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Original Article

Demonstration of Poly-*N*-acetyl Lactosamine Residues in Ameboid and Ramified Microglial Cells in Rat Brain by Tomato Lectin Binding[1]

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This study was designed to demonstrate the localization of poly-*N*-acetyl lactosamine residues in postnatal and adult rat brain, visualized by their specific binding to a lectin obtained from *Lycopersicon esculentum* (tomato). Lectin histochemistry was carried out on cryostat, paraffin, and vibratome sections and was examined by light microscopy. Selected vibratome sections were processed for electron microscopy. Our results showed that tomato lectin histochemistry was found in relation to blood vessels and glial cells in both postnatal and adult rat brain. Since tomato lectin-positive glial cells did not show GFAP immunoreactivity and displayed the same morphological features and overall distribution as nucleoside diphosphatase (NDPase)-positive cells, they were consequently identified as microglial cells. At the electron microscopic level, both ameboid and ramified microglial cells displayed intracytoplasmic and plasma membrane lectin reactivity. In postnatal brain, ameboid microglial cells always showed stronger binding of tomato lectin compared with ramified microglial cells in the adult brain. The putative significance of this decrease in poly-*N*-acetyl lactosamine from ameboid to ramified microglial cells and the possible role(s) of this sugar residue are discussed.
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KEY WORDS: Tomato lectin histochemistry; Ameboid microglia; Resting microglia; Rat brain; Double labeling; Microglial marker; Poly-*N*-acetyl lactosamine; NDPase, GFAP.

Introduction

In the last decade there has been an increasing interest in the use of lectins (carbohydrate binding proteins) to study the biochemical composition of membranes and to identify specific cell types in vivo and in vitro (Rademacher et al., 1988; Reading, 1984). These studies are based on findings showing that lectins interact specifically with sugar groups of both external and internal cell membranes in a variety of cells (Streit et al., 1985a; Lis and Sharon, 1973). In the nervous system, lectins interact with neurons and with other structures, such as myelin nodes and the outer myelin membrane (Ambalavanar and Morris, 1991; Streit et al., 1985a,b; Hatten et al., 1979), but lectin binding in neural tissue has usually been related to glial cells, with specific affinity for microglial cells. Therefore, several lectins such as wheat germ agglutinin (WGA), mistletoe lectin (ML-1), *Ricinus communis* agglutinin-I (RCA-I), and *Griffonia simplicifolia* B_4 isolectin (GSA-I B_4) have recently been proposed as microglial cell markers (Boya et al., 1991b; Streit, 1990; Suzuki et al., 1988; Yamamoto et al., 1988; Streit and Kreutzberg, 1987; Mannoji et al., 1986). Accordingly, these lectins have been used in studies of the morphology and distribution of microglial cells in normal tissue (Boya et al., 1991b; Streit, 1990; Suzuki et al., 1988; Mannoji et al., 1986), in different experimental lesion models (Marty et al., 1991; Kaur et al., 1990; Streit and Kreutzberg, 1987), and in a variety of pathological conditions (Esiri and Morris, 1991; Morris and Esiri, 1991; Cras et al., 1991).

A lectin obtained from *Lycopersicon esculentum* (tomato), with affinity for poly-*N*-acetyl lactosamine sugar residues (Zhu and Laine, 1989), has been reported to show a distinctive band of fiber-like staining in the rat trigeminal system (Ambalavanar and Morris, 1991). In the same study, the authors reported that tomato lectin binding was also found in relation to glial cells, although they were not able to identify the specific glial cell type. The aim of the present study was to examine the histochemical binding of this lectin in brain sections by light and electron microscopy to establish the nature of the glial cell type bearing poly-*N*-acetyl lactosamine residues.

Materials and Methods

Eight neonatal (5–6-day-old) and eight adult (approximately 200 g) Sprague–Dawley rats of both sexes were used for this study. Animals were distributed in groups and processed for light and electron microscopy.

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Animals were anesthetized by ether inhalation and fixed by intracardiac perfusion with one of the following fixatives: (a) 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for vibratome and paraffin sections; (b) 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for cryostat sections; and (c) 1% paraformaldehyde and 1% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4), for electron microscopy. After 10 min of perfusion, brains were removed and post-fixed for 3 hr at 4°C in the same fixative solution.

Tomato Lectin Histochemistry

Tomato lectin histochemistry was performed on vibratome, paraffin, and frozen sections.

