

Diverse Targets for Intervention during Inflammatory and Neurodegenerative Phases of Multiple Sclerosis

Minireview

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Multiple sclerosis (MS) is an autoimmune central nervous system (CNS) demyelinating disease that causes relapsing and chronic neurologic impairment. Recent observations have altered certain traditional concepts regarding MS pathogenesis. A greater diversity of cell types and molecules involved in MS is now evident. While remyelination can occur during the early inflammatory phase when damage may be reversible, it is impaired in the later stages, which involve axonal death. These observations have important therapeutic implications.

Multiple sclerosis (MS) is the most common CNS demyelinating disease, affecting 350,000 individuals in North America and more than 1.1 million worldwide. MS is multiphasic (Steinman, 2001). Approximately 85% of individuals begin with relapsing-remitting MS, a form characterized by attacks (exacerbations) of neurologic deficits with intervening periods of relative clinical stability. After 10 years, half of these individuals enter a secondary progressive phase, with persistent and advancing impairment. Another 10% of individuals, usually somewhat older, have an insidious “primary” progressive course from onset with few, if any, exacerbations. MS is considered to have an autoimmune basis. Women are affected at least twice as often as men, which is characteristic of other autoimmune diseases, such as rheumatoid arthritis or systemic lupus erythematosus, which also have a female gender bias.

Both genetic and environmental factors contribute to MS susceptibility. The prevalence varies between 50 to 100 per 100,000 in high-risk regions, which include the northern United States and the Scandinavian countries, to less than 5 per 100,000 in Japan and Africa. Individuals that migrate from high-risk to low-risk regions, or vice versa, after adolescence carry their native risk for contracting MS, suggesting that exposure to an environmental factor, possibly one of several different viruses, during childhood or adolescence contributes to MS susceptibility. Genetic studies have shown that the risk of developing MS is elevated 10- to 20-fold in first-degree relatives of individuals with MS and that the concordance rate among monozygotic (identical) twins is 30%–35%, but only 2%–5% in dizygotic (fraternal) twins. Chromosome 6p21 is one locus most consistently iden-

tified in MS susceptibility (Haines et al., 1996). This region contains the genes that encode the highly polymorphic human leukocyte antigen (HLA) D molecules used to present peptide antigens to CD4⁺ T cells. Individuals with the HLA-DR2 (DRB*1501, DQB*0602) allele carry a 3- to 4-fold risk for MS. Other genes within the HLA complex, including tumor necrosis factor (TNF)- α , components of the complement cascade, and myelin oligodendrocyte glycoprotein (MOG), are also involved in MS pathogenesis. However, genes outside the HLA complex also contribute to MS pathogenesis. In fact, genome-based studies of multiplex MS families (more than one family member affected) indicate that 10 to 15 chromosomal loci contribute to MS susceptibility. Multiple genes acting in concert may elevate the risk for MS.

Myelin-Specific CD4⁺ T Cells Initiate CNS Inflammation

Evidence indicates that myelin-specific T cells participate in the initial phase of MS. CD4⁺ T cells specific for either MOG, myelin basic protein (MBP), or proteolipoprotein (PLP), three candidate CNS autoantigens, can induce experimental autoimmune encephalomyelitis (EAE), an inflammatory CNS demyelinating disease model for MS. Lymphocyte activation is required for initial CNS entry. It is likely that an encounter with a microbe causes this activation, via both the innate and adaptive immune systems. Microbes containing non-coding CpG sequences trigger innate immunity and lead to secretion of proinflammatory mediators like interferon (IFN)- γ . Microbial organisms can also activate T cells via the adaptive immune system, through the T cell receptor. Microbial pathogens share many conserved structural gene and protein sequences with humans. Thus, invading organisms can activate lymphocytes that are crossreactive to self-antigens, a process called “molecular mimicry.” Epstein-Barr virus, herpesvirus 6, measles, and papilloma virus are examples of viruses that contain shared sequences with myelin proteins. MS relapses are often associated with microbial infections.

