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Blood–Brain Barrier Abnormalities in Longstanding Multiple Sclerosis Lesions. An Immunohistochemical Study

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Abstract. Thirty-five randomly selected plaques from five patients with longstanding multiple sclerosis were examined immunohistochemically for evidence of extravascular serum proteins. One lesion showed histological evidence of active demyelination and 34 were inactive. In the one active lesion and in 26 of the 34 inactive lesions, serum proteins were detected outside blood vessels in a distribution consistent with leakage during life. The findings suggest that the blood–brain barrier (BBB) is permanently damaged in many old plaques, although to a degree not often detectable by current gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA)-enhanced magnetic resonance imaging (MRI). The findings also suggest that in patients with multiple sclerosis, a breached BBB is not by itself sufficient to induce active demyelination. Continuous exposure of demyelinated axons and glia to cytokines, antibody or other factors present in the circulation might be important, however, in preventing oligodendrocyte regeneration and new myelin formation in longstanding lesions.

Key Words: Blood–brain barrier; Multiple sclerosis; Oligodendrocytes; Remyelination.

INTRODUCTION

Increased vascular permeability in histologically verified demyelinated multiple sclerosis plaques has been demonstrated during life using a variety of techniques including radionuclide brain scan (1), contrast enhanced computed tomography (1, 2), and gadolinium-diethylenetriamine pentaacetic acid (Gd-DTPA)-enhanced magnetic resonance imaging (MRI) (2, 3). Serial Gd-DTPA-enhanced MRI studies of patients with relapsing and remitting multiple sclerosis have shown that in 90% of new lesions there is evidence of breakdown of the blood–brain barrier (BBB) (4, 5) which lasts on average about 4 weeks, with recovery of function coinciding with restitution of the BBB (6). Enhancement following Gd-DTPA administration during this 4 week period is typically rapid and intense (5, 7). The fact that a similar pattern of rapid Gd-DTPA enhancement is seen in inflammatory lesions in experimental allergic encephalomyelitis (8) suggests that BBB breakdown may be an important and perhaps essential component of the acute inflammatory process that results in myelin destruction in new multiple sclerosis lesions (9).

MRI evidence of a defective BBB has also been observed in some longstanding lesions. Barnes et al (10), in a study using quantitative and Gd-DTPA-enhanced MRI techniques, found a slow, modest pattern of enhancement indicating impaired barrier function in 8 of 53 lesions shown by MRI to have been present for more than 3 years in four patients with clinically stable disease and 12 patients with progressive disease. Others have also described plaques exhibiting a similar pattern of slow enhancement, distinct from that observed in acute lesions (4, 11).

Although there have been a number of immunohistochemical studies demonstrating abnormally permeable blood vessels in multiple sclerosis plaques (12–18), how frequently this occurs in old lesions is not known and it has not been established whether the abnormal BBB in such lesions is associated with fresh activity or has some other explanation. The present immunohistochemical study was designed specifically to investigate BBB integrity in longstanding plaques and to correlate BBB changes with histological evidence of inflammation and ongoing myelin breakdown. The results indicate that the BBB is impaired in a high proportion of classic old plaques with typical "burnt out" histology, and that a defective BBB may not, by itself, be sufficient to cause active demyelination in patients with multiple sclerosis.

MATERIALS AND METHODS

Thirty-five plaques with adjacent unaffected white matter from five patients with multiple sclerosis were examined immunohistochemically. The age, sex, duration of clinical illness and number of plaques examined in each case are shown in Table 1. Postmortem intervals were 3 hours (case 1), 3.5 hours (case 5), 6 hours (case 2), 21 hours (case 3), and 39 hours (case 4). In each case there was evidence at autopsy of extensive disease affecting the brain and spinal cord. None had received other than supportive treatment for several years before death except case 1 who was treated with prednisone until 14 months before death. Three patients without neurological disease aged 49, 56, and 76 autopsied 14, 29 and 32 hours after death provided control tissue. Brain weights were less than 1,450 grams in all cases and in no case was there morphological evidence of generalized cerebral edema.

Six micron thick paraffin sections of tissue fixed for 2–3 weeks in buffered 10% formalin or 4% paraformaldehyde were

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TABLE 1
Plaque Histology and Immunohistochemical Findings

Case number	Sex Age	Duration (years)	Plaque number	Lipid macrophages	Macrophages containing LFB- or MBP-positive myelin	Perivascular mononuclear cells	Enlarged astrocytes*	Oligodendrocyte palisade	Contiguous zones of remyelination	Fibrinogen	IgM	Beta-Lipoprotein	Transferrin	Alpha-2 macroglobulin	IgG	Albumin
1	M	3.9	1	+	0	+	+	0	+	0	0	0	0	0		
	34		2	++	0	++	++	0	0	+	0	+	±	+		
			3	++	0	++	++	++	0	++	+	++	++	+		
			4	++	0	+	++	0	0	++	±		++	±		
			5	+++	0	+++	++	++	++	+				++		
			6	++	0	++	++	++	0	++	+	+++	+++	++	+++	(++)
			7	++	0	++	+++	+++	0	0	0		±		+	(++)
			8	+++	0	+++	+++	0	0	+++	+	+++	+++	++	+	
			9	+++	0	++	++	++	0	+	0	+++	+	0	++	
			10	+++	0	+	++	+	++	++	±		+	0		
			11	+++	0	+	++	0	+	+			+		+++	(++)
2	M	14	12	+++	0	0	+	+	+	++	+		++			
	59		13	±	0	0	+	+	0	++	+		++			
			14	0	0	+	0	0	0	++	++		++			
			15**	++	+	+	++	±	0	+	+	+	++		+	
			16	0	0	++	0	0	++	++	++		++		++	
			17	0	0	0	±	0	0	++	++		+		+	
			18	0	0	0	±	0	0	0	0	0	0		0	
			19	0	0	0	0	0	0	0	0	0		0	0	
3	M	20	20	0	0	0	+	0	++	++	++	++	++	++		
	55		21	0	0	0	0	±	+	++	++	++	++	++	++	
4	M	37	22	0	0	+	+	0	0		+				+	
	71		23	0	0	+	+	0	0		+		++			
			24	0	0	0	+	0	0	++	++	0	++		+	
5	M	40	25	0	0	0	±	0	0	++	0	0		0		
	63		26	0	0	±	++	0	+++	0	++	0		0		
			27	0	0	0	+	0	0	0	0	0		0		
			28	0	0	0	+	+	+++	0	0	0		0		
			29	0	0	0	+	0	++	++***	++	++	+	++		
			30	0	0	+	+	+	0	++	0	+	++	++		
			31	±	0	±	±	0	0	0	0	0		0		
			32	0	0	±	±	0	0	0	0	0	0	0		
			33	0	0	+	±	0	+++	0	++	+		+		
			34	0	0	±	±	0	+++	0		0				
			35	0	0	0	±	0	0	0	++	+	++	+		

0 = absent; ± = equivocal; +, ++, +++ = mild, moderate, marked change (histology) or increased staining of plaque glia cf distant white matter (immunohistochemistry). (++) = Moderately intense specific immunoreactivity but not more than distant white matter.

* Astrocytes substantially larger than small fibrous astrocytes, in most instances restricted to plaque margin.

** Thirty-four of the 35 lesions examined showed no evidence of active demyelination as determined by the presence of macrophages containing LFB- or MBP-positive myelin. Lesion 15 was the sole actively demyelinating lesion examined.

*** Dawson's fingers only.

The results indicate that extravascular serum proteins are detectable in a high proportion of old plaques with typical pathology (paucity of reactive astrocytes, minimal perivascular cuffing, no active demyelination, no edge build-up of oligodendrocytes).

collected onto poly-L-lysine-coated slides, deparaffinized and immunostained using an indirect immunoperoxidase procedure. Following treatment with 0.3% H_2O_2 in water or methanol to block endogenous peroxidase activity, sections were treated with 0.066% trypsin (Lipshaw, Detroit, MI) (10 minutes), then incubated sequentially in 10% normal rabbit, goat or horse serum (20 minutes), unlabeled primary antibody (24 hours), biotinylated rabbit anti-sheep, goat anti-rabbit or horse anti-mouse IgG (Vector Laboratories, Burlingame, CA) (60 minutes), and ABC horseradish peroxidase complex (Vector) (60 minutes) with Tris-buffered saline rinses between each step. Peroxidase was visualized using diaminobenzidine tetrahydrochloride (Polysciences, Warrington, PA) with 0.065% (w/v) nickel ammonium sulphate (4 minutes). Sections were mounted after dehydration and clearing in Permount (Fisher Scientific, Fairlawn, NJ), usually with no counterstain.

