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Modeling the Heterogeneity of Multiple Sclerosis in Animals

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Abstract

Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system manifested with varying clinical course, pathology, and inflammatory patterns. There are multiple animal models that reflect different aspects of this heterogeneity. Collectively, these models reveal a balance between pathogenic and regulatory CD4⁺ T cells, CD8⁺ T cells and B cells that influences the incidence, timing, and severity of central nervous system autoimmunity. In this review we discuss experimental autoimmune encephalomyelitis (EAE) models that have been used to study the pathogenic and regulatory roles of these immune cells, models that recapitulate different aspects of the disease seen in patients with MS, and questions remaining for future studies.

Keywords

EAE; Multiple sclerosis; Autoimmunity; Animal models; Neuroimmunology

Overview of multiple sclerosis

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system, estimated to affect up to two million people worldwide. Hallmarks of MS include focal inflammatory infiltrates, demyelinating plaques, and axonal damage. While the etiology of MS is not known, it is widely considered to be an autoimmune disease. Recent genome-wide association studies (GWAS) provided strong support for this notion by identifying >50 susceptibility loci associated with MS of which the vast majority represent genes with immune cell function [1]. A longstanding hypothesis is that environmental factors trigger MS in genetically susceptible individuals by promoting the activation of myelin-specific T cells that normally circulate in the periphery in a tolerant state. Once activated, these T cells can enter the CNS and initiate an autoimmune response. A major role for CD4⁺ T cells in this process is strongly supported by the observation that the strongest association of genetic susceptibility to MS is with MHC class II alleles that present antigen to CD4⁺ T cells. However, CD8⁺ T cells are also implicated in the pathogenesis of MS as CD8⁺ T cells outnumber CD4⁺ T cells in MS lesions [2, 3], and GWAS studies have indicated an association with some MHC class I alleles that present antigen to CD8⁺ T cells [1].

Developing therapies to treat or prevent MS requires an in-depth understanding of the pathogenesis of the disease. Mechanistic studies in MS are difficult because central nervous

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system tissue is difficult to access and immune responses within this tissue cannot be easily monitored. Therefore, animal models are essential in defining the mechanisms underlying MS. These models are important not only to discover new therapeutic targets, but also to test new therapies prior to translation to patients. One of the major challenges in developing animal models of MS is that patients with MS vary widely both in disease presentation and in response to therapeutics. Examples of such heterogeneity in disease include the type of clinical signs (which reflect location of inflammatory plaques [4]), disease course (occurrence of remissions and relapses) [5], and pathological features [6]. This extensive heterogeneity raises the question: does MS have a single etiology, made heterogeneous by genetics and environment, or is MS a syndrome resulting from various pathogenic pathways? The question remains to be answered, yet has important ramifications on treatment decisions. Developing animal models that recapitulate different aspects of the disease seen in patients with MS may shed important light on this issue.

A wide variety of animal models of CNS demyelination exist, including chemical-induced demyelination (cuprizone, ethidium bromide, lysolecithin) or infection with demyelinating viruses such as Theiler's murine encephalomyelitis virus (TMEV) or murine hepatitis virus [7]. Other models of demyelinating disease have been developed which constitutively express different cytokines in the CNS [8, 9] or employ diphtheria toxin to deplete oligodendrocytes [10, 11]. However, the oldest and most widely used animal model for MS is experimental autoimmune encephalomyelitis (EAE). EAE has been studied in a variety of animal species, including mouse, rat, rabbit, guinea pigs, and non-human primates. This review will focus on mouse models of EAE. EAE is induced either by immunization with myelin antigens (active induction) or by adoptive transfer of activated T cells isolated from animals immunized with myelin antigens (passive induction) [12, 13]. In addition, EAE can also develop spontaneously in certain mouse models engineered to express a myelin-specific, transgenic TCR [14, 15]. The manifestation of EAE can vary greatly depending on the strain or genotype of rodent and sometimes the mode of induction (active versus passive). Although differences between EAE models can occasionally confound results and make interpretations difficult, they also can shed light on the complexity and heterogeneity of CNS autoimmune disease. No single model replicates the full spectrum of inflammatory mechanisms and neurodegeneration seen in MS, just as individual patients manifest only a subset of the diverse features of the disease. The goal of this review is to describe how differing EAE models reflect specific aspects of MS, how these various models have influenced our current understanding of mechanisms of CNS autoimmunity, and questions remaining for future studies. We begin with a description of the heterogeneity in the human disease and identify different aspects of MS that are important to model in animals. We then discuss how animal models have contributed to our understanding of human disease, highlighting specific animal models that recapitulate particular components of MS (Table 1).

