REVIEW ARTICLE

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Multiple Sclerosis — The Plaque and Its Pathogenesis

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Substantial advances have occurred in the understanding of some of the central mechanisms underlying the inflammation, demyelination, and neurodegeneration that occur in multiple sclerosis since the topic was last reviewed in the *Journal*.[1] Accordingly, the available clinical strategies for the management of the disease have widened (Table 1).[2] However, the treatment options for the disease are most effective during the relapsing–remitting phase (relapsing–remitting multiple sclerosis), which is characterized by clinical exacerbations, inflammation, and evidence of plaques within the brain and spinal cord on magnetic resonance imaging (MRI). Less understood are factors that promote the transition from relapsing–remitting multiple sclerosis to treatment-resistant secondary progressive multiple sclerosis. Evidence now suggests that neurodegenerative mechanisms within the disease plaques constitute the pathologic substrate for the latter disabling phase.[3-5] Effector mechanisms that underlie the relapsing inflammatory and the progressive neurodegenerative phases of multiple sclerosis appear to be distinctly different.

This review focuses on the current knowledge of the pathogenesis of the inflammatory and neurodegenerative elements of the multiple sclerosis plaque.

Evolution of the Multiple Sclerosis Plaque

A central mission in multiple sclerosis research has been to determine the sequence of events underlying the development of the inflammatory plaque. It is generally held that this histopathological hallmark originates from a breach in the integrity of the blood–brain barrier in a person who is genetically predisposed to the disease. One hypothesis suggests that some forms of systemic infection may cause the up-regulation of adhesion molecules on the endothelium of the brain and spinal cord, allowing leukocytes to home to and traverse vessel walls to enter the normally immunologically privileged central nervous system. If lymphocytes programmed to recognize myelin antigen exist within the cell infiltrate, they may trigger a cascade of events resulting in the formation of an acute inflammatory, demyelinating lesion.[6] These lesions typically develop in white matter, where the primary targets are the myelin sheath and the myelinating cell, the oligodendrocyte (Fig. 1). However, gray-matter lesions, in which the primary target is also myelin, are known to occur.[7]

Cells Involved in the Pathogenesis of the Multiple Sclerosis Plaque

T Cells

Studies of animal models demonstrating that autoreactive T cells (CD4+ or CD8+) can result in inflammatory demyelination of the central nervous system support the

Table 1. Treatment Options for Multiple Sclerosis.*

Status	Treatment	Suggested Mechanism of Action	Uses and Range of Effects	Forms of Multiple Sclerosis Affected
Approved by the Food and Drug Administration	Interferon beta	Inhibits adhesion Inhibits synthesis and transport of MMPs Blocks antigen presentation	Treatment of relapses Slows progression Reduces lesions seen on MRI and brain atrophy Potential cognitive benefit	Relapsing
	Glatiramer acetate	Increases regulatory T cells Suppresses inflammatory cytokines Blocks antigen presentation	Treatment of relapses Reduces lesions seen on MRI	Relapsing–remitting
	Mitoxantrone	Reduces Th1 cytokines Eliminates lymphocytes	Treatment of relapses Reduces lesions seen on MRI Slows progression	Relapsing–remitting Secondary progressive Progressive relapsing
Possible adjunctive therapy	Corticosteroids (intravenous or oral formulations)	Inhibit synthesis and transport of MMPs Alter cytokine profile Reduce CNS edema	Treatment and prevention of relapses	Relapsing
	Azathioprine	Inhibits purine synthesis, affecting B cells, T cells, and macrophages	Treatment of relapses Slows progression	Relapsing–remitting Secondary progressive
	Methotrexate	Acts as folate antagonist, affecting DNA synthesis in immune cells	Slows progression	Secondary progressive
	Plasma exchange	Removes deleterious antibodies	Treatment of relapse	Relapsing
	Intravenous immune globulin	Has antiidiotypic effects Blocks Fc receptors Alters cytokine profile	Treatment and prevention of relapses	Relapsing

* This table is adapted from Goodin et al.[2] MMPs denotes matrix metalloproteinases, MRI magnetic resonance imaging, Th1 type 1 helper T cells, and CNS central nervous system. Natalizumab had been approved by the FDA for treatment of multiple sclerosis but was withdrawn from the market in February 2005, to allow assessment of the risk of progressive multifocal leukoencephalopathy.