Several discrete steps are involved in CNS entry and initial inflammation (Figure 1). Activated T cells must penetrate the blood-brain barrier, which is composed of a specialized nonfenestrated capillary endothelium. Activated proinflammatory (Th1) cells secrete IFN- γ and TNF- α , cytokines that induce expression of vascular cell adhesion molecule (VCAM) on inflamed endothelium. Activated T cells upregulate very late antigen (VLA)-4, the ligand for VCAM-1, as well as other adhesion molecules (selectins) that permit binding and transmigration through the endothelium. Antibody blockade of the interaction of VLA-4 with VCAM-1 was effective in prevention and reversal of relapsing EAE. A phase II trial demonstrated that anti-VLA-4 antibodies reduced the frequency of MS relapses and the mean number and volume of new lesions on brain MRI scans (Miller et al., 2003). IFN- β , an approved treatment for relapsing-remitting MS, also prevents upregulation of VLA-4 and VCAM-1.

Before entering CNS parenchyma, activated T cells must penetrate through an extracellular matrix com-

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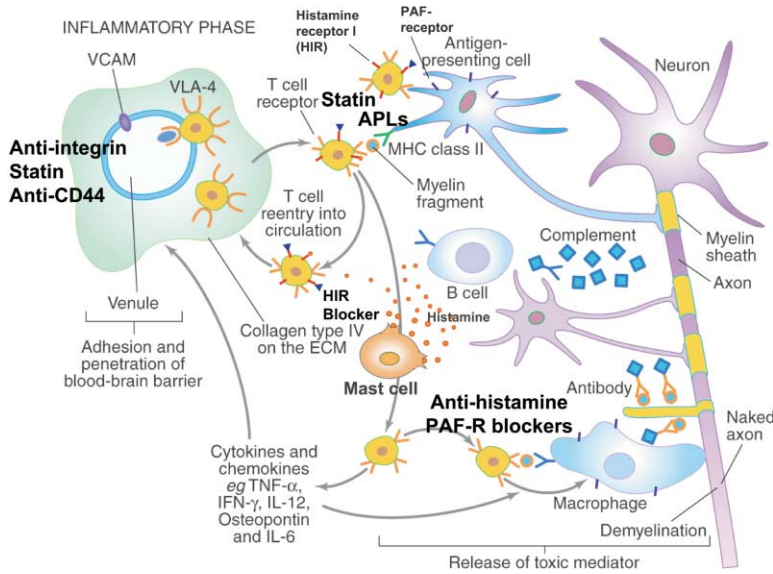


Figure 1. The Inflammatory Phase of MS

In EAE, T lymphocytes gain access to the CNS via their $\alpha 4$ integrins, recognizing VCAM on the blood vessel wall. The T cells diapedese through the endothelium, using matrix metalloproteases to cleave collagen IV in the extracellular matrix. Once within the CNS, they release inflammatory cytokines. The B cell and the mast cell also contribute to pathology. Complement plus antibody leads to activation of membrane attack complexes which attack the myelin sheath. EAE can be blocked by an $\alpha 4$ integrin antibody. Antegen, which recognizes human $\alpha 4$ integrin, has shown promise in phase II trials in MS. Statins and altered peptide ligands can interfere with the recognition of myelin fragments by inducible MHC class II in the CNS. Antihistamines and platelet activating receptor antagonists can block mast cell activity in EAE.

posed of type IV collagen. Matrix metalloproteases (MMPs) are a family of proteolytic enzymes that degrade this basement membrane. MMPs also have TNF- α convertase activity and cleave membrane-bound TNF- α to its soluble form. MMP-9 (Gelatinase B), which has an important role in basement membrane degradation, is secreted by activated, myelin-specific T cell clones from MS patients and encephalitogenic murine T cell clones. MMP-9 is also detectable in endothelial cells, pericytes, and astrocytes in MS lesions, as well as in spinal fluid from MS patients. T cell penetration of extracellular matrix can be blocked by tissue inhibitors of matrix metalloproteases (TIMPs), a family of proteins that bind and prevent MMP activity. IFN- β also inhibits MMP activity by T cells, providing another mechanism for its beneficial effects. Considerable effort is being devoted to the development and testing of other MMP inhibitors.

