Multiple sclerosis (MS) has traditionally been viewed as an autoimmune disease in which autoreactive CD4+ T lymphocytes migrate from the periphery to the central nervous system and initiate an immune response against components of myelin. Acute MS attacks were attributed to active inflammation, and progression of disability was generally believed to reflect chronic demyelination. Recent research has demonstrated that the pathophysiology of MS is more complex than was appreciated in the past and that the development of the acute MS lesion includes the participation of a large number of cell populations, including CD8+ T lymphocytes, B cells, Th17 cells (a population of helper T cells that secrete the inflammatory cytokine interleukin 17), macrophages/microglia, and astrocytes. Progressive MS is now thought to reflect axonal transection and degeneration, a process that begins at the earliest stages of MS and continues to evolve even in chronic inactive plaques. Specific cellular and molecular interactions have been identified that suppress the regeneration of transected axons within the adult central nervous system and prevent remyelination of MS plaques by oligodendrocyte precursors. Neuroimaging techniques and biologic disease markers are being developed for MS diagnosis and treatment, and for the study of the pathophysiologic processes that cause inflammation and demyelination. The identification of novel physiologic pathways that contribute to the clinical phenomena of MS will permit the development of new therapeutic strategies to prevent MS relapses and disease progression.

The clinical signs and symptoms of multiple sclerosis (MS) reflect a complex pathophysiology that includes central nervous system (CNS) inflammation, demyelination, and neurodegeneration. MS relapses are generally thought to reflect transitory inflammatory demyelination of the CNS, whereas the gradual progression of the disease has been attributed to processes that include chronic demyelination, gliosis, and gradually accumulating axonal loss.

Considerable recent research has helped to define how interactions among many different cell populations, cytokines, and receptors contribute to the demyelination and axon loss that are the hallmarks of the disease. New biochemical and neuroimaging techniques are helping to further characterize the pathophysiologic changes that occur in MS and to identify potential therapeutic strategies.

**Pathogenesis of MS: Inflammation and Axonal Loss**

Multiple sclerosis has generally been viewed as an autoimmune disease in which demyelinating lesions develop when myelin-specific T lymphocytes migrate from the periphery into the CNS. These peripherally activated T cells express cell-surface adhesion molecules that interact with ligands on vascular endothelial cells, cross the blood-brain barrier, and produce an inflammatory response that is directed against myelin epitopes. Peripheral T cells that are reactive to myelin components are common among healthy subjects, but they do not become activated or migrate into the CNS. Among individuals with MS, myelin-reactive T cells are more likely to exhibit a memory rather than a naïve phenotype, and they are more likely to secrete interferon (IFN) β or other inflammatory cytokines.

The precise steps that cause these self-reactive T cells to become activated among individuals with MS are not well understood, but may involve a process of mol-
ecular mimicry in which viral or bacterial antigens that structurally resemble myelin components are processed by antigen-presenting cells (APCs) and displayed to T lymphocytes, triggering an immune response against myelin. Although demyelination has traditionally been thought to occur primarily in white matter, gray matter lesions are becoming increasingly recognized with newer imaging techniques and higher field strength magnetic resonance imaging (MRI).

For many years, the pathogenesis of MS was generally attributed to the infiltration of CD4+ T cells into the CNS. This view was largely due to similarities between MS and CD4-mediated CNS inflammation that has been produced in experimental animal models (experimental autoimmune encephalomyelitis). Recent research has shown that many other populations of immune cells participate in the development of MS lesions. Involvement of CD8+ T cells in the pathogenesis of MS has been suggested by several lines of evidence, including the presence of CD8+ cells in inflammatory CNS lesions, increased numbers of CD8+ cells that recognize myelin in patients with MS, and a potential role of CD8+ T cells in increasing permeability of the blood-brain barrier. B lymphocytes have also increasingly been recognized as important in MS. Growth factors that stimulate B-cell survival may promote the loss of self-tolerance in patients with MS and other autoimmune diseases, and antibodies secreted by B cells may contribute to myelin loss. The importance of B cells in MS is also supported by the results of recent clinical studies in which rituximab, a monoclonal antibody directed against the B lymphocyte CD20 cell-surface marker, produced B-cell depletion and improved outcomes among patients with MS. Some experts have suggested that the initial stages of new lesion formation may reflect primarily an insult to oligodendrocytes and that immune-mediated myelin destruction is a secondary process. However, evidence from studies of genetic susceptibility to MS have identified several immune-related genes that confer increased risk of MS, including genes that code for receptors for the cytokines interleukin (IL)-2 and IL-7, in addition to portions of the major histocompatibility complex. These genetic studies suggest that immune dysregulation is in fact central to the pathogenesis of MS, and is not a secondary effect of oligodendrocyte loss.

