Immunopathogenesis of Multiple Sclerosis

Disruption of immune tolerance, inflammation, and blood-brain–barrier breach lead to demyelination and axonal injury.

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Of presumed autoimmune origin, multiple sclerosis (MS) is a chronic acquired inflammatory disorder of the central nervous system (CNS). Like most autoimmune disorders, MS is more prevalent in women and has a typical onset between the second and fifth decades. Although the etiology and pathogenesis remain largely unknown, MS is recognized as a consequence of complex host genetic and environmental factors resulting in immune-mediated CNS injury. Although many clinical subtypes are defined, biologically there are only 2 forms, relapsing and progressive. The immune system mediates much of the early injury and accounts for mechanisms underlying clinical relapses. In contrast, mechanisms of progression are less understood and may share similarities with other neurodegenerative disorders.

Pathogenesis of the Relapsing Form

The initial events leading to disruption of immune tolerance are unknown. Genome-wide association studies have identified more than 200 genes linked to MS. The HLA-DRB1*1501 allele has the strongest genetic association. In a susceptible individual, exposure to environmental factors at a critical age predisposes the person to MS. A specific pathogen may play a role, and exposure to Epstein-Barr virus has been implicated due to the higher incidence of infectious mononucleosis in patients with MS compared with the general population. Other factors implicated include vitamin D deficiency, smoking, and obesity. Recently, a pivotal role has been attributed to the gut microbiome as a site for immunotolerance disruption.

Disruption of Normal Immune Tolerance

Much of the information on immune mechanisms that mediate injury to the CNS in MS comes from studies using the animal model of MS, experimental autoimmune encephalomyelitis (EAE). In early studies of rats with EAE, adoptive transfer of T lymphocytes but not non-T–cell fractions (B cells had not been discovered at that time) could transfer disease. As a result, T lymphocytes became the focus of research in MS to the exclusion of B cells. The classic model of T-cell mediated pathogenesis starts with T-lymphocyte activation in the systemic immune compartment, presumably by an inciting environmental factor, such as a virus. Development of immunity against the agent results in autoimmunity because of shared homology between viral antigens and proteins of the CNS. Thought to be the main culprits of immune-mediated injury, T cells cross the blood-brain barrier (BBB), they are reactivated by antigen-presenting cells (APCs) expressing major histocompatibility class (MHC) II molecules (Figure). In the CNS these cells include microglia, macrophages, and B-cells. Studies in EAE have further identified the specificities of the CD4 cells. The pro-inflammatory CD4 cells belong to the Th1 or the Th17 subtypes, whereas the anti-inflammatory CD4 T cells belong to the Th2 subtype. These phenotypes are defined by the lymphokines they secrete. The Th1 cells secrete interferon γ and tumor necrosis factor-α (TNF-α) and are driven by the T-cell transcription factor TBET (initially termed T-box transcription factor). Th17 cells secrete interleukin (IL)-17 and are activated by IL23, secreted by the activated macrophages. Th2 cells are activated by the transcription factor GATA3, and secrete IL4, IL5, and IL10.

Well-designed clinical trials with negative results still have provided information about pathogenesis. Early clinical trials with human interferon γ showed deleterious effects and a possible role in mediating clinical exacerbations. When it was discovered that interferon γ knock-out mice could still develop EAE, the role of Th17 cells in mediating CNS injury became apparent. However, in a clinical trial of a monoclonal antibody to a subunit of IL17 and IL23 receptors, the intervention failed in patients with MS. Regulatory T-cells (Treg), another player in a complex immune system, counteract the pathogenic effects of Th1 and Th17 cells. Early treatments for persons with MS, using immunomodulators such as interferon β and glatiramer acetate, are postulated to affect the proposed immune mechanism described in favor of a Th2 response. Although exact mechanisms of the drugs remain unclear, clini-
cal effects on relapse rate reduction and decrease in enhancing lesions support an immunomodulatory role in MS.6,9

It was not until the unexpected success of rituximab, an antibody to CD20, that it became clear that B cells also played a significant role in the pathogenesis of MS.10 How B cells contribute to the pathogenesis of MS is largely unknown, however abundant evidence supports their role. Intrathecal IgG synthesis and presence of oligoclonal immunoglobulin bands in the CSF are found in over 85% of patients with MS. Evidence suggests antibodies with activated complement may contribute to myelin injury.11 Reduced antibody production by intrathecal B cells, however, does not appear to be the mechanism of action for anti-CD20 therapy because plasma cells do not express CD20, and penetration of monoclonal antibodies to CD20 into the intrathecal space is very limited. Outside the CNS, programmed B cells enhance autoreactive T-cell expansion by promoting antigen presentation to T cells, currently thought to be the main mechanism of action of anti-CD20 therapy—breaking this cycle to resume immunotolerance.