Vascular permeability for plasma proteins in most tissues is determined mainly by physical factors, especially the size of the protein as measured by its hydrodynamic radius (R) (19). In the central nervous system, diffusion of leaked protein away from blood vessels is also influenced by molecular size as the larger plasma proteins approach in their dimensions the width of the extracellular space which in this tissue is normally only 20 nm (20). Of the plasma proteins examined in the present study, albumin (MW 69,000, R 3.58 nm) and transferrin (MW 81,000, R 3.67 nm) were the smallest, IgG (MW 150,000, R 5.34 nm) and alpha-2 macroglobulin (MW 798,000, R 9.35 nm) were intermediate in size, and fibrinogen (MW 340,000, R 10.8 nm), IgM (MW 800,000, R 12.1 nm) and beta-lipoprotein (MW 2,239,000, R 12.4 nm) were the largest. The following unlabeled primary antibodies were used: sheep anti-human alpha-2 macroglobulin Ig fraction (Serotec, Kidlington, UK), rabbit anti-human IgM Ig fraction (Dako Labs Inc., Carpinteria, CA), rabbit anti-human beta-lipoprotein Ig fraction (Dako), sheep anti-human fibrinogen (Serotec), sheep anti-human transferrin Ig fraction (Serotec), rabbit anti-human myelin basic protein (MBP) Ig fraction (Dako), rabbit anti-human IgG heavy and light chain specific Ig fraction (Cappel Laboratories, Cochranville, PA), rabbit anti-human albumin Ig fraction (Cappel), rabbit anti-bovine glial fibrillary acidic protein (GFAP). Antisera were absorbed with mouse liver powder before use. Negative controls used were nonimmune rabbit or sheep serum (Cappel) or, in the case of anti-albumin, antiserum absorbed with human albumin (Cappel). Intravascular serum in each section was used as a positive control.

Evidence of BBB breakdown was determined blindly, the intensity of staining of extravascular tissue within each plaque being graded as none (0), equivocal (±), mild (+), moderate (++), or intense (+++) compared to distant white matter. Sections immunostained for MBP and sections stained with hematoxylin and eosin and for myelin with Luxol fast blue-periodic acid Schiff (LFB-PAS) were used for histological characterization. Active demyelination was judged to be present when macrophages containing both LFB-positive (light blue staining) and MBP-positive particles were present at the plaque margin. In the absence of such cells, even in lesions containing numerous foamy macrophages, the plaque was judged to be inactive.

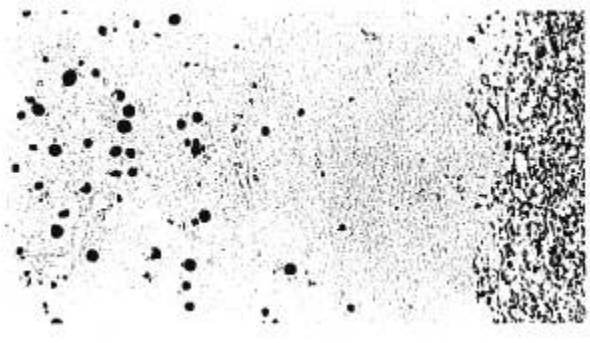

Fig. 1. Nonspecific immunostaining of corpora amylacea near the edge of an old plaque. Immunostained for MBP. No counterstain. Case 4, ×160.

RESULTS

Plaque histology and immunohistochemical findings in the five multiple sclerosis cases are summarized in Table 1. Only one of the 35 plaques examined (plaque 15, Table 1) showed evidence of active myelin breakdown indicated by the presence of macrophages containing MBP-positive, LFB-positive myelin particles located among deformed myelin sheaths at the plaque margin. None of the remaining 34 plaques examined, including 12 containing moderate to large numbers of foamy macrophages, revealed evidence of ongoing myelin destruction.

Nonspecific Immunostaining

In both the control brains and in multiple sclerosis tissue, variable staining was observed with nonimmune rabbit and sheep serum, anti-MBP antiserum and several of the anti-plasma protein antisera of walls of capillaries and larger blood vessels, the inner and outer lining of Virchow-Robin spaces, pia mater, and corpora amylacea which were sometimes numerous in plaques and in remyelinated shadow plaques (Fig. 1). In none of the 35 plaques examined were oligodendrocytes seen that showed strong specific staining for MBP, reported recently to be common in inactive plaques and in remyelinating shadow plaques (21, 22). Cell bodies of enlarged astrocytes and perivascular macrophages in some sections stained faintly with nonimmune sera. No nonspecific staining of intravascular contents was observed.

Agonal and Postmortem Leakage of Plasma Proteins

Where there was evidence in control and multiple sclerosis tissue of extravasation of whole blood, indicated by the presence of erythrocytes in perivascular spaces, a rare finding in two of the eight brains examined due presumably to mechanical deformation during brain removal, a distinctive pattern of plasma protein immunoreactivity was observed in which all tissue elements close to the extravasated red blood cells stained positively for all five plasma proteins studied (Fig. 2). The same distinctive

Fig. 2. Postmortem leakage of plasma proteins perivascularly in normal cerebral cortex. Immunostained for IgM. No counterstain. ×25.

pattern of intense staining of all parenchymal elements close to normal-appearing blood vessels was also observed in the absence of red cells. This was a rare finding; it was observed in both control and multiple sclerosis brains, and in the latter it was no more frequent in plaques than in distant unaffected white and grey matter.

Inactive Multiple Sclerosis Plaques

In contrast to the type of leakage just described, where multiple sclerosis plaques were observed to stain positively for plasma proteins, the staining was not perivascular in distribution but tended to be equally intense throughout the plaque, and usually did not include all plasma proteins investigated.

Fibrinogen: In histologically normal white and grey matter distant from plaques and in control cases, fibrinogen was detected only within blood vessels. Faint staining of subpial astrocytes was observed occasionally. In 19 of the 32 inactive plaques examined in which intravascular contents stained positively for fibrinogen, astrocyte cell bodies and processes throughout the plaque stained positively for fibrinogen, causing the plaque to appear at low magnifications more darkly stained than the surrounding tissue. Enlarged astrocytes outside the plaque usually remained unstained. Variable positive staining of macrophages and axons diffusely throughout the plaque, sometimes extending into the surrounding tissue, was present on occasion (Figs. 3, 6).

IgM: Eighteen of 32 inactive plaques in sections in which intravascular contents stained positively for IgM showed faint to moderate reactivity for IgM in a distribution similar to that of fibrinogen (Figs. 4–6). Around most of these lesions, adjoining and distant white and grey matter showed no staining for IgM except for intense staining of intravascular plasma. In one inactive lesion (and in the single actively demyelinating lesion in the study, *vide infra*), oligodendrocytes in bordering white matter stained positively for IgM. No IgM-positive plasma cells were observed in any lesion.

