting as well as reactive microglia were stained by RCA-I, but not by the other lectins.

Plasma membrane of normal white-matter oligodendrocytes showed strong staining by PNA. The perineuronal cortical oligodendrocytes were unlabelled with PNA, ConA and WGA. Myelin was stained by all four lectins. The most prominent staining was by PNA and somewhat less by RCA-I. Myelin was very weakly labelled with ConA and WGA. Results with PNA in myelin resembled those obtained with MBP (Figols et al., 1985) and with any of the classical stains for myelin (Spielmeyer, luxol fast blue, etc.).

Vascular endothelium and pericytes were intensely labelled with WGA and RCA-I. While pericytes were stained by PNA, endothelium was not labelled with this lectin. Ependyma and plexus choroideus epithelium showed intense binding to RCA-I and WGA and moderate binding to ConA. PNA did not show any labelling to this structure.

Pilocytic astrocytomas (grade I)

–ConA: mainly the cytoplasm in most fibrillar astrocytes was intensely stained. The «eosinophilic granular bodies» (EGB), which were especially numerous in tumors with prominent cystic changes, as was observed by Russell and Rubinstein (1989), were also intensely stained. Rosenthal fibres were negative in the centre and positive in the periphery (Fig. 1). Similar results were obtained with the GFAP procedure.

–WGA: only 50% of the tumoral astrocytes were labelled having a similar, but less intense pattern of binding to that of the former lectin.

–RCA-I: the individual cells, as well as the Rosenthal fibres were unlabelled, although some binding was detected in the tumoral intercellular matrix. The EGB, on the other hand, were intensely stained.

–PNA: about 25% of the tumoral astrocytes were weakly stained. The Rosenthal fibres, as well as the EGB, were unstained.

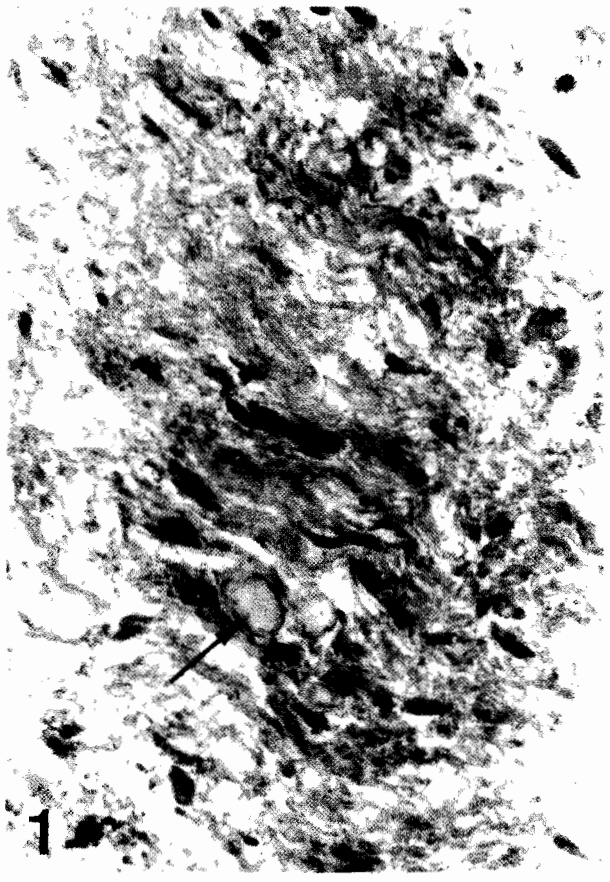

Fig. 1. Pilocytic astrocytoma. Elongated cells label with ConA especially perinuclear. Centre of Rosenthal fibres (arrow) is negative with a positive peripheral rim. Concavanalin A peroxidase labelled. × 475

Table 1. Lectins: Characteristics and dilutions

LECTIN	SOURCE	CARBOHYDRATE BINDING SPECIFICITY	INHIBITORY SUGAR	DILUTION
Concanavalin A Con A (Goldstein and Hayes, 1978; Hennigar et al., 1987)	*Canavalia ensiformis*	D-glucose, D-mannose	Methyl - α - mannopyranoside	20 μg/ml
Ricinus communis agglutinin RCA-1 (Baenzinger and Fiete, 1979)	*Ricinus communis*	D-galactose	D-galactose	25 μg/ml
Peanut agglutinin PNA (Lotan et al., 1975; Hennigar et al., 1987)	*Arachis hypogaea*	D-galactose, N-acetyl-galactosamine	D-galactose	15 μg/ml
Wheat germ agglutinin WGA (Bhavanandan and Katlic, 1979)	*Triticum vulgaris*	N-acetyl-glucosamine, sialic acid	N-acetyl-glucosamine	8 μg/ml

Table 2. Lectin positivity in other structures of the CNS.