Histopathological studies have shown the presence of ectopic B-cell follicles containing germinal centers in the cerebral meninges of patients with MS.12 These follicles have been
Implicated in pathogenesis of cerebral cortical plaques. Shared B-cell clones also have been identified both in the CNS as well as draining cervical lymph nodes (LNs). Most of the founding clones in cervical LNs indicate a bidirectional exchange of B cells between the CNS and the periphery and implicate B cells as the driving factor perpetuating disease activity.  

Myelin oligodendrocyte glycoprotein (MOG) induces disease in animals with EAE, but a role in patients with MS is less clear. The pattern of MOG-antibody associated disease is similar pathologically to a specific clinical subtype of MS (pattern II). However, clinically MOG antibody-associated disease is more similar to neuromyelitis optica (NMO) and may represent a different disease entity.  

**Autoimmune Adjuvant in CNS and Blood–Brain Barrier (BBB) Breach**

The BBB is composed of the tight junctions between the post capillary endothelial cells, the astrocyte foot processes, and the extracellular matrix (ECM). All immune cells that traffic to the brain first interact with the postcapillary venular endothelium where cells are attached via interaction of very late antigen-4 (VLA-4; α4β1 integrin) molecules on the mononuclear cells with vascular cell adhesion protein 1 (VCAM1). Cells are then internalized by the endothelium and extruded to the nonluminal perivascular space where they come in contact with the ECM. By secretion of matrix metalloproteinase 9 (MMP9), breakdown of ECM follows, and cells reach the CNS compartment. Breakdown of ECM is commensurate with breakdown of the BBB. Inflammation occurs as immune cells cross into the CNS across a leaky BBB. This is visualized clinically as gadolinium-enhancing lesions on conventional MRI. Interferon β and minocycline decrease production and function of MMPs and favorably influence relapses in MS. The importance of this early step is also highlighted by natalizumab efficacy for reducing clinical relapses and new lesion accumulation. Natalizumab is a humanized monoclonal antibody against the α4-integrin subunit of VLA-4 that blocks VLA-4 binding with VCAM-1 to reduce immune cell entrance, breakdown of the BBB, and overall CNS inflammation. Although BBB breach is generally attributed to T-cell–mediated mechanisms, recent studies in NMO identify mechanisms of BBB disruption that are not cell mediated. Antibodies to glucose regulated protein 78 (GRP-78), a heat-shock protein were shown to cause a BBB breach in NMO without involvement of T lymphocytes. Whether such mechanisms exist in MS is not clear, but pathologic studies suggest that complement activation by antibodies can be the initiating event in MS before any T cells are identified at the site of injury.

