Multiple Sclerosis: Microglia, Monocytes, and Macrophage-Mediated Demyelination

John W. Prineas, MD and John D.E. Parratt, MBBS, PhD

Abstract
This study examined the roles of microglia and monocytes in myelin destruction in patients with early multiple sclerosis (MS). Twenty-two cases were studied; the clinical duration was <9 weeks in 10 cases. Twenty myeloid cell subtypes or categories were identified including 2 cell types not known previously to occur in demyelinating diseases. Commencing myelin breakdown in plaques and in perivascular and subpial tissues occurred in the immediate presence of infiltrating monocytes and was effected by a homogenous population of IgG-positive Fc receptor-bearing early phagocytes interacting with abnormal myelin. Oligodendrocyte apoptosis was observed in intact myelinated tissue bordering areas of active demyelination. Capillaries in the cerebral cortex plugged by large numbers of monocytes were common in acute cases of MS and in a patient with a neuromyelitis optica variant and extreme systemic recruitment of monocytes. In an MS patient with progressive disease, microglial nodules centered on myelin breakdown products including 2 cell types not known previously to occur in demyelinating diseases. Commencing myelin destruction in patients with early multiple sclerosis (MS) cases (2). He noted that myelin breakdown was minimal or absent in early lesions and that the first and most important structural change was a commencing loss of myelin with relative preservation of axons, concluding that "...changes in the myelin sheath must be looked upon as the primary structural element attacked by...some noxious agent or effect ...from the blood or perivascular spaces." His suggestion as to the nature of the "causal agent" was that it could be a toxin, some unknown pathogen, or an enzyme. How fat granule cells, that is lipid macrophages, came to contain myelin breakdown products he suggested was that myelin in a soluble form was adsorbed by the cell. His illustration of the earliest structural change in newly forming lesions is a colored drawing of several small, lipid-filled fat granule cells located in otherwise normal looking myelinated tissue.

INTRODUCTION

Charcot and other early investigators, using stained sections and teased wet specimens of chronic plaques, noted that affected tissue consisted of packed glial fibers and nerve fibers without myelin sheaths (1) (Supplementary Data Fig. S1). Towards the edges of the lesions there were lipid-filled "fat granule cells," which were thought to be a form of cell death caused by lipid material accumulating within aging or diseased cells. Myelin loss with preservation of axons was attributed to, "strangulation" of myelinated nerve fibers by increasing glial fiber formation.

J.W. Dawson, in a remarkable 1916 monograph (with 155 references, 22 drawings, and 434 photographs and micrographs), written about 40 years after Charcot, summarized the work of French and German pathologists and results of his own study of 9 multiple sclerosis (MS) cases (2). He noted that myelin breakdown was minimal or absent in early lesions and that the first and most important structural change was a commencing loss of myelin with relative preservation of axons, concluding that "...changes in the myelin sheath must be looked upon as the primary structural element attacked by...some noxious agent or effect ...from the blood or perivascular spaces." His suggestion as to the nature of the "causal agent" was that it could be a toxin, some unknown pathogen, or an enzyme. How fat granule cells, that is lipid macrophages, came to contain myelin breakdown products he suggested was that myelin in a soluble form was adsorbed by the cell. His illustration of the earliest structural change in newly forming lesions is a colored drawing of several small, lipid-filled fat granule cells located in otherwise normal looking myelinated tissue.

Macrophage-Mediated Demyelination

Waksman showed that destruction of myelin in the T cell mediated autoimmune disease experimental allergic neuritis (EAN), which was produced by injecting intradermally peripheral nerve myelin in susceptible animal strains, occurred near blood vessels and in the immediate presence of blood born leukocytes, mostly macrophages together with other inflammatory cells (3). Later, electron microscopic studies showed that myelin breakdown in EAN and in experimental autoimmune encephalomyelitis (EAE), a T-cell-mediated autoimmune disease of CNS myelin, was effected by macrophages directly contacting myelin sheaths, that they removed myelin directly from myelin sheaths by phagocytosis, and that this occurred without the formation of extracellular myelin debris or engagement with other cells or membranes in the vicinity (4, 5). Referred to as macrophage-mediated demyelination, this pattern of myelin destruction has since been described in other PNS and CNS demyelinating diseases and experimental models including chronic inflammatory demyelinating poly-
neuropathy, several caused by viruses, and, in the PNS, by localized trauma (6–8).

Regarding MS, of the more than 70 electron microscopy (EM) studies published since 1964, in the few instances where suitably fixed early lesions have been available, commencing loss of myelin has followed this pattern with the additional detail that detached myelin lamellae are sometimes seen attached to receptor-rich areas on the macrophage surface (clathrin-coated pits) (9, 10). A subsequent study has shown the same pattern of macrophage myelin engagement in EAE (11). There are also reports that myelin engaged by macrophages in MS may appear vesiculated and that this may involve the full thickness of the sheath (12).

As to the origin of the macrophage population in MS and other conditions where myelin disruption occurs, the traditional view has been that in all acute forms of injury to white matter including traumatic, ischemic, and inflammatory lesions, lipid macrophages develop from a population of small unusually shaped cells with fine branching processes discovered and named microglia by del Rio Hortega (13). Ramon y Cajal (14) and most modern neuropathologists, while acknowledging the importance of Hortega’s discoveries, consider that macrophages including lipid macrophages derive in part or entirely from monocytes from the blood (15–18).

**Oligodendrocyte Loss**

Oligodendrocytes, sometimes in relatively large numbers, are present in typical postphagocytic plaques (19). The numbers, however, are less than in normal tissue which has encouraged the view that macrophage myelin engagement in MS may be due not to antibody or T cells targeting myelin but to macrophages responding via innate immune mechanisms to the presence of degenerate myelin secondary to oligodendrocyte loss. There are 2 contrasting current opinions regarding this possibility. Brück et al (20) examined oligodendrocytes in series of MS cases diagnosed by needle biopsy of single lesions in cases of clinical duration 11 days to 7.5 months. Some lesions showed oligodendrocytes largely preserved whereas in others oligodendrocyte loss was pronounced. In other series, it was determined that about one third of cases had lesions with oligodendrocyte loss, whereas in two thirds of cases biopsied lesions showed no loss of oligodendrocytes (21–23). Myelin destruction in the latter group was ascribed to the presence of antimyelin antibodies as indicated by the presence of activated complement and IgG on affected sheaths.

The other view, based on autopsy studies of multiple lesions in equally atypical cases of early MS, is that in all cases of MS there is a complete or almost complete loss of oligodendrocytes in newly forming lesions, that the relatively numerous oligodendrocytes sometimes seen are newly generated cells, and that deposition of activated complement (but not IgG) on myelin is common in early lesions in early MS (24–28). Others report no evidence of patient heterogeneity with respect to the presence of myelin immunoreactive for activated complement in patients with chronic disease (29).

Regarding the relative roles played by microglia and monocytes in myelin destruction in MS, some authors ascribe such activity to microglia, others noting that myelin phagocytes are mostly derived from activated monocytes (30–37). This study focuses chiefly on the different morphologically defined populations of microglia, monocytes, and other types of myeloid cells involved in initiating tissue breakdown and myelin destruction in newly forming lesions in patients with early MS.

**MATERIALS AND METHODS**

This is the second of 2 studies designed to investigate the pathogenesis of plaque formation in exceptionally early cases of MS. The first of the 2 studies reported that astrocytes as well as oligodendrocytes and myelin are destroyed by macrophages in newly forming lesions (38). In this study of the same group of patients, attention is focused specifically on the role played by microglia and monocytes in the destruction of myelin in newly forming lesions.

