Acta Neuropathol (Berl) (1985) 65:177–189

Acta Neuropathologica

Original Works

Immunocytochemical Study of Myelin-associated Glycoprotein (MAG), Basic Protein (BP), and Glial Fibrillary Acidic Protein (GFAP) in Chronic Relapsing Experimental Allergic Encephalomyelitis (EAE)*

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Summary. Chronic relapsing experimental allergic encephalomyelitis (EAE) lesions that resemble those seen in multiple sclerosis (MS) were produced in young Hartley and strain 13 guinea pigs (Lassmann and Wisniewski 1979). To study distributions of myelin-associated glycoprotein (MAG), myelin basic protein (MBP), and glial fibrillary acidic protein (GFAP) in these lesions, paraffin and semithin epon sections of CNS from eight of these guinea pigs were immunostained with antisera to these proteins according to the peroxidase-antiperoxidase (PAP) method. In lesions with active myelin sheath breakdown, changes in anti-MAG and anti-BP immunoreactivity corresponded closely. Abnormal and/or decreased anti-MAG staining did not extend beyond margins of lesions into surrounding areas containing myelin sheaths stained normally by anti-BP and by histological stains for myelin. GFAP-stained astrocyte processes were more numerous and much larger in more chronic lesions. Anti-MAG and anti-BP both stained regenerating myelin sheaths which were very numerous in both paraffin and epon sections. In the latter, anti-MAG also stained some myelin-forming oligodendroglia. The results are additional evidence suggesting that in chronic relapsing EAE, myelin sheaths are the primary target. Oligodendroglia appear to be relatively unaffected and remyelinate most of the demyelinated axons.

Key words: Guinea pigs – Chronic relapsing experimental allergic encephalomyelitis (EAE) – Myelin-associated glycoprotein (MAG) – Myelin basic protein (MBP) – Glial fibrillary acidic protein (GFAP)

Introduction

Chronic relapsing experimental allergic encephalomyelitis (EAE) has been an important model for studies of CNS myelin breakdown and regeneration because some of its features resemble those seen in multiple sclerosis (MS) (Lassmann and Wisniewski 1979; Lassmann 1983; Raine 1983). It has been produced in guinea pigs (Stone and Lerner 1965; Raine et al. 1974; Wisniewski and Keith 1977; Lassmann and Wisniewski 1979), rats (McFarlin et al. 1974), mice (Raine et al. 1980), and monkeys (Alvord 1980), and much is now known about the morphology of lesions. The clinical and pathologic features are variable and depend on the animals selected (age, species, strain), the constituents of the antigen-adjuvant mixture, and the injection procedure (dose, frequency, route, and number of sites).

The purpose of this investigation was to study distributions of myelin-associated glycoprotein (MAG), myelin basic protein (MBP), and glial fibrillary acidic protein (GFAP) in chronic relapsing EAE lesions that resembled those seen in MS. Such lesions have been produced by injecting mixtures of guinea pig spinal cord, complete Freund's adjuvant, and heat-killed *Mycobacterium tuberculosis* s.c. into young Hartley or strain 13 guinea pigs (Lassmann and Wisniewski 1979). Therefore, we selected blocks from representative guinea pigs and used antisera to the above proteins to immunostain paraffin and epon

* Supported in part by the Science Research Fund (Austria), project no. 525-07
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Table 1. Recovery indicates clinical improvement after last attack. Relapse indicates either no improvement or progression

Guinea pig	Chronic relapsing EAE			
	No. of attacks	Duration		Clinical state
		Total (days)	From onset of last attack (days)	
1	2	88	8	relapse
2	3	85	9	relapse
3	2	67	31	relapse
4	4	138	50	recovery
5	4	64	14	relapse
6	6	104	20	relapse
7	3	132	23	recovery
8	5	89	48	recovery

sections according to the peroxidase-antiperoxidase (PAP) technique. The results showed that distributions of MAG and BP corresponded closely; they were similar to those found in acute EAE (Itoyama and Webster 1982) and differed from those described in MS (Itoyama et al. 1980 b). A summary of these findings was presented and published by Shii et al. (1982).

Materials and Methods

Paraffin and epon-embedded tissue was selected from a large series of Hartley guinea pigs that had chronic relapsing EAE. The preparation of the antigenic mixture, the injection technique, and the tissue processing procedure have been described (Lassmann and Wisniewski 1979). In brief, at age 15–22 days, guinea pigs received 0.2 ml of an encephalitogenic emulsion that contained guinea pig spinal cord, physiologic saline, complete Freund's adjuvant (Difco), and *Mycobacterium tuberculosis* (Difco M37Ra).

Paraffin-embedded tissue included blocks of brain, brain stem, cerebellum, and spinal cord (multiple levels) from four guinea pigs; their clinical features are summarized in Table 1. At varying intervals after sensitization, these guinea pigs were perfused with a phosphate-buffered solution of 4% formaldehyde; brains and spinal cords were removed and processed according to standard procedures. Table 1 also includes data on four other guinea pigs. They were perfused with a phosphate-buffered solution containing 1.5% glutaraldehyde and 0.5% formaldehyde. After removal, blocks of CNS containing lesions were postfixed in OsO_4, dehydrated, and embedded in epon.

Serially cut sections (7 μm) were deparaffinized (Itoyama et al. 1980a) and plastic was removed from the serial semithin (1.0 μm) epon sections by pretreating them with sodium ethoxide and hydrogen peroxide (Baskin et al. 1979). The rabbit anti-rat CNS MAG, rabbit anti-GFAP (Dako Antibodies, Westbury, NY, USA), and goat anti-rabbit BP (gift of Dr. S. Cohen, described in Itoyama et al. 1980a; Omlin et al. 1982) were used to immunostain the paraffin and epon sections according to previously described modifications (Itoyama et al. 1980 a; Winchell et al. 1982) of the PAP technique (Sternberger et al. 1970). Staining specificity of each antiserum was tested by substituting preimmune serum or antiserum absorbed with excess antigen for primary antiserum in the PAP procedure.

To identify histological and cytologic features of lesions, paraffin sections were also stained with luxol fast blue and counterstained with hematoxylin and eosin (HE) or nuclear fast red. Others were stained with the Bodian method to demonstrate axons. Toluidine blue was used to stain one of the serially cut semithin epon sections.

Lesions were counted and assigned to groups to characterize those studied in immunostained sections. Most lesions (particularly those containing transversely cut fibers and vessels) were oval or circular in shape. To estimate their size, we used an ocular micrometer (magnification × 50) to measure maximum and minimum diameters and then calculated approximate areas. Lesions with areas of less than 0.03 mm^2 were called "small"; "large" lesions had areas of 0.03–0.05 mm^2 and the "largest" were those with areas of more than 0.5 mm^2.

Results

Histopathology

Paraffin sections of brain and spinal cord from the four guinea pigs we selected contained more than 500 lesions that were similar to those seen in the large group studied earlier (Lassmann and Wisniewski 1979). To correlate histological and immunostaining observations, we counted the lesions in each section, calculated their approximate size and noted whether they were located in white matter, gray matter, or both.