In the past, the progressive disability of MS was usually attributed to chronic demyelination. Many researchers now believe that MS progression is more closely related to axonal pathology, including axon swelling, transection, and Wallerian degeneration. Axonal loss within MS plaques may vary from approximately 20% to 80% of normal axon density in adjacent white matter, and is significantly related to disease progression. Axonal destruction in MS may occur by different mechanisms. Axons may be acutely damaged by inflammatory mediators that are released by CD8+ T lymphocytes and other immune cells. Chronic axonal injury may continue to accumulate in inactive plaques, as demyelinated axons lack neurotrophic factors that are ordinarily secreted by oligodendrocytes or other cells. Although axonal loss appears to underlie the transition from relapsing-remitting MS to progressive irreversible functional decline, it should be noted that axonal transections occur early in the course of the disease and are present in patients with a single demyelinating event suggestive of MS (clinically isolated syndromes [CIS]). Early axonal loss is generally clinically silent because of the ability of the remaining tissue to compensate for the initial stages of injury. The process of early axonal loss as a consequence of inflammatory demyelination early in the course of MS suggests that strategies that prevent demyelination may interrupt these processes, which may be one reason why early treatment is especially important. Several processes have been identified that suppress the regrowth of transected axons in the mammalian adult CNS. Nogo is a membrane protein present in CNS myelin that interacts with Nogo receptors on axons to strongly suppress neurite outgrowth. Since the initial identification of Nogo, several other molecules have been identified that prevent neurite outgrowth in the mature CNS, including myelin-associated glycoprotein and oligodendrocyte myelin glycoprotein, both of which also act at Nogo receptors. The suppression of axon regrowth by Nogo and related pathways may be part of an adaptive mechanism that prevents uncontrolled neurite outgrowth within the brain, which could result in the formation of aberrant connections. In MS, this mechanism may prevent transected axons from regrowth and repair of demyelinated tissue. Recovery from an acute MS attack does not reflect remyelination during the initial stages, but rather occurs as a result of the redistribution of sodium channels along chronically demyelinated axons, permitting some
degree of restoration of neuronal conduction (Figure).\textsuperscript{13}

Recent research also has focused on the role of astrocytes in the pathogenesis of MS. As noted previously, oligodendrocytes provide important trophic support to axons, and therefore, the loss of oligodendrocytes may indirectly contribute to axonal degeneration.\textsuperscript{3} One common feature of the MS lesion is a well-defined border, which is only partially penetrable by oligodendrocyte precursor cells.\textsuperscript{1} Of the few oligodendrocyte precursors that are able to enter the lesion, only a small proportion effectively remyelinate axons.\textsuperscript{18,19} Some investigators have argued that astrocytes may be at least partly responsible for the limited remyelination of MS lesions. Stimulation of astrocytes by the inflammatory cytokine transforming growth factor-β results in increased release of the ligand Jagged1, which interacts with a specific oligodendrocyte cell-surface receptor (the Notch1 receptor) that is normally activated during CNS development.\textsuperscript{19} Activation of Notch1 receptors suppresses oligodendrocyte maturation and reduces remyelination.\textsuperscript{19} Thus, the development of an astroglial scar creates an environment that prevents oligodendrocyte precursors

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**Figure. Demyelination and Axon Degeneration in MS**

- **Panel A**: Normal myelinated axon. Action potentials are propagated at high velocity along the axon to the postsynaptic neuron.
- **Panel B**: Acutely demyelinated axon. Loss of myelin blocks conduction of the action potential (black bar).
- **Panel C**: Chronically demyelinated axon. Conduction restored by increase in density of sodium channels along the demyelinated axon.
- **Panel D**: Degenerated axon. Conduction is permanently lost.

MS = multiple sclerosis.