**Clinical Material**

The neuropathologists and institutions that provided tissue together with clinical details of 12 acute cases and 10 chronic cases are shown in Table 1. Identifying case numbers are the same as those used in the astrocyte study (38). The study complies with the requirements of the Human Ethics Committee of The University of Sydney.

**Immunohistochemistry**

Areas of different histological age within lesions are defined as follows: myelinated prephagocytic areas, active demyelination (Luxol fast blue [LFB]-positive macrophages in partially demyelinated tissue), “immediate” postphagocytic zones (LFB-positive macrophages in completely demyelinated tissue), postphagocytic zones (LFB-negative lipid macrophages in demyelinated tissue), and “late” post-phagocytic areas (lipid macrophages numerous but located chiefly in perivascular spaces). Previously identified indications of imminent myelin breakdown in normally myelinated tissue bordering areas of active demyelination (prephagocytic changes), include chiefly loss or degenerative changes in oligodendrocytes, intramyelinic edema, and myelin sheaths immunoreactive for activated complement.

Paraffin sections 4–6 microns thick were stained using hematoxylin and eosin, LFB-PAS for myelin, and Bodian silver stain for axons. Frozen sections were prepared from mirror blocks of selected lesions and stained for neutral lipids using Oil Red O and hematoxylin. Immunohistochemical staining was performed as described in Supplementary Material using primary antibodies listed in Table 2, biotinylated or polymer-bound hors eradish peroxidase-labeled second antibodies (Vector ABC Elite Kit, Vector Laboratories, Burlingame, CA; EnVision+ and LSAB+ Kits, Dako Cytomation, Inc., Carpinteria, CA), and diaminobenzidine as chromogen. Antigens and lectins examined included: RCA-1 lectin (endothelial cells, microglia, macrophages), MRPI-4 (activation monocyte marker), HAM56 (macrophages), CD45 (myeloid cells), CD209 (dendritic cells), MHC-class II antigens, UCHL1 (CD45RO activated and memory T cells), lymphocytes (CD3, CD4, CD8, and CD20), IgG, complement proteins (C3d, monoclonal B7 anti-MAC 5b-9), and PCNA and Ki67 (proliferating cells). Also examined were sections immunostained using biotinylated normal and MS CSF IgG as described in Supplementary Material as well as sections pre-
pared during previous immunohistochemical studies of the same lesions. The latter included sections reacted for myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), CNPase, HNK-1 (immature oligodendrocytes and type 2 astrocytes), activated caspase-3, glial fibrillary acidic protein, and aquaporin 4 (AQP4).

Electron Microscopy

Micrographs from previous EM studies of plaques in Cases 19 and 20 were reviewed. In Case 19, the plaques examined were inactive lesions with few or no lipid macrophages. In Case 20, the lesion examined was an actively demyelinating plaque described previously by Raine et al (19).

| Case | Sex/age | Duration of Illness/Terminal Illness | Neuropathologist | Institution |
|------|---------|-------------------------------------|------------------|-------------|
| 1    | F 31    | 3 months/7 days                     | CWM Adams        | Guy’s Hospital Medical School, London, UK |
| 2    | F 20    | 2 weeks                             | CWM Adams        | Guy’s Hospital Medical School |
| 3    | M 25    | 29 days                             | S Pogacar        | Brown University Medical School, Providence, RI, USA |
| 4    | F 42    | 2.5 months/2 weeks                  | RD Terry         | Albert Einstein College of Medicine, The Bronx, NY, USA |
| 5    | M 14    | 18 days                             | J McLaughlin     | Royal Free Hospital, Hampstead, London, UK |
| 6    | F 70    | 21 days                             | S Love           | The National Hospital, Queen Square, London, UK |
| 7    | M 32    | 10/8 months                          | R Doshi          | The Maudsley Institute of Psychiatry, London, UK |
| 8    | F 48    | 18 days                             | CG Harper        | Royal Perth Hospital, Perth, WA, Australia |
| 9    | F 14    | 9 months/17 hours                   | GN Budzilovich   | New York University Medical Center, NY, USA |
| 10   | M 36    | 3 years/not known                   | DM Boehme        | VA Hospital, East Orange, NJ, USA |
| 11   | F 23    | 5 weeks                             | CJ Bruton        | Runwell Hospital, Wickford, UK |
| 12   | F 23    | 30 days/60 hours                    | BA Kakulas       | Royal Perth Hospital |
| 13   | F 27    | 32 months                           | RO Barnard       | Maida Vale Hospital for Nervous Diseases, London, UK |
| 14   | F 29    | 12 months                           | W Evans          | Oliver Latham Laboratory, Macquarie Hospital North Ryde, NSW, Australia |
| 15   | F 27    | 49 months                           | TH Moss          | Frenchay Hospital, Bristol, UK |
| 16   | M 34    | 3 years/11 months                   | DM Boehme        | VA Hospital, East Orange, NJ, USA |
| 17   | M 43    | 13 years                            | JW Prineas       | Dover General Hospital, NJ, USA |
| 18   | M 55    | “Long standing”                     | CJ Bruton        | Runwell Hospital |
| 19   | F 39    | 14 years                            | JW Prineas       | Concord Hospital, Sydney, Australia |
| 20   | F 30    | 11 years                            | CS Raine         | Albert Einstein College of Medicine |
| 21   | M 60    | 20 years                            | Eun-Sook Cho     | University Hospital, UMDNJ—New Jersey Medical School, Newark, NJ, USA |
| 22   | M 55    | 20 years                            | Dr Krumerman     | Jersey Shore Medical Center, Neptune, NJ, USA |

Remyelinating lesions were present in the same section or elsewhere in cases 1, 3, 4, 9, and 10. Cases 3, 7, 9, and 10 received corticosteroids. Case 3 received azathioprine. Prominent concentric bands were present in Case 8. Neurogenic pulmonary edema was present in cases 9 and 10. Herniation was present in cases and 8. Cases 16 and 17 received cyclophosphamide. Cases 1–12 are acute cases; cases 13–22 are less acute cases.

| Antibody | Clone | Dilution | Antigen Retrieval | Source |
|----------|-------|----------|-------------------|--------|
| CD45     | PD7-26,2B11 | 1:200     | Microwave/citrate  | Dako, Carpinteria, CA |
| CD68     | PG/M1   | 1:50      | Pronase            | Dako   |
| MAC-1    | HAM56   | 1:1000    | Proteinase K       | Dako   |
| MHC-class II | CR3/43 | 1:50      | Heat/high pH Dako  | Dako   |
| MRP14    | BMA-S36,48 | 1:25    | Microwave/citrate  | Accurate Chemical, Westbury, NY |
| RCA-1 lectin |      | 1:4000    |                   | Vector, Burlingame, CA |
| CD209 DC-SIGN |      | 1:100     | Heat/high pH Dako  | R and D Systems, Minneapolis, MN |
| IgG      | B7     | 1:2000    | Proteinase K       | BP Morgan, Cardiff University, Cardiff, UK |
| CD3      | C8/144B | 1:1000    | Microwave/citrate  | Novocastra, Newcastle Upon Tyne, UK |
| CD4      | UCHL1   | 1:150     | Microwave/citrate  | Novocastra |
| PCNA     | PC10    | 1:80      |                   | Dako   |
RESULTS

Myeloid Cells

Resident Ramified Microglia

These nonphagocytic (no phagosomes detected by light microscopy), IgG-negative, MHC-II-negative, small, ramified microglia in normal tissue remote from plaques appeared as depicted by del Rio Hortega and others in drawings and photomicrographs of normal and diseased tissue using particular silver impregnation techniques. Immunohistochemically, these CD45-positive cells stained negatively for all lymphocyte markers tested and they were negative for CD209 and the activation marker MRP-14 (Table 3). In some sections, ramified microglia stained positively for CD68. Ramified microglia were seen encircling (corralling) cell bodies of neurons and other cells in intact gray matter and white matter in several of the acute cases (Fig. 1).

