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ARTICLE

Nonspecific Labeling of Myelin with Secondary Antisera and High Concentrations of Triton X-100

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SUMMARY Triton X-100 is used in immunohistochemistry to make tissue permeable, to present certain antigens to antisera, and to prevent certain nonspecific interactions. This detergent is routinely dissolved in buffers at concentrations of 0.01–0.2%. Using high concentrations of Triton X-100 (0.2–2%) and anti-immunoglobulins G (anti-IgGs), labeling of myelin and microglia was detected in fixed brain tissue by indirect fluorescence and avidin–biotin–immunoperoxidase techniques. Differences were found between the species studied (mouse and rat), the type of anti-IgG (anti-mouse, anti-rabbit, anti-sheep, anti-rat, or anti-guinea pig), the detergent concentration, and whether Triton X-100 was included in the incubation media or applied as a pretreatment. Mouse brain displayed strong myelin labeling with all anti-IgGs but rat brain only with anti-rabbit or anti-sheep IgGs. Staining of ramified microglia occurred only in mouse tissue when anti-mouse IgG was used. Nonspecific staining of myelin was also intense in paraffin-embedded tissue and in human brain frozen sections. These results are significant for the prevention of undesirable staining in routine immunolabeling and they also provide a comparatively inexpensive, easy to perform strong labeling of myelin. In addition, the double marker signal (peroxidase and fluorescence) is useful for double labeling studies. **(J Histochem Cytochem 46:109–117, 1998)**

KEY WORDS
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The avidin–biotin complex (ABC) technique, a suitable method for most immunohistochemical laboratories, is used today largely because of the high affinity of its components, its stability, and the great signal amplification that the complex yields (Hsu 1990). Biotinylated anti-IgGs (α-IgGs; secondary antibodies or antisera) can be detected with the ABC method and with avidin-bound fluorochromes among others. Some reagents are common to both detection systems, making this technique flexible and economical. Moreover, a combination of the avidin–biotin technique with indirect immunohistochemistry or with other histochemical reactions can also be used for double staining for light microscopy (Weruaga–Prieto et al. 1996).

For successful application of immunohistochemistry to tissue sections, a high degree of binding of specific IgGs (primary antibodies or antisera) to the antigen epitopes is necessary, as well as unhindered penetration of all reactive molecules in the tissue. For the sake of complete penetration, light microscopic immunohistochemistry is usually carried out using media containing a detergent such as the Triton X series or Tween. These detergents remove lipids aggressively (Helenius and Simons 1975), enhancing reagent penetration through biological membranes (Hartman 1973; Neugebauer 1988; Wouterlood et al. 1988). The nonionic detergent Triton X-100, currently the most widely used in histo- and immunohistochemistry techniques, also reduces nonspecific staining (Hartman 1973; van Leeuwen 1980). Certain antigens that are embedded in membranes may even require exposure to detergent for successful immunostaining (Ribeiro-da-Silva et al. 1993; Pennell et al. 1994), and hence a short pretreatment with 0.25% Triton X-100 is recommended for antibodies that recognize intramembranous segments of the antigen (Pickel 1981). This treatment is also used to detect molecules in myelin, a lipid-enriched structure sheathing axons in the central and peripheral nervous systems. Immunohistochemical rec-

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ognition of some of these molecules requires higher concentrations of Triton X-100. Therefore, Berger and Frotscher (1994) used a pretreatment with 0.5% Triton to detect Rip antigen and myelin proteolipid protein in the myelin segments. Moreover, Friedman et al. (1989) added 2% Triton X-100 to the incubation medium to immunostain the myelin-producing cells with their entire processes. Apart from these high concentrations, Triton X-100 is largely used for immunohistochemistry in the central nervous system at concentrations ranging from 0.01% to 0.2% (for review and details see Cuello 1983,1993).

Despite the high specificity of monoclonal antibodies, background and non-specific staining can be detected in different tissues when immunohistochemical methods are employed. These reactions can be caused by endogenous tissue properties or subsequently applied reagents (Larsson 1993). One of these nonspecific stainings may be caused by hydrophobic interactions of immunoglobulins with components of the tissue and can be prevented by addition of a nonionic detergent such as Triton X-100 (Pool et al. 1983). Therefore, detergents used for immunohistochemistry play multiple roles, facilitating the penetration of antibodies and other molecules into the cell, exposing some membrane-bound antigens to antibodies, and reducing nonspecific background caused by hydrophobic interactions.

In this article we describe a particular staining caused by direct incubation with anti-IgG (secondary antisera) and high concentrations of Triton X-100. This staining was detected after specificity controls in aldehyde-fixed brain tissue in two common laboratory species, rat and mouse, and in human brain tissue stored for long periods in formalin. Secondary antisera were applied for immunohistochemistry after performance of a histochemical reaction in which high concentrations of Triton X-100 were used (unpublished data). In these reactions, myelin, microglial cells, or blood vessels were labeled without the use of any specific primary antiserum. The staining can be considered as undesirable when location of an antigen is sought, but it can also be employed as a neuroanatomic marker. The aim of this work is to describe this phenomenon in detail, providing data both to prevent undesirable labeling in routine immunostaining and to use it as a method for marking specific neural elements, such as myelin.