Heterogeneity of multiple sclerosis

The clinical disease course varies among patients with MS

Patients with MS can be subcategorized based on distinct clinical patterns. Patients may first develop an isolated episode of neurological disability termed clinically isolated syndrome (CIS) and may or may not go on to develop clinically definite MS [16]. About 85% of patients with MS develop relapsing-remitting MS (RR-MS), during which patients experience episodes of neurological disability but return to baseline following relapse. Approximately 50% of these patients develop a clinically distinct form of disease referred to as secondary progressive MS (SP-MS) within 10 years of onset of RR-MS [17]. In SP-MS, relapses still occur but clinical disability worsens over time and brain atrophy steadily increases [18]. The reasons for this shift to increasing clinical disability and lack of return to baseline are not known. Interestingly, even though patients may convert from RR-MS to SP-

MS at different times following the initial diagnosis, once a patient converts to SP-MS, the overall increase in disability and in brain atrophy proceed at the same rate [5]. This suggests that there is a mechanistic shift in the pathogenesis that occurs when a patient converts to SP-MS, and mechanisms underlying progressive disease have recently been proposed [5]. While inflammatory lesions detected by MRI during RR-MS decrease in SP-MS, some inflammatory immune cells may still be engaged in tissue destruction but are not detected by imaging because the blood brain barrier is no longer permeable to gadolinium.

There is also a form of MS called primary progressive (PP-MS), which comprises approximately 10–20% of all MS cases. Patients with PP-MS lack the acute exacerbations seen in patients with RR-MS; instead, they develop steadily worsening clinical disability from the time of their initial diagnosis [19]. Patients with PP-MS respond poorly to most commonly used immune-modulating MS therapeutics [20], suggesting that a different pathogenic pathway may be at play in these patients compared to those with RR-MS.

Inflammatory patterns differ in patients with MS

The locations of lesions within the CNS are the major determinant of clinical signs, and these are also variable among patients. The majority of lesions are found in the brain, particularly in the periventricular white matter, cerebellum, brainstem, and optic nerves. Many patients exhibit lesions in the spinal cord as well as the brain, while 2–10% of patients exhibit inflammation in the spinal cord and optic nerves without extensive involvement of the brain (referred to as opticospinal MS) [21, 22]. Opticospinal MS has particularly high prevalence in patients with MS of Asian descent, and there is currently some debate as to whether opticospinal MS is distinct from neuromyelitis optica (NMO) (otherwise known as Devic's disease). An antibody specific for aquaporin-4 is found in a high proportion of patients with NMO; recently, this antibody was also found in patients with opticospinal MS, but not in patients with MS from North America that exhibit the more common pattern of brain lesions [23]. Regardless of whether NMO and opticospinal MS are distinct entities, they share a predilection for inflammation localized to the spinal cord, in contrast to the majority of patients with MS, in which inflammation targets the brain in addition to the spinal cord. These distinct localization patterns suggest that mechanisms promoting inflammation in the brain may be distinct from lesions promoting inflammation in the spinal cord.

Patients with MS exhibit diverse pathological features

The CNS pathology in MS is characterized by inflammatory lesions, demyelination, remyelination, neurodegeneration, and glial scar formation [5]. Most MS lesions are dominated by T cells and macrophages. Beyond these fairly common characteristics, substantial heterogeneity has been observed in CNS pathology. A seminal study categorized the CNS lesions in patients with MS into different patterns based on the distribution of myelin loss, plaque geography and extension, pattern of oligodendrocyte injury, and immunopathological evidence of immunoglobulin and activated complement deposits [6]. Patterns I and II lesions share a perivenous distribution of plaques dominated by T cells and macrophages with preservation of oligodendrocytes. Pattern II is distinguished from pattern I by immunoglobulin and complement deposits within the lesions. Pattern III lesions are associated with oligodendrocyte apoptosis and preferential loss of myelin-associated glycoprotein (MAG). These lesions are not centered around veins and have been suggested to resemble hypoxic/ischemic white matter damage [24]. A small number of patients, all with PP-MS, demonstrated non-apoptotic oligodendrocyte cell death without preferential MAG loss, and were originally categorized in a fourth pattern, although this is quite rare.