Reactive Ramified Microglia

These enlarged ramified cells were present in increased numbers (microgliosis) near and distant from lesions. They

| TABLE 3. Immunohistochemical Profiles of Morphologically Defined Myeloid Cells in and Near Multiple Sclerosis Lesions |
|---------------------------------------------------------------|
| Antigen | Ramified | Early Activated | Phagocytic | Lipid | Residual | Monocytes | Plugs |
|---------|----------|----------------|------------|-------|----------|-----------|-------|
| CD45    | +        | +              | +          | 0–±   | +        | +         | +     |
| CD68    | ±–+      | +              | +          |       |          |           |       |
| HAM56   | 0–±      | +              | +          |       |          |           |       |
| RIC1    | 0–+      | +              | +          |       |          |           |       |
| MHC-II  | 0–±      | +              | ±          | ±     | +        | +         |       |
| MRP14   | 0        | (±)            | 0–±        | 0–±   | +        | +         | +     |
| CD209   | 0        | 0              | 0          | 0     | 0        | 0         | 0     |
| IgG     | 0        | 0              | 0          | ±     | 0–±      | +         | +     |

Ramified: resident and reactive microglia with thin branching processes. Early activated: irregularly shaped or elongated thin myeloid cells with no or equivocal branches. Phagocytic: cells containing particles of Luxol fast blue (LFB)-positive myelin. Lipid: LFB-negative cells distended with lipids. Residual: parenchymal phagocytes with distinctive secondary lysosomes, which are common in chronic multiple sclerosis cases. Currently there are no reliable immunohistochemical data on this population. Monocytes: typical appearance by light and electron microscopy (negative for all lymphocyte markers tested). Plugs: squeezed monocytes in gray matter capillaries.

Blank boxes indicate information lacking or uncertain. + = positive, ± = variable, 0 = negative, and (+) = Case 7.

FIGURE 1. Microglia corralling neurons in intact gray matter (cerebral cortex) (Case 7, CD45, ×280).

FIGURE 2. Reactive microglia. Enlarged ramified microglia in the molecular layer of the cerebellum. A blood vessel located in the subarachnoid space is cuffed by monocytes (Case 17, CD45, ×220).
were nonphagocytic (no phagosomes), IgG-negative, and MHC-II-negative (Table 3; Figs. 2 and 3). There was a single instance of ramified microglia that were MHC-II-positive and corralling unidentified cells in tissue bordering an early expanding lesion in Case 4 (Fig. 4).

Early Activated Microglia

These elongated cells, irregular in shape or thin, with no or equivocal branches were not phagocytic (no phagosomes by light microscopy), IgG-negative, variably MHC-II-positive, and located in intact tissue bordering lesions (Table 3). They were present in large numbers and were distributed diffusely or in the form of small clusters of IgG-negative microglia (microglial nodules). Although not proven, there was the impression that the blood brain barrier in such areas was intact, (that is no IgG in astrocytes or elsewhere; Fig. 5, Supplementary Data Figs. S3 and S4). This population corresponds roughly to Hortega’s nonphagocytic “rod cells” and “flat cells” (13). In the absence of monocytes, myelin and oligodendrocytes in the vicinity usually appeared normal with no evidence of commencing myelin breakdown.

Nodules of Early Activated Microglia Centering on C3d-Positive Myelin

Microglia clustered alongside myelin sheaths immunoreactive for activated complement (C3d), were located in intact tissue bordering lesions (Fig. 6). Such nodules were observed in 2 patients with secondary progressive MS (Cases 16 and 17). The cells were MHC-II-positive, CD16-positive, MRP-14-negative, and IgG-negative. The nodules were seen only in myelinated tissue, never in demyelinated tissue. This and reports that the C3d immunoreactivity may be nodal or paranodal, that paranodes at plaque margins show immunohistochemical evidence of extensive damage as well as EM evidence of detachment of lateral loops from the axolemma, support other evidence that a hypothetical MS antigen may prove to be a nodal/paranodal protein (28, 39–41).

Nodules Composed of Microglia and Monocytes

These were common in intact tissue bordering some lesions, especially in Cases 7 and 17. These nodules were not seen in demyelinated tissue (Supplementary Data Fig. S2).

Nodules Composed of Monocytes, Early Activated Microglia, and MHC-II-Positive Capillaries

Nodules with these characteristics were present in moderate to large numbers in the cerebral cortex in a case of secondary progressive MS (Case 16; Fig. 7). Microglia within each nodule appeared to be encircling (corralling) an MHC-class II-positive capillary containing a monocyte (Figs. 8 and 9). Neurons in the vicinity were intact but showed increased immunostaining of neurofilaments. As described below,

FIGURE 3. Reactive microglia in the molecular layer of the cerebellum. Cell bodies consist of little more than a nucleus, with almost all cytoplasm located in thickened branching processes. Extremely fine secondary branches are also present. This is the appearance of ramified microglia in tissue that is neither white matter nor gray matter (Case 17, CD45, ×770).
monocytes located in capillaries in normal gray matter structures including the cerebral cortex was a common finding in most of the acute MS cases. The nodules described here, however, were observed only in Case 16, a patient with chronic progressive disease, ongoing recruitment of monocytes, and lesions with tight knots of unidentified MHC-class II-positive cells located in the glial wall of plaques. This is the second report of upregulation of MHC-class II antigen expression in lesion capillaries in MS (42–44).

Monocytes were present in large numbers, in and around all early lesions examined in 11 acute MS cases, in Case 7 and in Cases 16–18 (Fig. 10). Morphologically, these cells appeared to be typical CD45-positive monocytes; they were MHC-II-positive, MRP14-positive, and stained negatively for all lymphocyte markers tested. Monocytes were not detected in any of a large number of lesions examined in Case 19, a chronic case of 14 years duration with almost no lesions with lipid macrophages present in edge zones. Monocytes were lo-
cated in perivascular spaces, in the walls of small blood vessels, in the lumen of blood vessels, in actively demyelinating lesions including small perivascular lesions (Figs. 11–14 and Supplementary Data Figs. S5–S7), and as cuffs around vessels in the subarachnoid space, cerebral sulci and on the surface of the brain (Supplementary Data Fig. S8).

Mononuclear leukocytes underdoing mitosis were present in perivascular spaces in several cases where there were numerous PCNA-positive and Ki67-positive early macrophages nearby. The presence of monocytes in developing lesions including prephagocytic perivascular lesions was closely associated with the appearance in the tissue of myelin phagocytes as no lesion in any of the acute cases showed evidence of commencing myelin phagocytosis where they were absent. As with microglia, we were unable to identify an intermediate stage in their transition to phagocytes.

Perivascular cuffs composed entirely or almost entirely of monocytes were common. Lymphocytes were present in small numbers in perivascular spaces and in the parenchyma in all acute cases. Large cuffs predominantly of lymphocytes were also present but these were relatively rare. Importantly, with one exception, no polymorphonuclear leukocytes were seen in lesions of any age in any case. The single exception was the atypical Case 7 in which they were relatively numerous. No meningeal B-cell follicles containing plasma cells were identified in this series of acute cases (45). Plasma cells were absent in prephagocytic tissue and in all 6 actively demyelinating lesions examined in the 2 most acute cases in the series (Cases 1 and 2).