theory that multiple sclerosis is an immune-mediated disorder involving one or more antigens located in the myelin of the central nervous system.[8-10] Patients with multiple sclerosis and healthy persons appear to have similar numbers of T cells in peripheral blood that react to myelin. Nevertheless, these two groups have substantial qualitative differences in responses mediated by circulating mononuclear-cell populations (B cells, T cells, and macrophages). Myelin-reactive T cells from patients with multiple sclerosis exhibit a memory or activated phenotype, whereas these same antigen-specific cells in healthy persons appear to have a naive phenotype.[11,12] Marked differences in the cytokines secreted and the specific chemokine receptors expressed suggest that myelin-reactive T cells from patients with multiple sclerosis are relatively more inflammatory.[13,14] Further, myelin-specific CD8+ T cells appear to be more abundant in patients with relapsing multiple sclerosis than in healthy persons or in those with secondary progressive disease.[13,15]

Perhaps the most convincing evidence that myelin-reactive T cells lead to inflammatory demyelination came from a clinical trial in which an altered peptide ligand was used as a putative disease-modifying treatment in patients with multiple sclerosis.[16] In this study, either clinical exacerbations or an increase in disease activity, as measured by MRI, unexpectedly developed in several patients treated with the ligand (a peptide developed to stimulate autoreactive T cells and render them inactive). These changes coincided with marked increases in T cells responding to a specific component of myelin basic protein (signifying immune-cell activation rather than inactivation). In contrast, in another study, a lower dose of this peptide ligand actually reduced evidence of disease activity on MRI.[17] This treatment strategy is currently being studied in a phase 2 clinical trial.

The cytokine-producing phenotype of myelin-specific T cells determines the ability of these cells to cause inflammation in the central nervous system.[13] Organ-specific autoimmune diseases such as multiple sclerosis are thought to be mediated by type 1 helper T cells (Th1) that produce interferon-γ.[9] Abundant data also sug-

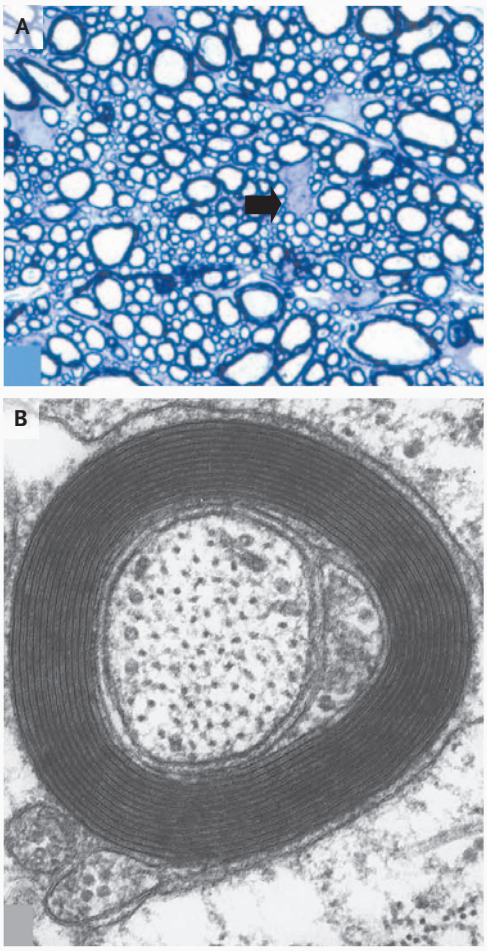

Figure 1. Cross Section of White-Matter Lesions Targeting the Myelin Sheath and Oligodendrocytes.

In Panel A, light microscopy reveals myelin sheaths (dark blue rings) around axons in a cross section of myelinated white matter (toluidine blue). Two darker-staining oligodendrocytes, the cells that make and maintain myelin, lie to the right of center (arrow). In Panel B, an electron micrograph reveals the myelin sheath in cross section to be a spirally wrapped membrane beginning in the lower left as an outer (oligodendroglial) "tongue" of cytoplasm and spiraling counterclockwise to terminate at an inner tongue inside the myelin sheath to the right of the axon. Microtubules and neurofilaments can be seen cut in cross section within the axoplasm.

gest that inflammatory immune responses or delayed hypersensitivity responses are primarily mediated by inflammatory Th1 cells, which produce lymphotoxin and interferon-γ, but little interleukin-4.[18] Alternatively, CD4+ type 2 helper T cells (Th2) represent an antiinflammatory population of lymphocytes that produce large amounts of immunoregulatory cytokines (e.g., interleukin-4 and interleukin-5). Myelin-reactive T cells from patients with multiple sclerosis produce cytokines more consistent with a Th1-mediated response, whereas myelin-reactive T cells from healthy persons are more likely to produce cytokines that characterize a Th2-mediated response.[13] Cytokines such as interleukin-12 and type 1 interferons such as interferon-β can activate the transcription factor Stat-4 in human T cells, thus causing the cells to differentiate into pathogenic Th1 lymphocytes.[19] Interferon beta, which has been used to treat patients with multiple sclerosis (Table 1), was thought to cause a shift from a Th1-mediated to a Th2-mediated response.[20] However, microarray studies indicated that a number of genes in patients with multiple sclerosis that are upregulated by this cytokine are associated with differentiation into Th1 rather than Th2 lymphocytes, suggesting that such a shift may not be the mechanism of action of interferon beta.[21]