In the smallest lesions, mononuclear cell infiltrates and/or myelin sheath changes surrounded one vessel and were called "single" lesions. Borders of lesions containing multiple vessels suggested derivation from multiple single lesions. These lesions were called "confluent" and often were larger. Finally, lesions were grouped according to stage of demyelination. "Active" lesions contained prominent mononuclear infiltrates, numerous LFB-positive myelin sheath fragments and ovoids in macrophages, and relatively little fibrous astrogliosis. Myelin sheath loss and fibrous astrogliosis characterized lesions that we called "inactive"; these lesions did not have margins or other areas that contained mononuclear cell infiltrates and LFB-positive ovoids in macrophages or numerous myelin sheath fragments. Lesions with both "active" and "inactive" features were included in a third group.

The distribution of lesions is shown in Fig. 1. In all groups, most of the lesions were in white matter. Also, confluent, small lesions with "active" demyelination were more numerous than other lesion types.

Acitve Demyelination

As noted above, perivascular inflammatory cell infiltrates were prominent in paraffin sections of lesions

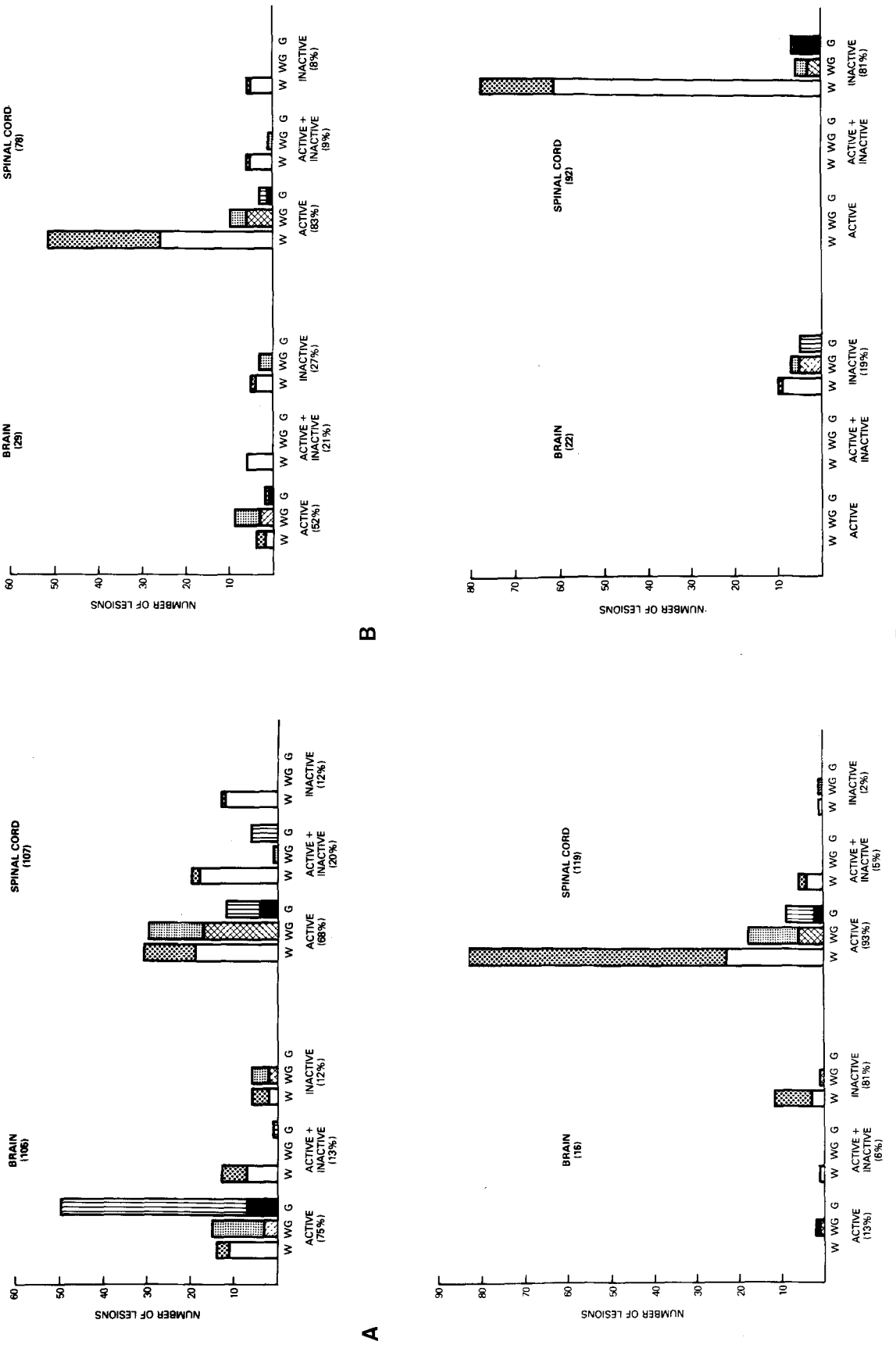

Fig. 1. Distribution of active, active and inactive, and inactive lesions in brain and spinal cord sections of four guinea pigs killed 8 (**A**), 9 (**B**), 31 (**C**), 50 (**D**) days after their last attack of chronic relapsing EAE. In white matter (*W*), white and gray (*WG*), and gray matter (*G*), *upper parts of bars* are single lesions, *lower parts* are confluent lesions. Different *shadings* are used to show numbers of single and confluent lesions in *W*, *WG* and *G* (see text and Table 1)

with active demyelination and included mononuclear cells, lymphocytes, plasma cells, and occasional macrophages (Fig. 2 A). Myelin sheaths surrounding these infiltrates were fragmented and LFB-positive ovoids were present extracellularly and in nearby macrophages and astrocytes. In sections treated with anti-GFAP serum, there were relatively few densely stained hypertrophic astrocytes (Fig. 2 B). Anti-BP immunoreactivity was reduced or lost in the same areas that contained fragmented or reduced numbers of LFB stained myelin sheaths. Most abnormal, fragmenting myelin sheaths and ovoids were intensely stained by BP antiserum (Fig. 2 C). Fewer ovoids and myelin sheaths remnants had anti-MAG immunoreactivity, but areas of decreased and absent staining by anti-MAG (Fig. 2 D) and anti-BP (Fig. 2 C) were similar in size and contour. In surrounding white matter where myelin sheaths appeared normal in LFB-stained sections, there were no changes in anti-MAG or anti-BP immunoreactivities.

In semithin epon sections stained with toluidine blue, localized changes in myelinated fibers were easier to identify (Fig. 3 A) and resembled those described earlier (Lassmann 1983). Mononuclear cells and macrophages were present in areas with fragmented myelin sheaths and ovoids. Splitting and vacuolation were common focal sheath abnormalities. Both macrophages and astrocytes contained osmiophilic myelin ovoids. Myelin sheaths that appeared normal in toluidine blue-stained sections (Fig. 3 C) often were represented by thicker rings of immunoreactivity in adjacent BP-stained sections (Fig. 3 A). When normal sheaths were compared in adjacent BP-stained sections (Fig. 3 A) and anti-MAG (Fig. 3 B), rings of anti-MAG reaction product were thinner in large sheaths. In small sheaths, there was little difference. Anti-BP and anti-MAG both stained abnormal sheaths undergoing breakdown. As in paraffin sections, anti-BP immunoreactivity was present in myeline fragments and ovoids located in macrophages, astrocytes, or extracellularly. Staining of these fragments and ovoids by anti-MAG was much less common. Astrocyte processes, identified by anti-GFAP immunoreactivity, were small and were evenly distributed throughout active lesions.