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from entering and remyelinating the MS lesion, resulting in the loss of trophic support for surrounding neurons and enhanced axonal degeneration. It has also been proposed that astrocytes, rather than microglia, may be the principal APCs that stimulate the activation of self-reactive T lymphocytes. Astrocytes may also facilitate the entry of T lymphocytes into the CNS by expressing adhesion molecules that are used by T cells to migrate into the CNS, and by releasing matrix metalloproteases that break down tight junctions between vascular endothelial cells.20

Finally, recent research has focused on cytokines that regulate effector mechanisms of T cells or other cells of the immune system. Cytokines of the IL-12/IL-23 family regulate T-cell responses in autoimmune diseases, including MS. IL-23 induces the proliferation of a population of T cells that secrete IL-17 (Th17 cells), and many researchers believe that these cells are central to the pathogenesis of MS. IL-23 and IL-17 are expressed in MS lesions, and increased expansion of Th17 cells significantly enhances the infiltration of myelin-specific T cells into the CNS.21 In an animal model of MS, the suppression of Th17 lymphocytes has been shown to reduce the activity of autoreactive Th1 lymphocytes and ameliorate neurologic dysfunction.22

**NEUROIMAGING TECHNIQUES IN MS**

Conventional MRI imaging techniques are useful for the diagnosis of MS and for evaluating the effects of treatment. MRI assessment can also help to assess the likelihood of conversion to clinically definite MS in patients with CIS.24 T2 hyperintense lesions are nonspecific markers of tissue injury that may reflect several causes, including edema, astrogliosis, axonal injury, demyelination, and remyelination.25 Therefore, T2 MRI images provide little information about specific pathologic processes occurring within the CNS, and are only weakly predictive of the future course of illness.25-27

Other imaging techniques provide more specific information about the pathologic changes that occur in patients with MS.25 Gadolinium (Gd)-enhancing lesions reflect the leakage of contrast material through the cerebrovascular endothelium, which is caused in part by the migration of immune cells into the CNS. Therefore, Gd-enhancing lesions indicate regions of active inflammation and loss of the integrity of the blood-brain barrier. Chronic T1 hypointense lesions (T1 black holes) are a more specific measure of axonal swelling or loss, and are more closely correlated with disease progression than T2 lesions.25-26 Magnetization transfer MRI (MT MRI) is a technique that examines the interactions between protons in free fluid and protons that are bound to macromolecules.28 Studies conducted over the last decade have demonstrated that MT MRI is able to detect neuronal injury in normal-appearing gray and white matter, and that MT MRI lesions in these tissues often precede macroscopic lesion formation.29 Diffusion tensor MRI (DT MRI) distinguishes healthy from injured tissue based on the diffusion of water molecules.29 In patients with MS, changes in water diffusivity on DT MRI have been associated with demyelination and axonal loss in lesions present on T1 or T2 imaging and in normal-appearing white matter.29 In patients with progressing MS, diffusion abnormalities identified using DT MRI have been shown to predict the worsening of MS disability over time.30 Another technique, proton magnetic resonance spectroscopy, uses the spectra of magnetic resonance signals to quantify several specific chemical compounds, including choline, creatine, lactate, and N-acetylaspartate (NAA, a marker of axonal integrity) in MS lesions or normal-appearing tissue.31 NAA in the mature brain is found almost exclusively in neurons and neuronal processes, and NAA concentrations have been used to evaluate axonal density and to demonstrate widespread alterations in gray matter in patients with MS.31-32 Elevated choline concentrations occur in acute MS lesions as a consequence of the release of membrane phospholipids during myelin degradation.31 Optical coherence tomography (OCT) is commonly used to evaluate the size and number of retinal ganglion cells in patients with glaucoma and other retinal diseases.32 Optic neuritis in patients with MS is associated with decreased macular volume and thinning of the retinal nerve fiber axon layer, which may be quantified using OCT. Macular volume loss is especially pronounced in patients with secondary progressive MS.33 OCT has emerged as a potential marker for axonal injury in patients with MS and has been shown to correlate strongly with brain atrophy and disability.33-34

