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BRIEF REVIEWS

Mechanisms of Immunopathology in Murine Models of Central Nervous System Demyelinating Disease[1]

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Many disorders of the CNS, such as multiple sclerosis (MS), are characterized by the loss of the myelin sheath surrounding nerve axons. MS is associated with infiltration of inflammatory cells into the brain and spinal cord, which may be the primary cause of demyelination or which may be induced secondary to axonal damage. Both the innate and adaptive arms of the immune system have been reported to play important roles in myelin destruction. Numerous murine demyelinating models, both virus-induced and/or autoimmune, are available, which reflect the clinical and pathological variability seen in human disease. This review will discuss the immunopathologic mechanisms involved in these demyelinating disease models. ***The Journal of Immunology,*** **2006, 176: 3293–3298.**

The axons of many vertebrate neurons are surrounded by a myelin sheath, which increases the speed at which the axon can conduct electrical impulses. Numerous inflammatory and metabolic disorders of the nervous system result in loss of the myelin sheath, with symptoms ranging from speech and visual disturbances to paralysis. Many of these disorders are associated with immune infiltrates into the nervous system, normally considered to be an immunologically privileged site. These inflammatory infiltrates may be the primary cause of the demyelination; alternatively, infiltrates may amass at sites of prior injury and contribute to progressive tissue damage. Multiple sclerosis (MS) is the most prevalent human demyelinating disease of the CNS (1). The loss of myelin in MS is thought to be autoimmune in nature because it is associated with elevated levels of $CD4^+$ T cells specific for the major myelin proteins (2–4), as well as with the presence of myelin-specific Abs (5, 6).

Genetic and environmental factors (particularly exposure to virus or bacterial infections) are postulated to interact to varying degrees depending on disease type to initiate autoimmune demyelination. The many described murine models of CNS demyelination reflect the diversity of clinical manifestations in humans. Although none are exact replicates of the human disease, they share many similarities and have provided insight into the pathobiology of the human diseases they model.

Experimental autoimmune encephalomyelitis (EAE)

EAE is a frequently studied autoimmune model of MS. EAE is induced in mice by active priming with whole myelin proteins or specific myelin peptide epitopes in adjuvant; the specific myelin epitopes able to induce EAE varies with the strain of mouse used. Demyelination and paralytic episodes are associated with infiltration of myelin-specific inflammatory Th1 $CD4^+$ T cells into the CNS (7). EAE can also be induced by adoptive transfer of myelin-specific $CD4^+$ T cells, confirming the importance of T cells in disease induction (8). The symptoms of EAE in mice are varied and mimic different clinical manifestations seen in human MS (9). Disease can be monophasic, involving an acute paralytic episode followed by complete recovery; relapsing-remitting, which involves multiple cycles of attack interspersed by full or partial recovery; or chronic, where disease symptoms of the initial attack either stabilize at peak levels or gradually worsen over time. In the monophasic and relapse-remitting forms, recovery from disease is associated with clearance of inflammatory infiltrates from the CNS. Susceptibility to either the monophasic or relapse-remitting subtypes has been mapped to distinct genetic loci (10). Similarly, studies suggest that these two MS disease subtypes are genetically distinct entities (11, 12).

A primary hallmark of the relapsing-remitting and chronic subtypes of EAE is the phenomenon of epitope spreading, which is the diversification of the initial immune response, secondary to acute myelin destruction, to include reactivity to endogenous CNS determinants (13). In EAE, spreading can occur to different epitopes within the same myelin protein used to initiate the disease (intramolecular spreading) or to epitopes within a different myelin protein (intermolecular spreading). For example, there is a sequential and hierarchical order of epitope spreading seen in the relapse-remitting disease of SJL mice primed with $PLP_{139-151}$ (myelin proteolipid protein)

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[3] Abbreviations used in this paper: MS, multiple sclerosis; CSF, cerebrospinal fluid; DC, dendritic cell; EAE, experimental autoimmune encephalomyelitis; HI-TMEV, *Haemophilus influenzae*-TMEV; i.c., intracerebral; MBP, myelin basic protein; MHV, murine hepatitis virus; MOG, myelin oligodendrocyte glycoprotein; PLP, proteolipid protein; PLP, proteolipid protein; SFV, Semliki Forest virus; SV, Sindbis virus; TMEV, Theiler's murine encephalomyelitis virus; TMEV-IDD, TMEV-induced demyelinating disease.