Early Myelin Regeneration

In paraffin sections of these lesions, perivascular infiltrates were smaller and most of the cells were macrophages containing myelin remnants stained by anti-BP but not by anti-MAG or LFB (Fig. 4 A, B). Other smaller round profiles in macrophages resembled lipid droplets and did not react with anti-BP. Central areas of lesions were demyelinated and contained large GFAP-positive astrocytic processes, many of which formed thick cuffs around vessels (Fig. 4 C). Among these processes, there were small groups of fibers with thin sheaths stained by LFB, anti-BP, and anti-MAG. Foci of active myelin breakdown often were present around other vessels in these lesions or at their margins. Inactive areas of lesions usually had more sharply defined borders.

In semithin epon sections, fibers partially or completely surrounded by thin BP and MAG stained myelin sheaths were much easier to identify (Fig. 4 D, E). They occurred individually, in small clusters, or in large groups among GFAP-stained astrocytic processes (Fig. 4 G). Regenerating myelin sheaths were found in central and peripheral parts of lesions. No preferential location (edge or center of lesion, perivascular, subpial, or subependymal regions) for early oligodendroglial remyelination was identified. As noted above, astrocytes and their processes were closely associated with regenerating CNS sheaths; no regenerating sheaths were surrounded by infiltrating mononuclear cells or macrophages. Near root entry zones of the spinal cord, axons were also remyelinated by Schwann cells. BP and MAG antisera stained these regenerating sheaths of PNS origin too (Fig. 4 F).

MAG antiserum stained perikarya of oligodendroglia producing new myelin sheaths; neither these MAG-positive oligodendroglia nor others within groups of regenerating sheaths were stained by anti-BP (Figs. 5, 6). Many axons near MAG-positive oligodendroglia were partly or completely surrounded by rings of BP and MAG immunoreactivtiy (Figs. 5 A, B; 6 A, B). Thicker sheaths with more than 5 compact lamellae were seen as complete, equally thick rings of BP and MAG staining in adjacent semithin sections (Figs. 5, 6). Sheaths with fewer lamellae and/or only partly compaction were represented by thinner, partial rings of BP and MAG immunostaining that often did not match precisely. Some very thin partial rings of MAG staining surrounded axons that lacked periaxonal BP staining. No thin BP-stained sheaths were identified that were unstained by anti-MAG in the adjacent semithin section.

Advanced Myelin Regeneration

Advanced myelin regeneration made many lesions difficult to identify in paraffin sections (Fig. 7). Absence of myelin sheaths was limited to a narrow perivascular zone that contained hypertrophic astrocytic processes and macrophages or showed capillary fibrosis. Elsewhere, there were numerous thin myelin sheaths readily stained by anti-BP (Fig. 7 A), anti-MAG (Fig. 7 B), and LFB. Because of their size and density, boundaries of lesion areas often

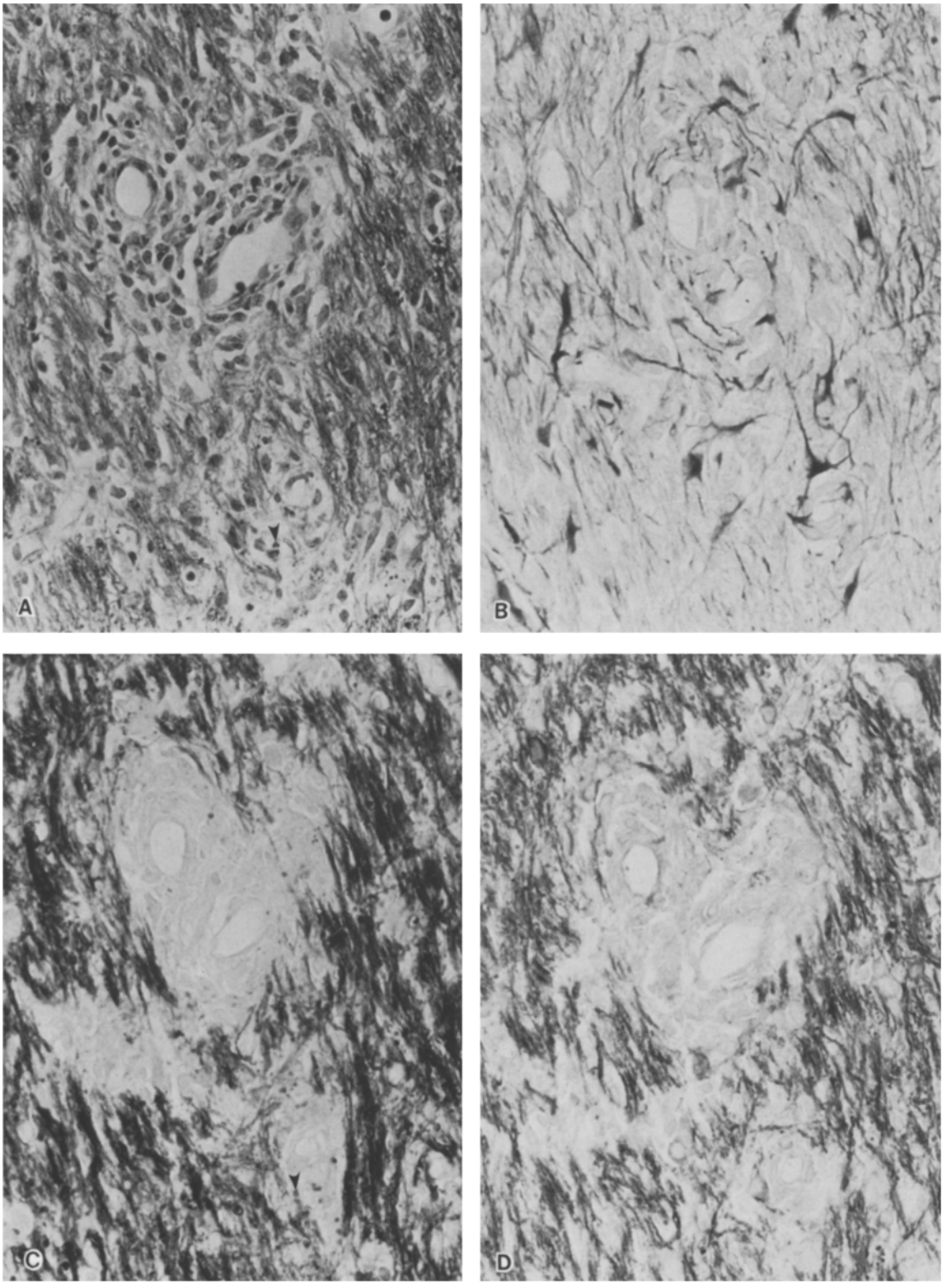

Fig. 2 A–D. Active demyelination, chronic EAE. Adjacent paraffin sections of subcortical white matter stained with LFB (**A**), 1:250 anti-GFAP (**B**), 1:500 anti-BP (**C**), and 1:250 anti-MAG (**D**). A few LFB- and BP-stained ovoids (*arrowheads*) are located next to fragmented myelin sheaths. Areas of decreased and absent MAG and BP staining correspond closely. GFAP-stained astrocytic processes appear normal in size and density. × 360