While lymphocyte activation facilitates CNS entry, antigen-specific recognition is not required at this stage. However, myelin recognition is required for initial CNS inflammation. Antigen recognition by CD4⁺ T cells requires expression of MHC class II molecules on antigen-presenting cells (APC), yet the normal CNS is nearly devoid of class II expression. Through secretion of IFN- γ , which is a potent stimulator of class II expression on resident CNS APC including microglia and astrocytes, it is thought that Th1 cells drive this stage of pathogenesis (Slavin et al., 2001). IFN- γ secretion is driven by osteopontin, a cytokine produced by neurons and glia in inflamed CNS (Chabas et al., 2001) (Figure 1). IFN- γ and its associated downstream genes are increased in MS lesions (Lock et al., 2002). Intact myelin proteins are not recognized by T cells but undergo proteolytic processing into peptide fragments by APC before binding MHC class II molecules and presentation to CD4⁺ T cells. It was observed that mutant mice containing APC that expressed class II molecules and could present myelin peptide but not process native (intact) myelin protein were completely resistant to EAE (Slavin et al., 2001), indicating that myelin peptides are not normally exposed in normal CNS without processing.

MMPs and other proteolytic enzymes, including cathepsins, a family of cysteine and aspartyl proteases, are secreted by microglia in response to IFN- γ activation and participate in processing of myelin proteins. Proteolytic 13-16 amino acid peptide fragments that bind MHC class II molecules on resident CNS APC are recognized by the antigen-specific T cell receptors expressed on myelin-specific CD4⁺ T cells. This causes de novo CNS T cell activation and further secretion of IFN- γ , amplifying myelin autoantigen recognition and T cell activation. IFN- β , which inhibits IFN- γ -inducible class II expression, may also suppress antigen recognition by pathogenic T cells. One goal in MS therapy has been to develop agents that selectively inhibit pathogenic myelin-reactive T cells. Altered peptide ligands (APL), which are synthetic mutant myelin peptides that are designed to alter antigen/MHC recognition and promote T cell secretion of antiinflammatory (Th2) cytokines are being tested in relapsing-remitting MS (Kappos et al., 2000). Glatiramer acetate (Copaxone, Cop-1), a medication used in treatment of relapsing-remitting MS, is a synthetic random copolymer composed of tyrosine, glutamic acid, alanine, and lysine that appears to preferentially affect T cells specific for CNS autoantigens, possibly in a manner similar to APL (Duda et al., 2000).

T Cells, B Cells, and Myelin-Specific Autoantibodies Promote CNS Demyelination

Most MS and EAE studies have focused on the role of myelin-specific CD4⁺ T cells. Other T cell subtypes can participate in CNS demyelination. Clonal expansions of CD8⁺ T cells also occur in demyelinating MS and EAE lesions. Two groups reported that myelin-specific CD8⁺ T cells, which recognize antigen in association with MHC class I molecules, could induce EAE (Huseby et al., 2001; Sun et al., 2001). The pathology was not the same as in EAE induced by CD4⁺ T cells, but in some ways it was more reminiscent of MS. CD8⁺ T cells may have more potential CNS targets than CD4⁺ T cells. For example, oligodendrocytes can upregulate MHC class I but not class II molecules. While those two studies have sparked wide interest in elucidating the role of CD8⁺ T cells in

MS pathogenesis, it should be recognized that in general CD8⁺ T cell expansion requires help from CD4⁺ T cells (Stuve et al., 2002), and elimination of CD8⁺ T cells has caused more EAE relapses. It is likely that therapies targeting CD8⁺ myelin-specific T cells will also need to suppress the activity of CD4⁺ T cells. Natural killer T (NKT) cells, although much less abundant than CD4⁺ or CD8⁺ T cells, may have a role in CNS demyelination. Unlike conventional T cells that recognize protein antigens, NKT cells recognize glycolipids, major constituents of myelin. NKT cells bear an invariant T cell receptor and recognize α -galactoceramide, the prototype glycolipid in the context of a nonpolymorphic MHC class I-like molecule known as "CD1d." One study showed that an analog of α -galactoceramide suppressed clinical EAE (Miyamoto et al., 2001), suggesting that "altered lipid ligands" might also be an attractive means for intervention in MS.