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(14). The first relapse is associated with Th1-type $CD4^+$ T cell reactivity to $PLP_{178-191}$ and the second to MBP_{84-104} (myelin basic protein). Myelin destruction during the acute disease episode creates an inflammatory environment, leading to the infiltration of peripheral myeloid dendritic cells (DCs) to the CNS, which locally present endogenously acquired myelin epitopes to naive T cells (15). In both SJL and (SWR × SJL)F_1 EAE models, tolerance to the primary spread epitope after induction of EAE prevented relapses and/or disease progression (16, 17). However, using a mouse transgenic for a single myelin-specific TCRs, Jones et al. (18) showed that disease relapses could occur in the absence of reactivity to spread epitopes. Epitope spreading has been shown in mice double transgenic for human TCR and MHC class II molecules associated with susceptibility to MS (19, 20). The ability to clearly assess the role of epitope spreading in MS is hampered somewhat by the heterogeneity of the disease and time it takes for disease progression in humans. A recent study showed that patients with long-term disease recognized more myelin epitopes than those with recent-onset disease, but there was no certain correlation between number of epitopes recognized and disease severity (21). This supports previous studies demonstrating epitope spreading in MS patients (22, 23).

Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease

TMEV is a natural mouse pathogen than can cause CNS demyelination in susceptible mouse strains. TMEV is an appealing model to study the potential role of pathogenic agents in the development of MS. Numerous epidemiological studies have shown a link between the environment and MS development (24–26), and MS relapses are often preceded by infections (27, 28). Although most reports linking infection with MS are circumstantial, efforts are ongoing to identify specific pathogens that may be important in disease development. In one study, Ags derived from human herpesvirus type 6 were found in MS plaques but not in tissues from patients with other neurological conditions (29). In another, cerebrospinal fluid (CSF) from MS patients was reported to show a marked increase in levels of the intracellular bacteria *Chlamydia pneumoniae* as compared with CSF from patients with other neurological diseases (30). The significance of these observations to MS initiation remain to be determined.

There are two subgroups of TMEV. One subgroup causes an acute and often fatal encephalitis, and the other causes and initial acute gray matter disease followed by a chronic progressive demyelination in the white matter of the spinal chord known as TMEV-induced demyelinating disease (TMEV-IDD) (31–33). The latter subgroup (Thieler's Original) is made up of TMEV-Daniel's strain and TMEV-BeAn; although the induced disease is slightly different depending on which strain is used, they key characteristics remain the same. Intracerebral (i.c.) injection of virus leads to persistent CNS infection in susceptible mouse strains. Although the level of infectious virus is low during the chronic phase, abundant amounts of viral RNA and viral Ag can be detected throughout the lifetime of the mouse (34–36). Importantly, TMEV-IDD is characterized by immune infiltration into the CNS that is responsible for the demyelination and resulting clinical symptoms (abnormal gait and spastic hindlimb paralysis). The immune response is initiated by the presentation of persistent viral Ags by CNS-resident APCs to Th1-type $CD4^+$ T cells (37). Proinflammatory cytokines released by these T cells recruit monocytes and macrophages into the CNS, which cause damage to myelin. This subsequent release of myelin Ags and uptake by APCs lead to the emergence of myelin-specific $CD4^+$ T cells. Initial studies showed that immune responses to TMEV were detectible 5–7 days postinfection but that reactivity to myelin did not appear until after the onset of clinical symptoms (30–35 days postinfection) (15, 38–40). Thus, the chronic phase of TMEV-IDD is autoimmune caused by epitope spreading from viral determinants to self-myelin determinants. Tolerance to multiple myelin proteins after virus infection significantly inhibits TMEV-IDD, which demonstrates the importance of the myelin-specific immune response in disease progression (41). In SJL mice, reactivity appears to multiple myelin epitopes starting with the immunodominant epitope $PLP_{139-151}$ and spreading at later time points to other subdominant myelin determinants in a hierarchical manner similar to what is seen in EAE (15, 37, 42).