	ConA	WGA	RCA-I	PNA
Endothelium	–	+++	+++	–
Neurons	++	++	–	–
Microglia	–	–	++	–
Ependyma Plexus choroideus	++	+++	+++	–
Myelin	+	+	++	+++

– = scarce or no reactivity at all
\+ = weak reactivity
++ = moderate reactivity
+++ = intense reactivity

Table 3. Lectin reactivity in gliomas

GLIOMAS	LECTINS CONA Intensity	RCA-1 Intensity	PNA Intensity	WGA Intensity
Pilocytic astrocytoma (I) – Tumoral cells	+++	–	+	++
Fibrillary astrocytoma (II) – Tumoral cells	+++	++	+	++
Gemistocytic and/or protoplasmic astrocytoma (II) – Tumoral cells	++	++	++	++
Malignant astrocytoma (III) – Differentiated tumoral cells – Undifferentiated tumoral cells	+++ –	++ –	– –	+ –
Glioblastoma (IV) – Differentiated tumoral cells – Undifferentiated tumoral cells	++ –	+++ –	– –	– –
Oligodendroglioma (I-II) – "Clear" cells – Trapped astrocytes	– +++	– +++	+++ –	+ –
Malignant oligodendrogliomas (III-IV) – Undifferentiated cells	++	–	–	–

– = unreactive
\+ = weak reactivity
++ = moderate reactivity
+++ = intense reactivity

Grade II astrocytomas

–ConA: both the cytoplasm and the processes of all tumoral astrocytes were intensely stained (Fig. 2). Also, the EGB showed intense binding to this lectin. The intensity of labelling was especially strong in gemistocytic astrocytomas (Fig. 3).

–WGA: similar to ConA, but only 50% of the cells were labelled.

–RCA-I: tumoral astrocytes, especially of the gemistocytic type, were stained, although the intensity of reaction was less than with ConA. EGB were intensely positive.

–PNA: only about 20-25% cells of fibrillary astrocytomas were labelled but only weakly, while 100% of the tumoral astrocytes in protoplasmatic astrocytomas were moderately stained.

Anaplastic astrocytomas (grade III-IV) and glioblastomas

–ConA: lectin staining was only localized in the cytoplasm of well-differentiated cells. Small undifferentiated cells were mostly unlabelled, with occasional stained cells which were also mostly GFAP-positive. The bizarre giant cells of glioblastomas were mostly stained by this lectin (Fig. 4).

–WGA: less than 10% of the well-differentiated cells were weakly stained in anaplastic astrocytomas and unstained in glioblastomas.

–RCA-I: the pattern resembled that obtained with ConA, both in astrocytomas and glioblastomas. The labelling of this lectin to giant cells was very intense (Fig. 5).

–PNA: tumoral cells were unlabelled in both tumor types.

Oligodendrogliomas

–ConA: well-differentiated tumoral oligodendrocytes (typical «clear cells») were unstained. Trapped

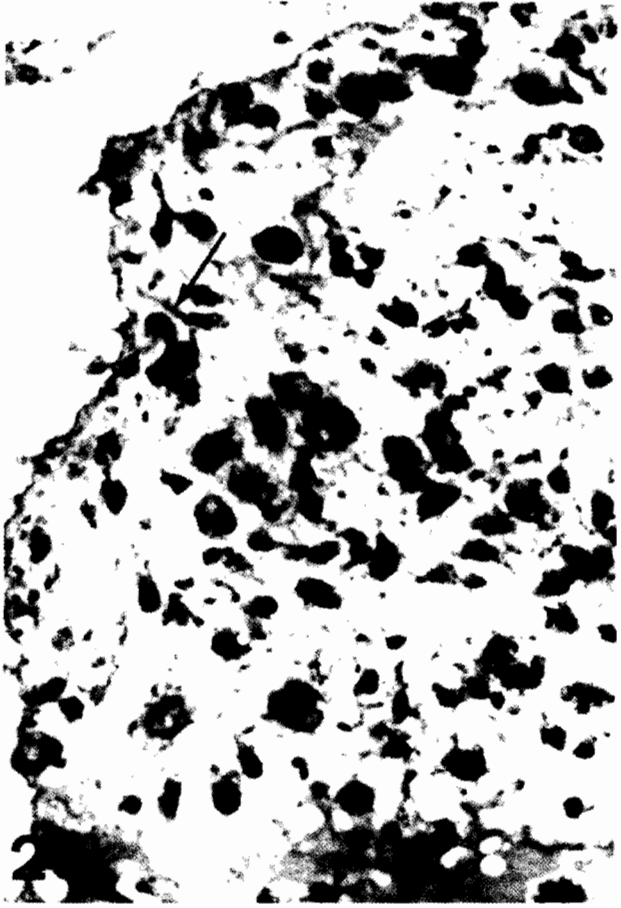

Fig. 2. Fibrillary astrocytoma grade II. Cytoplasmic perinuclear positivity for ConA of 100% of cells. Some processes are also labelled (arrow). Concavanalin A peroxidase labelled. × 350