IgG-positive MRP14-positive MHC-class II-positive monocytes squeezed into elongated sausage-shaped forms in multiple capillaries in normal gray matter (pontine nuclei, cerebral cortex, granular layer of the cortex of the cerebellum) (capillary monocyte plugs) was a common finding in Cases 2, 4, 7, 9, 12, and 17 (Figs. 15 and 16).

**Secondary Progressive MS**

In 2 secondary progressive MS cases (Cases 16 and 17) and in Case 18, there was evidence of continuing recruitment of monocytes into and around plaques where active myelin breakdown was no longer evident (i.e., lipid macrophages present but no LFB-positive macrophages). The monocytes were located amongst lipid macrophages in demyelinated tissue, in the glial wall and in lesser numbers in the surrounding parenchyma (Fig. 17). The 3 cases were also unusual in having microglial nodules in bordering white matter; some of these contained not only early activated microglia but also monocytes.

**Phagocytic Macrophages**

Early macrophages, (cells referred to in routine diagnostic neuropathology as motile, ameboid, or pleomorphic microglia or phagocytes), were located in areas of commencing myelin breakdown and had a histochemical profile typical of tissue histiocytes (Table 3). They also stained positively for IgG (Supplementary Data Figs. S9 and S10).
FIGURE 8. Microglia-monocyte-capillary nodules. Comparison with Figure 9 shows that MHC class II-positive capillaries are unstained in nodules stained for CD45 and CD68 (Case 16, A: CD45; B: CD68; A, B, ×560).

FIGURE 9. Microglia-monocyte-capillary nodules. Thin processes of ramified microglia enclose MHC-class II-positive cells associated with filmy, net-like MHC-class II-positive capillaries. Capillaries elsewhere in the cortex were MHC-class II-negative (Case 16, MHC-class II, ×560).
Contents ranged from no discernible phagosomes (Fig. 18) to large cells containing particles of myelin (Fig. 19). Myelin sheaths contacted by infiltrating phagocytes or in their immediate vicinity appeared spongy due in part to the presence of intramyelinic edema; they reacted positively for activated complement (C9neo) (Fig. 20); and they stained positively with biotinylated normal and MS CSF IgG. The latter was especially apparent near expanding lesions in Cases 3 and 10.

In partially demyelinated tissue close to the edge of the lesion illustrated in Figure 19 (Case 20), myelinated fibers contacted by myelin phagocytes showed sheaths of reduced thickness, focal lysis of superficial lamellae and detached fragments of compact myelin (Supplementary Data Fig. S12). Vesiculated myelin, an important change noted in some EM studies of macrophage-myelin engagement in MS, was not observed in this case. Counts of oligodendrocytes identified by EM in tissue bordering the edge of the same lesion (10) showed some reduction in oligodendrocyte numbers in areas where macrophages and lymphocytes were observed in contact with degenerate oligodendrocytes and unidentified cells (Fig. 21 and Supplementary Data Fig. S11).

None of the large number of lesions examined by EM in the longstanding case of MS (Case 19) showed evidence of recent myelin breakdown or the presence of monocytes. Plaque margins showed occasional small phagocytes with lipid

**FIGURE 10.** Perivascular monocytes. (A) Two large, circumscribed areas of pale but intact myelin are seen on the left of the figure. (B) Perivascular space mononuclear leukocytes are present around a vessel in one of the lesions. Higher magnifications showed few if any lymphocytes in the cuff (Case 12, A, B, Luxol fast blue; A, ×5, B, ×260).

**FIGURE 11.** Monocyte infiltration. Skip serial sections of a blood vessel in intact tissue bordering a (postphagocytic) plaque. Apart from some vacuolation, myelin is intact. Monocytes are entering the parenchyma from the perivascular space where lymphocytes, if present, are inconspicuous. Extravascular IgG, chiefly in astrocytes, signifies an open blood brain barrier (Case 3, A, MRP14; B, CD45; C, IgG, A–C, ×230).
vacuoles and secondary lysosomes composed of membrane bound stacks of distinctive filamentous material but no recognizable particles of myelin. A few such microglia/macrophages were seen attached via clathrin-coated pits to loosened myelin lamellae on the surface of otherwise normal appearing myelin sheaths (Fig. 22 and Supplementary Data Fig. S13).

An important difference in the 2 cases relates to the number of plasma cells present in lesions. In the actively demyelinating lesion in Case 20, no plasma cells were observed in the albeit small amount of tissue examined. In Case 19, where there was little evidence of recent demyelinating activity, counts of perivascular and parenchymal plasma cells determined in semithin epoxy sections showed plasma cells to be numerous in plaque tissue and in surrounding intact tissue. Actual numbers were 1772 plasma cells per cubic mm in
demyelinated tissue and 389 per cubic mm in the surrounding white matter. Plasma cell counts in a control case of motor neuron disease showed no plasma cells (46, 47).

Lipid Macrophages

Lipid macrophages comprised a population of large IgG-positive cells (Fig. 23). These cells were swollen with Oil Red O-positive metabolized myelin and were located behind a phalanx of active phagocytes at plaque margins. In the case of nonenlarging plaques, (that is no LFB-positive macrophages), they were located in demyelinated tissue towards the plaque rim. Large fragments of MBP-positive, MAG-positive myelin were attached to the surfaces of some lipid macrophages. This population of cells showed clustering of surface IgG (Figs. 24 and 25) (48). These cells correspond to the lipid macrophages

FIGURE 15. Monocytes. (Left) An intracapillary monocyte in an old white matter plaque. Typical of white matter capillaries there is a prominent perivascular space. (Right) Four squeezed monocytes (plugs) in normal gray matter capillaries in intact tissue remote from a plaque. (Left: Case 19, electron micrograph, original magnification: ×2800. Right: Case 7, MRP14, ×260).

FIGURE 16. Monocyte plugs in normal gray matter. Those in capillaries appear squeezed (Case 7, MRP14, ×60).

FIGURE 17. Secondary progressive multiple sclerosis. (A, B) Demyelinated tissue in 2 chronically inflamed nonexpanding lesions (no Luxol fast blue-positive macrophages) shows a mixture of lipid macrophages and recently recruited monocytes. Monocytes appear not to be distributed randomly with respect to the lipid macrophages, a disproportionate number locating close to a macrophage (A, Case 16, CD45. B, Case 17, CD45. A, B, ×240).
designated M2 anti-inflammatory macrophages that have been described in postphagocytic plaques (50, 51).

Small Lipid Macrophages

Small spindle-shaped cells containing small amounts of lipid identified in frozen sections were observed in some of newly forming gray matter plaques. These cells correspond to the small lipid containing cells observed by Dawson in normally myelinated tissue bordering early lesions.

Macrophages and Oligodendrocytes

Microglia/macrophages phagocytosing apoptotic oligodendrocytes were observed in Case 9. In Case 20, macrophages were seen contacting degenerate oligodendrocytes (Supplementary Data Figs. S11 and S14).

Macrophages and Astrocytes

Phagocytosis of large stellate AQP-positive astrocytes in recently demyelinated tissue was observed in several of the cases of clinically early MS, as reported previously (38). This activity was selective in the sense that AQP4-negative gemistocytic astrocytes in the immediate vicinity were spared.