Certain members of the interleukin-12 family of proteins probably have a role in the regulation of T-cell responses that have potential relevance to multiple sclerosis.[22] In experimental models of inflammatory demyelination, such as experimental autoimmune encephalomyelitis in mice, the likelihood of disease development depends on which interleukins are functional. For example, experimental autoimmune encephalomyelitis did not develop in mice deficient in both interleukin-12 and interleukin-23, but severe disease developed in animals with a deficiency of interleukin-12 alone. Other studies indicate that interleukin-23 probably has an essential role in brain inflammation.[23] For instance, interleukin-23–deficient mice are resistant to experimental autoimmune encephalomyelitis but have a normal Th1 response.[24] Such studies in mice may be directly relevant to patients with multiple sclerosis. Interleukin-23 causes T cells to produce interleukin-17, which some investigators believe is the chief determinant of brain inflammation, rather than interferon-γ. Recent microarray studies of lesions of multiple sclerosis from patients demonstrated increased expression of interleukin-17, suggesting that it may be an important factor in the development of inflammatory demyelination.[25] Studies of experimental autoimmune encephalomyelitis in mice have recently shown that the T-bet and Stat-4 (necessary for Th1 differentiation) transcription factors are important in the differentiation of autoimmune T cells.[26-28] Studies in

humans are now needed to determine whether similar transcriptional programs determine the mechanism underlying the pathogenic potential of myelin-reactive T cells in multiple sclerosis.

B CELLS

It has long been recognized that intrathecal synthesis of immunoglobulins is increased in patients with multiple sclerosis, as evidenced by the presence of oligoclonal bands on agarose-gel electrophoresis and an increased IgG index or synthesis rate. Many studies have suggested that these antibodies recognize myelin antigens, but only recently has it become possible to characterize the antibody response on a molecular level in the cerebrospinal fluid of patients with multiple sclerosis. Perhaps not surprisingly, the demonstration in the cerebrospinal fluid of B-cell proliferation and increased mutations in B-cell receptors, a process called somatic hypermutation, suggest that a B-cell response to a specific antigen is occurring in the central nervous system, whereas corresponding clones are absent from the peripheral circulation.[29,30] Examination of these B-cell clones also indicated that some B cells had undergone a process called receptor revision, or editing, in which these cells recognize the body's misguided capability to manufacture autoantibodies and subsequently remove this capacity.[31] Investigations are now under way to determine which specific central nervous system antigens are recognized by the autoantibodies generated by clonally expanded B cells in patients with multiple sclerosis. The observed overexpression of immunoglobulin genes and Fc receptors in lesions of this disease suggests that targeting the B-cell component of the immune response (e.g., with rituximab) may represent an attractive therapeutic strategy (Table 2).

OTHER IMMUNE CELLS

It is likely that still other types of cells play a role in the pathogenesis of multiple sclerosis. For example, regulatory cells, such as CD4+/CD25+ and CD8+ regulatory T cells, appear to be deficient in patients with this disease.[32,33] Glatiramer acetate, a treatment for multiple sclerosis that may increase the numbers of these regulatory cells, may provide a means of reconstituting tolerance to self-antigens (Table 1).

DISEASE INITIATION AND PATHOGENESIS

There is substantial evidence to support the hypothesis that genetics has an important role in a person's susceptibility to multiple sclerosis, prob-

Table 2. Neuroprotective and Restorative Strategies for Multiple Sclerosis.*

Strategy	Rationale or Mechanism	Preliminary Observations
Combinations of approved agents	Targets multiple injury mechanisms	Evidence of reduced activity on MRI Reduced relapses
Rituximab	Depletes B cells	Clinical trials under way
Chemokine-receptor antagonists	Reduce entry of lymphocytes into CNS	Clinical trials under way
Riluzole	Blocks *N*-methyl-D-aspartate and sodium channels	Reduced spinal-cord atrophy Reduced number of hypointense lesions on T_1-weighted MRI
Phenytoin and flecainide	Block sodium channels	Neuroprotective in animals Clinical trials under way
Blockers of neurite outgrowth inhibitor	Promote axonal sprouting	Studies in animals under way
Blockers of NG2, LINGO-1, Notch, and Jagged	Promote oligodendrocyte differentiation	Studies in animals under way
Activation of oligodendrite transcription factor 1	Promotes oligodendrocyte differentiation	Under development
Stem cells	Initiate myelin repair	Established efficacy in animal models Early trials in humans under way
Growth factors	Promote neuronal survival	Under development
Antiapoptosis factors	Promote survival of neurons and oligodendrocytes	Studies in animals under way