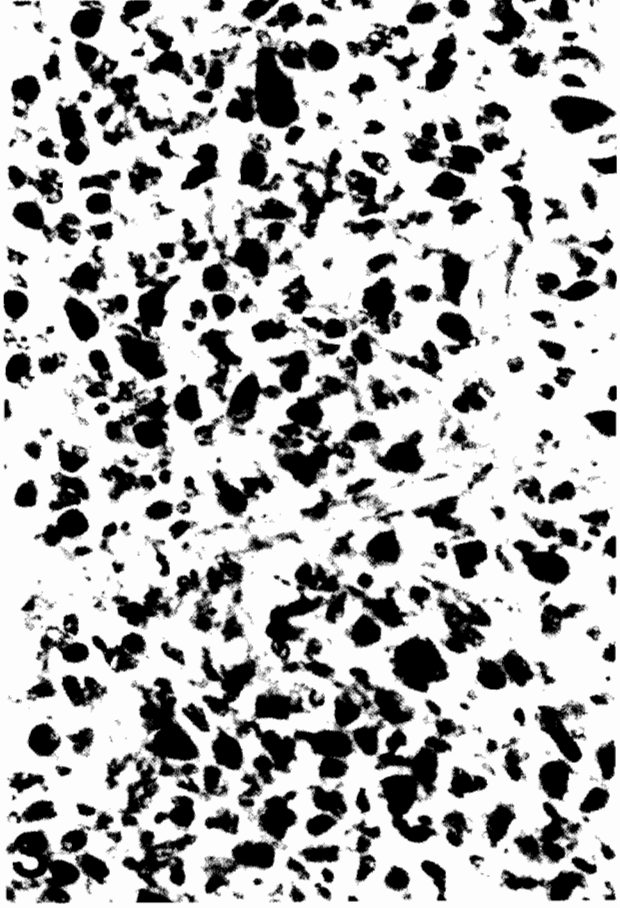

Fig. 3. Gemistocytic astrocytoma (grade II). Strong ConA cytoplasmic labelling. Concanavalin A peroxidase labelled × 350

Fig. 4. Anaplastic astrocytoma (grade III). ConA labels only few cells (arrows). Concanavalin A peroxidase labelled. × 350

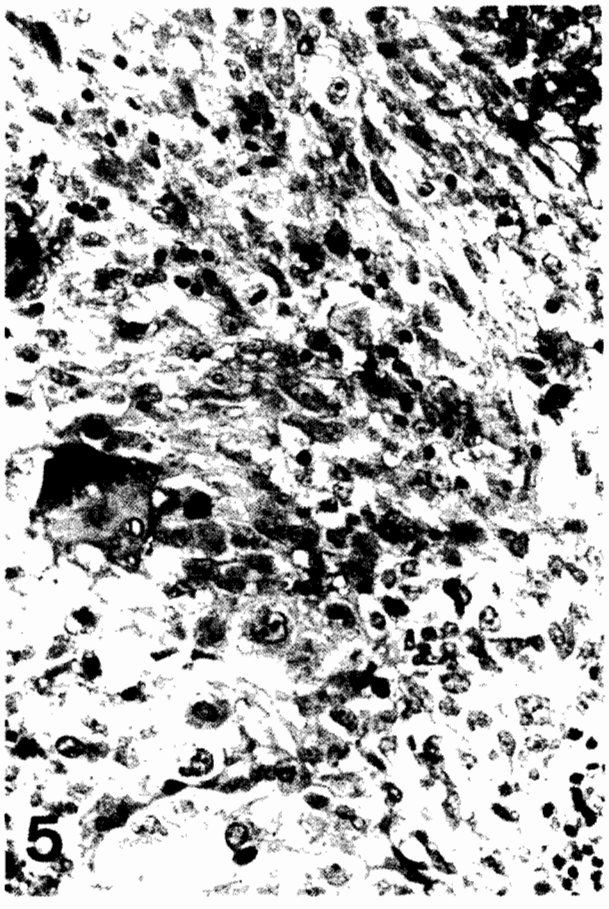

Fig. 5. Glioblastoma. RCA-I labels a giant bizarre cell. *Ricinus communis* agglutinin peroxidase labelled. × 350