Once the integrity of the blood-brain barrier is compromised during initial CNS inflammation, other mononuclear cells, including B cells and macrophages, penetrate into the CNS. B cells may have a dual role. B cells serve as APC and may expose additional myelin autoantigens, permitting the immune response to diversify. Myelin-specific B cells that differentiate into plasma cells secrete antibodies (Abs) (Figure 1), which are also detected in active demyelinating lesions in MS and EAE (Genain et al., 1999; Warren et al., 1995). While passive transfer of myelin-specific Abs alone does not initiate CNS inflammation or demyelination, they do promote demyelination once T cells establish CNS inflammation. A B cell-depleting monoclonal Ab is currently being tested in MS. Activated components of the complement cascade, including its terminal components, the "membrane attack complex," are also detected along nerve fibers in active demyelinating lesions and periplaque white matter (Prineas et al., 2001). In some cases, complement components are detected without the presence of IgG Ab, indicating that Ab-independent complement activation also occurs and may promote demyelination in a nonspecific manner.

Mast cells are also present in MS lesions (Pedotti et al., 2003). Analysis of mRNA from MS lesions revealed increased transcripts for several genes encoding molecules traditionally associated with allergic responses (Chabas et al., 2001; Pedotti et al., 2003). Histamine receptors 1 and 2 (H2R) are present on inflammatory cells in EAE brain lesions, and histamine receptor genes confer susceptibility to EAE (Ma et al., 2002). Encephalitogenic Th1 cells express more H1R and less H2R than Th2 cells. An H1R antagonist blocked EAE, and a PAFR antagonist reduced the severity of EAE. EAE was attenuated in mast cell-deficient mice. Taken together, these data reveal involvement of elements associated with allergy in autoimmune demyelination. The role of mast cells presents a challenge to our understanding of the pathophysiology of these autoimmune disorders, previously thought to be diametrically opposite to allergy. Pathogenesis of demyelination must now be viewed as encompassing elements of both Th1 and "allergic" responses: allergy and autoimmunity are not antipodal.

Pathologic Heterogeneity in MS

Just as there is heterogeneity in clinical MS, there is also marked histologic heterogeneity. In analysis of acute

demyelinating MS lesions, Lucchinetti and colleagues distinguished four distinct histologic patterns (Lucchinetti et al., 2000). The first two patterns were similar and characterized by perivenular demyelination with predominance of macrophages and T cells within inflammatory lesions. IgG deposition and complement distinguished the second, most common, pattern and both of these patterns resembled lesions in EAE. Demyelination was not perivenular in the two other patterns. While demyelinating lesions of these latter two patterns contained T cells and macrophages, IgG and complement were absent. These two patterns were also characterized by loss of oligodendrocytes, suggesting a primary oligodendrogliopathy. Interestingly, lesions within a given individual were homogeneous. However, most tissue samples in this study were obtained by needle biopsies. Larger scale autopsy studies revealed considerable heterogeneity even within a single focus, with a spectrum of activity ranging from acute inflammation to gliosis (Lock et al., 2002). In comparison of specimens between individuals, Lucchinetti and colleagues observed marked heterogeneity. Pathologic heterogeneity clearly has profound implications for MS treatment.

Demyelination in early MS is not necessarily permanent. Remyelination by oligodendrocytes has been well documented in relapsing-remitting MS. While myelin is the primary target for damage in early MS, axonal degeneration accumulates in chronic MS. Axonal loss, which is irreversible, is detected within demyelinating lesions as well as in normal-appearing white matter, likely secondary to Wallerian degeneration. Axonal degeneration correlates with both permanent disability and brain atrophy in advanced MS. One study identified the presence of oligodendrocyte progenitors in chronic lesions, and they appeared to interact with dystrophic naked axons but were unable to remyelinate them (Chang et al., 2002). These results suggest that axons in chronic MS are not receptive to remyelination and raise concern regarding transplantation of oligodendrocyte precursors (Hohlfeld, 2002). TGF- β , which can be secreted by inflammatory or glial cells during CNS demyelination, induces astrocytes to produce jagged1, which binds the Notch1 receptor expressed on oligodendrocyte precursors. As a consequence of Notch-mediated signaling, oligodendrocyte precursors failed to extend processes and replace degenerating myelin. In contrast, one group observed migration of neural progenitor cells in EAE from the subventricular zone (SVZ) to the olfactory bulb and mobilization of progenitor cells in periventricular white matter, a region frequently involved in MS (Picard-Riera et al., 2002). Progenitor cells in periventricular white matter gave rise to oligodendrocytes and astrocytes, indicating the SVZ is a source of newly generated oligodendrocytes that might promote remyelination.