Residual Microglia

Microglia/macrophages with distinctive cytoplasmic inclusions consisting of membrane bound stacks of curved linear profiles were common in autopsy tissue examined by EM in Case 19. The same inclusion-bearing cells have been noted in MS by many authors and, according to some, in unrelated diseases. Usually located in partially myelinated tissue these cells are also seen crossing into perivascular spaces where they are phagocytosed by resident perivascular space macrophages. That the inclusions are metabolized myelin is unproven and it may be that that these cells are not myelin phagocytes but normally functioning microglia (52). This study provided little reliable immunohistochemical data related to these cells, particularly regarding CD45 and MHC-II antigen immunoreactivity.

Perivascular Space Macrophages

Macrophages located in this mesodermal compartment have unusually large primary lysosomes, and, in contrast to resident microglia, are actively phagocytic and express IgG Fc gamma receptors (53). In MS Case 19, they were seen phagocytosing debris-laden cells that had crossed the glial limiting membrane and entered the space.

Strangely Shaped IgG-Positive Mononuclear Leukocytes

The single instance where these were seen was in a brain stem section with 3 very early newly forming lesions in Case 2 (Figs. 12 and 26). They were present in perivascular spaces, in capillaries (plugs), and in the parenchyma near and at a dis-
tance from the 3 developing plaques. The cytoplasm stained evenly for IgG without edge accentuation. Except for those located in capillaries, the unusual shape was maintained whether they were located in open perivascular spaces or in compact tissue. Clinical and other details of the case are described in Supplementary Data Case 2.

Small Elongated MRP14-Positive Cells

Small elongated cells were detected in intact tissue bordering cerebral hemisphere lesions in a patient with a severe neuromyelitis optica (NMO) spectrum variant characterized by infiltration of affected tissues by both monocytes and neutrophils (Figs. 27–29; Supplementary Data Fig. S19, and Supplementary Data Case 7). The true size and shape of the cells could not be determined as the cells were identified only in sections stained for MRP14. In such sections, the only parts of the cell that were visible were those parts staining positively for MRP14.

CD209-Positive Dendritic Cells

These cells were common in the walls of small venules and in cuffs of inflammatory cells in newly forming MS lesions.

Microglia Undergoing Mitosis

Mitoses in microglia were observed in otherwise unaffected tissue in Case 7, a patient with a fulminant spinal and cerebral variant of NMO (Supplementary Data Fig. S18).
Other CD45-Positive Myeloid Cells

These included macrophages contacting via clathrin-coated pits complement-positive mineral inclusions in the chorioid plexus, and ramified microglia in contact with the glial limiting membrane around blood vessels and at the pial surface. No nodules of lymphocytes of the type seen in patients with paraneoplastic encephalomyelitis and antibodies against intracellular antigens were seen in any MS case (54).

Oligodendrocytes and Astrocytes

Sections of the medulla in Case 9 showed oligodendrocytes with fragmenting pyknotic nuclei located amongst normal looking nerve cells in intact myelinated gray matter close to the floor of the fourth ventricle and at a second site (Figs. 30–32 and Supplementary Data Fig. S3). The presence of apoptotic nuclear bodies located in tags of cytoplasm confirmed this to be classical apoptosis of oligodendrocytes. The location of degenerate oligodendrocytes amongst normal neurons in myelinated gray matter adds to existing evidence that oligodendrocyte loss in MS precedes and probably determines loss of myelin in MS. Nothing similar has been noted in studies of early postphagocytic plaques containing large numbers of oligodendrocytes. Other types of oligodendrocyte injury are illustrated in Supplementary Data Figures S11, S14–S16. Large astrocytes with pale cytoplasm and unusually pale nuclei were observed in intact myelinated tissue bordering some acutely expanding plaques (Supplementary Data Fig. S17).

Progressive Gliosis

Two patients with longstanding MS and no evidence of ongoing myelin breakdown (Cases 18 and 22) showed marked over expression of AQP4 in demyelinated tissue as well as in surrounding intact tissues. In 1 of the 2 cases, this was exaggerated to the point that affected plaques were indiscernible in sections stained for AQP4.

DISCUSSION

Using a staining procedure considered by some to be specific for microglia, del Rio Hortega and others reported...
that phagocytes including lipid macrophages that are present in focal brain lesions of various sorts develop from resident small branching microglia. There is an equally longstanding alternative view, namely that brain macrophages develop chiefly from infiltrating monocytes and other leukocytes. This study shows that monocytes are a major source of phagocytes in MS lesions.

Microglia and Other Myeloid Cells

This study identified 20 myeloid cell subtypes or categories including 2 cell types not known previously to occur in demyelinating diseases. The following is a classification of CD45-positive myeloid cells in lesions of different histological age in patients with early and late MS: (i) Microglia - resident, reactive, mitotic, and residual. (ii) Microglial nodules - C3d-positive, monocyte-positive, and capillary-positive. (iii) Monocytes - capillary plugs, vessel walls, perivascular spaces, parenchyma, and nodules. (iv) Macrophages phagocytic - of myelin, oligodendrocytes, and astrocytes. (v) Macrophages nonphagocytic - lipid. (vi) Mesenchymal macrophages - perivascular spaces, meninges, and choroid plexus. (vii) Strange IgG-positive mononuclear leukocytes. (viii) Small elongated MRP14-positive cells in a patient with an NMO variant.

Macrophage-Mediated Demyelination

MS plaques, small perivascular lesions and subpial strips of demyelination, the 3 main forms of focal myelin loss in MS, develop in relation to perivascular infiltrates of inflammatory cells, initially chiefly monocytes together with lymphocytes and other inflammatory cells. Myelin loss is selective, leaving axons relatively intact. There is evidence that this selective loss is caused not by oxidative stress or some other nonspecific effect of aggregates of inflammatory cells but to a particular property of the inflammatory response in MS (55).

Although the event that initiates commencing loss of myelin is unknown, the proximate cause of myelin destruction involves the appearance in the tissue of what seems to be a homogeneous population of early IgG-positive Fcγ receptor-bearing macrophages in tissue that normally lacks such cells and in which there is no IgG (56, 57). Accompanying changes present from the beginning include signs of a disrupted blood-brain barrier, deposition of activated complement and other degenerative changes in oligodendrocytes and myelin, loss of astrocyte foot processes, and an increase in number, size and shape, and other evidence of activation of microglia. The population of active phagocytes changes in time into a population of nonphagocytic lipid macrophages that exit the plaque via mesodermal perivascular spaces.

Little is known about this population of early phagocytes except that the cells are motile, IgG-positive myeloid-derived macrophages that appear in developing lesions at the same time as recruited monocytes. It can be assumed that they are Fcγ receptor-positive (lipid macrophages are FcR-positive) (56, 57), but there are no genomic or other identifies known at this time.

Regarding the origin of early phagocytes, whether largely from monocytes or from both microglia and monocytes remains uncertain (58). Both cell types are present together in the parenchyma at the time early macrophages begin appearing, with both disappearing as the proportion of phagocytes increases. How they combine, assuming that this is what happens, to produce the population of early macrophages is unknown. Although the rounded monocyte-type cells in perivascular spaces and in the parenchyma are likely monocytes from the circulation, it is difficult to be absolutely sure that all large active phagocytes originate from monocytes rather than transitioning rounded or ameboid cells of microglial origin.

Myelin Autoantibodies

The current view, that macrophages interact with myelin in MS under the direction of T lymphocytes, is based largely on the EAE model as reviewed by Hohlfeld et al (59, 60). That
FIGURE 25. Arrested phagocytosis. (A) Lipid macrophages contacting a still largely intact myelinated nerve fiber have myelin fragments attached to the ends of the cells. (B) A section from the same region of the plaque stained for IgG shows surface IgG on aligned lipid microglia/macrophages in the form of polar caps (Case 16, A, MBP, ×700; B, IgG, ×890). Reproduced from (48).