Materials and Methods

Adult female rats (Wistar) and mice (CD-1 and BALB/cJ strains; Criffa SA, Barcelona, Spain) were used for this study. After deep anesthesia with ketamine, the blood was rinsed with a solution containing 0.9% (w/v) NaCl and 5 IU/ml heparin. Then a fixative containing 4% (w/v) depolymerized paraformaldehyde and 15% (v/v) saturated picric acid in 0.1 M phosphate buffer (pH 7.3) was perfused for 20 min. The brains were dissected out and immersed for 2 hr in the same fixative at 4C. After rinsing overnight with phosphate buffer, the tissue was cryoprotected with 30% (w/v) sucrose in phosphate buffer. Brains were serially cut with a cryostat in 40-μm-thick sections at the coronal plane. Human cervical spinal cord kept for long periods in formalin was also cryosectioned at the same thickness. In addition, mouse and rat brains were paraffin-embedded (Paraplast; Sherwood, St Louis, MO), and cut serially at 10 μm in parasagittal planes.

The reactions were carried out at room temperature. All sera and antisera used (Table 1) were diluted in PBS, pH 7.3, and the sections were thoroughly rinsed (four times for 10 min) with the same solution between each step. Three different experimental groups were assayed: (a) without detergent; (b) with a Triton X-100 (Prolabo #28 817.295; Paris, France) pretreatment for 1 hr; and (c) with Triton X-100 in the incubation media. When added, Triton X-100 was diluted at the following concentrations: 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, and 2.0% (v/v). In addition, different normal sera (Table 1) were tested in the secondary antisera incubation media (10% v/v).

To reveal secondary antisera binding, the avidin–biotin–immunoperoxidase (ABC) method was used. Biotinylated secondary antisera diluted 1:200 in PBS were applied for 2 hr. The Vectastain Elite ABC kit (Vector, Burlingame, CA; #PK-61000) was used at 1:200, being applied for 3 hr. Tissue-bound peroxidase was revealed by incubating the sections in a solution containing 0.002% (w/v) 3,3′-diaminobenzidine (DAB; Sigma, St Louis, MO) and 0.003% (v/v) H_2O_2 in 0.05 M Tris-HCl buffer, pH 7.6. The reaction was

Table 1 Normal sera and antisera

Secondary antiserum	Normal serum and antiserum source	Antiserum code[a]	Normal serum code[a]
Biotinylated α-mouse IgG	Horse	#BA-2000	#S-2000
Biotinylated α-rabbit IgG	Goat	#BA-1000	#S-1000
Biotinylated α-sheep IgG	Rabbit	#BA-6000	#S-5000
Biotinylated α-rat IgG	Rabbit	#BA-4000	#S-5000
Biotinylated α-guinea pig IgG	Goat	#BA-7000	#S-1000
Fluorescein α-mouse IgG	Horse	#FI-2000	#S-2000
Fluorescein α-rabbit IgG	Goat	#FI-1000	#S-1000
Fluorescein α-sheep IgG	Rabbit	#FI-6000	#S-5000

[a]From Vector Labs, Burlingame, CA.

controlled under the microscope and detained with cold PBS. The sections were mounted, dehydrated, and coverslipped with Entellan (Merck; Darmstadt, Germany).

The nonspecific staining of myelin or microglia was first detected and systematically studied on frozen sections of mouse and rat brains. The combination of Triton X-100 concentrations/secondary α-IgGs, which produces optimal staining of myelin, was selected for the study in the human and paraffin-embedded sections (see Results).

Controls of the ABC–peroxidase immunohistochemical procedure included (a) elimination of secondary antisera, (b) incubation of the sections with DAB/H_2O_2 solution alone, and (c) use of indirect immunofluorescence. For the latter purpose, fluorescein-conjugated secondary antisera (Vector) (Table 1) were diluted 1:100 and applied for 3 hr. Sections were rinsed with PBS, pH 7.3, once with PBS, pH 8.0, mounted on gelatin-coated slides, and coverslipped with phosphate-buffered glycerol (pH 8.2).

Labeling with specific markers for the suspected elements (i.e., myelin or microglia; see Results) was carried out on adjacent serial sections to confirm their nature. Sections from mouse brain were used to perform these controls because the best visualization of the nonspecific staining was always achieved in this material. In the case of myelin, odd sections were stained with biotinylated α-sheep IgG after pretreatment with 0.8% Triton X-100 and the ABC method, whereas even sections were colored with antibodies against myelin oligodendrocyte glycoprotein (MOG; Dyer et al. 1991). The primary α-MOG antibodies (Chemicon, Temecula, CA; 1:5000 in PBS) were applied for 72 hr at 4C, followed by biotinylated α-mouse IgG and the ABC method as described above, but without addition of detergent. In the case of microglia, odd sections were stained with α-mouse IgGs jointly diluted with 1.0% Triton X-100, and the even ones with specific markers for microglial cells: tomato lectin histochemistry (Acarín et al. 1994) or histochemical demonstration of nucleoside diphosphatase (Vela et al. 1995).

We also performed a test to determine whether the nonspecific staining of microglial cells was due to a specific binding of the IgG to Fc receptors on the surface of such cells. Frozen sections of mouse brain were incubated with α-mouse immunoglobulin $F(ab)_2$ fraction conjugated with fluorescein (Silenus Labs, Hawthorn, Australia; 1:300 in PBS, 3 hr) jointly with 1.0% (v/v) Triton X-100, rinsed thoroughly, and directly inspected under convenient fluorescent light.