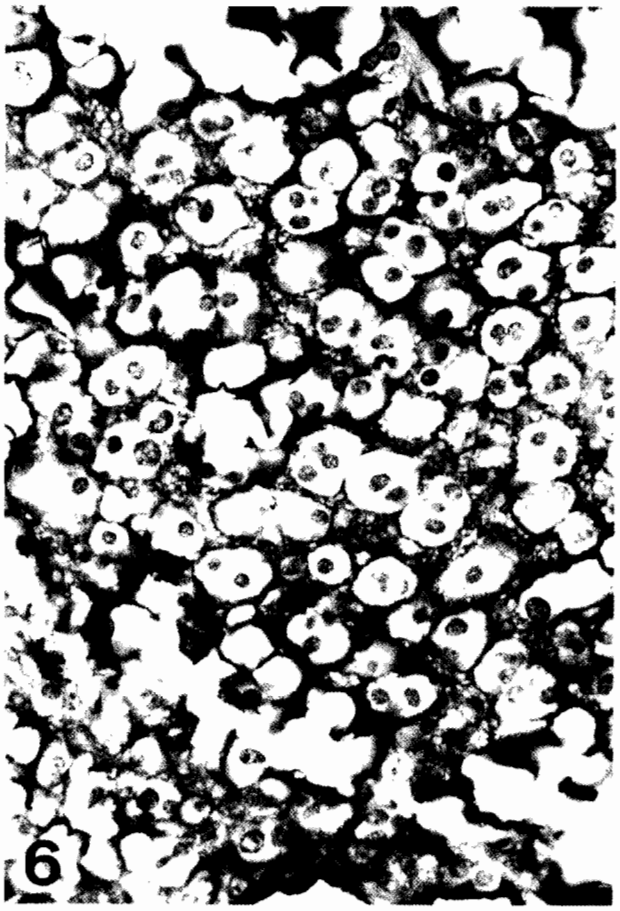

Fig. 6. Oligodendroglioma grade II. Strong and selective plasma membrane labelling of the tumoral cells with PNA. Intercellular spaces are also labelled. Peanut agglutinin peroxidase labelled. × 350

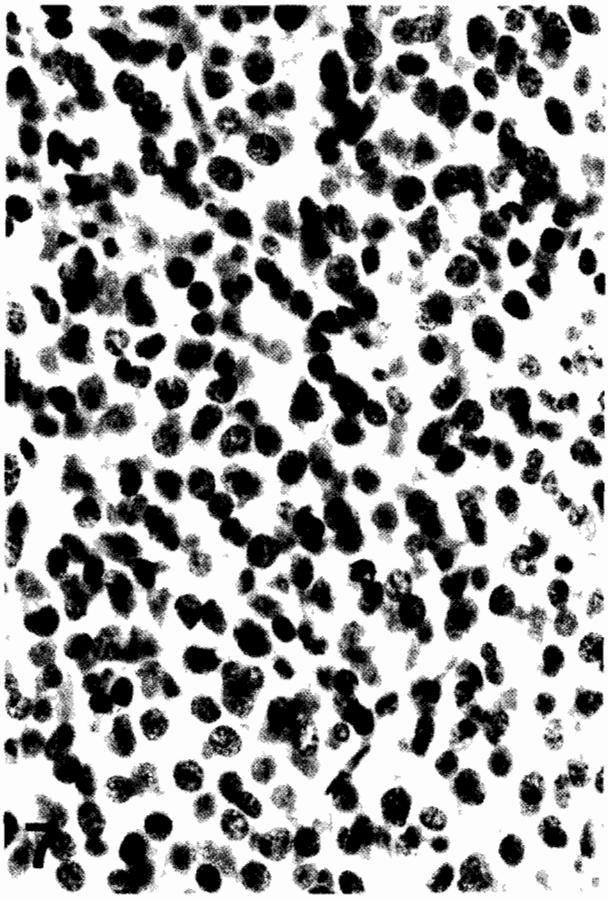

Fig. 7. Oligodendroglioma grade III with dense cellularity and high mitotic rate. PNA is negative. Peanut agglutinin peroxidase labelled. × 475

astrocytes showed an intense binding. About 50% of tumor cells of the undifferentiated (anaplastic) oligodendrocyte type showed stained cytoplasm.

–WGA: plasma membranes of well-differentiated oligodendrocytes were weakly labelled.