Recombinant TMEV model of molecular mimicry-induced demyelination

Among the ways in which a virus may trigger autoimmune reactions, molecular mimicry is the mechanism most often proposed. Molecular mimicry involves immunological cross-reactivity between self-epitopes and epitopes from a foreign pathogen. Activated MBP-specific T cells have been found in MS patients (43–45); the subsequent identification of pathogen-derived mimics capable of activating human MBP-specific T cell lines reinforces the theory that pathogens may induce MS via molecular mimicry (46, 47). Pathogen-derived mimics capable of cross-activating murine MBP-specific T cells have also been identified, including several mimics capable of inducing EAE in mice transgenic for an MBP-specific TCR (48, 49).

In the first attempt to create a murine model of infection-induced CNS disease via mimicry, vaccinia virus was engineered to express rat PLP (VVplp) (50). Although disease could not be directly induced by infection with this recombinant virus, VVplp-infected mice later challenged with encephalitogenic myelin peptides had enhanced EAE-type disease as compared with mice infected with control vaccinia virus. Later, we infected SJL mice with rTMEV engineered to express the immunodominant SJL myelin epitope $PLP_{139-151}$ (PLP-TMEV) (51). Mice infected with this virus developed a demyelinating disease that was early onset (7–10 days postinfection) as compared with wild-type TMEV (30–40 days). This early onset was associated with inflammatory PLP_{13-151}-specific $CD4^+$ T cell responses that arose concomitantly with responses to the virus epitopes around day 7–14 postinfection. Hence, in contrast to wild-type TMEV, where disease is the result of epitope spreading from virus to myelin Ags, disease in the rTMEV is a result of initial priming of myelin-specific T cells. Another rTMEV was constructed using a previously identified mimic epitope of $PLP_{139-151}$ derived from the bacteria *Haemophilus influenzae* (HI-TMEV) (52, 53). Mice infected with HI-TMEV also developed early-onset gait abnormality associated with early induction of $PLP_{139-151}$-specific $CD4^+$ T cell responses, and the disease could be inhibited by prior induction of tolerance to either the HI mimic epitope or the self $PLP_{139-151}$ epitope. Significantly, infection with this mimic-expressing virus was able to induce disease, whereas priming with the mimic epitope in adjuvant could not (52, 53). This

suggests that innate immune signals provided by the virus are important for disease induction. Subsequent studies showed that the relatively mild disease induced by infection with HI-TMEV could be exacerbated by giving mice a second dose of virus 2 wk following initial infection (54); the increase in clinical symptoms was associated with increases immune infiltrates into the CNS. This supports the hypothesis that while a single infection may not be sufficient to precipitate autoimmune demyelination, several infections over a person's lifespan may eventually lead to disease.

Murine hepatitis virus (MHV)

MHV, like TMEV, is an infection-induced murine model of inflammatory CNS demyelination (55). In this model, mice are inoculated i.c. or intranasally with the neurotropic strains, JHMV or MHV-A59. CNS infection results in an influx of immune cells that for the most part will clear the virus, although the virus does persist in low amounts (56). In contrast to TMEV, susceptible mice infected with MHV have a single major symptomatic episode (ataxia, hindlimb paresis, paralysis) from which the majority will recover (57). Demyelination begins about 1 wk postinfection and peaks at week 3, after which lesion repair and remyelination occurs, although new areas of demyelination can occur throughout the lifetime of the mouse (58–60).

The exact mechanism of demyelination is somewhat controversial, but there is extensive evidence suggesting that immune responses are critical to this process. MHV infects and replicates within oligodendrocytes, the myelin-synthesizing cells of the CNS (61, 62), and it can be argued that oligodendrocyte damage or death is the major mechanism of demyelination (63, 64). However, mice exposed to immunosuppressive doses of irradiation following JHM strain of mouse hepatitis virus infection showed little demyelination despite the presence of virus in oligodendrocytes and reconstituting irradiated mice with splenocytes from unirradiated-infected mice restored demyelination (65). Similarly, T and B cell-deficient RAG1-deficient mice, which were resistant to demyelination, developed histological disease after adoptive transfer with splenocytes from MHV-inoculated mice, which involved the recruitment of activated macrophages/microglia to sites of demyelination in the spinal cord (66). Chemokine receptor knockout mice ($CCR5^{-/-}$) showed reduced demyelination that correlated with reduced macrophage but not T cell infiltration into the CNS (67). Taken together, these studies suggest that macrophages are primarily responsible for myelin destruction but that T cells are required to recruit macrophages into the CNS. Other studies indicate that the presence of either $CD8^+$ or $CD4^+$ T cells, but not both subsets at the same time, is required for demyelination. Both β_2-microglobulin- and MHC class II (I-A^b)-deficient mice display demyelination after MHV infection (68–70). However, in two reports, CD4-deficient mice showed less severe disease than CD8-deficient mice, which again correlated