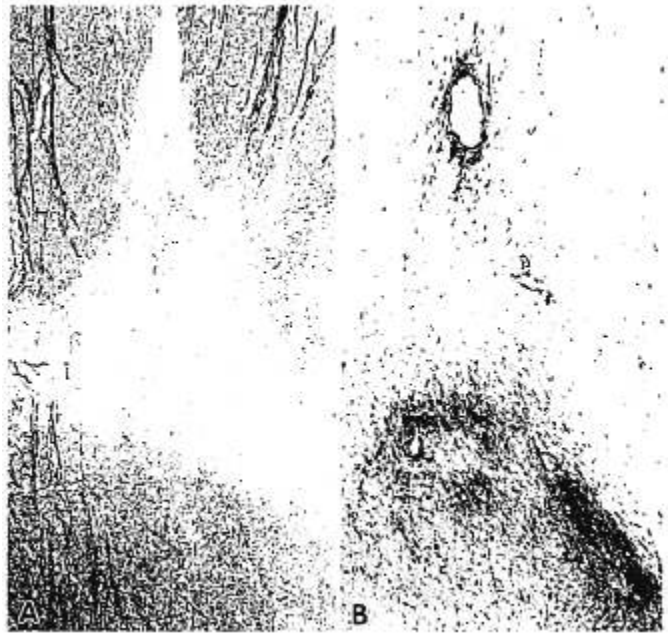

Fig. 3. Extravascular fibrinogen in an inactive plaque. A: Immunostained for MBP. B: Immunostained for fibrinogen. No counterstains. Case 1, ×25.

Transferrin: Vascular contents and a variable proportion of oligodendrocytes stained positively for transferrin in unaffected white matter distant from plaques and in control tissue. Variable staining of axons, neurons and astrocytes was also observed in some sections. In 19 of 24 inactive plaques in sections in which intravascular plasma stained positively for transferrin, astrocytes

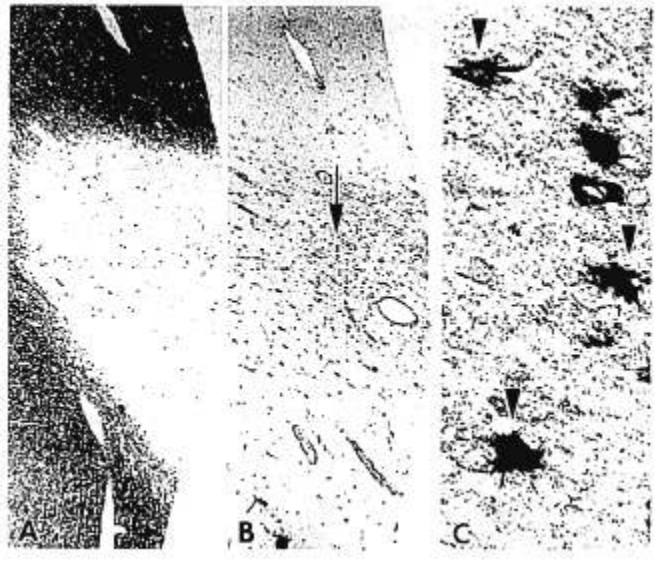

Fig. 4. Small astrocytes throughout an old plaque react positively for IgM. The area indicated by the arrow in B is shown enlarged in C. Arrow points = astrocytes. A: Immunostained for MBP. B, C: Immunostained for IgM. No counterstains. Case 5, A, B ×25, C ×440.

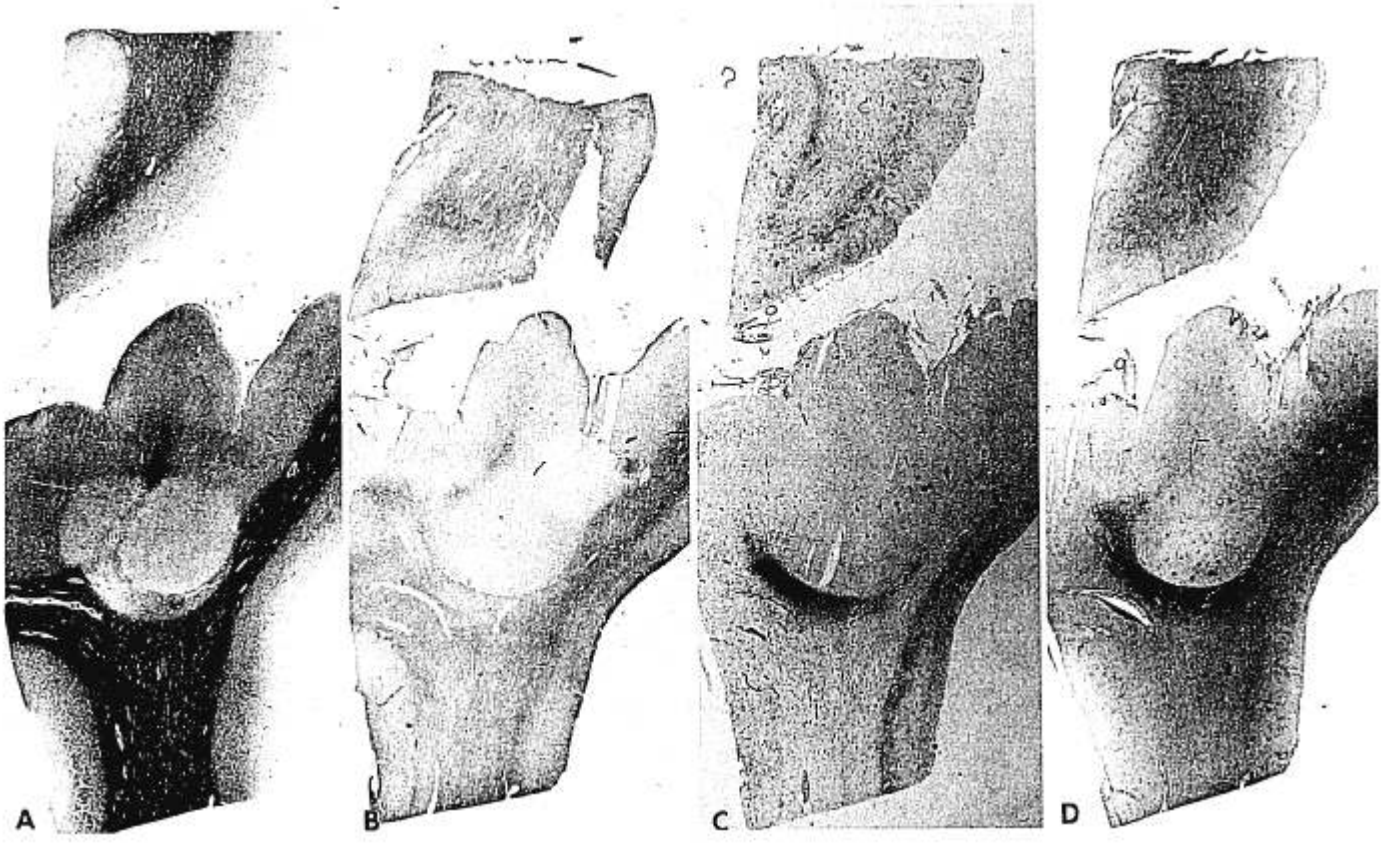

Fig. 5. An old lesion overlapping cortex and subcortical white matter stains positively for IgM (C) and transferrin (D). A: LFB-PAS. B: Control section reacted with nonimmune rabbit serum. A part of this lesion is shown enlarged in Figure 6. No counterstains. Case 3, ×4.3.