Vibratome Sections. Brains were rinsed three times for 30 min in 0.1 M cacodylate buffer (pH 7.4) with 7.5% sucrose and sections 40 μm thick were obtained in a vibratome (Vibracut) and kept in the same buffer. Some of these vibratome sections were processed for histoenzymatic demonstration of nucleoside diphosphatase (NDPase), a specific microglial marker (Castellano et al., 1991b), as described below. The rest of the sections were kept in 0.05 M Tris-buffered saline (TBS) (pH 7.4) and processed for tomato lectin histochemistry as follows. Endogenous peroxidase activity was blocked with 2% H_2O_2 in 100% methanol for 10 min. After rinsing in TBS (three times for 10 min) sections were immersed either in TBS containing cations (0.1 mM $CaCl_2$, $MgCl_2$, and $MnCl_2$) and 0.1% Triton X-100 for 15 min or in TBS with 0.1% Triton X-100 without cations for the same length of time. These alternative methods were used to determine which one was more appropriate. Then, vibratome sections were incubated free-floating overnight at 4°C, with biotinylated tomato lectin (L-9389; Sigma, St Louis, MO) diluted to 10 μg/ml either in TBS or in TBS with cations. After rinsing in TBS (three times for 10 min) the sections were incubated for 1 hr at room temperature (RT) with avidin-labeled peroxidase (Dakopatts; Glostrup, Denmark) in a 1:400 dilution in TBS. Sections were then rinsed in TBS (three times for 10 min) and the peroxidase reaction product was visualized with 50 mg of 3,3'-diaminobenzidine (DAB) with 33 μl of H_2O_2 in 100 ml TBS. Some sections were counterstained with cresyl violet. Finally, the sections were rinsed in TBS, mounted on gelatin-coated slides, dehydrated, cleared in xylene, and coverslipped in DPX. Control of tomato lectin specificity was carried out incubating some sections in a 0.1 M solution of *N*-acetyl lactosamine (Sigma A-7791) for 30 min before incubation with the biotinylated lectin in a sugar solution.

Paraffin Sections. After fixation, the brains were rinsed in TBS (three times for 30 min), dehydrated in ethanol, cleared in toluene, and embedded in paraffin. Sections 10 μm thick were cut in a microtome (Leitz, Germany) and mounted on gelatin-coated glass slides. Tissue sections were then dewaxed and rehydrated. Lectin histochemistry was carried out overnight with the biotinylated lectin at a concentration of 20 μg/ml in TBS. A final counterstaining was carried out with Harris' hematoxylin on some sections. Sections were then dehydrated, cleared in xylene, and coverslipped with DPX.

Frozen Sections. After fixation, brains were rinsed in 0.1 M phosphate buffer (pH 7.4) (three times for 30 min) and immersed in 30% sucrose in phosphate buffer until they sank. Brains were quickly frozen with CO_2 and 30 μm-thick sections were obtained in a cryostat (Leitz, Germany). Sections were processed free-floating for tomato lectin histochemistry, as follows. Endogenous peroxidase was blocked with 2% H_2O_2 in 100% methanol. After rinsing (twice for 10 min in TBS and once in TBS + 1% Triton X-100), sections were incubated with the biotinylated lectin diluted to 6 μg/ml in TBS + 1% Triton X-100, either overnight at 4°C or for 2 hr at RT. Sections were rinsed once in TBS + 1% Triton X-100 and twice in TBS, and incubated with avidin–peroxidase (Dakopatts; Carpinteria, CA) in a 1:400 dilution in TBS. Reaction product was visualized with DAB as described above.

Electron Microscopy. After fixation, brains were rinsed in 0.1 M cacodylate buffer (pH 7.4) (three times for 30 min) and 50–100 μm-thick vibratome sections were processed for lectin histochemistry following the same procedure described for vibratome sections, but the biotinylated lectin was diluted in TBS + 1% Triton X-100 to facilitate penetration. Visualization of the reaction product was carried out with nickel–cobalt-enhanced DAB (Adams, 1981). After lectin histochemistry, sections were post-fixed in 2.5% glutaraldehyde and 5% sucrose in 0.1 M cacodylate buffer (pH 7.4) for 30 min and washed in 0.1 M cacodylate buffer (pH 7.4) containing 7.5% sucrose (three times for 30 min). Sections were post-fixed with 1% OsO_4 in 0.1 M cacodylate buffer (pH 7.4) for 1 hr, dehydrated in acetone, cleared in propylene oxide, and embedded in epon. Semi-thin sections 1 μm thick were obtained in an ultramicrotome (Jeol) and stained with toluidine blue. Ultra-thin sections 500–700 Å thick were lightly stained with uranyl acetate and lead citrate and examined with the electron microscope (Hitachi).

Histoenzymatic Demonstration of NDPase

For microglial demonstration, some vibratome sections were stained with the NDPase method (Castellano et al., 1991b). Vibratome sections were incubated for 25 min at 38°C in a medium described by Novikoff and Goldfischer (1961) containing 7 ml distilled water, 10 ml 0.2 M Trizma-maleate (Sigma) (pH 7.4), 5 ml 0.5% $MnCl_2$, 3 ml 1% $Pb(NO_3)_2$, and 25 mg sodium salt of 5'-inosine diphosphate (I-4375, Sigma) as substrate. After rinsing (three times for 10 min) in 0.1 M cacodylate buffer with 7.5% sucrose (pH 7.4), visualization of the reaction product was achieved by treating the sections for 2 min in 2% ammonium sulfide. Finally, sections were mounted, dehydrated, and coverslipped in DPX. Control sections were incubated in a medium lacking substrate.