FIGURE 26. Unidentified IgG-positive mononuclear leukocytes. These were present in perivascular spaces, as “capillary plugs,” and in the parenchyma, in and around newly forming lesions in the medulla in an exceptionally early case of multiple sclerosis (Case 2, IgG, ×720).
Specific antibodies may play a role is suggested by the reduction in clinical exacerbations seen in patients receiving treatment with monoclonal antibody therapies targeting the B-cell antigen CD20 (61).

Perivascular spaces in old MS lesions contain lymphoid-like tissue consisting of plasma cells, reticular cells, macrophages, lymphocytes, and lymphocyte–macrophage contacts resembling a type of immune synapse (46, 47). The same structures, termed ectopic lymphoid-like B cell follicles, are present in the leptomeninges and cerebral sulci close to demyelinated tissue on the surface of the brain, especially in cases of secondary progressive MS (45, 62, 63) leading to the view that these plasma cell aggregates act as a source of pathogenic autoantibodies that contribute to the formation of new plaques, perivascular lesions and subpial strips of demyelination (64). Against this is the fact that there are no plasma cells in most newly forming lesions and it may be that the clinical improvement accompanying treatment with anti-CD20 monoclonal antibodies is due not to suppression of local antibody production by plasma cells but to some other mechanism.

The present findings are consistent with reports that myelin sheaths in some early MS lesions stain positively for activated complement (C9neo). The study also shows that in fixed tissue myelin sheaths in tissue bordering areas of active demyelination bind biotinylated normal IgG and MS CSF IgG.

**IgG Fcγ Receptor-Dependent Mechanisms**

If the IgG from lymphoid-like structures or other sources is a pathogenic autoantibody its mode of action (MOA) is likely to be antibody-dependent cellular phagocytosis (ADCP) (65). Other “classical pathway” MOAs, that is complement-dependent cyto-
toxicity, antibody-dependent cellular cytotoxicity, and programmed cell death (apoptosis) following binding of the antibody to the surface of the target cell (PCD), are other possibilities (66, 67). Activation of FcRs following Fc engagement by macrophages requires clustering of FcRs and the displacement of inhibitory receptors. Clustering of IgG on macrophages does occur in MS, which supports a role for ADCP in the disease.

**Innate Immunity**

On present evidence it is not known if the population of early macrophages involved in the destruction and removal of degenerate myelin, oligodendrocytes, astrocytes, and neurons in ischemic infarcts or traumatic brain injury differs from the population of phagocytes that effect destruction of myelin in MS. In experimental brain damage where the blood-brain barrier is disrupted, studies show that the great majority of phagocytes that invade for example, a small stab wound are monocytes that transform rapidly into early phagocytes as they emerge from blood vessels and later transform into typical nonphagocytic lipid macrophages (68, 69). In MS, selective interaction via innate immune mechanisms of macrophages with myelin sheaths and not with other cells and cell membrane in the same location, could be accounted for by changes in myelin determined by an oligodendrocyte lesion with the resulting uptake of degenerate myelin by phagocytes utilizing scavenger and complement receptors.

**Bystander Demyelination**

NMO is an inflammatory demyelinating disease affecting initially optic nerves and the spinal cord. During the course of the disease autoantibodies of different specificities sometimes develop. These include lupus antibodies, Sjogren’s antibodies, AQP4 antibodies, and MOG antibodies and it is the characteristics of the autoantibody that determines the clinical and pathological features that distinguish each of the several NMO variants. Demyelination with axonal preservation in some NMO cases is associated with conspicuous oligodendrocyte apoptosis (70). It is possible that in MS the cause of demyelination and oli-
godendrocyte apoptosis is not mediated by an adaptive immune mechanism targeting oligodendrocytes and myelin, but to some mechanism similar to that responsible for demyelination in NMO.

Secondary Progressive MS

The nodules composed of monocytes, MHC-II-positive capillaries and microglia observed in the cerebral cortex in Case 16 can be added to the list of gray matter lesions associated with progressive disease (71). Sobel et al in an electron immunocytochemical study of MS biopsy tissue noted that capillaries near white matter lesions in patients with clinically active disease but not in patients with inactive disease were immunoreactive for class II MHC antigens. MHC-II-positive capillaries were also noted in initial stages of lesion formation in EAE (42–44). These findings implicate this phenomenon of upregulation of the expres-
ision by capillaries of MHC-II antigens in the pathogenesis of gray matter lesions in secondary progressive MS.

Monocyte Encephalopathy

Misshapen monocytes that is, plugs, located in capillaries in normal-appearing gray matter in most of the patients with early MS were present in numbers large enough to raise the possibility of compromised capillary circulation in affected tissues. Normal monocytes are larger than other leukocytes measuring between 16 and 22 μm in diameter (neutrophils are 9–15 μm in diameter). The diameter of a capillary lumen, on the other hand, is 3–10 μm “...barely wide enough for an erythrocyte to squeeze through” (72). The mismatch may be even greater in conditions where monocytes are atypical (73). Measurement of the diameter of capillaries and other small blood vessels in Case 19 using semithin toluidine blue-stained epoxy sections (46) showed that vessels measuring <23 μm in diameter were more than twice as numerous in cortical gray matter than in subcortical white matter, which could account for plugging affecting chiefly gray rather than white matter.

The apparent absence of any reactive tissue changes associated with the presence of large numbers of monocytes in cortical capillaries in early active MS suggests that any disturbance in function that might be associated with their presence is minor or transient. However, in Case 16, a patient with secondary progressive MS, microglial nodules centered on abnormal capillaries containing monocytes were present in numbers large enough to suggest the possibility of more serious cerebral cortical dysfunction.

The phenomenon of squeezed monocytes in gray matter capillaries present in numbers large enough to be of possible clinical significance is not restricted to MS. In Case 7, a patient with a severe progressive NMO variant (Supplementary Data Case 7) and who developed severe unexplained cognitive deficits terminally, monocyte capillary plugs in the cerebral cortex were excep-
tionally common. Whether other conditions with increased numbers of circulating activated monocytes, unexplained cortical dysfunction and normal MRI imaging show monocyte capillary plug formation (74) is yet to be determined.

Remodeling MS
In lesions sampled very early in their formation, (i.e., within hours or a few days), myelin loss is accompanied by a loss of oligodendrocytes and astrocytes. This suggests that the disease may not be a disease associated with a mechanism that specifically targets myelin. In patients with longstanding disease, failure of repair of the oligodendrocyte lesion is common. Failure of repair of the astrocyte lesion is also not uncommon, the latter manifesting itself as a permanent opening of the blood-brain barrier and ongoing progressive gliosis. In inflammatory demyelinating diseases in the peripheral nervous system, myelin destruction by macrophages occurs in the absence of microglia; in MS, 2 cell types seem to be involved in the process. It is uncertain regarding the degree, or the manner microglia contribute to the population of myelin phagocytes. The occurrence of microglia corolling monocytes in secondary progressive MS suggests differing roles for these 2 cell types in disease pathogenesis.

Summary
Myelin breakdown is initiated by a population of IgG-positive macrophages contacting largely intact myelin sheaths that stain positively for activated complement (C9neo). The appearance of this population occurs in the presence of a disrupted blood-brain barrier and is associated closely with commencing recruitment into the tissue of IgG-positive blood monocytes. Microglia and early activated microglia, in the absence of recruited monocytes, are nonphagocytic. How monocytes combine with nonphagocytic microglia to generate a population of phagocytes is unclear. The result, however, is the abrupt appearance in tissue that normally has no IgG and no Fc receptor-bearing cells of a largely apparently homogeneous population of IgG-positive Fc receptor-bearing phagocytes that target myelin sheaths, apoptotic oligodendrocytes and astrocytes but leave nerve cells and axons relatively untouched. It is unclear how much of this macrophage activity is mediated by innate and adaptive immune mechanisms.