Documentation

Both fluorescence and DAB labelings were photographed in an Axiophot Zeiss Microscope equipped with an epifluorescence lamp and appropriate filter sets. For low-magnification pictures, sections were inserted into a photographic enlarger and directly printed on photographic paper (without the use of any photographic film), thus producing negative images of enhanced contrast (histographs).

Results

The two methods used to reveal the tissue-bound secondary antisera presented basically the same results, but differences in both the degree of the staining and the background were found and are detailed below.

Incubation of the sections with DAB and H_2O_2 did not produce any staining. When the secondary antisera steps were omitted from the ABC method, a residual nonspecific DAB product could be detected throughout all regions of the brain. Furthermore, this brownish background was absent in the main tracts, such as the optic and olfactory tracts, the corpus callosum, the white matter of both cerebral and cerebellar cortex, and the columns of the spinal cord (Figure 1). This nonspecific staining was increased by incubating the sections with Triton X-100 and was drastically reduced but still present when serum was added to the incubation media in the ABC method. This background staining appeared in both mouse strains and in the rat. When immunofluorescence was used, this type of nonspecific staining was practically nonexistent.

Secondary antibodies diluted in media with high concentrations (0.2–2%) of Triton X-100 and without any specific primary antiserum enabled us to distinguish three elements, identified by their morphological features as blood vessels, myelin sheaths, and microglial cells. They did not appear when Triton X-100 was omitted from the incubation medium.

Positive staining was found on the walls of blood vessels. This staining was more clearly seen in the cerebellar and cerebral cortices. With high concentrations of Triton X-100 (Table 2), a remarkable staining was localized mainly in myelinated tracts (Figure 2) such as the optic nerve and tract, the olfactory tracts, the corpus callosum, the fimbria hippocampi, inferior colliculi, pons, most brainstem tracts, and the white mat-

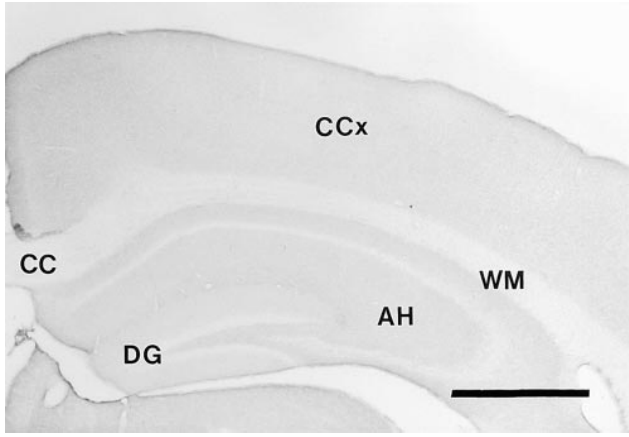

Figure 1 Coronal section through the hippocampus and cerebral cortex of a BALB/c mouse. Note the differential background staining between gray matter regions (stronger) and white matter as the result of applying only the ABC complex and DAB/H_2O_2 reaction. AH, Ammon's horn; CC, corpus callosum; CCx, cerebral cortex; DG, dentate gyrus of the hippocampus; WM, white matter of the cerebral cortex. Bar = 1 mm.

Table 2 Minimum concentrations of Triton X-100 for myelin labeling to appear

Secondary antiserum	Type of Triton X-100 treatment[a]	Triton X-100 concentration (%)[a]	
		Mouse brain	Rat brain
α-mouse	P	0.4	—
	T	1.5	—
α-rabbit	P	0.4	0.8
	T	—	—
α-sheep	P	0.2	0.6
	T	1.5	—
α-rat	P	0.2	—
	T	1.5	—
α-guinea pig	P	0.2	2.0
	T	—	—

[a]P, Triton X-100 was applied as pre-treatment; T, Triton X-100 was diluted jointly with the secondary antiserum; —, no myelin staining at the concentrations used.

ter of the cerebral and cerebellar cortices, among others. This dense and strong staining was continued into these last regions within the pertaining gray matter, gradually losing its packaged appearance. In the cerebral cortex, this labeling continued to Layer III, was practically absent in Layer II, and was again present in Layer I (Figure 3A). In the cerebellar cortex, this characteristic staining could be detected sparsely among the granular layer and more rarely in the molecular layer (Figure 3B). In these gray matter regions solitary sheath-like profiles could be better distinguished, being identified as myelinated fibers (Figure 3C and 3D). Sections stained for MOG exhibited the same labeling pattern as the adjacent ones incubated only with α-mouse IgGs and a pretreatment with Triton X-100, confirming the myelinated nature of the stained elements (Figure 4).