Double Staining with GFAP–Tomato Lectin

Immunocytochemical detection of glial fibrillary acidic protein (GFAP) has been carried out for specific demonstration of astrocytes (Bignami et al., 1972). GFAP immunocytochemistry was performed alone or in combination with tomato lectin histochemistry on frozen sections. For single labeling, sections were treated with 10% fetal calf serum (FCS) in PBS (pH 7.4) for 1 hr, and incubated in the primary rabbit anti-GFAP antibody (Dakopatts) overnight at 4°C in a 1:1000 dilution in 10% FCS in PBS. After washing (four times for 10 min) with PBS, the sections were incubated at RT for 1 hr with an anti-rabbit Ig–biotinylated antibody (Gibco; Grand Island, NY) in a 1:500 dilution in 10% FCS in PBS. Blocking of endogenous peroxidase was carried out with 1% H_2O_2 in PBS for 30 min. Sections were then rinsed in PBS (three times for 10 min) and incubated for 1 hr at RT with peroxidase-conjugated streptavidin (Dakopatts) at 1:400 dilution in 10% FCS in PBS. After rinsing in PBS (three times for 10 min), the peroxidase reaction product was visualized by incubating the sections in 100 ml of Tris buffer containing 50 mg DAB, 2.6 g nickel ammonium sulfate, and 33 μl H_2O_2. For double labeling, GFAP-stained sections were rinsed with Tris buffer (three times for 10 min), remaining peroxidase blocked for 10 min with 2% H_2O_2 in methanol, and tomato lectin histochemistry was carried out. as previously detailed for frozen sections. Finally, sections were mounted on gelatin-coated slides, dehydrated in alcohol, cleared in xylene, and coverslipped in DPX.

Results

Our results showed that poly-*N*-acetyl lactosamine residues can be demonstrated in postnatal and adult rat brain by tomato lectin histochemistry on paraffin, cryostat, and vibratome sections (Figure 1). In our experience, the presence of cations commonly used in lectin histochemistry, both in the pre-incubation solution and

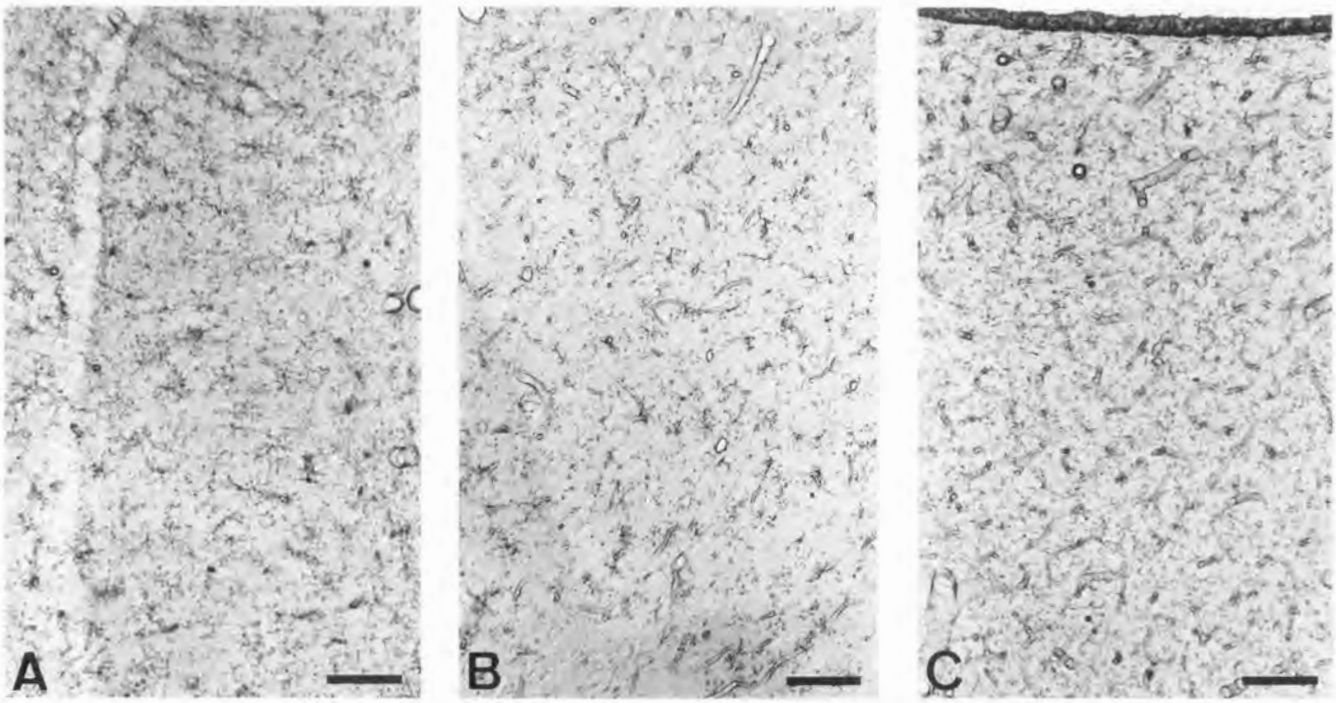

Figure 1. Microglial cells stained with tomato lectin in adult rat brain by different tissue processing techniques. (**A**) Vibratome section, hippocampus. (**B**) Paraffin section, pons. (**C**) Cryostat section, hypothalamus. Original magnification × 96. Bars = 100 μm.

in the medium containing the biotinylated lectin, yielded more background and did not improve lectin staining. Better results were obtained by pre-incubating the sections with TBS and detergent and incubating the lectin with TBS only. Staining was uniform over the entire sections, and the same quality of staining was seen with the different processing techniques, the thickness of the sections being the only difference between paraffin and cryostat or vibratome sections.