* CNS denotes central nervous system, NG2 neuron-glia antigen 2, and LINGO-1 leucine-rich–repeat and immunoglobulin-domain–containing neurite outgrowth inhibitor receptor–interacting protein 1.

ably in conjunction with environmental factors. Although some investigators argue for a direct causal link between various infectious agents and this disorder, such agents may merely provide the appropriate milieu for the development of an autoreactive immune response directed against central nervous system myelin. Recent work in experimental autoimmune encephalomyelitis has focused on pathogens that can stimulate toll-like receptors, highly conserved receptors that recognize pathogen-associated molecular patterns. These patterns are important for the initiation of disease and the production of interleukins, specifically interleukin-12 and interleukin-23, which lead to the differentiation of T cells into autoreactive effectors.[34,35] Infectious agents may also have a role in the central mechanism that culminates in the interaction between T cells and the cerebrovascular endothelium by up-regulating adhesion molecules important for immune-cell recruitment into the central nervous system.[36]

Studies of experimental autoimmune encephalomyelitis in mice demonstrated the importance of T-cell expression of a family of cell-surface receptors (the integrins) that promote adhesion and transport mechanisms. Such studies led to the development of a therapeutic antagonist of integrin, natalizumab, a monoclonal antibody specifically against α_4 integrin.[37] This agent significantly reduced both clinical relapses and the formation of gadolinium-enhancing lesions in patients with multiple sclerosis.[38] Despite its early promise, the development of progressive multifocal leukoencephalopathy in a few patients receiving natalizumab in combination with interferon, or with azathioprine and infliximab, resulted in its withdrawal from the market and a halting of all clinical trials in February 2005; whether it will return to the market is unknown as of January 2006.[39-41] These observations underscore the principle that strategies interfering with the recruitment of leukocytes in the pathogenesis of multiple sclerosis may also interfere with routine immunosurveillance functions of the central nervous system.

Several additional targets for potential study and therapeutic intervention have been identified with the use of microarray techniques. For instance, this approach led to the discovery that osteopontin was overexpressed in multiple sclerosis lesions and subsequently to the finding that it has an important role in the progression of experimental autoimmune encephalomyelitis.[42]

PATHOGENESIS OF MULTIPLE SCLEROSIS LESIONS REVISITED

In the light of the current consensus that the pathogenesis of the lesions of multiple sclerosis is heterogeneous, it is not surprising that no single predominant mechanism for this disease has emerged. Indeed, with a condition that includes fulminant as well as chronic forms with such a wide-ranging phenotype, multiple pathogenetic mechanisms have been proposed.[43] In fact, the pattern of the lesions appears to be totally unpredictable; both acute and chronic cases have old as well as new lesions, illustrating the dynamic nature of the disease process. Regardless of this innate variability, the end-point chronic silent lesion (without active inflammation) is a constant and pathognomonic feature of multiple sclerosis.

NEUROPATHOLOGY

The histologic features of lesions of acute multiple sclerosis (Fig. 2A, 2B, and 2C) include an indistinct margin, hypercellularity, intense perivascular infiltration by small lymphocytes (Fig. 2D and 2E), parenchymal edema, loss of myelin and oligodendrocytes, widespread axonal damage (Fig. 2F and 2G), plasma cells, myelin-laden macrophages, hypertrophic astrocytes, and little or no astroglial scarring. Demyelination in acute lesions may be due to an antimyelin antibody–mediated phenomenon in which normal lamellar myelin is transformed into vesicular networks (Fig. 2H and 2I), coated with antimyelin oligodendrocyte glycoprotein or antimyelin basic protein immunoglobulin, and phagocytosed in the presence of complement by local macrophages.[44] Remyelination is occasionally seen.

Lesions of chronic active multiple sclerosis display a sharp edge; along the edge are perivascular cuffs of infiltrating cells, lipid-laden and myelin-laden macrophages, hypertrophic astrocytes, and some degenerating axons, and demyelination is occurring (Fig. 3A). In contrast to acute lesions, demyelination in chronic active lesions is associated with the deposition of immunoglobulin and the dissolution of myelin into droplets, which undergo phagocytosis once they become attached to macrophages.[45,46] An increase in the number of oligodendrocytes and some remyelination are not uncommon in chronic lesions. The centers of such lesions are hypocellular and contain naked axons embedded in a matrix of scarring (fibrous) astrocytes, lipid-laden macrophages,

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