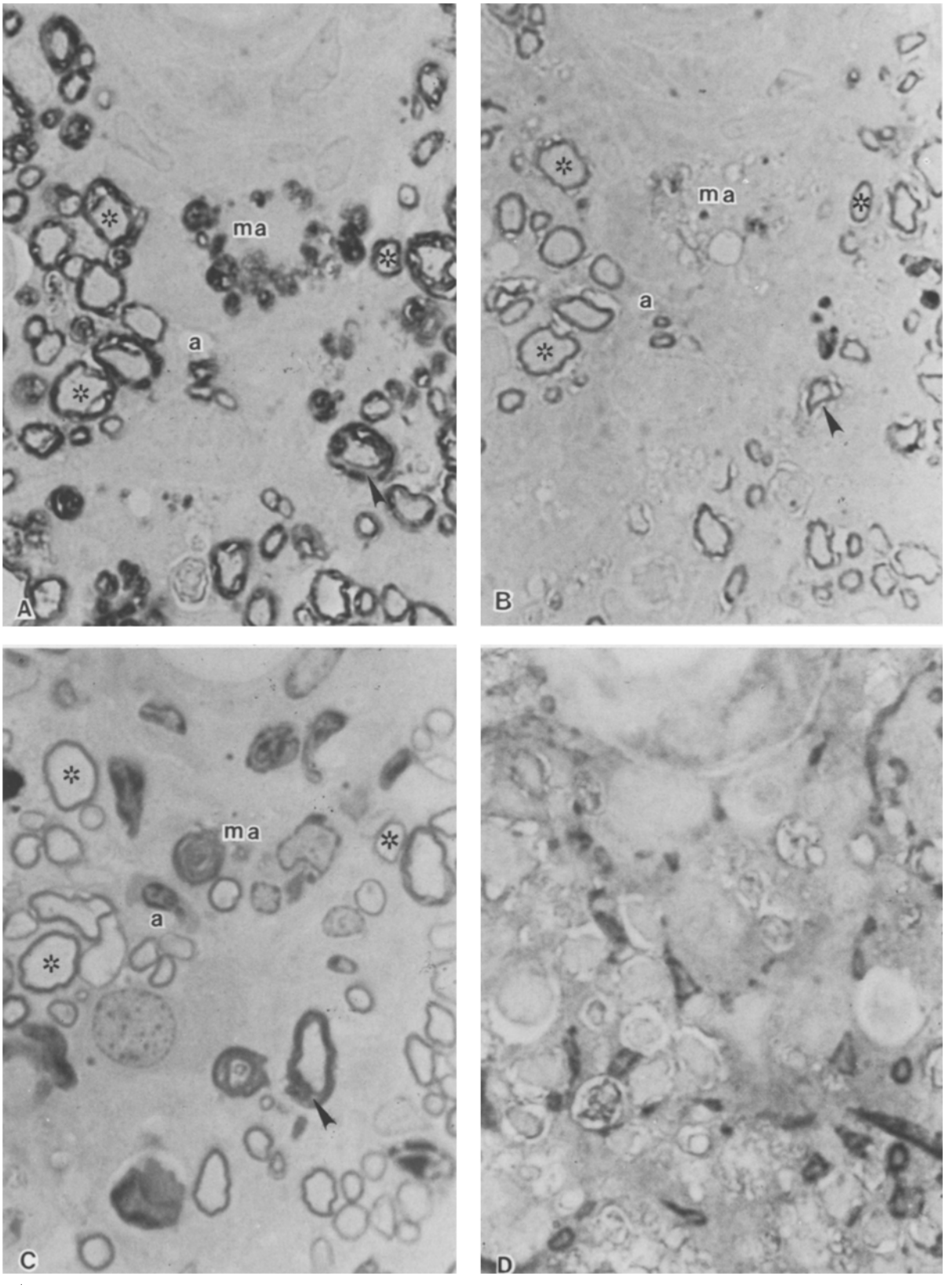

Fig. 3A–D

were poorly defined with these methods when sections were examined at low magnification. In anti-GFAP-treated sections, hypertrophy and hyperplasia of astrocytes were very marked (Fig. 7 C). They were particularly striking around vessels where adjacent processes often formed a dense thick cuff.

BP- and MAG-stained regenerating myelin sheaths were thicker and more densely packed in epon sections of these lesions (Fig. 7D, E). Macrophages were found perivascularly and scattered among regenerating sheaths. They often contained BP-positive ovoids. GFAP-stained astrocytic processes were observed between regenerating sheaths (Fig. 7F). In epon sections, their prominence around vessels varied.

Controls

In most experiments, additional paraffin and epon sections were treated with preimmune serum instead of primary antiserum. These control sections were unstained and resembled those illustrated in reports by Itoyama et al. (1980a, b) and Winchell et al. (1982).

Discussion

Our histological analysis and counts show that lesions found in the paraffin and epon sections of guinea pigs selected for this immunocytochemical study are similar in appearance and distribution to those described earlier in a much larger series of guinea pigs with chronic relapsing EAE (Lassmann and Wisniewski 1979; review in Lassmann 1983). Active demyelinating lesions were characterized by perivascular mononuclear cell infiltrates, focal breakdown and loss of myelin sheaths, LFB-stained fragments of myelin in macrophages, and only minor changes in astrocytes. Generally, lesions were most numerous in spinal cord white matter, but it is interesting that many small active lesions were present in the gray matter of one guinea pig brain. Active lesions also often contained some thin regenerating myelin sheaths. In contrast, inactive lesions had areas of myelin sheath loss, did not have LFB-stained myelin fragments in macrophages, contained prominent astrocytic processes, and otherwise were hypocellular. Regenerating myelin sheaths were thicker, more densely packed, and more numerous than in active lesions. Lesions often had active and inactive regions, especially when they were large. All of these lesion features also were found in the semithin epon sections that had been stained with toluidine blue.

Our light-microscopic immunocytochemical results on BP and MAG distributions in normal appearing adult guinea pig white matter confirm and extend data obtained earlier in rat (Sternberger et al. 1978, 1979) and human (Itoyama et al. 1980 a, b) CNS. The entire thickness of all myelin sheaths is stained by BP antiserum. The previously described periaxonal localization of MAG is demonstrated most easily in large sheaths. But in smaller sheaths, distributions of MAG and BP match more closely, suggesting that MAG is in compact myelin. Also, in recent electron-microscopic studies (Webster et al. 1983; Favilla et al. 1984), anti-MAG reaction product was found on all myelin lamellae, not just those located periaxonally. Methods used to obtain this result also were tested with anti-BP. Identical localizations of BP and MAG as well as tests of pretreatment procedures led us to conclude that MAG is localized in both developing and adult compact CNS myelin. Even though this localization is supported by biochemical evidence (Quarles et al. 1973; Sato et al. 1982), it is controversial (Quarles and Trapp 1984) and deserves further study (Webster 1984).