In paraffin, vibratome, and frozen sections, tomato lectin revealed ameboid microglial cells in postnatal brain and stellate ramified cells widely distributed in all regions of adult brain. In addition, ependymal cells and blood vessels were also stained with tomato lectin histochemistry (Figure 2). Ameboid and ramified microglial cells visualized by tomato lectin histochemistry showed the same morphological features and overall distribution as NDPase-positive cells stained in parallel vibratome sections (see Figure 3C). Accordingly, ameboid and ramified lectin-positive cells were identified as microglial cells.

Sections double labeled with GFAP immunocytochemistry and tomato lectin histochemistry allowed simultaneous visualization of astroglial cells showing a bluish-black staining and microglial cells showing a brown staining corresponding to tomato lectin binding (Figure 3D). All lectin-positive microglial cells examined were negative for GFAP, and no GFAP-positive astroglial cells showed lectin staining. Of note is that some microglial processes were found in close relation to astrocytic processes.

In adult rats, ramified tomato lectin-positive cells displayed the typical morphology of resting microglial cells, with an elliptical or elongated cell body with several processes branching out from the perinuclear cytoplasm. Processes were usually subdivided into spindle-like thinner processes. Distal parts of microglial processes often appeared to end in proximity to blood vessel walls (Figures 3A and 3B) or in relation to neurons.

In 5-day-old postnatal rats, ameboid tomato lectin-positive cells showed a different morphology according to their developmental state. Round ameboid microglial cells were devoid of processes and

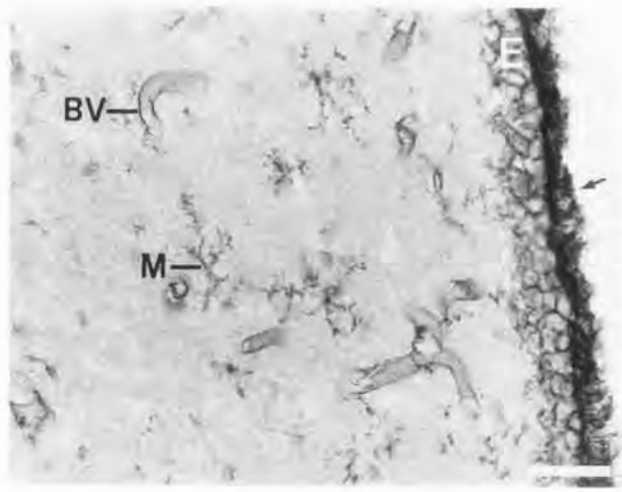

Figure 2. Paraffin section stained for tomato lectin histochemistry. Microglial cells (M) are selectively stained. Blood vessels (BV), ependymal cells (E), and their cilia (arrows) are also positive. Original magnification × 350. Bar = 30 μm.