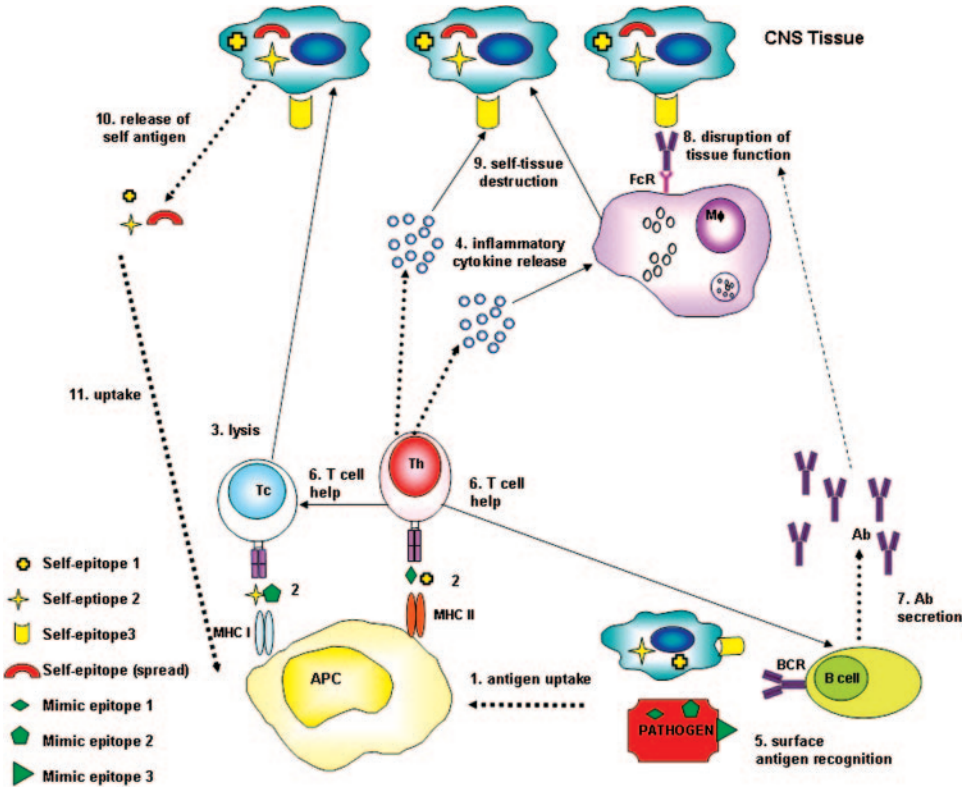

FIGURE 1. Cells of the immune system potentially involved in demyelination. APCs can take up Ag from a foreign source (such as an invading pathogen) or from self-tissue (myelin or oligodendrocyte proteins) (no. 1). Ag is processed into peptides, which are loaded onto MHCs and presented to T cells via the TCR (no. 2). Activated cytolytic T cells (Tc, activated by MHC class I on APCs) cause damage by direct lysis of the target (no. 3). Th cells (activated by MHC class II) release inflammatory cytokines that are directly damaging to tissue and also activate monocytes/macrophages (Mφ) (no. 4). T cells may be specific for self-tissue (direct damage), specific for a tissue-resident pathogen (bystander damage), or cross-reactive with pathogen and self-epitopes (molecular mimicry). Surface Ag (foreign or self) is recognized by B cells via the BCR (no. 5). Upon receiving T cell help (no. 6), the B cell secretes Abs specific for self or dual specific for foreign and self-epitopes (molecular mimicry) (no. 7). The binding of Ab to tissue may interfere with biological function (no. 8). Abs can also simultaneously bind to and activate Mφ via its FcR (Fc), which mediate tissue damage (no. 9). Damaged tissue releases self-Ag, including new Ags not involved in the initial activation (no. 10), which are taken up by APCs (epitope spread) (no. 11). This further propagates the self-reactive immune response and leads to additional tissue damage.

with recruitment of macrophages to the CNS (71, 72). There is no evidence of self-specific immunity in the CNS of MHV-infected mice (73). Therefore, the primary mechanism of demyelination in murine MHV infection appears to be bystander myelin destruction by the immune response initially recruited to the CNS to control viral infection. The implication of this model to human disease is that a pathogen may cause demyelination in an Ag-nonspecific manner if it is tropic for cells within the nervous system.