In this EAE model, MAG and BP abnormalities in both active and inactive lesions are similar morphologically, have the same distribution in adjacent sections, and correspond to demyelinative changes that have been well characterized in studies using both light and electron microscopy (Lassmann and Wisniewski 1979; Lassmann 1983). In small, active lesions thought to represent earlier stages of myelin breakdown, losses of MAG and BP are focal, where one or several axons are demyelinated. As demyelination progresses, areas of MAG and BP loss increase in size. BP- and MAG-stained myelin remnants increase in number; they are located extracellularly, in macrophages, and in astrocytes. Later on, they are smaller and less numerous, and inactive lesions usually only contain BP-stained ovoids in perivascular macrophages. Morphologically normal myelin sheaths in white matter around lesions

Fig. 3 A–D. Active demyelination, chronic EAE. Adjacent semithin (1 μm) epon sections of spinal cord white matter stained with 1:500 anti-BP (**A**), 1:250 anti-MAG (**B**), toluidine blue (**C**), and 1:250 anti-GFAP (**D**). A demyelinated axon (**A**) and a myelin sheath showing focal loss of BP staining (*arrowhead*) lie below a macrophage (*ma*) containing many BP- and fewer MAG-stained ovoids. When larger, normal appearing myelin sheaths (*left asterisks*, **A–C**) are compared, thickness of anti-MAG and toluidine blue staining are similar; both are thinner than corresponding profiles of BP staining. In smaller sheaths (*right asterisks*), thickness of anti-BP, anti-MAG, and toluidine blue staining are about the same. This area contains relatively few GFAP-stained astrocytic processes (**D**). ×2,000

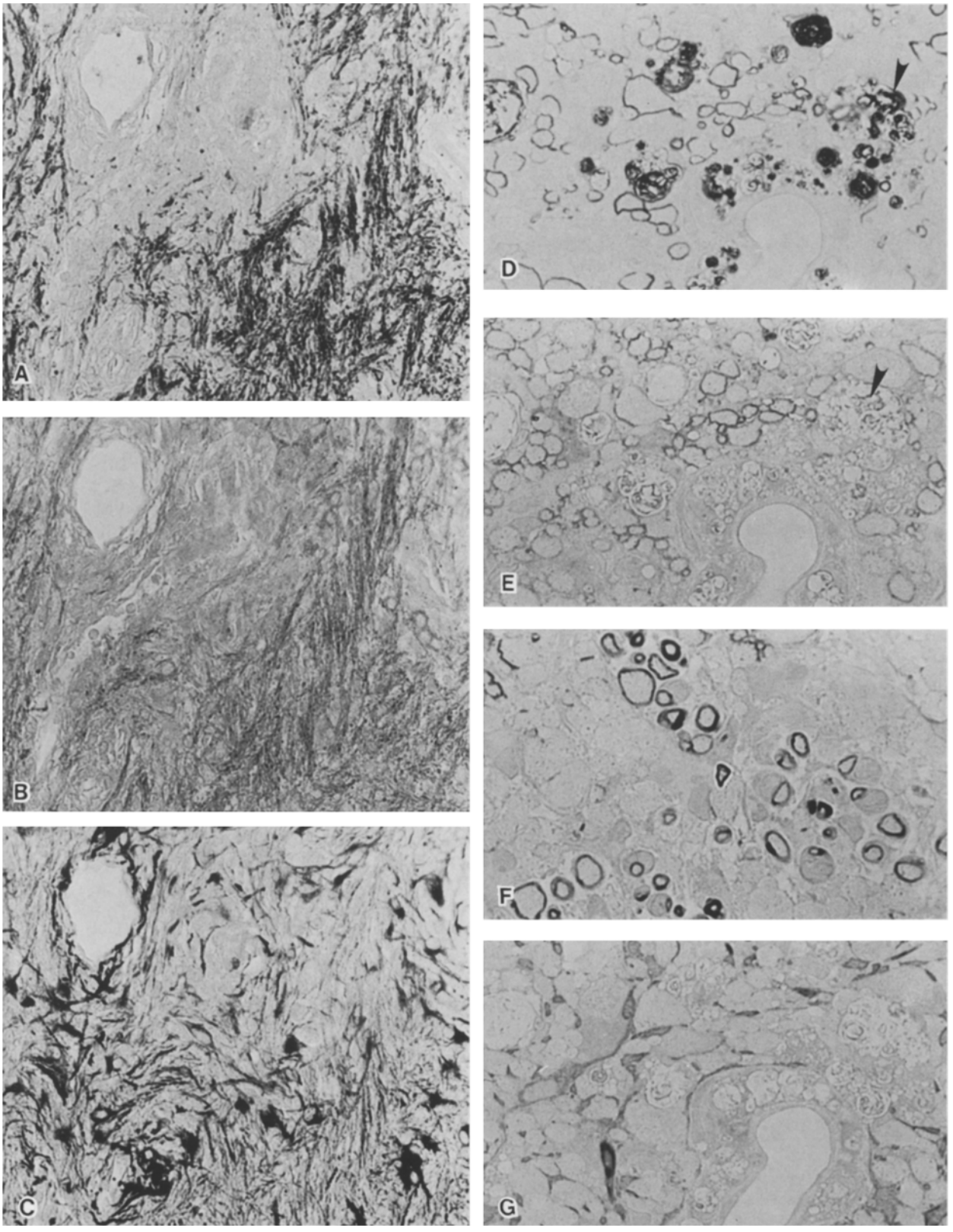

Fig. 4. Paraffin (**A—C**) and semithin epon (**D—G**) sections of inactive EAE lesions stained with 1:500 anti-BP (**A, D, F**), 1:250 anti-MAG (**B, E**), and 1:250 anti-GFAP (**G**). In adjacent sections (**A—C, D, E, G**) areas of MAG and BP loss match closely, macrophages contain few MAG- and many more BP-stained ovoids (*arrowheads*, **D, E**), and GFAP-positive astrocytic processes are prominent (**C, G**). Numerous regenerating BP-stained (**D**) and MAG-stained (**E**) regenerating CNS myelin sheaths are present. BP-stained regenerating PNS sheaths and Schwann cells also are seen in spinal cord lesions (**F**), **A—C** ×320; **D—G** ×800