The other type of staining using secondary antisera and high concentrations of Triton X-100 was clearly cellular. Elements dispersed throughout the brain displayed polygonal (predominantly triangular) somata with a few long, sparsely branched processes (Figure 5). The secondary branches were clearly shorter than the primary ones. In general, these cells were disposed at higher densities in the cerebral gray matter, displaying shorter processes. In contrast, in the fiber tracts

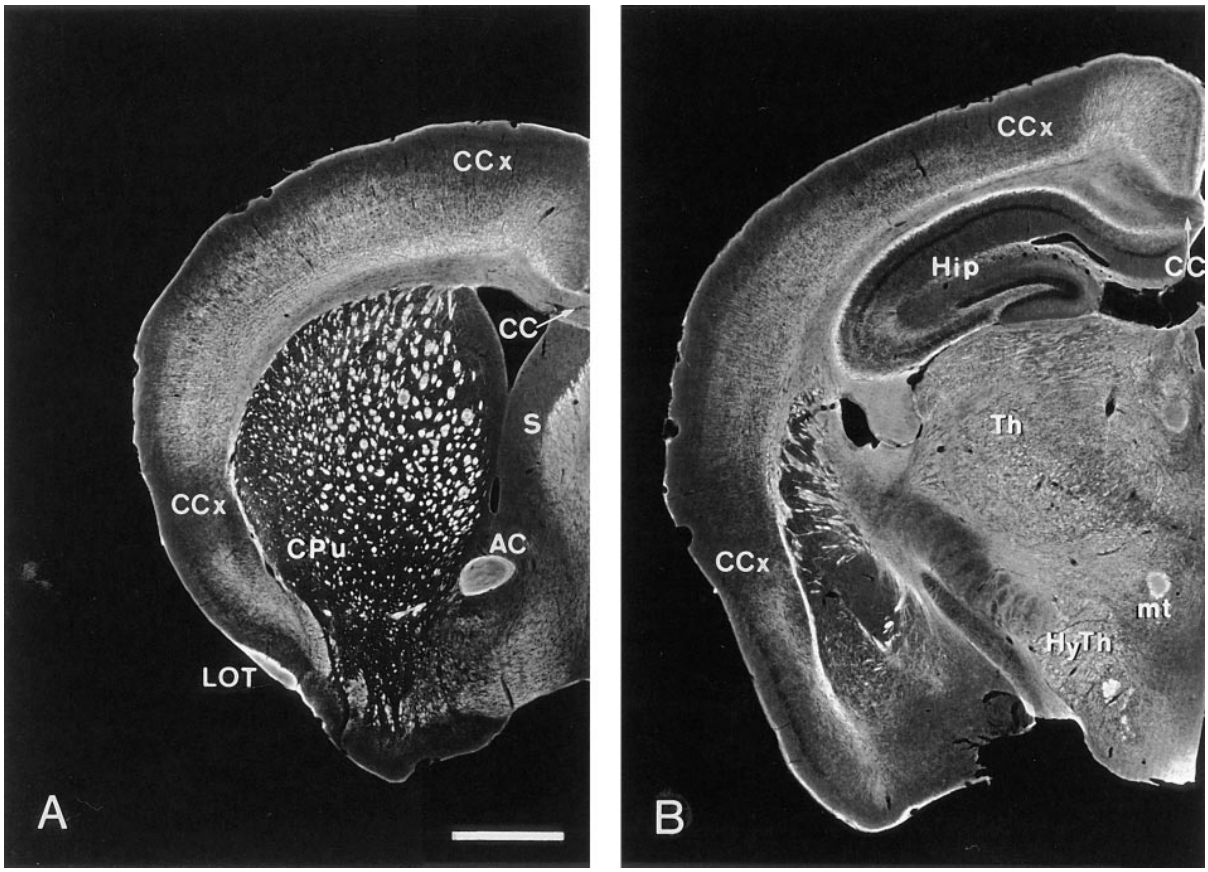

Figure 2 (**A,B**) Histographs of coronal sections of a BALB/c mouse brain subjected to a 0.8% pretreatment with Triton X-100, and subsequently incubated with α-sheep IgG and rabbit serum. Staining (white profiles, corresponding to DAB reaction product) is located in the main myelinated tracts. **A** is rostral to **B**. AC, anterior commissure; CC, corpus callosum; CCx, cerebral cortex; CPu, caudate putamen; Hip, hippocampus; HyTh, hypothalamus; LOT, lateral olfactory tract; mt, mammillothalamic tract; S, septum; Th, thalamus. Bar = 1 mm.