–RCA-I: the staining pattern was similar to that of ConA of well-differentiated tumors.

–PNA: well-differentiated tumoral oligodendrocytes were intensely labelled (plasma membrane binding) (Fig. 6). Some cases of grade I oligodendrogliomas showed intense staining of the neuropil. Macrophages with myelin and/or cellular debris were also intensely marked. In the undifferentiated and highly cellular tumors, the cells were unstained (Fig. 7).

The results are summarized in Tables 2 and 3.

Discussion

ConA and WGA bound specifically to neuronal membranes in our material, which agrees with observations of other authors (Hampson and Poduslo, 1987). In our cases, ConA also labelled to normal and reactive astrocytes as well as ependymal and choroid plexus epithelium as it was previoulsy reported by Schwechheimer et al. (1984a,b). The two latter structures were more intensely stained with RCA-I and WGA than with PNA, which contrasts with the findings of Schwechheimer et al. (1984b). The different staining procedures and the different types of marked lectins utilized may explain this disparity.

RCA-I was also found by us to be a very good marker of normal and tumoral endothelium. Few authors have emphasized this peculiarity of RCA-I (Gerhart et al., 1986: Szumanska et al., 1987; Seitz et al., 1988). In our experience, RCA-I gives better results in vessels than *Ulex europaeus* (UEA-I), the use of which is much more widespread (Ordonez and Batsakis, 1984; Nag, 1985).

ConA was claimed to permit the differentiation between benign and malignant cells in actinic skin lesions, such as nevi, premalignant solar lesions, malignant melanomas and epidermoid carcinomas (Klein et al., 1977; Newman et al., 1979). These claims are based on characteristics of the surface glycoproteins, which are monomeric in normal cells, and dimeric in tumoral ones (Bramwell and Harris, 1978).

Our results have shown, however, that ConA is a

good marker of well-differentiated cells in astrocytomas and PNA in well-differentiated oligodendrogliomas. ConA also bound to the well-differentiated cellular components of malignant (grade III-IV) astrocytomas, as well as to the bizarre giant cells of glioblastomas. The small undifferentiated cells of these tumors are negative. This fact, which has been reported by others (Schwechheimer et al., 1984a; Wang et al., 1989), confirms the notion that ConA is a marker of cellular differentiation in astrocytomas rather than a marker of malignancy. Since ConA has been used as a malignancy marker in lymphomas and some carcinomas (Louis et al., 1981; Koch and Smith, 1983; Raedler and Raedler, 1985), the discrepancy indicates that neural tissue displays different characteristics in lymphomas and carcinomas.

Our results with ConA (and to a lesser degree with RCA-I and WGA) agree with those of other investigators regarding the staining of cellular membranes. Plasma membranes of both normal and tumor cells undergo cyclic changes: normal cells in interphase and malignant cells in mitotic activity are not bound by ConA. However, malignant cells in interphase and normal cells in mitosis are bound by this lectin (Shoham and Sachs, 1974). In our cases, the absence of staining by ConA in undifferentiated astrocytes in anaplastic astrocytomas might correspond to these changes in lectin binding occurring in undifferentiated tumor cells. Recent papers (Fischer et al., 1989) reported possible differences in the localization of lectin binding sites of tumor and normal cells. These binding sites, which could be found in definite cellular localizations in normal and well-differentiated tumor cells, appeared in different localizations in undifferentiated tumor cells.

The constant plasma membrane positivity for PNA in well-differentiated oligodendrogliomas was systematically found in our cases, and thus this lectin also appears to be related to cellular differentiation in these gliomas. This fact was mentioned by some authors (Schwechheimer et al., 1983), but denied by others (Dickson et al., 1986; Wang et al., 1989). These discrepancies and other peculiarities in lectin staining in oligodendrogliomas deserve further investigation.

Our results concerning RCA-I and WGA in astrocytomas are in partial agreement with those obtained by Wang et al. (1989). In contrast to results reported by these authors, however, we found a regular intense RCA-I-labelling in giant/bizarre cells of glioblastomas, while small undifferentiated cells of these tumors remained unstained. These findings suggest that the positivity for RCA-I in gliomas is also related to cellular differentiation. Similar findings (non-reactivity of undifferentiated tumor cells) have been obtained by Langbein et al. (1989) with RCA-I and with WGA in rhabdomyosarcomas.

The fact that the lectins ConA, WGA, RCA-I and PNA can serve as markers of cellular differentiation, might help pathologists to establish a more accurate prognosis as a complement to the widely used immunohistochemical markers (GFAP, Vimentine, etc.).

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