**Demyelination and Axonal Injury**

Demyelination is the hallmark of MS but the mechanisms of injury remain poorly understood. Class 1 or 2 histo-compatibility antigens are not expressed on myelin, and therefore direct T-cell–mediated injury to myelin cannot occur. Much of the myelin injury results from antibody production, complement activation, and the subsequent removal of myelin by activated macrophages. Vesiculation of myelin is the initial event leading to myelin phagocytosis by macrophages. Lymphokines are toxic to myelin as well as oligodendrocytes, especially TNF-α. Focal demyelination occurs not only in white matter but also in gray matter of the cerebral cortex and the deep gray nuclei. This can occur as an extension of subcortical white matter plaque known as a leukocortical plaque. Intracortically, perivascular CD3- and CD8-reactive cells were identified in over 75% of plaques when such examination was possible from early brain biopsies. Subpial demyelination in relation to meningeal B-cell follicles was found to span across gyri. MS was the focus of MS research for many years, because axonal injury was considered only as a consequence of demyelination. It was not until the elegant demonstration of axonal transection in early MS lesions with meticulous confocal microscopy that the importance of early axonal injury became evident. In acute early MS lesions, as many as 11,000 axons per mm² were severed. Axonal injury can occur directly by CD8+-cytotoxic lymphocytes because Class 1 antigens are expressed on axons especially at the nodes of Ranvier. Primary axonal injury by T cells may be an important mechanism of axonal injury not secondary to demyelination. In clinical practice, conventional MRI readily detects focal demyelination as increased T2/fluid-attenuated inversion recovery (FLAIR) signal in lesions; in contrast, axonal injury is only seen late in the process, when “black holes” emerge on T1 imaging. Reversible axonal injury or irreversible axonal transection can be detected by spectroscopy early, in normal appearing white matter using N-acetyl-aspartate, a marker produced exclusively in neuronal mitochondria. Brain atrophy, which is accelerated in MS, is a reflection of cortical neuron loss and white matter axon and myelin loss. Microglia are the resident macrophages in the CNS and are part of the innate immune system. They serve as the initial APCs when immune cells cross the BBB. The expansion of these immune cells orchestrated by the microglia results in the initial injury causing the acute plaque. These proinflammatory microglia, termed M1 type, secrete a number of proinflammatory cytokines as well as nitric oxide, and function under anerobic conditions because mitochondrial functions are dampened by nitric oxide in the inflammatory milieu. During repair, arginine-rich M2 microglia become active and activate the Krebs cycle, promoting remyelination and repair. Microglial activation, especially the M1 type that is a hallmark of progressive MS, is a phenomenon characteristic of a variety of neurodegenerative disorders including Alzheimer’s and Parkinson’s disease.
Mechanisms of Remission

The molecular mechanisms mediating functional recovery are also poorly understood. Clinical recovery is seldom a consequence of remyelination, which occurs poorly in the CNS. If axons are spared, during recovery Na⁺ channels realign and occupy the demyelinated internode, which probably requires synthesis of new Na⁺ channels. Equally important in recovery is the closure of axonal K⁺ channels that have been laid open following demyelination. This closure helps to restore resting membrane potential that allows the segment of axolemma to become once again, excitable.25

Pathogenesis of the Progressive Form

Progressive MS is best defined as a decline in neurologic function in the absence of exacerbations.26 Primary progressive MS (PPMS) begins with progression without relapses and is experienced by 10% to 15% of all patients with MS. Affecting men and women equally, PPMS is a disorder that has a later onset. Spinal cord involvement is common, sometimes with no or nearly no brain lesions on MRI. When progression occurs in a person whose disease course previously included relapses, the disorder is described as secondary progressive MS (SPMS).

Although relapsing-remitting MS and both forms of progressive MS seem to lie on a clinical spectrum, there are clear differences in the predominant underlying mechanisms of relapsing vs progressive disease.27 Years of clinical trials also support the distinction between relapsing and progressive forms, as most treatments are beneficial for relapsing but not progressive MS. Individuals with progressive disease tend to have fewer gadolinium-enhancing lesions, probably because immune-mediated injury is not the main mechanism of injury.28 Pathologically, abnormal proliferation of microglia occurs; and inflammatory cells, particularly T lymphocytes are few, especially where cortical lesions are evident. Cortical involvement is more prominent in progressive disease, most notably in the subpial cortical layers occurring near areas of meningeal immune cell collections.29 Resident CNS cells including activated microglia and astrocytes have been implicated in active demyelinating and neuronal-axonal injury through the release of toxic mediators including nitric oxide (NO), oxygen radicals, and glutamate release further driving progressive disease.30

Mitochondrial abnormalities also play a role in the pathogenesis of progression.21 Early oxidative injuries to mitochondrial DNA accumulate over time, similar to the phenomenon observed in aging. When mitochondria with defective DNA predominate in a neuron, oxidative phosphorylation is impaired resulting in a state of virtual hypoxia that impairs neuron function.30 Neurons with defective mitochondria undergo apoptosis. Over time, neuron attrition leads to accelerated brain atrophy, a hallmark of progression Mitochondrial injury is seen in other neurodegenerative diseases including stroke, Alzheimer’s disease, Parkinson’s disease, and amyotrophic lateral sclerosis, although initial triggers may vary.21