ABSTRACT

Many cell types, chemical mediators, and target receptor molecules contribute to the pathophysiologic processes that cause demyelination and axon degeneration in multiple sclerosis (MS). The specific cell populations and molecular mediators that produce MS lesions vary depending on lesion type and age. Acute MS lesions are characterized by an inflammatory central region, an indistinct boundary, and large numbers of CD4+ T-lymphocytes and oligodendrocytes. The resolving MS lesion has a clearly defined boundary of microglia and remyelination that depends on the absence of immune cells. The secondary progressive lesion is characterized by a preponderance of CD8+ lymphocytes and few oligodendrocytes. In chronic active MS lesions, remyelination appears to be suppressed by the action of astrocytes, which maintain oligodendrocytes in an immature, remyelinating state. Glutamate excitotoxicity, a feature of many disorders of the central nervous system, may also be important in the pathogenesis of MS. In vitro and autopsy studies have suggested the balance of glutamate synthesis and metabolism is disordered in MS lesions, resulting in an accumulation of high concentrations of extracellular glutamate. Preclinical studies also suggest agents that block the activity of glutamate at receptors (in particular, receptors of the AMPA/kainate type) prevent some of the pathologic changes that occur in experimental demyelinating model systems that resemble the demyelination that occurs in MS.

the pathophysiology of M.S. These lesions are characterized by a relatively well-defined boundary separating the lesion from surrounding tissue, possibly with some remyelination at the edge. Although nothing about the structure of the lesion obviously suggests why the edge has formed where it has, the clear boundary between lesion and nonlesion tissue suggests that some mechanism prevents the spread of the lesion beyond a certain point.

**CHARACTERISTICS OF MS LESIONS**

**THE ACUTE LESION**

The acute M.S. lesion is characterized by an inflammatory central region and an indistinct margin. Lymphocytes, primarily CD4+, are present within the lesion. Nerve fibers appear to have highly vesiculated myelin. Deep within the lesion there is a distinct zone of surviving oligodendrocytes, which may be stained by markers suggesting they are immature oligodendrocytes. Macrophages are common, many of which contain lipids or myelin debris. Many of the surviving oligodendrocytes appear unhealthy and to be undergoing cytolysis. The oligodendrocytes do not appear to undergo apoptosis at this stage. Transected axons are common in the acute lesion. Toward the edge of the lesion, more myelin debris and macrophages are apparent, and at the advancing edge are large numbers of macrophages. In the early acute lesion it may be difficult to tell whether these cells are monocytes or reactive microglia, but over time they begin to exhibit features of reactive microglia. The monocyte component of the macrophage force leaves the lesion early, and microglia become the predominant cell type. At the advancing edge of the lesion, the myelin sheaths of axons exiting the lesion breaks down into a vesiculated appearance, with local macrophages, probably reactive microglia, taking up myelin debris (Figure 1).

Studies from animal models and tissue samples obtained at autopsy from patients with M.S. suggest autoantibodies directed against one or more myelin-associated antigens are important in the demyelination process in these active lesions. Antigen-specific antibody reactions in M.S. were examined using a primate model of M.S. (experimental allergic encephalomyelitis [EAE] in the marmoset) and in tissue samples from patients with recently active M.S. lesions. EAE was induced in marmosets by sensitizing them either to whole white matter or to myelin/oligodendrocyte glycoprotein (MOG). The marmoset EAE model, in which primary demyelination and grossly visible CNS plagues develop after exposure of the animals to either white matter or MOG, is the closest experimental analogue of human M.S. The development of anti-MOG autoantibodies corresponds to the onset of demyelination that resembles the demyelination observed in patients with acute M.S. lesions. The animal and human autopsy specimens exhibited similar patterns of myelin breakdown, with transformation of lamel-
lar myelin sheaths to a characteristic pattern of vesiculated networks. In both EAE and the MS lesion samples, a zone of myelin vacuolization was noted at the edge of the lesions; within the lesions, the myelin sheaths were transformed into vesiculated membranous networks that were separated from axons by processes of macrophages. Using immunogold labeling of antigenic peptides applied to sections to recognize antibodies to the peptides, autoantibodies to myelin antigens were identified in the lesions in tissue samples from EAE and MS (Figure 2) and were associated with the vesiculated myelin networks. In the human MS samples, autoantibodies to both MOG and myelin basic protein were detected in the lesion tissue, suggesting that more than one antigen may be involved in the autoimmune response that culminates in the demyelination of MS. These results suggest that early lesion formation is the result of immunoglobulin-mediated destruction of myelin. Other mediators (e.g., proteolytic enzymes, cytokines) may be more important in older established lesions.