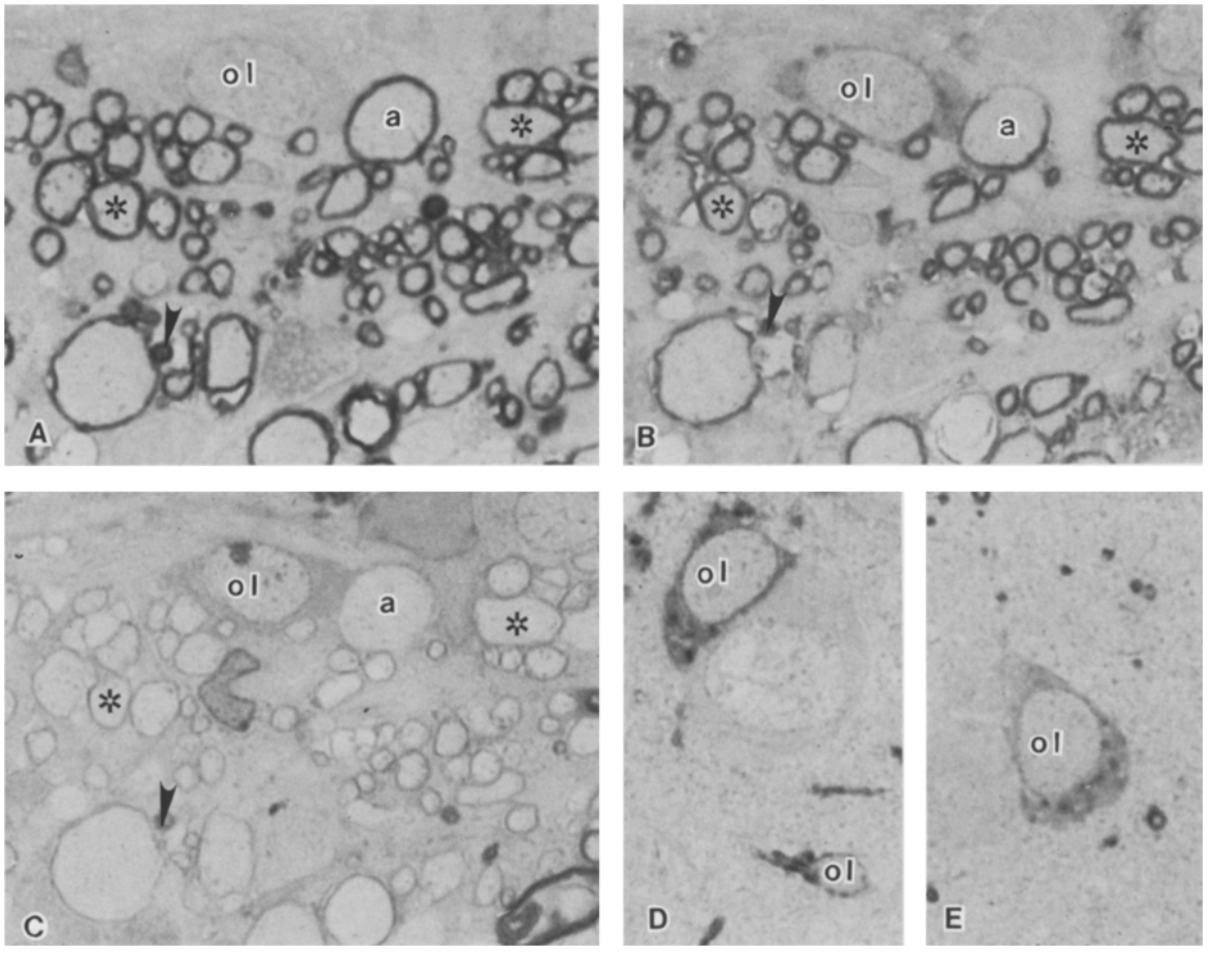

Fig. 5. Semithin epon sections of inactive EAE lesions stained with 1:500 anti-BP (**A**), 1:250 anti-MAG (**B, D, E**), and toluidine blue (**C**). BP- and MAG-stained ovoids (*arrowsheads*, **A—C**) are next to a partially fragmented, degenerating sheath. Oligodendroglia remyelinating nearby axons (i.e., *a*) are stained by anti-MAG (**B, D, E**) but not by anti-BP (**A**). Individual regenerating sheaths, when compared in adjacent sections, are thinnest when stained by toluidine blue (**C**). Rings of BP and MAG immunoreactivity are thicker (**A, B**) and match closely. × 1,600

also appear normal when immunostained with anti-MAG and anti-BP. There is no selective decrease or loss of MAG. Thus, in this demyelinative process, MAG and BP changes appear together, progress in parallel, correspond closely at each stage of lesion development, and suggest that myelin sheaths are the primary target. Similar, closely matched changes have been reported in acute EAE (Itoyama and Webster 1982) and measles encephalitis (Gendelman et al. 1984).

Myelin regeneration was assessed most easily in the MAG- and BP-stained semithin sections. As demyelination progresses, perikarya of oligodendroglia enlarge and nearby naked axons are surrounded by thin MAG- and BP-stained myelin sheaths. These newly formed sheaths with less than six lamellae in transverse sections generally have equally thick rings of anti-MAG and anti-BP immunoreactivity. This suggests that neither BP or MAG appears before the other during remyelination. However, more precise timing of MAG and BP appearance in regenerating myelin would require more systematic comparisons of many more remyelinating axons in adjacent semithin sections. As remyelination progresses, regenerating sheaths become thicker and more numerous. Anti-MAG and anti-BP staining match closely and are localized on entire thicknesses of these regenerating sheaths. This suggests that MAG also is a constituent of compact myelin during early stages remyelination. Later, when regenerating sheaths become more numerous, thicker, and more densely packed in clusters or large areas, anti-MAG reaction product is concentrated periaxonally, especially in thick sheaths that surround large axons.

Perikarya and large processes of oligodendroglia near thin, newly formed myelin sheaths are not stained