More recently, functional MRI (fMRI) has been used to assess brain function during different tasks by quantifying regional differences in the concentration of deoxyhemoglobin, which reflects blood flow and
studies using fMRI have demonstrated that patients with MS exhibit recruitment of brain areas that are not normally activated during a particular task. For example, a simple hand movement task elicits widespread activation of sensory and motor cortical regions among patients with MS that are not activated in control subjects. This recruitment of additional brain regions may provide an explanation for at least some of the fatigue that is experienced among patients with MS, as heightened brain activity may significantly increase total body energy expenditure. Another recent development has been the use of MRI magnets with higher field strength than the 1.5-Tesla (T) magnets that are used in routine medical imaging. Conventional MRI magnets detect only a small fraction of cortical gray matter lesions that are identified histopathologically. Studies that have used MRI magnets with field strengths as high as 8 T have demonstrated that many more lesions are present in the cortex than are observed with a typical 1.5-T magnet. More powerful magnets have made it possible to look at MS lesions at very high levels of magnification. One insight from these studies has been that nearly every MS lesion is penetrated by a blood vessel, which provides additional support for the hypothesis that MS is mediated by an immune process. High-power magnets have also been used to help to define changes that occur within the CNS when patients undergo treatment. For example, there have been several studies of the immune-modulating agent cladribine in which treatment produced a dramatic effect on MRI activity using conventional field strengths, yet patients continued to exhibit progression of MS. Studies that have used more powerful magnets have shown that there is probably significantly more disease activity than was previously suspected, much of which was not apparent with conventional MRI imaging. In the BECOME clinical trial, which compared the efficacy of IFNβ-1b versus glatiramer acetate (GA), an imaging study was conducted using MRI with 3-T magnets and triple-dose Gd. The investigators expected to demonstrate superiority of IFNβ-1b over GA on disease activity measured with MRI. However, imaging results obtained with the high-power magnet revealed no significant differences between the 2 treatments.

**Biological Markers of MS**

Reliable biological markers of MS severity are potentially useful for the diagnosis of MS, for assessing prognosis, or for evaluating the effects of therapy. Cerebrospinal fluid (CSF) markers, such as oligoclonal bands or immunoglobulin G index, are often present in patients with MS but also occur in patients with other inflammatory CNS conditions. Potential markers of disease with greater sensitivity and specificity are being evaluated in laboratory and clinical studies. As noted previously, Nogo inhibits axonal sprouting and regrowth. Several Nogo variants have been identified in different tissues: Nogo A is found primarily in the CNS, including oligodendrocytes and neurons; Nogo B is expressed throughout the body; and Nogo C is found primarily in muscle. Nogo A in CSF has been proposed as a sensitive and specific biomarker for MS, including both relapsing and progressing forms, and new and established disease. An examination of Nogo A concentrations in CSF found a soluble Nogo A fragment in samples from 110 of 114 patients with MS (96%), but in no samples obtained from more than 150 control subjects with other CNS disorders, including meningencephalomyelitis and other CNS autoimmune diseases. The utility of Nogo A as a biomarker for MS awaits independent confirmation.

Studies of the molecular changes that occur in MS may also lead to the rational design of new treatments. Currently, natalizumab, which binds to the α4 integrin receptor on lymphocytes, thereby preventing their entry into the CNS, is the only MS therapy developed rationally from preclinical models of disease to target a specific pathologic process believed to be important in MS. Recently, a number of technical advances have enabled the study of widespread molecular alterations within MS lesions, providing important information about the nature of the immune response in individuals with MS, and about how this response differs from that in healthy subjects. This information may suggest approaches to selectively adjust immune functioning to induce a state that is more similar to that of healthy subjects. For example, a recent proteomic analysis was conducted to identify proteins unique to different types of MS lesions (ie, acute plaques, chronic active plaques, and chronic plaques). Surprisingly, this analysis identified abnormally expressed proteins that normally participate in the coagulation process (ie, protein C and tissue factor) within MS lesions. Additional experiments revealed that in addition to their effects on coagulation, the presence of activated protein C suppressed the production of inflammatory
cytokines by Th1 and Th17 lymphocytes. Studies such as this one are important because they provide the opportunity to rationally develop treatments that are based on the biological mechanisms of illness. Techniques, such as proteomics and metabolomics (the study of small molecules involved in metabolic pathways), are beginning to create the potential to identify additional potential therapeutic targets beyond cell-surface molecules or cytokines that have traditionally been the targets of drug design. Potential targets of new MS treatments include transcription factors that are important in the development of pathogenic T cells. This approach is already being used in other areas of medicine. For example, peroxisome proliferator-activated receptor γ agonists, which are used to treat type 2 diabetes, activate transcription factors to alter glucose metabolism and alleviate hyperglycemia.11

CONCLUSIONS

Understanding of the pathogenesis of MS has expanded considerably in the past few years. A growing body of research has described how a diverse population of immune cells trigger inflammatory demyelination and axon loss, and has begun to define potential mechanisms that suppress axonal regeneration and remyelination. Neuroimaging techniques are useful for the diagnosis and treatment of MS, and new techniques are being developed that reveal specific pathologic processes within the CNS. The identification of biological markers of MS may help to improve diagnostic accuracy while also suggesting potential new targets for therapeutic intervention.

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