THE RESOLVING LESION

The resolving lesion is characterized by a clear boundary. Staining for CD45 reveals the presence of immune cells at the lesion edge. Remyelinated lesions, however, exhibit no evidence of immunoreactivity, which suggests that remyelination may occur if immune cells are not present. The lesion is bordered by a sharp layer of actively dividing microglial cells, suggesting that the microglia contain the lesion and serve to create its sharp edge (Figure 3). The microglia may be beneficial in the resolving lesion by containing the lesion, although these same cells may have a deleterious effect in active lesions.

THE SECONDARY PROGRESSIVE LESION

Staining for CD phenotype in a secondary progressive lesion usually identifies a predominance of CD8+ cells, which are both perivascular and, more commonly, distributed throughout the parenchyma. The edge is less clearly defined, with an irregular appearance and sometimes vacuolated profiles. There are few oligodendrocytes and little myelin. In some lesions, what initially appears as myelin vacuolization appears at higher magnification to be vacuolated oligodendrocytes undergoing cytolysis (Figure 4). The predominance of the CD8+ infiltrates supports the cytolytic loss of oligodendrocytes from the
edge of the secondary progressive MS lesion. Lymphocytes are seen returning to the Virchow-Robin space, leaving the CNS and returning to the bloodstream. The edge of the lesion is a line of microglia, which can occasionally be seen absorbing myelin debris. Reactive microglia are also present along stretches of myelinated axons where the myelin sheath is being broken down. Electron microscopy confirms the presence of vacuolated oligodendrocytes. Apoptosis is either not evident or is very unusual in oligodendrocytes in these lesions, suggesting a cytolytic mechanism for the oligodendrocyte loss.

The Chronic Active Lesion

The chronic active MS lesion appears to be influenced primarily by astrocytes rather than microglial cells. These lesions are characterized by an indistinct edge, with recent infiltrates at perivascular sites and at the lesion edge. At higher magnification, the edge of the lesion is often demarcated by the presence of hypertrophic astrocytes, and in association with these cells there is extensive oligodendroglial hyperplasia (Figure 5). These oligodendrocytes stain for myelin antigens, but they also stain for the markers O4, NG2, and PDGF-alpha. Thus, they have markers for both adult and immature oligodendrocytes. There is very little remyelination, which is surprising because of the large number of premyelinating oligodendrocytes present. Microscopic studies of chronic MS lesions, however, have noted that these premyelinating oligodendrocytes associate with axons but do not myelinate them, raising the possibility that inhibitory signals produced within the lesion prevent the remyelination of axons by oligodendrocytes.

Recent research suggests astrocytes are particularly important in maintaining demyelination and suppressing remyelination by oligodendrocytes in chronic MS lesions. One pathway that appears to be important in this process is the activation of Notch1 receptors, which are located on premyelinating oligodendrocyte precursors, by the astrocyte-derived ligand Jagged1. The activation of Notch1 receptors by Jagged1 induces the downstream molecule Hes5, which inhibits the maturation of oligodendrocytes. The role of the Hes5 signaling pathway was examined in a study of the adult CNS in multiple sclerosis. The incubation of human astrocytes in vitro with transforming growth factor (TGF)-beta-1, a cytokine that is upregulated in MS, was associated with the induction of Jagged1 on reactive astrocytes. Jagged1 was not upregulated by interleukin-1 or interferon-gamma. In autopsy samples from 10 patients with MS, Jagged1 was also found in hypertrophic astrocytes in high concentrations within MS lesions and in the surrounding tissue, and Notch1 and Hes5 were found in cells that resembled immature oligodendrocytes. TGF-beta-1 was evident in the extracellular matrix surrounding the blood vessels. Although Jagged1 was increased in active lesions, no Jagged1 was observed in remyelinated lesions. Thus, within the MS lesion, high levels of TGF-beta-1 may stimulate the production of the Jagged1 ligand by astrocytes, which suppress maturation by activation of the Hes5 pathway within oligodendrocytes. This pathway may provide a potential therapeutic target for improving remyelination in MS.

Glutamate Excitotoxicity in MS

The excitatory neurotransmitter glutamate is one of the most common neurotransmitters in the CNS. Under some circumstances extracellular glutamate can injure neurons and produce neurodegeneration. Glutamate is produced in large quantities by activated leukocytes as the result of the enzymatic conversion of glutamine, and in inflammation there is also a decrease in glutamate removal by astrocytes. The enzymes that are most important in the regulation of extracellular glutamate concentration are glutaminase, which pro-