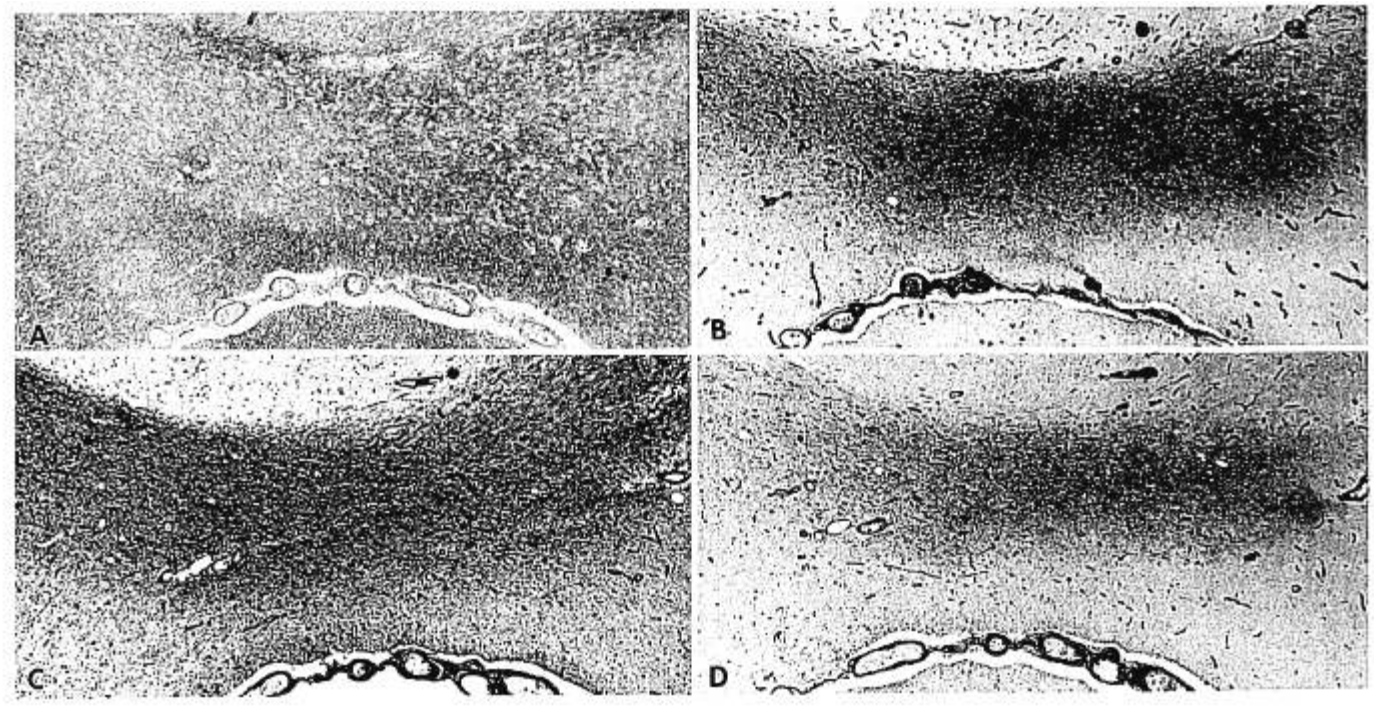

Fig. 6. Enlarged view of lesion illustrated in Figure 5 showing positive staining for extravascular IgM (B), transferrin (C), and fibrinogen (D). A: Control section reacted with nonimmune rabbit serum. No counterstains. Case 3, ×28.

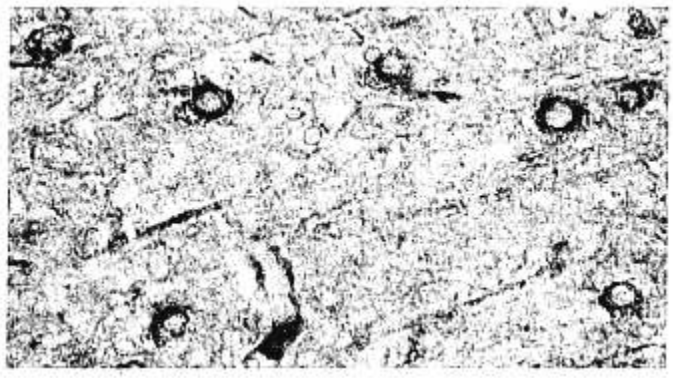

Fig. 7. Transferrin-positive large oligodendrocytes in white matter close to a plaque. No counterstain. Case 3, ×400.

throughout the plaques exhibited enhanced reactivity for transferrin, with axons and macrophages in the same lesions also usually transferrin-positive (Figs. 5, 6). When present, the large oligodendrocytes sometimes seen close to plaque margins consistently exhibited intense transferrin reactivity around plaques both with and without evidence of vascular leakage (Fig. 7).

Beta-Lipoprotein: In contrast to fibrinogen and IgM, normal white matter in multiple sclerosis subjects and controls contained elements, in addition to intravascular plasma, that stained positively for beta-lipoprotein, namely small and large oligodendrocytes and small granules associated with myelin sheaths. Subpial astrocytes and vessel walls also exhibited variable positive staining. In 11 of 22 plaques in sections with positive staining of vascular contents, astrocytes, macrophages and occasionally also axons within the plaque and in surrounding white matter reacted positively for beta-lipoprotein. Large oligodendrocytes in white matter bordering these lesions and in white matter adjacent to plaques without evidence of increased vascular permeability frequently exhibited intense beta-lipoprotein reactivity (Fig. 8).

Alpha-2 Macroglobulin: In unaffected white matter in both multiple sclerosis and control cases, vascular contents stained positively for alpha-2 macroglobulin with variable staining also of myelin, astrocytes and blood vessel walls. In 11 of 22 inactive plaques in sections with positively stained vascular contents, astrocytes within the plaques stained more intensely for alpha-2 macroglobulin than astrocytes elsewhere. Macrophages and axons were also positive in some of the latter lesions (Fig. 9).

IgG and Albumin: Both IgG and albumin are normally present in relatively high concentrations in cerebrospinal fluid and are therefore of uncertain value in determining BBB integrity immunohistochemically. In the three inactive plaques examined for albumin, each with different degrees of vascular permeability as judged by the presence of other plasma proteins (plaques 6, 7, 11, Table 1), albumin was detected inside blood vessels and within all

Fig. 8. Small and large oligodendrocytes (arrow points) in white matter close to a plaque stain positively for beta-lipoprotein. Smaller positively stained structures in myelinated tissue are also evident. No counterstain. Case 4, A ×440, B ×920.

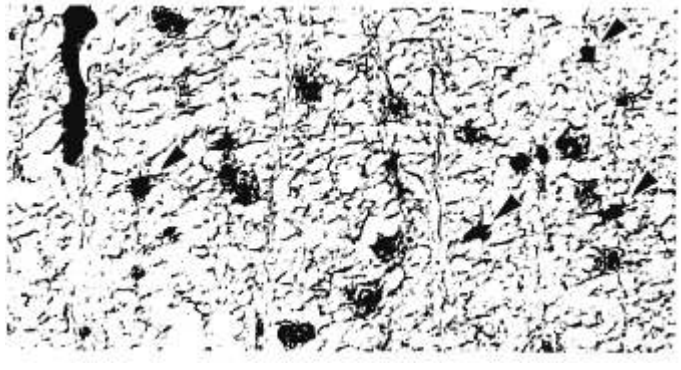

Fig. 9. Center of a plaque immunostained for alpha-2 macroglobulin shows positive staining of a blood vessel, astrocytes (arrow points) and macrophages. No counterstain. Case 2, ×270.

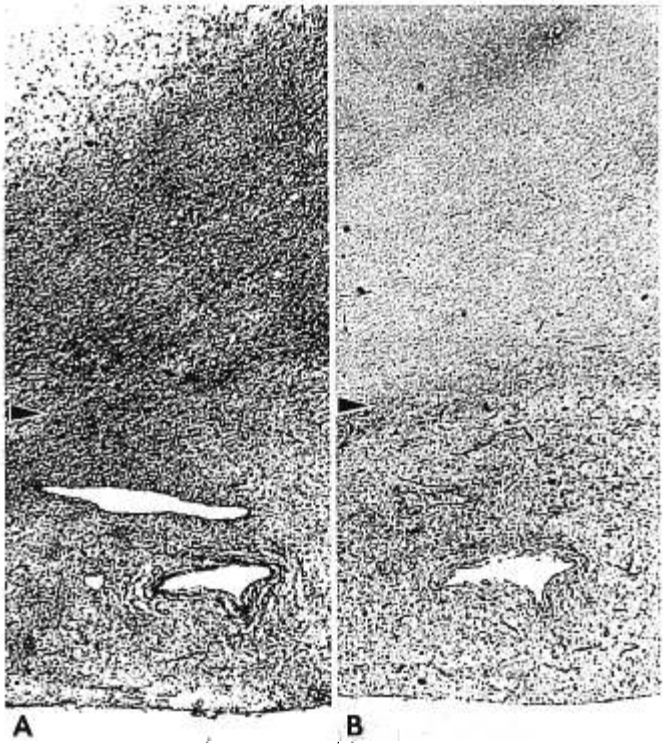

Fig. 10. A periventricular plaque immunostained for GFAP (A) and IgG (B) showing extravascular IgG reactivity restricted to astrocytes within the plaque. Arrow points indicate edge of plaque. No counterstain. Case 1, ×26.

tissue elements including axons and oligodendrocytes inside and outside each plaque with no gradation in staining intensity moving away from the plaque or across the plaque margin. Macrophages showed slightly more intense staining for albumin than astrocytes or axons as did swollen-body oligodendrocytes in surrounding white matter. Staining was abolished by prior absorption of the antiserum with human but not guinea pig albumin.