Semliki Forest virus (SFV)

SFV is a neuroinvasive and neurotropic virus that infects CNS neurons and oligodendrocytes (74, 75). In adult C57BL/6 and BALB/c mice, the virus is for the most part cleared from the CNS by day 6 postinfection. This is followed by demyelination that peaks around day 14 and then wanes, with sporadic and mild clinical symptoms (76, 77). SFV is thus an attractive model of "monosymptomatic" MS, where patients experience a single clinical episode (78).

The demyelination in SFV-infected mice is T cell mediated because demyelination is not seen in nude or SCID mice (76, 79). In BALB/c mice, depletion of $CD8^+$, but not $CD4^+$, T cells virtually abolished demyelinating lesions (80). This may suggest that T cell lysis rather than cytokine secretion is responsible for CNS pathology. Other studies in this model have shown that Th1-type cytokines are involved in viral clearance but not demyelination (81, 82). Although not definitively proven, it is thought that demyelination is due to cytolytic damage of virus-infected oligodendrocytes. Morphological changes were shown in optical nerve oligodendrocytes at peak of disease in BALB/c mice (83). In C57BL/6 mice, molecular mimicry may also play a role in demyelination. Infected mice have proliferative T cell responses to MBP (84), and Abs reactive to MBP and myelin oligodendrocyte protein (MOG) (85). Computer algorithms have uncovered homology between an epitope in the SFV surface protein E2 and MOG_{18-32} (86). Mice primed with either the E2 or MOG peptide develop an EAE-like disease whose histopathology resembles that of mice infected with SFV. It was concluded that the demyelinating lesions were due mainly to Ab responses, which were cross-reactive between MOG and the SFV E2 protein.

Sindbis Virus (SV)

Although not extensively studied as a model of demyelination, SV infection of mice provides further proof-of-principle that pathogen infection can lead to autoimmune disease. The AR339 strain replicates primarily in neurons of the brain and spinal cord, and infection is rapidly controlled by the immune response, with infectious virus becoming undetectable 7–8 days postinfection (87). In contrast to BALB/c mice, which normally remain asymptomatic following infection, SJL mice develop EAE-like paralysis starting at day 6 and continuing up to 8 wk postinfection (88). Cyclophosphamide treatment ameliorates symptoms despite increasing CNS viral titers, indicating that the paralysis induced in SJL mice is due to the immune response. CNS lymphocytes taken day 7 postinfection were specific for SV but not for MBP (89). However, MBP-specific T cells and Ab responses were detected in the periphery at 8 wk postinfection, indicating that as in TMEV-IDD, anti-myelin responses may arise due to bystander damage via epitope spreading (88). The fact that symptoms occur rapidly following SV infection indicates that, unlike TMEV-IDD, demyelination is not the primary cause of paralysis but may contribute to chronic disease. CNS inflammation resolves by 2 wk postinfection in BALB/c mice but persists for a longer period of time and is more severe in SJL mice (88, 89). CNS lymphocytes isolated from SJL mice appear to be less prone to apoptosis and local mechanisms of regulation than those from BALB/c mice (90). Comparison of CNS lymphocytes from both strains of mice showed that SJL mice had a higher percentage of $CD4^+$ T cells during chronic infection, fewer NK cells, higher expression of IL-10, and lower expression of IL-4 (89). The significance of this differential profile to disease progression remains to be determined, as does the true contribution of the anti-myelin response.

Conclusion

As outlined in Fig. 1, there are multiple pathways by which immune-mediated demyelination can occur in humans. Each of the murine models discussed above is different with respect to the underlying mechanisms thought to be responsible for myelin destruction. Moreover, the different clinical manifestations of each model reflect the spectrum of symptoms experienced by patients. Although no individual system precisely models the pathology and clinical course of human CNS disease, as a whole, these models have led to significant advances in understanding disease mechanisms and for designing novel therapies.

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