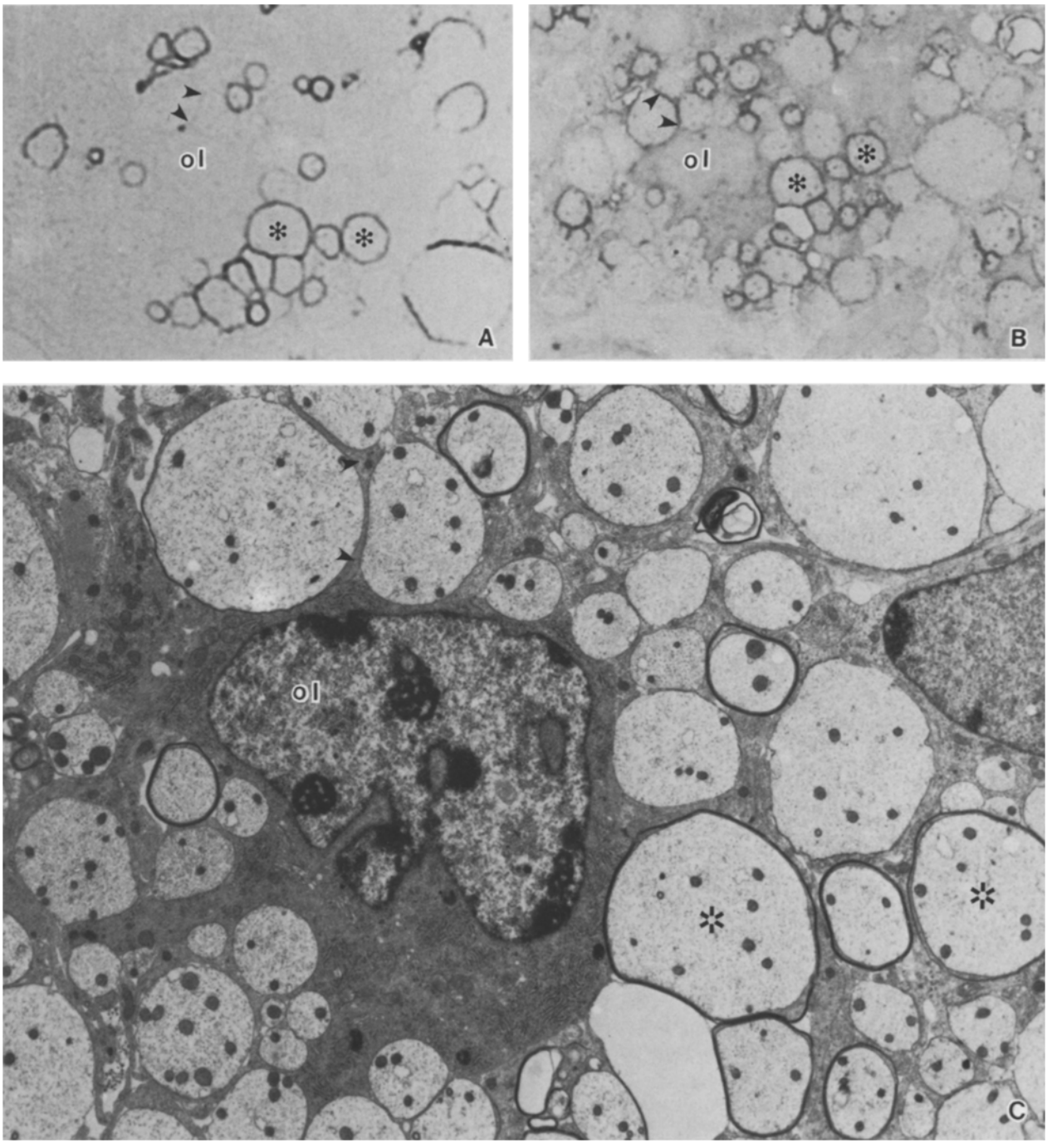

Fig. 6. Light (**A, B**) electron (**C**) micrographs of the same EAE lesion area in adjacent sections. An oligodendrocyte (*ol*) is not stained by 1:500 anti-BP **(A)**. After 1:250 anti-MAG treatment, there are some small densely stained granules, one densely stained thin process (*between arrowheads*), and faint, diffuse cytoplasmic staining **(B)**. For thin, regenerating myelin sheaths (* for example), rings of BP and MAG immunoreactivity are similar in thickness and contour. **A, B** ×1,700; **C** ×25,000

by anti-BP but do contain granular deposits of MAG similar to those seen during development (Sternberger et al. 1979). However, morphologically similar oligodendroglia in remyelinating areas of MS (Itoyama et al. 1980 b) and acute EAE (Itoyama and Webster 1982) lesions were stained by anti-BP but not by anti-MAG. This difference was observed repeatedly after using the same immunostaining method and the same antisera and it could be due to differences in tissue processing and in the species studied (acute EAE stud-

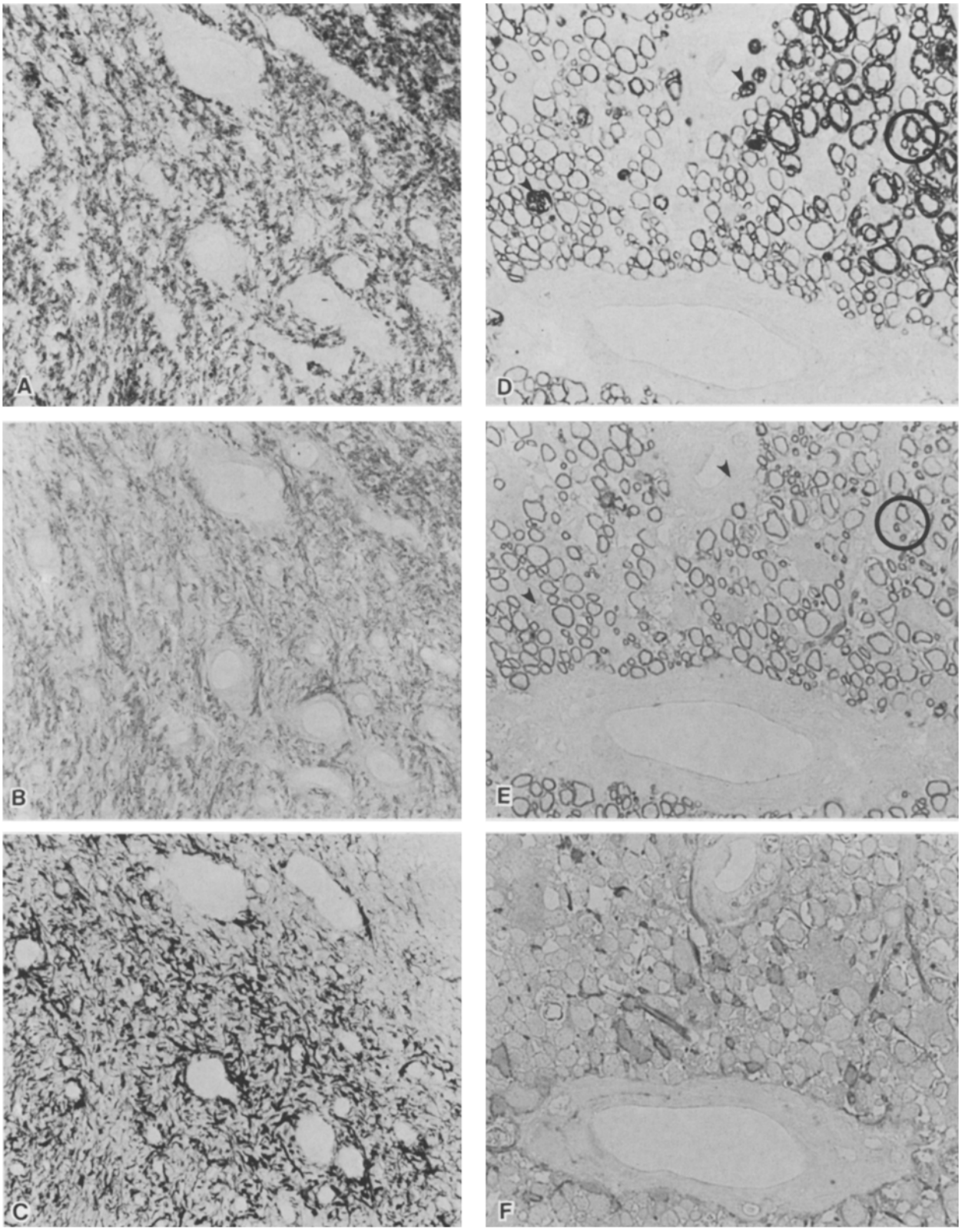

Fig. 7. More advanced myelin regeneration in paraffin (**A—C**) and semithin epon (**D—F**) sections of inactive EAE lesions treated with 1:500 anti-BP (**A—D**), 1:250 anti-MAG (**B, E**), and 1:250 anti-GFAP (**C, F**). Many densely packed regenerating myelin sheaths and hypertrophied processes of fibrous astrocytes are present. Rings of anti-BP and anti-MAG immunoreactivity are of about the same thickness in regenerating sheaths and in small normal-appearing fibers at the lesion's edge (*circle*, **D, E**). In larger sheaths, anti-BP is thicker than anti-MAG (large fiber in *circle*, **D, E**). **A—C** ×200; **D—F** ×800

ied by Itoyama and Webster was produced in Lewis rats. Also, oligodendroglial processing of MAG and BP may differ during remyelination in chronic EAE, acute EAE, and MS. Finally, some of the anti-BP staining of oligodendroglia might be due to early demyelinating changes and subsequent intracytoplasmic accumulation of immunoreactive BP fragments (Stoner 1984).