Ten of the 12 inactive lesions examined for IgG immunoreactivity in sections with positively stained vascular contents showed increased IgG reactivity compared to distant white matter. In contrast to albumin, IgG immunoreactivity was much more intense in astrocytes and axons within positively staining plaques than in surrounding tissue, with loss of staining occurring in some lesions abruptly at the plaque margin (Figs. 10, 11). Macrophages, lymphocytes, and plasma cells within these lesions were also IgG-positive. In surrounding white matter, swollen-body oligodendrocytes and perivascular plasma cells stained positively for IgG, and in tissue distant from plaques, IgG reactivity was observed in subpial astrocytes, ependymal cells, plasma cells and some axons and microglial cells.

Actively Demyelinating Lesion

The single lesion in the study (plaque 15, Table 1) in which active myelin breakdown was observed was a

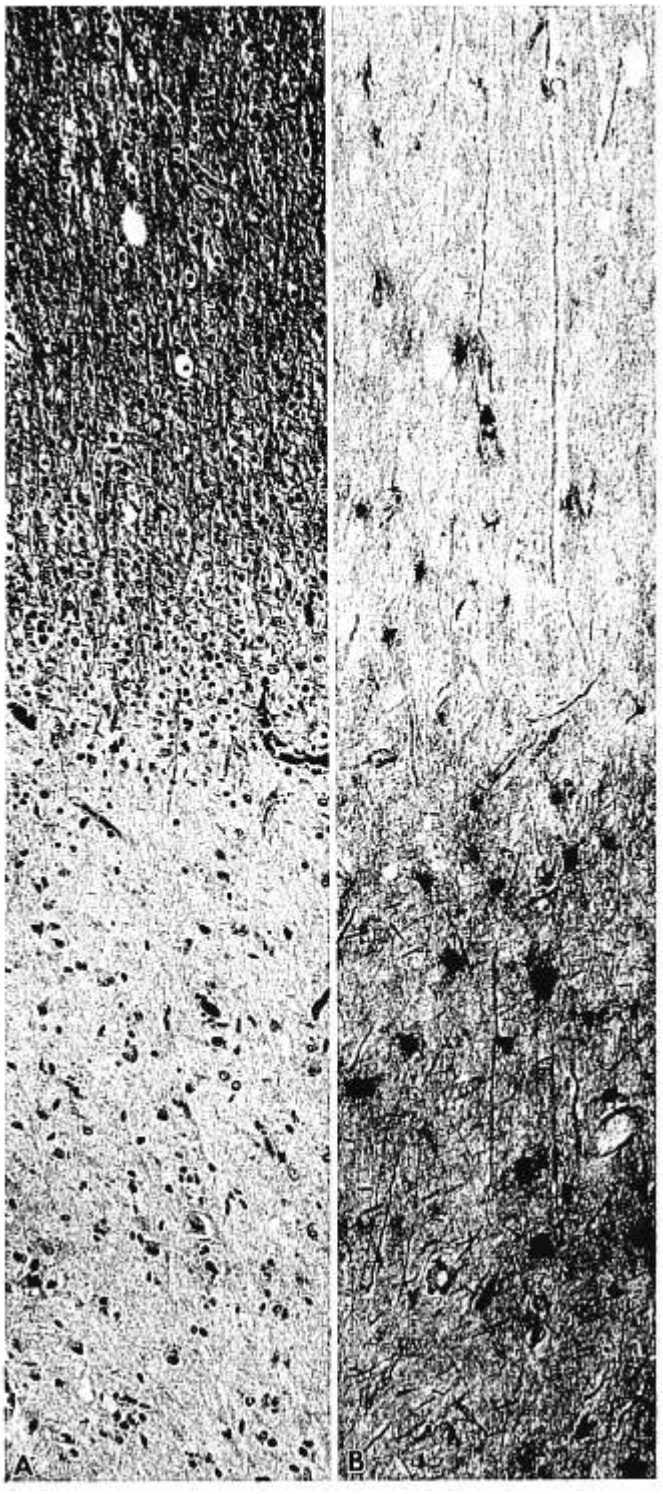

Fig. 11. Edge of an old inactive plaque stained for myelin (A) and immunostained for IgG (B) showing IgG-positive astrocytes and axons in the demyelinated zone with an abrupt decrease in IgG immunoreactivity at the plaque margin. The number of oligodendrocytes may be slightly increased at the edge of the lesion (oligodendrocyte palisade) but are absent from the demyelinated zone. A: LFB-PAS. B: Immunostained for IgG, no counterstain. Case 3, ×140.

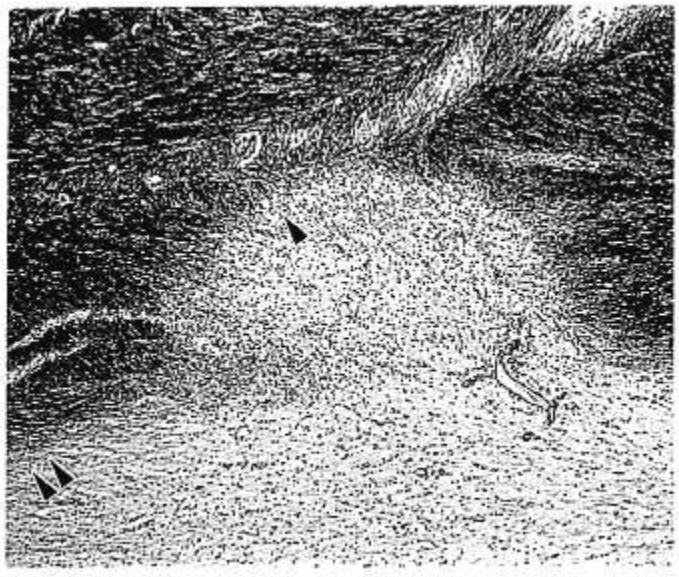

Fig. 12. A small actively demyelinating lesion located at the edge of an old inactive lesion. The whole of the semicircular edge of this lesion showed evidence of ongoing myelin breakdown. The areas indicated by the arrow points are shown enlarged in Figures 13 and 14. LFB-PAS. Case 2, ×24.

round lesion 2 mm in diameter located close to a blood vessel at the edge of a 1 cm long irregularly shaped old plaque (Fig. 12). It was totally demyelinated except for a narrow zone of fragmenting myelin sheaths infiltrated by macrophages containing LFB-positive, MBP-positive myelin fragments at the very edge of the lesion (Figs. 13, 14). The demyelinated center contained moderate numbers of astrocytes and LFB-negative, MBP-negative foamy macrophages but no oligodendrocytes. Nearer the margin astrocytes were observed closely contacting beta-lipoprotein-positive, HNK-1-positive oligodendrocytes.

As in many of the inactive plaques, macrophages and astrocytes within the lesion stained positively for fibrinogen, IgM, transferrin, and IgG. Scattered IgG-positive plasma cells were also present in the lesion. No evidence was seen of IgG deposition on deformed myelin sheaths.

In bordering intact tissue, serum protein immunoreactivity was restricted to small and large oligodendrocytes. In contrast to most of the inactive plaques examined, some large oligodendrocytes bordering the plaque showed intense IgM reactivity.

DISCUSSION

More than 50 percent of all inactive plaques examined in the present study, including those with few or no macrophages present, showed evidence of extravascular fibrinogen, IgM and other serum proteins. These findings support recent MRI evidence of increased vascular permeability in some old lesions (10) and are consistent with earlier autopsy studies that used radiolabeled serum proteins and trypan blue to demonstrate increased vascular permeability in plaques in patients with longstanding disease (23, 24).