During CNS inflammation, glutamate is released by activated leukocytes and microglia. Glutamate excitotoxicity mediated by AMPA/kinate receptors causes damage to neurons and oligodendrocytes. One AMPA/kinate antagonist suppressed clinical EAE (Pitt et al., 2000). While treatment with this AMPA/kinate receptor antagonist did not alter CNS inflammation or proliferation by encephalitogenic T cells, treatment was associated with increased oligodendrocyte survival and reduced axonal loss. These observations underscore the

importance of identifying neuroprotective agents and developing strategies to promote oligodendrocyte differentiation.

Therapeutic Considerations

Few classes of medications are approved for MS treatment. These agents, which are partially effective, are associated with side effects and potential toxicities. The number of different types of medications used is likely to increase as several novel therapies already described are tested in clinical trials. Recent results have ignited interest in the potential application of cholesterol-lowering HMG-CoA reductase inhibitors ("statins") in MS therapy. In contrast with currently approved MS treatments, which are administered parenterally, statins are given orally and are well tolerated. One study showed that atorvastatin (Lipitor®) could prevent or reverse chronic and relapsing EAE (Youssef et al., 2002). In vivo atorvastatin treatment induced T cell secretion of anti-inflammatory Th2 cytokines and suppressed secretion of Th1 cytokines. In vitro atorvastatin promoted Th0 cells to differentiate into Th2 cells, which could adoptively transfer protection to recipient mice. Atorvastatin suppressed CNS class II expression on microglia and, when tested in vitro, it inhibited IFN- γ -inducible APC expression of costimulatory and class II molecules. Thus, atorvastatin has immunomodulatory effects on both APC and on T cells. Statins also inhibit MMP-9 secretion, indicating that they might retard T cell migration into the CNS. Lovastatin, which partially suppressed acute EAE in rats, inhibited production of iNOS and TNF- α , proinflammatory molecules that are neurotoxic, suggesting statins might be beneficial in the chronic phase of MS. At this time there is one open-label trial testing simvastatin in relapsing-remitting MS. Another trial, which should begin soon, will test whether atorvastatin treatment reduces the risk of developing MS in patients that have experienced their CNS demyelinating attack, a "clinically isolated syndrome." Statins have pleiotropic actions that appear to be beneficial in other CNS diseases. Statins reduce the risk of stroke, an effect that appears to be independent of cholesterol reduction. Statin use has been associated with a reduced risk of developing Alzheimer's dementia (AD). Data indicate the β -secretase and γ -secretase processing of amyloidogenic A β 40 and A β 42 peptides is dependent upon cholesterol and suggest that statins may reduce A β accumulation. Clinical trials testing statins in AD are underway.

While one important goal is to develop more effective MS therapeutics, some medications may be beneficial when given in combination. Theoretically, medications chosen for combination therapy should produce an additive or synergistic effect but not have overlapping toxicities (Soos et al., 2002). MS medications that have different modes of action, possibly acting on different parts of the pathogenic cascade, may be preferred. Certain combinations are being tested. IFN- β exerts its effects in an Ag-independent manner and is being tested with glatiramer acetate, which appears to affect myelin-reactive T cells. Other combinations are being considered. As for treatment of neoplastic conditions, it can be envisaged that multidrug therapy may be employed in MS. Unraveling multiple mechanisms involved in the pathogenic cascade is clearly proving advantageous for the development of MS therapeutics.

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