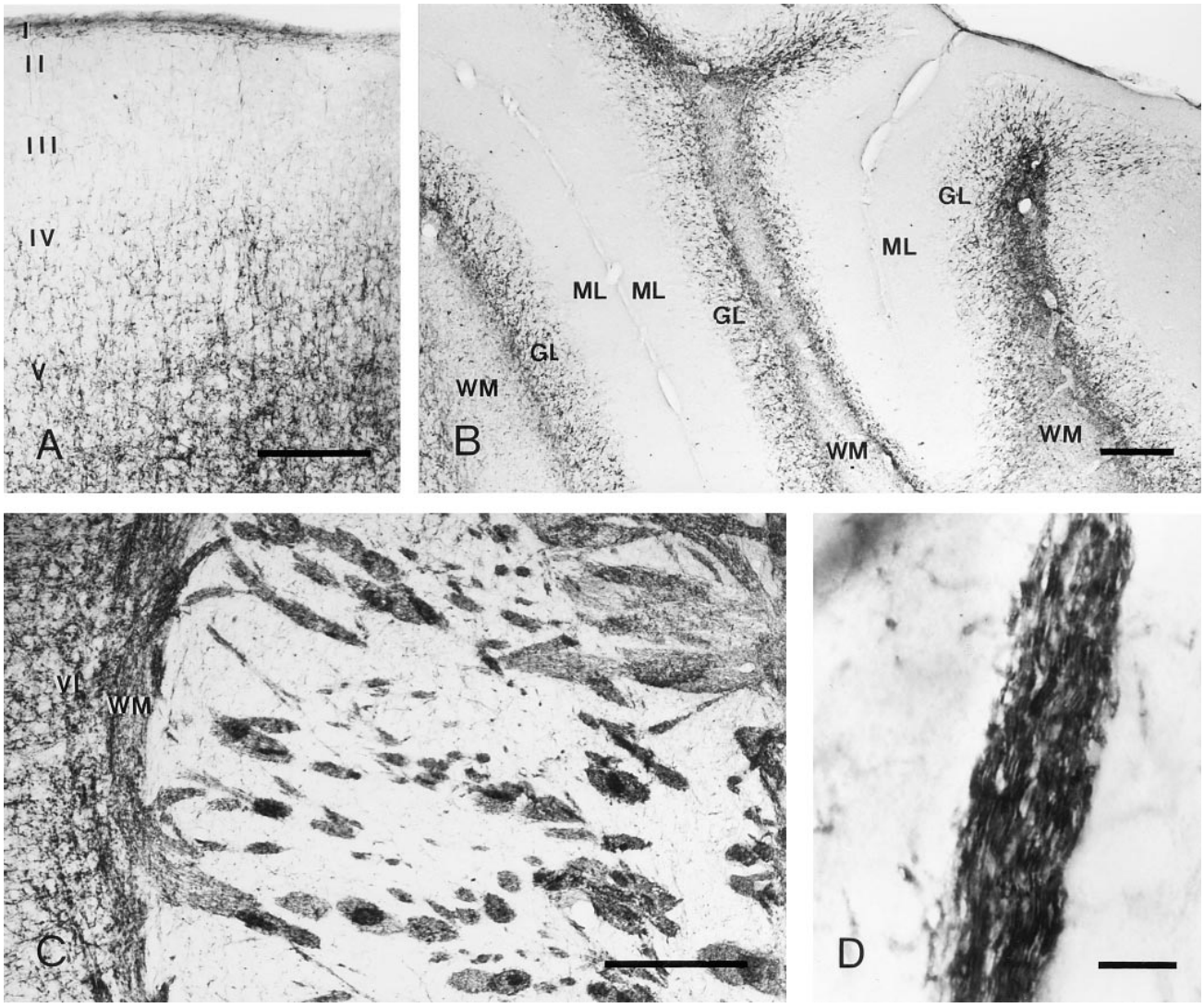

Figure 3 Sections similar to those shown in Figure 2, photographed with brightfield microscopy. DAB reaction product is seen as dark elements. (**A**) Cerebral cortex showing myelin staining in Layers I, III, IV, and V. (**B**) Cerebellar section in the molecular layer (ML). The myelin staining is practically absent, but is present in the granular layer (GL) and white matter (WM), as with incubating the tissue with only secondary antisera. (**C**) Micrograph showing myelinated tracts at a more caudal level of the caudate putamen. Layer VI and white matter (WM) of the cerebral cortex are also indicated. Bars = 200 μm. (**D**) Detail of a myelinated tract. Note myelinated fibers as stained parallel profiles. Bar = 10 μm.

and in the cerebellum, these cells were less densely distributed and had longer processes (Figures 5A–5D). In accordance with their morphological characteristics, they were identified as microglial cells (Figures 5C–5F). The microglial nature of these cells was confirmed in adjacent sections stained for specific microglial markers such as NDPase histochemistry. These sections displayed labeled elements with the same distribution, density, and morphological characteristics (size, shape, and branching pattern) as those observed after the nonspecific staining (Figure 6). Incubation of sections with only the $F(ab)_2$ fragments of immunoglobulin resulted in the staining of ramified microglial cells, similar to the labeling obtained with the complete α-mouse IgG.

The three different types of stained elements depended on four different factors: (a) the concentration of the detergent, (b) whether the detergent was applied before or together with the secondary antisera, (c) the diverse secondary antisera utilized, and (d) the species employed. There were no differences in the staining of both mouse strains studied in this work. When normal serum was added to the media, the three types of secondary antiserum labeling were reduced (especially the myelin staining) but they were still present. This characteristic was common to both methods em-