It is unlikely that the observed diffuse leakage of plasma proteins into plaque tissue occurred postmortem or as an agonal event. As in most previous similar studies in which the distribution of plasma proteins in paraffin-embedded autopsy tissue was used as a measure of BBB integrity during life, control cases without central nervous system disease showed no or only rare evidence of leakage and this was restricted to perivascular tissue (25–31). Positive immunostaining for extravascular plasma proteins is normally seen, however, in such cases in astrocytes close to cerebrospinal fluid pathways, ependymal cells, certain neuronal cell bodies and fiber tracts in the spinal cord, brain stem and diencephalic nuclei, and in

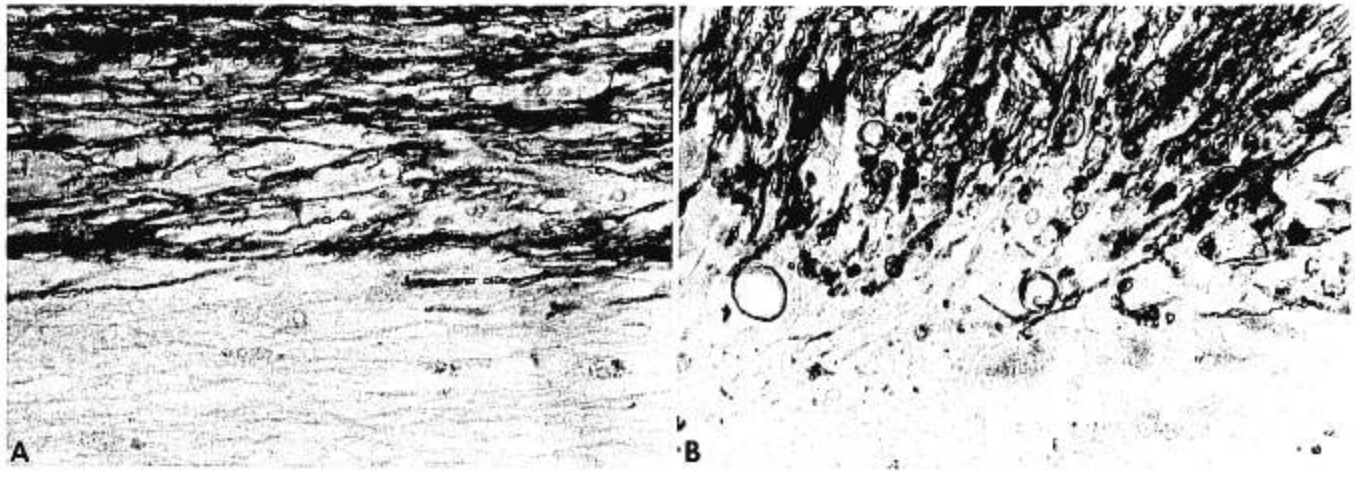

Fig. 13. A, B: Enlarged views of areas indicated by arrow points in Figure 12 in a serial section immunostained for MBP. A: Normal-appearing myelin sheaths at the edge of the quiescent lesion (double arrow points, Fig. 12). B: Distorted fragmenting myelin sheaths at the edge of the active lesion (single arrow point, Fig. 12). No counterstain. Case 2, ×370.

the leptomeninges (30–32), and transferrin has been detected in human autopsy tissue in oligodendrocytes, ependymal cells and choroid plexus epithelium (33). Animals sacrificed by whole body perfusion show a similar distribution of extravascular plasma proteins in the brain and spinal cord supporting the conclusion that during life there is a continuous flux of some plasma proteins into the central nervous system (25, 34–38). IgM is not found in the central nervous system in normal animals (38).

Inflammatory cells and stimulated glial cells synthesize a number of plasma proteins including immunoglobulins (plasma cells); alpha-2 macroglobulin, complement, clotting and coagulation components (macrophages); and other acute phase proteins (astrocytes) (12, 39). The absence of signs of inflammation in many of the plaques that reacted positively for extravascular proteins argues against this explanation for the present findings. Also, IgM was one of the marker proteins most frequently detected in affected plaques while IgM-positive plasma cells were extremely rare in all cases.

The widened extracellular space present in some lesions (10, 40), by increasing exposure of plaque glia via perivascular spaces (41) to plasma proteins normally present in the cerebrospinal fluid, including immunoglobulins, albumin, and transferrin (33), is a further possible source of plaque plasma proteins. This is unlikely as affected plaques frequently stained much more intensely for IgM, fibrinogen and some of the other plasma proteins than did distant astrocytes close to cerebrospinal fluid pathways (subpial astrocytes).

While the findings in the present multiple sclerosis cases differ from those observed in patients without neurological disease, they resemble those reported in autopsy studies of other conditions associated with focal BBB abnormalities including brain tumors, ischemic infarcts, central pontine myelinolysis, subacute sclerosing panencephalitis and herpes simplex encephalitis, all of which have been reported to show immunohistochemical evidence of focal uptake by enlarged astrocytes of immunoglobulins, transferrin and/or fibrinogen (15, 31, 42, 43). In animals with experimentally induced focal BBB abnormalities, immunohistochemical studies also show that extravasated plasma proteins are taken up by astrocytes, microglia, leptomeningeal macrophages, neurons, intact axons, and oligodendrocytes near the site of injury (44–46) where they remain detectable for several weeks after restitution of the BBB (47).

If the BBB is abnormally permeable in many chronic plaques as the present findings suggest, this points to a persistent or permanent change in the endothelium of blood vessels within affected plaques (48). As there was little histological evidence of inflammation in many of the affected lesions, it is probable that this endothelial abnormality resulted from earlier damage. This occurs, for example, in chronic experimental allergic encephalomyelitis (CEAE) where immunohistochemical studies of extravasated serum proteins (49) and studies employing Evans blue-albumin and peroxidase tracers have shown that following massive leakage during the acute stage of the disease, old demyelinated lesions, with and without cuffs of mononuclear cells, not infrequently continue to exhibit increased vascular permeability (50, 51). Quantitative studies of immunoglobulin and albumin in the central nervous system of animals with CEAE studied up to 250 days post-sensitization have shown evidence

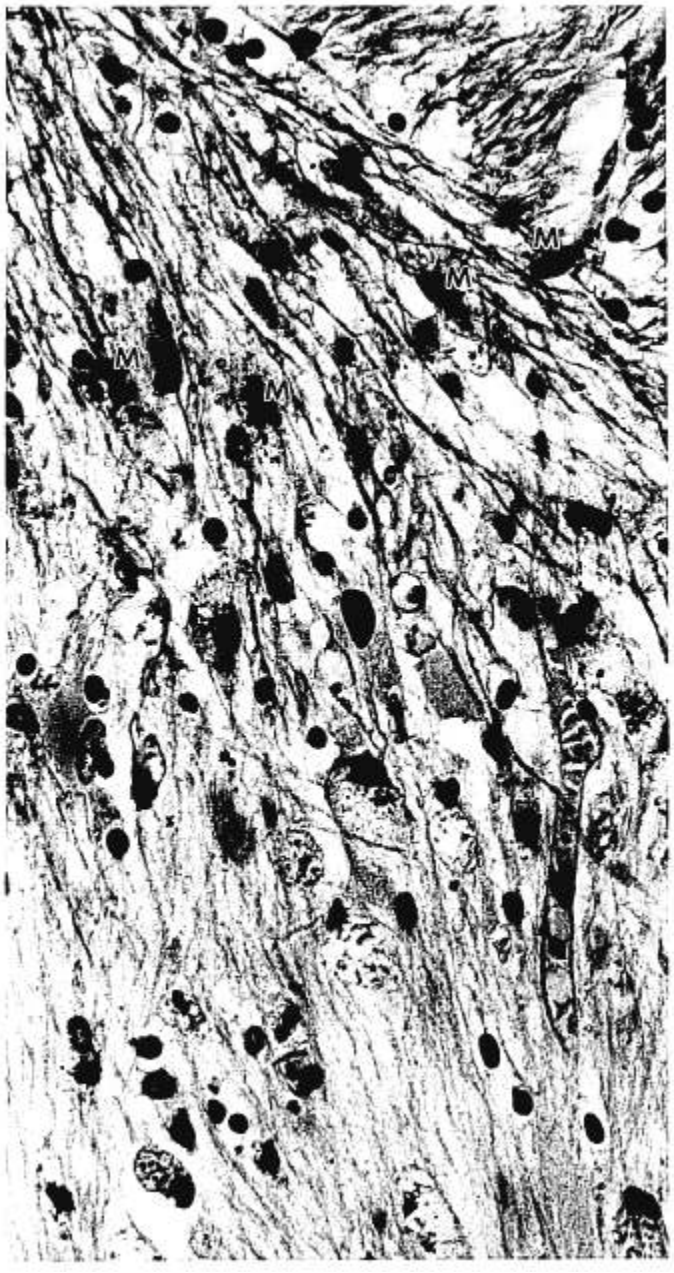

Fig. 14. Active demyelination. Macrophages (M) located among myelin sheaths at the edge of the lesion illustrated in Figures 12 and 13 contain particles that are MBP-positive and that stain light blue with LFB-PAS. LFB-PAS. Case 2, ×550.