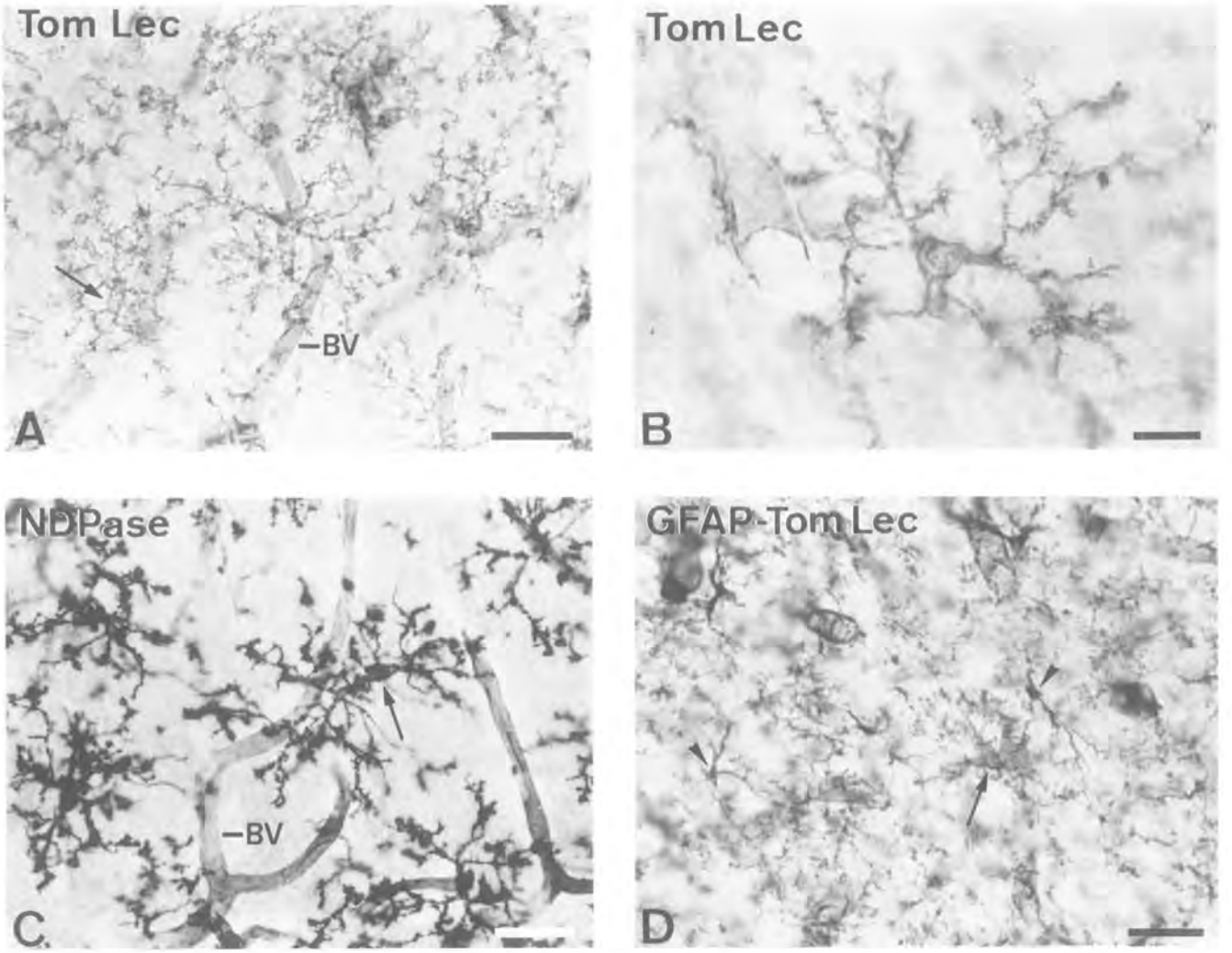

Figure 3. Microglial cells in adult rat hippocampus. (**A**) Tomato lectin (Tom Lec) stains microglial cells (arrow) and blood vessels (BV); vibratome section. (**B**) Tomato lectin-positive microglial cell at higher magnification. Thin processes are well stained; vibratome section. (**C**) Nucleoside diphosphatase (NDPase) histochemistry shows microglial cells (arrow) and blood vessels (BV); vibratome section. (**D**) Combination of GFAP immunocytochemistry and tomato lectin histochemistry allows simultaneous visualization of microglial cells (arrow), originally in brown, and astrocytes (arrowhead), originally in bluish-black; cryostat section. Original magnifications: **A**,**C** × 380; **B** × 960; **D** × 515. Bars: **A**,**C** = 30 μm; **B** = 10 μm; **D** = 20 μm.

displayed strong staining (Figure 4A). However, when microglial cells became ramified there was a slight decrease in lectin staining intensity until they reached the same intensity observed in adult ramified microglial cells (Figure 4B).

In semi-thin sections and at the ultrastructural level (Figures 5A–5D), lectin reactivity was clearly found in the cytoplasm and the plasma membrane of both ameboid and ramified microglial cells. In none of the cases did microglial nuclei show lectin staining. In addition, tomato lectin binding was found on the luminal plasma membrane and inside the cytoplasm of endothelial cells (Figure 5D).

Discussion

Our results with tomato lectin histochemistry revealed that poly-*N*-acetyl lactosamine residues in the rat brain are closely associated with endothelial cells, ependyma, and with a specific population of glial cells identified as ameboid and ramified microglial cells. Neurons and other glial cells, such as astrocytes and oligodendrocytes, never exhibited tomato lectin binding. The importance of tomato lectin as a new microglial marker and the putative role(s) of poly-*N*-acetyl lactosamine structures in microglial cells will be discussed separately.

Tomato Lectin as a New Microglial Marker

Increasing interest in the study of microglial cells during the last decade has encouraged the search for new specific markers for this glial population. Several methods are now commonly used in different laboratories.