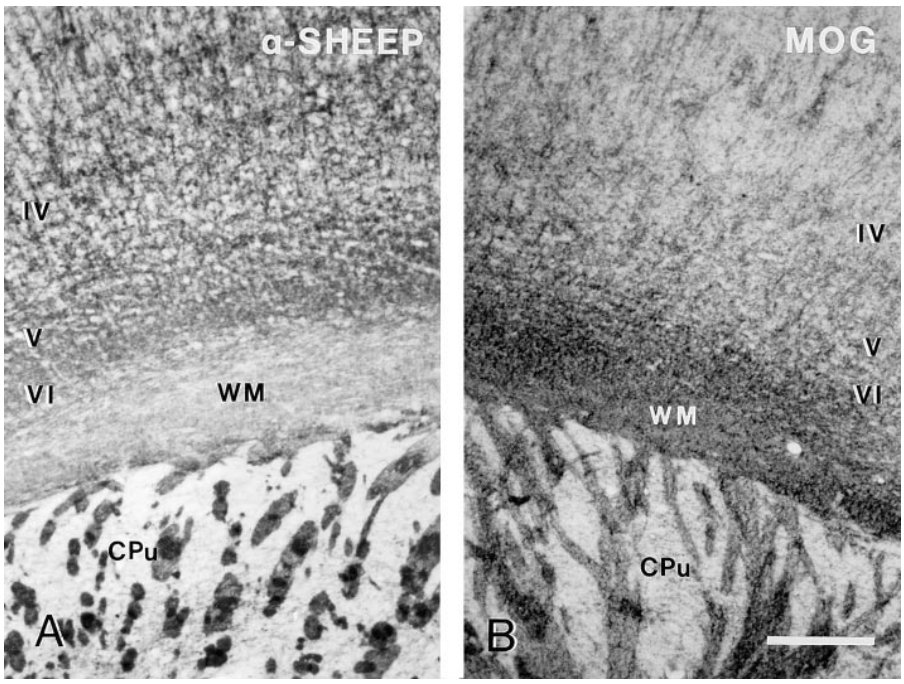

Figure 4 Comparison between nonspecific myelin staining as in Figure 2 (**A**, α-sheep) and the specific marker myelin oligodendrocyte glycoprotein (**B**, MOG) in mouse brain. Both fields correspond to the infragranular layers of the cerebral cortex and the rostral part of the caudate putamen. CPu, caudate putamen; IV, V, VI, layers of the cerebral cortex; WM, white matter of the cerebral cortex. Bar = 200 μm.

ployed, ABC and immunofluorescence. However, differences were detected in the intensity of the staining. It was always stronger after the use of immunofluorescence compared with the ABC method. Moreover, when Triton X-100 was applied as pretreatment, staining was stronger than when Triton X-100 was included in the incubation media together with the secondary antiserum. This aspect was more clearly observable in the myelin staining, so that the same degree of myelin labeling was obtained by using Triton X-100 within the media in concentrations five- to tenfold higher than those used as a pretreatment, in the same species and with the same secondary antiserum.

The most diverse results were obtained with the different combinations of species-type secondary antisera. Blood vessel labeling occurred with the use of α-mouse in mouse and rat, and α-rat only in rat. In human tissue, these elements were negative with all the antisera used.

Microglial labeling was unusual because it was detected only by using α-mouse in mouse. These cellular elements were marked when 0.1% of Triton X-100 was used as pretreatment or with 0.2% of Triton X-100 if diluted within the incubation media. Optimal microglial staining was obtained by diluting 1% Triton X-100 in the media, because pretreatment with Triton X-100 also led to myelin labeling.

Myelin staining occurred in mouse brain tissue with all species types of α-IgGs. It was observable only in rat tissue when α-rabbit, α-sheep, or α-guinea pig antisera were used, at the Triton X-100 concentrations used in this work. Minimal concentrations of Triton X-100 with which myelin staining appeared are detailed in Table 2. Optimal myelin staining was achieved only in mouse brain at the concentrations of Triton X-100 chosen for this work. The best labeling was obtained by pretreatment with the detergent at the following concentrations: 1% with α-rabbit; 0.6% with α-sheep; 0.8% with α-rat; and 1% with α-guinea pig secondary antisera. With the use of α-mouse IgG and Triton X-100 as pretreatment, optimal labeling of myelin was not possible because microglial cells were also labeled in all cases. Those combinations of IgGs/Triton X-100 for optimal myelin labeling produced similar results in both rat and mouse brains compared with the cryostat sections, although the background was lower after paraffin-embedding. Those combinations were also tested on cryostat sections of human cervical spinal cord. Myelin tracts were also noticeable, the staining with all IgGs employed being comparable.

Discussion

The present work describes the labeling of myelin and microglia in rodent brain tissue as the result of nonspecific binding of secondary anti-immunoglobulins originated by addition of high concentrations of Triton X-100 to the incubation media.

Nonspecific binding of immunoglobulins to the tissue (other than IgG antigen) may occur during immunohistochemical procedures. The microglial and myelin labelings described in this work are certainly due to