ACKNOWLEDGMENTS
Control tissue was received from the Australian Brain Donor Programs NSW Tissue Resource Centre which is supported by The University of Sydney and the National Health and Medical Research Council of Australia. Also received was tissue and cerebrospinal fluid from The National Neurological Research Specimen Bank, VAMC Wadsworth Division, Los Angeles, CA 90073, which is sponsored by NINDS/NIMH, National Multiple Sclerosis Society, Hereditary Disease Research Foundation, Comprehensive Epilepsy Program, Tourette Syndrome Association, Dystonia Medical Research Foundation, and Veterans Health Services and Research Administration, Department of Veterans Affairs. We thank Dr. B. Paul Morgan for a monoclonal anti-MAC antibody B7, Dr. C.S. Raine and Dr. B.A. Kakuks for autopsy tissue from patients with early MS, and Dr. H.L. Lipton and Dr. P. Rieckmann for helpful discussion. Technical assistance was provided by S. Lee, E. Kwon, J. Baverstock, and L. Garyfallos.

REFERENCES
1. Charcot JM. Disseminated Sclerosis. Pathological Anatomy. In: Translated by Sigerson, G. Lectures on Diseases of the Nervous System Delivered at La Salpetrière. London: The New Sydenham Society 1877; 157–81
2. Dawson JW. The histology of disseminated sclerosis. Trans R Soc Edinb 1916;50:517–740
3. Waksman BH. Atlas of Experimental Immunobiology and Immunopathology, New Haven and London: Yale University Press 1970.
4. Lampert FW. Electron microscopic studies on ordinary and hyperacute experimental allergic encephalomyelitis. Acta Neuropathol 1967;9:99–126
5. Wisniewski H, Prineas JW, Raine CS. An ultrastructural study of experimental demyelination and remyelination. Acute experimental allergic encephalomyelitis in the peripheral nervous system. Lab Invest 1969;21:105–18
6. Saida T, Saida K, Silberberg DH, et al. Experimental allergic neuritis induced by galactocerebrosides. Ann Neurol 1981;9(Suppl):87–101
7. Blakemore WF, Welsh CJ, Tonks P, et al. Observations on demyelinating lesions induced by Théiler’s virus in CBA mice. Acta Neuropathol 1988;76:581–9
8. Spencer PS, Weinberg HJ, Raine CS, et al. The perinuclear window—a new model of focal demyelination and remyelination. Brain Res 1975;96:323–9
9. Prineas JW, Connell F. The fine structure of chronically active multiple sclerosis plaques. Neurology 1978;28:68–75
10. Prineas JW, Kwon EE, Cho ES, et al. Continual breakdown and regeneration of myelin in progressive multiple sclerosis plaques. Ann N Y Acad Sci 1984;436:11–32
11. Epstein LG, Prineas JW, Raine CS. Attachment of myelin to coated pits on macrophages in experimental allergic encephalomyelitis. J Neurol Sci 1983;61:341–8
12. Raine CS, Cannella B, Hauser SL, et al. Demyelination in primate autoimmune encephalomyelitis and acute multiple sclerosis lesions: A case for antigen-specific antibody mediation. Ann Neurol 1999;46:144–60
13. del Rio-Hortega P. Microglia. In: Penfield, W, eds. Cytology and Cellular Pathology of the Nervous System, Vol. 2. New York: Paul B Hoeber 1932:483–534
14. Ramon y Cajal S. Cajal’s Degeneration and Regeneration of the Nervous System. In: May RM, ed. Vol. 2, 1959 edition. New York: Hafner Publishing Co. 1928:497, 714
15. Adams RD, Sidman RL. Introduction to Neuropathology. New York, Toronto, Sydney: McGraw-Hill 1968:36–7
16. Dolman CL, Microglia. In: Davis RL Robertson DM, eds. Textbook of Neuropathology. Baltimore, Hong Kong, London: Williams and Wilkins 1991:141–63
17. Brück W, Sommermeier N, Bergmann M, et al. Macrophages in multiple sclerosis. Immunobiology 1996;195:588–600
18. Esiri MM, Oppenheimer DR. Diagnostic neuropathology. In: A Practical Manual. Oxford, London, Edinburgh, Boston, Melbourne: Blackwell Scientific Publications 1989:54–5
19. Raine CS, Scheinberg L, Waltz JM. Multiple sclerosis. Oligodendrocyte survival and proliferation in an active established lesion. Lab Invest 1981;45:534–46
20. Brück W, Schmied M, Suchanek G, et al. Oligodendrocytes in the early course of multiple sclerosis. Ann Neurol 1994;35:65–73
21. Lassmann H. Comparative Neuropathology of Chronic Experimental Allergic Encephalomyelitis and Multiple Sclerosis. Berlin, Germany: Springer-Verlag 1983
22. Ozawa K, Suchanek G, Breitschopf H, et al. Patterns of oligodendroglia pathology in multiple sclerosis. Brain 1994;117(Pt 6):1311–22
23. Lucchinetti C, Brück W, Parisi J, et al. Heterogeneity of multiple sclerosis lesions: Implications for the pathogenesis of demyelination. Ann Neurol 2000;47:707–17
24. Prineas JW, The neuropathology of multiple sclerosis. In: Koetsier JC, ed. Handbook of Clinical Neurology. Amsterdam: Elsevier 1985:3:213–57
25. Prineas JW, Kwon EE, Goldenberg PZ, et al. Multiple sclerosis. Oligodendrocyte proliferation and differentiation in fresh lesions. Lab Invest 1984;49:49–80
26. Prineas JW, Barnard RO, Kwon EE, et al. Multiple sclerosis: Remyelination of nascent lesions. Ann Neurol 1993;33:137–51
27. Barnett MH, Prineas JW. Relapsing and remitting multiple sclerosis: Pathology of the newly forming lesion. Ann Neurol 2004;55:458–68
28. Prineas JW, Parratt JD, Oligodendrocytes and the early multiple sclerosis lesion. Ann Neurol 2012;72:18–31
29. Bjoel BC, Brink BP, Veethas R, et al. Homogeneity of active demyelinating lesions in established multiple sclerosis. Ann Neurol 2008;63:16–25
30. Park C, Ponath G, Levine-Ritterman M, et al. The landscape of myeloid and astrocyte phenotypes in acute multiple sclerosis lesions. Acta Neuropathol Commun 2019;7:130
31. Voet S, Prinz M, van Loo G. Microglia in central nervous system inflammation and multiple sclerosis pathology. Trends Mol Med 2019;25:112–23
32. Ransohoff RM, El Khoury J. Microglia in health and disease. Cold Spring Harb Perspect Biol 2015;8:a020560
33. Stratoulias V, Venero JL, Tremblay ME, et al. Microglial subtypes: Diversity within the microglial community. EMBO J 2019;38:e101997
34. Aujere B, Steinman L. Nonclassical monocytes: Are they the next therapeutic targets in multiple sclerosis? Immuno Cell Biol 2018;96:125–7
35. Chu F, Shi M, Zheng C, et al. The roles of macrophages and microglia in multiple sclerosis and experimental autoimmune encephalomyelitis. J Neuroimmunol 2018;318:1–7
36. O’Loughlin E, Madore C, Lassmann H, et al. Microglial phenotypes and functions in multiple sclerosis. Cold Spring Harb Perspect Med 2018;8:
37. Zirzya V, Hametner S, Wimmer I, et al. Loss of ‘homeostatic’ microglia and patterns of their activation in active multiple sclerosis. Brain 2017;140:1900–13