Astrocytes probably also play an important role in the development and progression of EAE lesions. These cells can present BP as antigen to encephalitogenic T-cell lines under in vitro conditions (Fontana et al. 1984). Also, increased GFAP staining in white matter astrocytes occurs early in the course of acute EAE (Smith et al. 1983). The material used for this study did not permit assessment of early astrocytic abnormalities and the later changes found as demyelination progressed and remyelination began are well documented in electron-microscopic studies of EAE (see reviews by Lassmann 1983; Rainer 1983).

We also found many GFAP-stained cell processes in areas containing oligodendroglia and regenerating myelin sheaths. Most of them clearly belonged to astrocytes, but we did not study enough serially sectioned areas to determine if a few oligodendroglial processes. Oligodendroglia could express GFAP transiently during myelin regeneration as they have been shown to do during myelin sheath development (Choi and Kim 1984).

Acknowledgements. The authors thank Dr. S. Cohen, Dept. of Neurology, Johns Hopkins Medical School, for his gift of BP antiserum, Ms. M. Gallo, and Ms. S. Carducci for making the publication prints, and Mrs. M. Hynan for typing the manuscript.

References

Alvord EC, Jr (1980) Chronic relapsing experimental allergic encephalomyelitis induced in monkeys with myelin basic protein. J Neuropathol Exp Neurol 39:338

Baskin DG, Erlandson SL, Parsons JA (1979) Immunocytochemistry with osmium-fixed tissue. I. Light-microscopic localization of growth hormone and prolactin with the unlabeled antibody-enzyme method. J Histochem Cytochem 27:867–872

Choi BH, Kim RC (1984) Expression of glial fibrillary acidic protein in immature oligodendroglia. Science 223:407–409

Favilla JT, Frail DE, Palkovits CG, Stoner GL, Braun PE, Webster H deF (1984) Myelin-associated glycoprotein (MAG) distribution in human central nervous tissue studied immunocytochemically with monoclonal antibody. J Neuroimmunol 6:19–30

Fontana A, Fierz W, Wekerle H (1984) Astrocytes present myelin basic protein to encephalitogenic T-cell lines. Nature 307:273–276

Gendelman HE, Wolinsky JS, Johnson RT, Pressman NJ, Pezeshpour GH, Boisset GF (1984) Measles encephalitis: Lack of evidence of viral invasion of the central nervous system and quantitative study of the nature of demyelination. Ann Neurol 15:353–360

Itoyama Y, Sternberger NH, Kies MW, Cohen SR, Richardson EP, Jr, Webster H deF (1980 a) Immunocytochemical method to identify myelin basic protein in oligodendroglia and myelin sheaths of the human nervous system. Ann Neurol 7:157–166

Itoyama Y, Sternberger NH, Webster H deF, Quarles RH, Cohen SR, Richardson EP, Jr (1980 b) Immunocytochemical observations on the distribution of myelin-associated glycoprotein and myelin basic protein in multiple sclerosis lesions. Ann Neurol 7:167–177

Itoyama Y, Webster H deF (1982) Immunocytochemical study of myelin-associated glycoprotein (MAG) and basic protein (BP) in acute experimental allergic encephalomyelitis (EAE). J Neuroimmunol 3:351–364

Lassmann H (1983) Comparative neuropathology of chronic experimental allergic encephalomyelitis and multiple sclerosis. Springer, Berlin Heidelberg New York

Lassmann H, Wisniewski HM (1979) Chronic relapsing experimental allergic encephalomyelitis. Clinicopathological comparison with multiple sclerosis. Arch Neurol 36:490–497

McFarlin DE, Blank SE, Kibler RF (1974) Recurrent experimental allergic encephalomyelitis in the Lewis rat. J Immunol 113:712–715

Omlin FX, Webster H deF, Palkovits CG, Cohen SR (1982) Immunocytochemical localization of basic protein in major dense line regions of central and peripheral myelin. J Cell Biol 95:242–248

Quarles RH, Everly JL, Brady RO (1973) Evidence for the close association of a glycoprotein with myelin in rat brain. J Neurochem 21:1177–1191

Quarles RH, Trapp BD (1984) Localization of myelin-associated glycoprotein (Letter to the Editor). J Neurochem 43:1773–1774

Raine CS (1983) Multiple sclerosis and chronic relapsing EAE: Comparative ultrastructural neuropathology. In: Hallpike J, Adams CWM, Tourtelotte WW (eds) Multiple sclerosis – pathology, diagnosis and management. Chapman and Hall, London, pp 413–460

Raine CS, Barnett LB, Brown A, Behar T, McFarlin DE (1980) Neuropathology of experimental allergic encephalomyelitis in inbred strains of mice. Lab Invest 43:150–157

Raine CS, Snyder DH, Valsalmis MP, Stone SH (1974) Chronic experimental allergic encephalomyelitis in inbred guinea pigs – an ultrastructural study. Lab Invest 31:367–380

Sato S, Quarles RH, Brady RO (1982) Susceptibility of the myelin-associated glycoprotein and basic protein to a neutral protease in highly purified myelin from human and rat brain. J Neurochem 39:97–105

Shii H, Webster H deF, Lassmann H (1982) Distribution of myelin-associated glycoprotein (MAG), myelin basic protein (MBP), and glial fibrillary acidic protein (GFAP) in chronic relapsing experimental allergic encephalomyelitis (EAE). Neurosci Soc Abstr 8:249

Smith ME, Somera FP, Eng LF (1983) Immunocytochemical staining for glial fibrillary acidic protein and the metabolism of cytoskeletal proteins in experimental allergic encephalomyelitis. Brain Res 264:241–253

Sternberger LA, Hardy PH, Cuculis JJ, Meyer HG (1970) The unlabeled antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. J Histochem Cytochem 18:315–333

Sternberger NH, Itoyama Y, Kies MW, Webster HdeF (1978) Myelin basic protein demonstrated immunocytochemically

in oligodendroglia prior to myelin sheath formation Proc Nat Acad Sci USA 75:2521–2524

Sternberger NH, Quarles RH, Itoyama Y, Webster HdeF (1979) Myelin-associated glycoprotein demonstrated immunocytochemically in myelin and myelin-forming cells of developing rat. Proc Nat Acad Sci USA 76:1510–1514

Stone SH, Lerner EM (1965) Chronic disseminated allergic encephalomyelitis in guinea pigs. Ann NY Acad Sci 122:227–241

Stoner GL (1984) Faulty myelin basic protein phosphorylation could precipitate demyelination. Trans Am Soc Neurochem 15:269

Webster H deF (1984) Myelin-associated glycoprotein localization: evidence and interpretations (Letter to the Editor). J Neurochem 43:1774–1777

Webster H deF, Palkovits CG, Stoner GL, Favilla JT, Frail DE, Braun PE (1983) Myelin-associated glycoprotein: electron-microscopic immunocytochemical localization in compact developing and adult central nervous system myelin. J Neurochem 41:1469–1479

Winchell KH, Sternberger NH, Webster H deF (1982) Myelin-associated glycoprotein localized immunocytochemically in periaxonal regions of oligodendroglia during hexachlorophene intoxication. Brain Res 239:679–684

Wisniewski HM, Keith AB (1977) Chronic relapsing experimental allergic encephalomyelitis – an experimental model of multiple sclerosis. Ann Neurol 1:144–148

Received July 24, 1984/Accepted September 12, 1984