of persistent modest BBB impairment during the whole of the chronic phase in the majority of animals (52). Also Snyder et al (53) describe extensive fine-structural changes (thinning of endothelial cells, numerous fenestrations, excessive pinocytotic vesicles) in the vascular endothelium in demyelinated lesions 12 months after sensitization in strain 13 guinea pigs with CEAE. Although there are no comparable ultrastructural studies of old multiple sclerosis lesions, fibrotic thickening of vessel walls and other vascular alterations have been described (54).

Antibodies and other large protein tracers infused locally into brain parenchyma readily diffuse through the normal extracellular space (35, 47). The present observation that in some plaques extravascular IgG in particular but also IgM and fibrinogen were present in much higher concentrations in astrocytes and axons inside the plaque than immediately outside it suggests an impediment to free diffusion at the edge of the lesion and the possibility that some lesions become sealed off from the surrounding brain tissue.

What role residual BBB abnormalities in longstanding plaques may play in disease progression is uncertain. The fact that no active myelin breakdown was observed in the great majority of such lesions suggests that increased vascular permeability is not sufficient by itself to precipitate new lesion formation, even in patients with actively demyelinating lesions elsewhere. An open BBB, however, might be a factor preventing normal remyelination in longstanding lesions. Perry and Lund (55) and Hildebrand et al (56), noting a paucity of myelination at sites in the mammalian central nervous system where the BBB is normally absent (pituitary stalk, median eminence and subfornical organ, olfactory fiber layer), have suggested that plasma-derived components might influence oligodendrocyte differentiation and that BBB abnormalities might contribute to failed remyelination in pathological states since Raff and coworkers (57) determined that 0-2A precursor cells *in vitro* differentiate to form astrocytes rather than oligodendrocytes when serum is present in the culture medium. There are also factors present in the blood in multiple sclerosis reported to be toxic for oligodendrocytes and mitogenic for astrocytes such as tumor necrosis factor (58, 59), IL-1 (59, 60), and complement (61).

The present study provides additional evidence that cells with relatively large round nuclei and non-vacuolated cytoplasm observed in white matter around multiple sclerosis plaques (62) are of oligodendrocyte lineage in that they were found to be transferrin-positive both near plaques with and without evidence of plasma protein leakage. Transferrin has been detected immunohistochemically in normal central nervous system tissue in oligodendrocytes, choroid plexus epithelium and ependymal cells, but not astrocytes or microglia (33). Neither these cells nor typical small oligodendrocytes with vacuolated cytoplasm showed any appreciable reactivity for MBP, and we were unable to identify in any of the inactive lesions examined a population of oligodendrocytes reported previously to stain intensely for both MBP and heat shock protein and to be most numerous in demyelinated tissue in old inactive lesions and in remyelinating shadow plaques (21, 22). Corpora amylacea, which are common in old multiple sclerosis plaques and shadow plaques, are known to stain nonspecifically with monoclonal and polyclonal antibodies and have been mistaken in the past for oligodendrocytes (63), which may explain these contradictory findings.

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REFERENCES

1. Warren KG, Ball MJ, Paty DW, Banna M. Computed tomography in disseminated sclerosis. J Can Sci Neurol 1976;3:211–16
2. Rodriguez M, Scheithauer BW, Forbes G, Kelly PJ. Oligodendrocyte injury is an early event in lesions of multiple sclerosis. Mayo Clin Proc 1993;68:627–36
3. Katz D, Taubenberger JK, Cannella B, McFarlin DE, Raine CS, McFarland HF. Correlation between magnetic resonance imaging findings and lesion development in chronic active multiple sclerosis. Ann Neurol 1993;34:661–9
4. Gonzalez-Scarano F, Grossman RI, Galetta S, Atlas SW, Silberberg DH. Multiple sclerosis disease activity correlates with gadolinium-enhanced magnetic resonance imaging. Ann Neurol 1987;21:300–6
5. Kermode AG, Tofts PS, Thompson AJ, et al. Heterogeneity of blood–brain barrier changes in multiple sclerosis: An MRI study with gadolinium-DTPA enhancement. Neurology 1990;40:229–35
6. Youl BD, Turano G, Miller DH, et al. The pathophysiology of acute optic neuritis. An association of gadolinium leakage with clinical and electrophysiological deficits. Brain 1991;114:2437–50
7. Miller DH, Rudge P, Johnson G, et al. Serial gadolinium enhanced magnetic resonance imaging in multiple sclerosis. Brain 1988;111: 927–39
8. Hawkins CP, Munro PMG, Mackenzie K, et al. Duration and selectivity of blood–brain barrier breakdown in chronic relapsing experimental allergic encephalomyelitis studied by gadolinium-DTPA and protein markers. Brain 1990;113:365–78
9. McDonald WI, Miller DH, Barnes D. The pathological evolution of multiple sclerosis. Neuropathol Appl Neurobiol 1992;18:319–34
10. Barnes D, Munro PMG, Youl BD, Prineas JW, McDonald WI. The longstanding MS lesion. A quantitative MRI and electron microscopic study. Brain 1991;114:1271–80
11. Larsson HBW, Frederiksen J, Kjaer L, Henriksen O, Olesen J. *In vivo* determination of T1 and T2 in the brain of patients with severe but stable multiple sclerosis. Magn Reson Med 1988;7:43–55
12. Tourtellotte WW, Kokmen E, Tavolato B, Kruger PS. Fluorescent cell tracing in multiple sclerosis brain tissue. Trans Am Neurol Assoc 1971;96:318–20
13. Vandvik B, Reske-Nielsen E. Immunochemical and immunohistochemical studies of brain tissue in subacute sclerosing panencephalitis and multiple sclerosis. Acta Neurol Scand 1972;48(Suppl 51): 413–16
14. Tavolato BF. Immunoglobulin G distribution in multiple sclerosis brain. An immunofluorescence study. J Neurol Sci 1975;24:1–11
15. Esiri MM, Taylor CR, Mason DY. Application of an immunoperoxi-