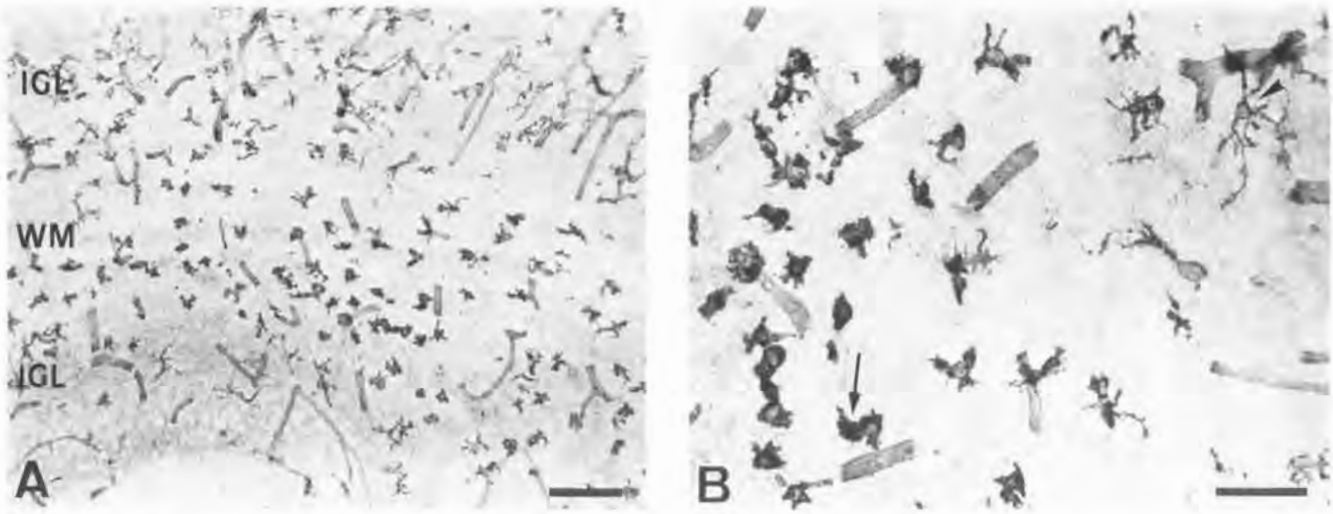

Figure 4. Tomato lectin histochemistry in 5-day-old rat cerebellum; vibratome sections. (**A**) Stained ameboid microglial cells are mainly found in white matter (WM). IGL, internal granular layer (developing gray matter). (**B**) Ameboid microglial cells (arrow) differentiate to primary ramified microglia (arrowhead). Original magnifications: **A** × 96; **B** × 240. Bars: **A** = 100 μm; **B** = 50 μm.

Monoclonal antibodies for the demonstration of microglial cells have been developed in recent years. These include the OX-42 antibody (specific for Type 3 complement receptor) (Perry and Gordon, 1987; Robinson et al., 1986) and the MUC 101 and 102 antibodies (Gehrmann and Kreutzberg, 1991) for rat microglial cells, the anti-MAC-1 antibody (specific for Fc receptors) (Ohno et al., 1992) and anti-F4/80 antibody (Perry et al., 1985) for mouse microglia, and the EMB-11 antibody, which labels human microglia (Esiri and McGee, 1986). All these monoclonal antibodies (MAb) are species-specific and their use is therefore restricted. Moreover, because most of them have been developed against macrophage epitopes, they usually give stronger labeling of ameboid and reactive microglial cells than of resting ramified microglia.

Alternatively, enzyme histochemistry for the demonstration of NDPase (Castellano et al., 1991b; Castellano et al., 1984; Murabe and Sano, 1982a,b), purine nucleoside phosphorylase (Castellano et al., 1990), or thiamine pyrophosphatase (Castellano et al., 1984; Murabe and Sano, 1981) has been used for visualization of resting microglial cells in in vivo and in vitro studies. These histoenzymatic methods allowed the demonstration of microglial cells in different species (Castellano et al., 1991a; Wisniewski et al., 1991; Schnitzer, 1989; Fujimoto et al., 1987; Vorbrodt and Wisniewski, 1982), but their use is restricted to vibratome sections because of enzymatic sensitivity, and poor labeling is obtained when cryostat and paraffin sections are used (unpublished observations).

In recent years, lectin histochemistry has provided several new and reliable microglial markers (see Table 1). Glycoconjugates are not as sensitive as enzymatic activities, implying that lectin histochemistry can be performed even in paraffin sections. Lectins more commonly used, such as *Ricinus communis* agglutinin I (RCA-I), *Griffonia simplicifolia* isolectin B_4 (GSA-IB_4), and soybean agglutinin (SBA), have been used to label different functional states of microglial cells in different species such as rat, mouse, rabbit, and human (Boya et al., 1991a,b; Streit and Kreutzberg, 1987; Mannoji et al., 1986). Lectin histochemistry also gives good results for labeling microglial cells in cell cultures (Colton et al., 1992).

As we show in this work, tomato lectin histochemistry provides a new and advantageous microglial marker, allowing visualization of the complete morphology of microglial cells including very thin microglial processes. Moreover, we have demonstrated its specific binding to ameboid and ramified microglial cells, as a useful tool to visualize these different microglial types in normal rat brain.

As stated in Results, no difference between NDPase and tomato lectin histochemistry was observed, strongly suggesting that tomato lectin histochemistry stains all microglial cells, as previously described for NDPase. However, to rule out the possibility that tomato lectin histochemistry labels a subpopulation of microglia, further studies combining this technique with all the other microglial markers could be useful.

Presence of Poly-N-acetyl Lactosamine in Microglial Cells

There is evidence indicating that complex carbohydrate structures are important in biological recognition processes. In this context, plasma membrane glycoconjugates have been shown to play key roles in receptor activity, intercellular recognition, and adhesion (Spicer and Schulte, 1992; Reading, 1984). These recognition processes imply a carbohydrate binding protein and a complex carbohydrate receptor.