Figure 5. Chronic Active MS Lesion

Along the margin of this lesion is a zone comprising hypertrophic astrocytes (large pale blue cells), accompanied by many smaller, rounder cells (hypertrophic oligodendrocytes). x1000.
duces glutamate from glutamine; and the enzymes glutamate dehydrogenase and glutamine synthetase, which inactivate glutamate. Two types of glutamate receptors that directly regulate the permeability of the neuron cell membrane to extracellular cations have been identified in the CNS: N-methyl-D-aspartate-sensitive receptors and AMPA/kainate receptors. Some evidence suggests that the AMPA/kainate receptors may be particularly important in the mechanisms that produce degeneration in a number of neurologic conditions, including MS. For example, Pitt and colleagues examined the effects of the AMPA receptor antagonist NBQX in a mouse model of EAE. Mice were sensitized to white-matter antigens and were treated with either NBQX (3 subcutaneous injections of 300 µg) or vehicle. NBQX treatment reduced the severity of disease, increased the survival of oligodendrocytes, and reduced the abnormal dephosphorylation of heavy-chain neurofilament (neurofilament H), a marker of axonal damage (Figure 6). NBQX did not affect inflammation or T-cell function. This study suggested that glutamate toxicity is an important mechanism of oligodendrocyte loss and neuronal injury that occurs in autoimmune-mediated demyelination similar to that occurring in MS, and that glutamate receptor antagonists may provide one means of preventing axonal injury.

A subsequent study examined several of the factors that are important in glutamate regulation in tissue specimens from patients with MS. Tissue from patients and white matter control tissue was stained using markers that identified sources of glutamate production (glutaminase enzyme), glutamate transporters, glutamate metabolism (glutamate dehydrogenase and glutamine synthetase), as well as axonal damage and cell types. In active lesions, high concentrations of glutaminase were identified in macrophages and microglia close to dystrophic axons. Glutaminase was also noted in white matter samples obtained in other inflammatory conditions but not in samples from noninflammatory controls. Enzymes that remove glutamate (GS and GDH) were found at low levels in active and chronic silent MS lesions, and one subtype of glutamate transporter was also found in abnormally low numbers. The loss of GDH and GS enzymes from the lesion area appeared to be related to the loss of oligodendrocytes. These findings suggest abnormalities in the production and removal of glutamate.

Figure 6. Glutamate Excitotoxicity in Mouse EAE

Note the large lesion in an anterior column of the lumbar spinal cord in the vehicle-treated EAE mouse (upper left), which is intensely positive for glutaminase immunoreactivity (dark blue). The presence of this enzyme denotes the presence of glutamate production by macrophages. In the lower panel, higher magnification shows many damaged, dystrophic axons that are positively stained for SM132. In EAE mice treated with an inhibitor of AMPA/kainate receptors (NBQX), although there is still an abundance of glutaminase immunoreactivity (blue), there is great reduction in the number and size of SM1-positive dystrophic axons. x1000.

EAE = experimental allergic encephalomyelitis.
glutamate within MS lesions resulting in higher than normal glutamate concentrations.

Another study examined the uptake of glutamate by oligodendrocytes. Several high-affinity glutamate transporters have been identified. This study examined the relative importance of astrocytes and oligodendrocytes in white matter on glutamate uptake in human tissue and in cultured fetal human oligodendrocytes. In fetal cultured cells, glutamate transporters (EAAT-1 and EAAT-2) were detected on nearly all mature oligodendrocytes (but not on immature oligodendrocytes) and glutamate uptake by these cells was observed. Glutamate uptake by these cells was inhibited by the proinflammatory cytokine TNF-alpha. In autopsy samples from patients with MS, glutamate transporters were largely absent from the central portion of the lesions where oligodendrocytes were depleted. This confirms findings from previous studies that oligodendrocytes are important in the removal of glutamate and that abnormalities in the processing of glutamate by these cells may be important in MS.

**SUMMARY AND CONCLUSIONS**

The results of studies that have examined glutamate synthesis and metabolism in MS suggest the model shown in Figure 7. Macrophages in the active MS lesion produce excess extracellular glutamate. Glutamate is absorbed and inactivated by oligodendrocytes, and the uptake of glutamate is impeded by products from T-cells such as reactive oxygen species, nitric oxide, or TNF-alpha. Glutamate uptake is also inhibited during oxidative stress. These decrease the ability of the glutamate transporter on the oligodendrocyte to take up glutamate, which increases oligodendrocyte damage. Extracellular glutamate activates AMPA/kainate glutamate receptors (which are present on the oligodendrocytes and also present on the axon and on the neuron), producing excitotoxicity of axonal components as well as of the oligodendrocyte, due to excessive calcium influx. Studies of experimental animal models suggest that therapeutic approaches that target glutamate excitotoxicity (e.g., blocking AMPA/kainate receptors) may reduce the demyelination and neurodegeneration typical of the MS lesion.

**REFERENCES**