dase method to a study of the central nervous system: Preliminary findings in a study of human formalin-fixed material. Neuropathol Appl Neurobiol 1976;2:233–46
16. Mussini J-M, Hauw J-J, Escourolle R. Immunofluorescence studies of intracytoplasmic immunoglobulin binding lymphoid cells (CILC) in the central nervous system. Report of 32 cases including 19 multiple sclerosis. Acta Neuropathol 1977;40:227–32
17. Esiri MM. Immunoglobulin-containing cells in multiple sclerosis plaques. Lancet 1977;2:478–80
18. Esiri MM. Multiple sclerosis: A quantitative and qualitative study of immunoglobulin-containing cells in the central nervous system. Neuropathol Appl Neurobiol 1980;6:9–21
19. Van Lis MJ, Jennekens FGI. Plasma proteins in human peripheral nerve. J Neurol Sci 1977;34:329–41
20. Brightman MW, Shivers RR, Prescott L. Morphology of the walls around fluid compartments in nervous tissue. In: Cserr HF, Fenstermacher JD, Fencl V, eds. Fluid environment of the brain. New York: Academic Press, 1975:3–29
21. Selmaj K, Brosnan CF, Raine CS. Colocalization of lymphocytes bearing γδ T-cell receptor and heat shock protein hsp 65^+ oligodendrocytes in multiple sclerosis. Proc Natl Acad Sci USA 1991;88:6452–6
22. Selmaj K, Brosnan CF, Raine CS. Expression of heat shock protein-65 by oligodendrocytes *in vivo* and *in vitro*: Implications for multiple sclerosis. Neurology 1992;42:795–800
23. Broman T. Supravital analysis of disorders in the cerebral vascular permeability. 2. Two cases of multiple sclerosis. Acta Psychiatr Neurol Scand 1947;46(Suppl 46):58–71
24. Gonsette R, André-Balisaux G. La perméabilité des vaisseaux cérébraux. IV-Étude des lésions de la barrière hémato-encéphalique dans la sclérose en plaques. Acta Neurol Psychiat Belg 1965;65: 19–34
25. Sparrow JR. Immunocytochemical localization of plasma proteins in neuronal perikarya. Brain Res 1981;212:159–63
26. Wisniewski HM, Kozlowski PB. Evidence for blood–brain barrier changes in senile dementia of the Alzheimer type (SDAT). Ann NY Acad Sci 1982;386:119–29
27. Mann DMA, Davies JS, Hawkes J, Yales PO. Immunohistochemical staining of senile plaques. Neuropathol Appl Neurobiol 1982;8: 55–61
28. Mihaly A, Bozóky B. Immunohistochemical localization of extravasated serum albumin in the hippocampus of human subjects with partial and generalized epilepsies and epileptiform convulsions. Acta Neuropathol 1984;65:25–34
29. Alafuzoff I, Adolfsson R, Grundke-Iqbal I, Winblad B. Blood brain barrier in Alzheimer dementia and in non-demented elderly. Acta Neuropathol 1987;73:160–6
30. Liu HM, Atack JR, Rapoport SI. Immunohistochemical localization of intracellular plasma proteins in the human central nervous system. Acta Neuropathol 1989;78:16–21
31. Petito CK, Cash KS. Blood–brain barrier abnormalities in the acquired immunodeficiency syndrome: Immunohistochemical localization of serum proteins in postmortem brain. Ann Neurol 1992;32:658–66
32. Löberg EM, Torvik A. Plasma proteins in normal neurons. Acta Pathol Microbiol Scand 1992;100:431–6
33. Connor JR, Benkovic SA. Iron regulation in the brain. Histochemical, biochemical and molecular considerations. Ann Neurol 1992;32(Suppl):51–61
34. Oehmichen M. Mononuclear phagocytes in the central nervous system. Berlin: Springer-Verlag, 1978
35. Lee JC. Anatomy of the blood–brain barrier under normal and pathological conditions. In: Haymaker W, Adams RD, eds. Histology and histopathology of the nervous system. Springfield: Charles C. Thomas, 1982:798–870
36. Tourtellotte WW. The cerebrospinal fluid in multiple sclerosis. In: Vinken PJ, Bruyn GW, Klawans HL, Koetsier JC, eds. Handbook of clinical neurology. Demyelinating diseases. Amsterdam: Elsevier 1985;47:79–130
37. Fabian RH, Ritchie TC. Intraneuronal IgG in the central nervous system. J Neurol Sci 1986;73:257–67
38 Fishman PS, Savitt JM. Selective localization by neuroglia of immunoglobulin G in normal mice. J Neuropathol Exp Neurol 1989;48:212–20
39. Werb Z. Phagocytic cells: Chemotaxis and effector functions of macrophages and granulocytes. In: Stites DP, Stobo JD, Fudenberg HH, Wells JV, eds. Basic and clinical immunology. Fourth ed. Los Altos, CA: Lange Medical Publications, 1982:109–23
40. Périer O, Grégoire A. Electron microscopic features of multiple sclerosis lesions. Brain 1965;88:937–52
41. Brightman MW. The intracerebral movement of proteins injected into blood and cerebrospinal fluid of mice. Prog Brain Res 1968;29: 19–40
42. Brett M, Weller RO. Intracellular serum proteins in cerebral gliomas and metastatic tumours. An immunoperoxidase study. Neuropathol Appl Neurobiol 1978;4:263–72
43. Gocht A, Lohler J. Changes in glial cell markers in recent and old demyelinated lesions in central pontine myelinolysis. Acta Neuropathol 1990;80:46–58
44. Klatzo I, Miquel J. Observations on pinocytosis in nervous tissue. J Neuropathol Exp Neurol 1960;19:475–87
45. Klatzo I, Miquel J, Otenasek R. The application of fluorescein labelled serum proteins (FLSP) to the study of vascular permeability in the brain. Acta Neuropathol 1962;2:144–60
46. Blakemore WF. The fate of escaped plasma protein after thermal necrosis of the rat brain. An electron microscope study. J Neuropathol Exp Neurol 1969;28:139–52
47. Baloyannis SJ, Gonatas NK. Distribution of anti-HRP antibodies in the central nervous system of immunized rats after disruption of the blood brain barrier. J Neuropathol Exp Neurol 1979;38:519–31
48. Reese TS, Karnovsky MJ. Fine structural localization of a blood–brain barrier to exogenous peroxidase. J Cell Biol 1967;34:207–17
49. Grundke-Iqbal I, Lassmann H, Wisniewski HM. Chronic relapsing experimental allergic encephalomyelitis. Immunohistochemical studies. Arch Neurol 1980;37:651–6
50. Kristensson K, Wisniewski HM. Chronic relapsing experimental allergic encephalomyelitis. Studies in vascular permeability changes. Acta Neuropathol 1977;39:189–94
51. Lassmann H. Comparative neuropathology of chronic experimental allergic encephalomyelitis and multiple sclerosis. Berlin: Springer, 1983
52. Mehta PD, Lassmann H, Wisniewski HM. Immunologic studies of chronic relapsing EAE in guinea pigs: Similarities to multiple sclerosis. J Immunol 1981;127:334–8
53. Snyder DH, Hirano A, Raine CS. Fenestrated CNS blood vessels in chronic experimental allergic encephalomyelitis. Brain Res 1975;100:645–9
54. Adams CWM. Color atlas of multiple sclerosis and other myelin disorders. New York: Sheridan House Inc., 1989:184–95
55. Perry VH, Lund RD. Evidence that the lamina cribrosa prevents intraretinal myelination of retinal ganglion cell axons. J Neurocytol 1990;19:265–72
56. Hildebrand C, Remahl S, Waxman SG. Axo-glial relations in the retina-optic nerve function of the adult rat: Electron microscopic observations. J Neurocytol 1985;14:597–617
57. Raff M, Miller R, Noble M. A glial progenitor that develops *in vitro* into an astrocyte or an oligodendrocyte depending on culture medium. Nature 1983;303:390–6
58. Robbins DS, Shirazi Y, Drysdale BE, Lieberman A, Shin HS, Shin ML. Production of cytotoxic factor for oligodendrocytes by stimulated astrocytes. J Immunol 1987;139:2593–7
59. Merrill JE, Strom SR, Ellison GW, Meyers LW. *In vitro* study of

mediators of inflammation in multiple sclerosis. J Clin Immunol 1989;9:84–96
60. Tyor WR, Glass JD, Griffin JW, et al. Cytokine expression in the brain during AIDS. Ann Neurol 1992;31:349–60
61. Compston A, Scolding N, Wren D, Noble M. The pathogenesis of demyelinating disease: Insights from cell biology. Trends Neurosci 1991;14:175–82
62. Prineas JW, Kwon EE, Goldenberg PZ, et al. Multiple sclerosis: Oligodendrocyte proliferation and differentiation in fresh lesions. Lab Invest 1989;61:489–503
63. Heinonen O, Syrjänen S, Mäntyjarvi R, Syrjänen K, Riekkinen P. JC virus infection and Alzheimer's disease: Reappraisal of an *in situ* hybridization approach. Ann Neurol 1992;31:439–41

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