Lectins are carbohydrate binding proteins considered important for their ability to histochemically visualize specific glycoconjugates to which the lectin binds. In this way, several studies have demonstrated specific sugar residues on the plasma membrane of microglial cells, including galactose (by SBA, ML-1, RCA-I, PNA, and GSA-IB_4 lectin binding), *N*-acetyl neuroaminic acid (by WGA binding), and mannose (by concanavalin A binding). The demonstration of tomato lectin binding in microglial cells reveals poly-*N*-acetyl lac-

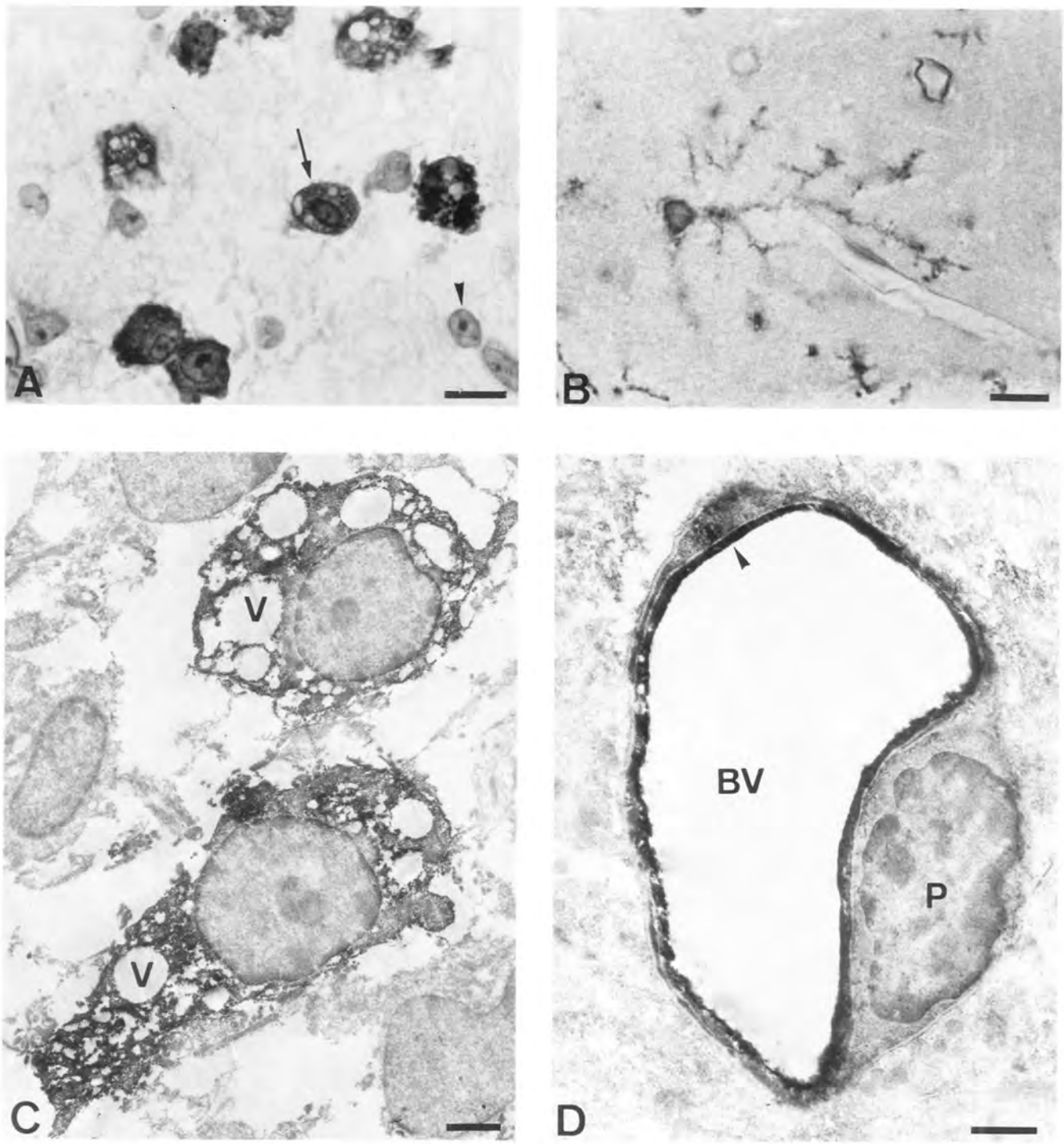

Figure 5. Detailed localization of tomato lectin binding. (**A**) Semi-thin section of corpus callosum from a 5-day-old rat brain. Note the strong lectin staining of ameboid microglial cells (arrows) in front of negative cells visualized by toluidine blue counterstaining (arrowhead). (**B**) Semi-thin section of stratum radiatum of adult rat brain hippocampus. Lectin binding is seen all along the processes and surrounding the nucleus. (**C**) Electron micrograph of ameboid microglial cells from a 5-day-old rat brain showing intracellular localization of lectin binding. Intracytoplasmic vacuoles (V) are unstained. (**D**) Blood vessel (BV) shows intense lectin staining. Reaction product is seen in the endothelial cell cytoplasm (arrow), whereas pericytes (P) are lectin-negative. Original magnifications: **A**,**B** × 960; **C** × 8800; **D** × 10,300. Bars: **A**,**B** = 10 μm; **C**,**D** = 1 μm.