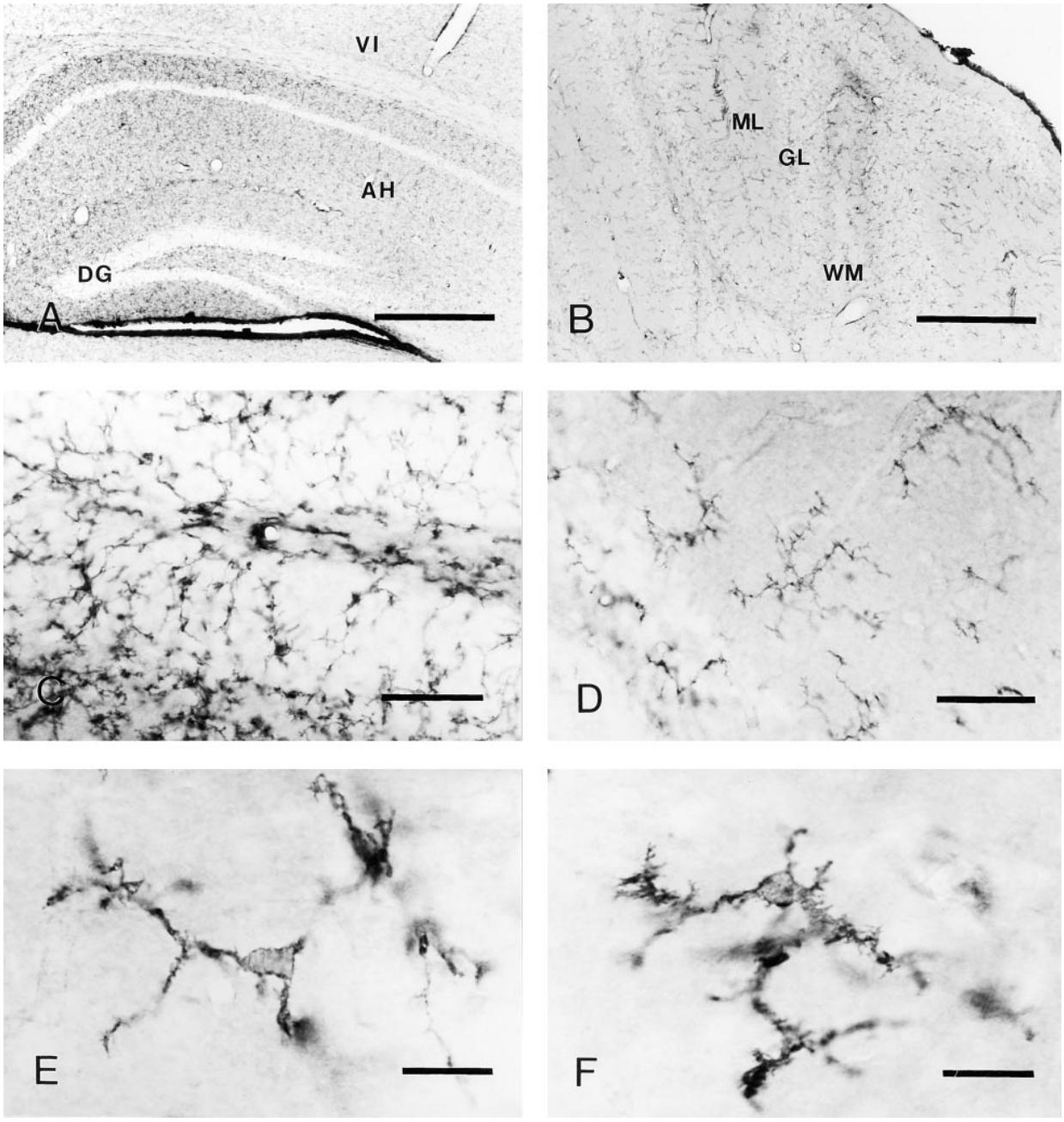

Figure 5 CD-1 mouse brain tissue incubated with α-mouse IgG together with 1% Triton X-100 and horse serum. The reaction was revealed using the ABC technique and DAB as chromogen. Microglial staining was evident in all brain regions. Microglial cells in the hippocampus (**A**) are more densely distributed than in the cerebellum (**B**). AH, Ammon's horn; DG, dentate gyrus; GL, granule cell layer of the cerebellum; ML, molecular layer of the cerebellum; VI, Layer VI of the cerebral cortex; WM, white matter of the cerebellum. Bars = 500 μm. (**C**,**D**) Higher magnifications of **A** and **B**, respectively. Microglial cells in the hippocampus have shorter branches than the microglia in the cerebellum. Bars = 50 μm. (**E,F**) Two microglial cells of the cerebellum at higher magnification. Note the polygonal somata and the long and ramified processes. Bars = 20 μm.

nonspecific binding of secondary immunoglobulins, because this coloring disappeared completely when the different secondary antisera were omitted from the ABC method. Moreover, the staining cannot arise from the combined incubation of the secondary antiserum with the avidin–biotin–peroxidase complex, because indirect fluorescence (with only fluorescein linked to α-IgGs) provided the same labeling as the complete ABC method.

Electrostatic binding of IgGs with basic proteins present in the tissue can result in nonspecific staining.