38. Prineas JW, Lee S. Multiple sclerosis: Destruction and regeneration of astrocytes in acute lesions. J Neuropath Exp Neurol 2019;78:140–56
39. Prineas JW, Kwon EE, Cho ES, et al. Immunopathology of secondary-progressive multiple sclerosis. Ann Neurol 2000;50:646–57
40. Wolswijk G, Balesar R. Changes in the expression and localization of the paranodal protein Caspr on axons in chronic multiple sclerosis. Brain 2003;126:1638–49
41. Prineas JW, Connell F. Remyelination in multiple sclerosis. Ann Neurol 1979:5:32–31
42. Sobel RA, Blanchette BW, Colvin RB. Pre-inflammatory expression of human fibronectin (Fn) and Ia in acute experimental allergic encephalomyelitis (EAE): Modulation of endothelial cells (EC) in the immune response detected by quantitative immunoperoxidase studies using monoclonal antibodies (Mab). Prog Clin Biol Res 1984;146:81–6
43. Sobel RA, Natele JM, Schneeweber EE. The immunopathology of acute experimental allergic encephalomyelitis. IV An ultrastructural immunocytochemical study of class II major histocompatibility complex molecule (Ia) expression. J Neuropathol Exp Neurol 1987;46:239–49
44. van der Maesen K, Hinojoza JR, Sobel RA. Endothelial cell class II major histocompatibility complex molecule expression in stereotactic brain biopsies of patients with acute inflammatory/demyelinating lesions. J Neuropathol Exp Neurol 1999;58:346–58
45. Seraffini B, Rosicarello B, Magliozzi R, et al. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. Brain Pathol 2004;14:164–74
46. Prineas JW, Wright RG. Macrophages, lymphocytes, and plasma cells in the perivascular compartment in chronic multiple sclerosis. Lab Invest 1978;38:409–21
47. Prineas JW, Multiple sclerosis: Presence of lymphatic capillaries and lymphoid tissue in the brain and spinal cord. Science 1979;203:1123–5
48. Prineas JW, Graham JS. Multiple sclerosis: Capping of surface immunoglobulin G on macrophages engaged in myelin breakdown. Ann Neurol 1981;10:149–58
49. Prineas JW, Kwon EE, Sternbergh NH, et al. The distribution of myelin associated glycoprotein and myelin basic protein in actively demyelinating multiple sclerosis lesions. J Neuroimmunol 1984;6:251–64
50. Boven LA, Van Meurs M, Van Zwam M, et al. Myelin-laden macrophages are anti-inflammatory, consistent with foam cells in multiple sclerosis. Brain 2006;129:517–26
51. Grajchen E, Hendriks JJA, Bogie JFJ. The physiology of foamy phagocytes in multiple sclerosis. Acta Neuropathol Commun 2018;6:124
52. Raine CS. Multiple sclerosis: The resolving lesion revealed. J Neurommunol 2017:304:2–6
53. Oehmichen M. Receptor activity on some mesenchymal cells in CNS of normal rabbits. Indications of the monocytic origin of intracerebral perivascular cells, epiphysen cells and mononuclear phagocytes in the subarachnoid space. Acta Neuropathol 1976;35:205–18
54. Bien CG, Vincent A, Barnett MH, et al. Immunopathology of autoantibody-associated encephalitides: Clues for pathogenesis. Brain 2012;135:1622–38
55. Junker A, Wozniak J, Voigt D, et al. Extensive subcortical demyelination is specific to multiple sclerosis. Brain Pathol 2020;30:641–52
56. Nyland H, Maret R, Mork S. Fc receptors of microglial lipophages in multiple sclerosis. N Engl J Med 1980;302:120–I
57. Nyland H, Mork S, Maret R, In-situ characterization of mononuclear cell infiltrates in lesions of multiple sclerosis. Neuropathol Appl Neurobiol 1982:8:403–11
58. Hendrickx DAE, van Eden CG, Schuurman KG, et al. Staining of HLA-DR, Iba1 and CD68 in human microglia reveals partially overlapping expression depending on cellular morphology and pathology. J Neuroimmunol 2015;7:10:12–22
59. Hohlfeld R, Dormair M, Meinl E, et al. The search for the target antigens of multiple sclerosis, part 1: Autoreactive CD4+ T lymphocytes as pathogenic effectors and therapeutic targets. Lancet Neurol 2016;15:198–209
60. Hohlfeld R, Dormair M, Meinl E, et al. The search for the target antigens of multiple sclerosis, part 2: CD8+ T cells, B cells, and antibodies in the focus of reverse-translational research. Lancet Neurol 2016;15:317–31
61. Hauser SL, Bar-Or A, Comi G, et al. Ocrelizumab versus interferon beta-1a in relapsing multiple sclerosis. N Engl J Med 2017;376:221–34
62. Reali C, Magliozzi R, Roncaroli F, et al. B cell rich meningeal inflammation associates with increased spinal cord pathology in multiple sclerosis. Brain Pathol 2020;30:779–93
63. Griffiths L, Reynolds R, Evans R, et al. Substantial subcortical demyelination in progressive multiple sclerosis: Have we underestimated the extent of cortical pathology? Neuroimmunol Neuroinflammation 2021;7:51–67
64. Kuerten S, Lanz TV, Lingampalli N, et al. Autoantibodies against central nervous system antigens in a subset of B cell-dominant multiple sclerosis patients. Proc Natl Acad Sci U S A 2020;117:21512–8
65. Alvord EC. Acute disseminated encephalomyelitis and allergic neuro-encephalopathies. In: Vinken PJ and Bruyn GW eds. Handbook of Clinical Neurology, Vol 9. Amsterdam: North-Holland 1970:500–71
66. Herbrand U, Surowy T. A neglected stepchild. Eureka blog. Available at: https://eureka.criver.com/a-neglected-stepchild/. Accessed May 26, 2015.
67. Patel KR, Roberts JT, Barb AW. Multiple variables at the leukocyte cell surface impact Fc gamma receptor-dependent mechanisms. Front Immunol 2019;10:223
68. Hurley JV. Autoimmune inflammation. In: Chapter 14, Chronic Inflammation, second edition. Edinburgh, London, Melbourne: Churchill Livingstone 1983:133–47
69. Gehrmann J, Kreutzberg GW, Microglia in experimental neuropathology. In: Kettenmann H and Ransom BR, eds. Neuroglia. New York, Oxford: Oxford University Press 1995:383–904
70. Parratt JD, Prineas JW. Neuroimmunology: A demyelinating disease characterized by acute destruction and regeneration of perivascular astrocytes. Mult Scler 2010;16:1156–72
71. Zuroff LR, Benjamins JA, Bar-Or A, et al. Inflammatory mechanisms underpinning cortical injury in progressive multiple sclerosis. Neuroimmunol Neuroinflammation 2021;8:111–33
72. Rhodin JAG, Histology A Text and Atlas. London, Toronto: Oxford University Press 1974:104–6,352.
73. Lombardi A, Trombeta E, Cattaneo A, et al. Early Phases of COVID-19 are characterized by a reduction in lymphocyte populations and the presence of atypical monocytes. Front Immunol 2020;11:560330
74. Hosp JA, Dressing A, Blazhenets G, et al. Cognitive impairment and altered cerebral glucose metabolism in the subacute stage of COVID-19. Brain 2021;144:1263–76