Table 1. *Lectin histochemistry in glial cells*

Lectin	Sugar specificity	Method	Species	Cell labeling	References
WGA (wheat germ agglutinin)	*N*-acetyl neuroaminic acid, β *N*-acetyl-glucosamine, and sialic acid	Paraffin	Human (Alzheimer)	Glia (no specific type described)	Rozemuller et al., 1986
		Paraffin	Rat	Perineuronal glial nets	Brauer et al., 1984
		TEM	Rat (E18)	Ameboid microglia Astrocyte-like processes	Brückner et al., 1985
		Cell culture	Rat	Ameboid microglia	Colton et al., 1992
ML-1 (mistletoe lectin)	D-galactose	Paraffin and frozen sections	Rat and human	Ramified microglia	Yamamoto et al., 1988 Suzuki et al., 1988 Konno et al., 1989
RCA-I (*Ricinus communis* agglutinin-I)	D-galactose	Paraffin and frozen sections	Human (normal and Alzheimer)	Brain macrophages Reactive and ramified microglia	Mannoji et al., 1986 Yamamoto et al., 1988 Sasaki et al., 1991 Suzuki et al., 1988 Morris and Esiri, 1991 Esiri and Morris, 1991 Cras et al., 1991
		Paraffin and frozen sections	Rat (adults and embryos)	Ramified and ameboid microglia	Yamamoto et al., 1988 Suzuki et al., 1988 Boya et al., 1991a,b
		Cell culture	Rat	Ameboid microglia	Colton et al., 1992
PNA (peanut agglutinin)	β-galactose	TEM	Rat (E18)	Ameboid microglia Astrocyte-like processes	Brückner et al., 1985
		Paraffin	Rat	Perineuronal glial nets	Brauer et al., 1984
GSA-IB_4 [*Griffonia* (*Bandeiraea*) *simplicifolia* B_4 isolectin)	α D-galactose	Paraffin, vibratome, frozen sections, and TEM	Rat, rabbit, and mouse	Ameboid, ramified, and reactive microglia	Streit and Kreutzberg, 1987 Ashwell, 1990 Marty et al., 1991 Kaur and Ling, 1991 Boya et al., 1991a,b
		Cell culture	Rat	Ameboid microglia	Colton et al., 1992
ConA (concanavalin A)	α-glucose and mannose	Paraffin	Rat	Perineuronal glial nets	Brauer et al., 1984
		Cell culture	Rat	Ameboid microglia	Colton et al., 1992
SBA (soybean agglutinin)	*N*-acetyl galactosamine	Frozen sections	Rat	Perineuronal glial nets	Lüth et al., 1992
		Cell culture	Rat	Ameboid microglia	Colton et al., 1992
Tomato lectin (*Lycopersicon esculentum*)	Poly-*N*-acetyl lactosamine	Vibratome, paraffin, frozen sections, and TEM	Rat	Ameboid and ramified microglia	Present work

tosamine residues in this glial cell population, although the exact role of poly-*N*-acetyl lactosamine structures and other sugar residues in microglial cells is not yet known.

Tomato lectin has commonly been used in affinity chromatography techniques because of its specific binding to long poly-*N*-acetyl lactosamine chains containing three or more linear units of the repeating disaccharide (3Galβ1, 4GlcNAcβ1) (Wang et al., 1991; Lee et al., 1990; Zhu and Laine, 1989; Merkle and Cummings, 1987). Several studies have demonstrated that this sugar residue is not usually found in glycoproteins (GPs), and is mainly associated with membrane GPs. The major GPs carrying poly-*N*-acetyl lactosamine are certain lysosomal membrane GPs called lamps, but these residues can also be found in cell surfaces (Carlsson et al., 1988).

Polylactosaminoglycans in cell membranes play important recognition functions, carrying various antigenic structures such as blood group antigens (Feizi, 1985), serving in monocytes and gran-

ulocytes as ligands for adhesive molecules (ELAM-1) on endothelial cells and platelets (Phillips et al., 1990), or forming part of the insulin receptor (Edge et al., 1990).

The presence of poly-*N*-acetyl lactosamine in cell membranes is not uniform. Several studies have demonstrated drastic changes under different conditions. In HL-60 granulocytes, poly-*N*-acetyl lactosamine on their plasma membrane is increased during cell differentiation, implying phagocytic activity and stabilization of lysosomes (Fukuda and Fukuda, 1984). In natural killer cells, tomato lectin binding is increased after activation by interleukin-2 (McCoy et al., 1990). Other studies indicate that macrosialin, a macrophage-restricted membrane GP, shows differential glycosylation on activation, presenting polylactosamine structures only in response to inflammatory stimuli (Rabinowitz and Gordon, 1991).

These findings are consistent with our results, suggesting that differences seen in the intensity of tomato lectin binding by ameboid and ramified microglial cells may be attributed to their stage of differentiation. Ameboid microglial cells, putative phagocytic cells during development, displayed a cytoplasm full of secretory granules and lysosomes, and accordingly showed high levels of poly-*N*-acetyl lactosamine. As ameboid microglial cells differentiate, losing their phagocytic activity and becoming ramified cells, there is a significant decrease of poly-*N*-acetyl lactosamine residues, reflected by a decrease in tomato lectin binding.

It should be noted that despite this reduction of poly-*N*-acetyl lactosamine residues, ramified microglial cells in normal adult brain still conserve these residues, suggesting that they may play an important role. Further studies using tomato lectin as a microglial cell marker in different pathological conditions or in experimental lesion models may give more information about the specific role of this lectin receptor.

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