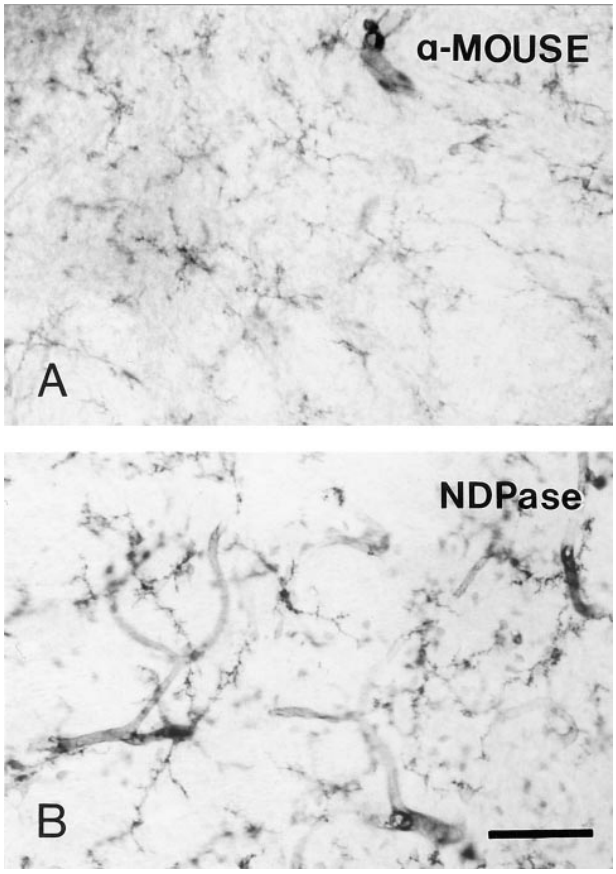

Figure 6 Microglial staining in mouse cerebellar white matter by incubation with only α-mouse secondary antiserum jointly with 1% Triton X-100 (**A**, α-mouse) or with the NDPase histochemical procedure (**B**, NDPase). Ramified microglia are noted with both methods with the same morphological pattern and density distribution. Bar = 50 μm.

This problem can be partially solved by incubating the sections with preimmune serum (normal serum) from the species that served as source for the secondary antibody (Sternberger 1979). As described in the Results, when sections were incubated in the presence of normal serum, the nonspecific staining was significantly reduced with both methods employed, ABC and indirect immunofluorescence, but it was still present. Other problems could derive from ionic interactions and might be reduced by enhancing the ionic strength of the buffer (Capel 1974; Grube 1980). The PBS employed for the incubation media in our experiments may solve this problem.

Interactions of IgGs with other molecules could also be hydrophobic, and to prevent them dilution of the antisera with a detergent is recommended (Pool et al. 1983). Triton X-100 is known to act in immunohistochemical techniques in this way (Hartman 1973; van Leeuwen 1980; Léránth and Pickel 1989). Furthermore, Triton X-100 also facilitates the penetration of reagents into the tissue by dissolving lipids (Neugebauer 1988; Wouterlood et al. 1988), and it allows access to antigenic epitopes that otherwise would be immersed in and masked by biological membranes. This would explain why the particular staining of myelin and microglia described appears only when a treatment of Triton X-100 is applied to the tissue and why the intensity of staining increased with the detergent concentration.

After addition of Triton X-100 to the incubation media, the myelin labelling was drastically reduced with all types of secondary antisera (see Table 2), but the microglial staining remained unaltered. Therefore, both kinds of staining presumably derive from different IgG–tissue interactions. In the former case, IgGs may display strong hydrophobic interactions with myelin components, but not with molecules present in microglial cells. The binding of α-IgGs to microglial components is stronger because labeling was not drastically reduced when either normal serum or detergent was added to the incubation medium. Fishman and Savitt (1989) found selective accumulation of IgGs in microglial-shaped cells in normal mouse brain. This does not appear to be nonselective phagocytosis of extracellular fluid, because serum albumin could not be detected in those microglial cells. On the other hand, Fc receptors have been demonstrated on the surface of rodent microglial cells in vitro and in vivo (Vedeler et al. 1994). We achieved positive staining of ramified microglial cells by using only $F(ab)_2$ fragments in the incubation medium. This indicates that the nonspecific staining of microglial cells by α-mouse IgGs enhanced with Triton X-100 is not specific for binding of the Fc fragment to the respective receptor but rather for IgGs present on the cells.

Differences exist in the crossreaction of the different α-IgGs with other IgGs raised in different species. This fact could be reflected in the diversity of results obtained with the different anti-immunoglobulins tested in the present study. Moreover, antibody activity after marker binding is different between biotinylation and fluorescein conjugation (for details see specifications of the supplier, Vector). This is in accordance with the different degree of staining obtained with either biotinylated or fluorescein conjugated antibodies. Furthermore, differences in the expression of cell surface markers and myelin proteins have been found between mouse and rat (Duchala et al. 1995). This concurs with our results, in which substantial differences between mouse and rat brain sections were found in the myelin staining (the latter species displayed labeling only with three of the IgGs employed).

Several immunocytochemical markers are used to stain myelin in brain tissue. Because α-mouse and α-rabbit secondary IgGs are used for detection of most of these molecules, it is interesting that the addition of

high concentrations of Triton X-100 (at least 1%) greatly enhances the labeling in the mouse brain. Non-immunological techniques are also used to demonstrate myelin in brain tissue. Luxol Fast Blue is the most usual, but others, such as Sudan Black B (Gerrits et al. 1992), and the rapid fluorescent stain for myelin membranes with tetracycline hydrochloride (Pereyra and Roots 1988), also provide adequate staining. An advantage of the technique described in this article is that it is highly reproducible, and it is possible to obtain both peroxidase/DAB and fluorescent signals.

In conclusion, we recommend care in the use of Triton X-100 when immunocytochemistry is performed for any antigen in the brain (not more than 0.1–0.2% added to the incubation media if possible), or the use of other techniques to facilitate penetration of immunoreagents, such as the freeze-thawing method. In addition, we propose the use of high concentrations (1–2%) of Triton X-100 without the use of any specific primary antisera for labeling myelin in frozen or paraffin sections.

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