An important finding that emerged from this study is that the active plaques within an individual all appeared to belong to the same pattern. This observation suggested that the different patterns of lesion pathology seen in patients with MS reflect the operation of distinct pathogenic pathways among individuals [6]. If this hypothesis is true, the development of animal models that recapitulate distinct pathogenic pathways is critically important to define treatment protocols that target the specific pathogenic mechanisms relevant to an individual's disease. However, an alternative hypothesis has been proposed that postulates that distinct lesion characteristics correspond to different stages of lesion development dictated by a common pathogenic pathway. This hypothesis is based on reports describing multiple lesion types within the same individual patient [25], as well as some reports of lack of heterogeneity in the immunopathological appearance of lesions in patients with established MS [26]. The reason for different assessments of lesion homogeneity within individuals is unclear, but a recent study suggested that the overlap of certain pattern types in patients with NMO may contribute to the controversy [27]. Development of new animal models that generate distinct lesion patterns may shed additional light on this controversy.

Modeling immune-mediated mechanisms in EAE

The “classic” EAE model of MS focuses on the role of CD4⁺ T cells

Mechanistic insights into the pathogenesis of MS have relied extensively on the EAE model. The origin of the model dates back to 1925 with the discovery that rabbits immunized with human spinal cord homogenate exhibited spinal cord inflammation [28]. The immunization protocol activates primarily CD4⁺ T cells, and subsequent studies demonstrated that adoptive transfer of CD4⁺ T cells from mice immunized with myelin antigens was sufficient to induce EAE [29]. Identification of CD4⁺ T cells as a major effector cell in EAE provided an explanation for the strong correlation of MS susceptibility to particular MHC class II alleles. Initial murine EAE models were developed in B10.PL or PL/J mouse strains, in which an epitope of myelin basic protein (MBP1–11) is targeted, as well as in SJL mice in which encephalitogenic T cell responses to both MBP and proteolipid protein (PLP) are generated [30]. Subsequently, C57BL/6 mice were discovered to be susceptible to EAE via immunization with a peptide from myelin oligodendrocyte glycoprotein (MOG35–55) [31], and this model is now extensively used in order to exploit the many genetic models on the C57BL/6 background. In all of these models, inflammation predominantly targets the spinal cord, and mice develop subsequent symptoms of ascending flaccid paralysis. Thus, while these models have provided many insights into CNS autoimmune disease, the relative lack of involvement of the brain is a distinct difference from most patients with MS.

Spontaneous EAE models provide insight into the pathogenesis of MS

In the first EAE model expressing a transgenic TCR specific for a MHC class II-restricted myelin epitope, the transgenic T cells were not subjected to central or peripheral tolerance, allowing the peripheral repertoire to be dominated by CD4⁺ T cells specific for MBP. Spontaneous EAE occurred in these TCR transgenic mice, providing the first spontaneous model of MS [32]. Several other myelin-specific, MHC class II-restricted TCR transgenic models were subsequently generated that exhibit varying degrees of spontaneous disease [33–36]. These models confirmed the ability of naïve, myelin-specific CD4⁺ T cells to be activated at some stochastic frequency and initiate CNS autoimmunity without additional experimental intervention.

EAE models identify distinct pathogenic CD4⁺ T cell effector subsets

IFN- γ -expressing Th1 cells were initially considered to be the effector CD4⁺ T cell subset that induced EAE [37]. Adoptive transfer of Th1 clones can induce EAE, and mice deficient in Tbet (a transcription factor required for Th1 cell differentiation) are reported to be

resistant to EAE [38–40]. However, the discovery that IL-23 (and not the Th1-promoting cytokine IL-12) was required for EAE development [41] led to the identification of the IL-23-dependent Th17 subset that can also induce EAE [42]. In many EAE models, Th17 cell-initiated disease appears more clinically severe than Th1 cell-initiated EAE [43, 44]. These observations stimulated many studies in both MS and EAE on the role of IFN- γ and IL-17, as well as the T cells that produce them. Both IFN- γ -producing and IL-17-producing T cells have been identified in the CNS and CSF of patients with MS [45–48]; however, it is not yet clear whether/how these subsets correlate with different types of disease pathology and/or inflammatory patterns. One study in patients with RR-MS comparing the prevalence of Th1 and Th17 cells during remission and relapses found that, while the majority of CSF T cells produced IFN- γ , the number of Th17 but not Th1 cells significantly increased during relapse compared to those in remission [49].

Adoptive transfer of Th1- versus Th17-skewed cells demonstrated that these T cell subsets influenced both the composition of infiltrates as well as the localization of inflammation within the CNS. Th1 cells induced production of chemokines CXCL9 and CXCL10 in the CNS, correlating with a predominantly monocytic CNS infiltrate. Th17 cells, however, induced CXCL1 and CXCL2, correlating with a higher proportion of neutrophils in the CNS infiltrate [50]. Additionally, the clinical symptoms of Th1- and Th17-mediated EAE were found to be distinct: Th1 cells induced classic EAE, while Th17 cells induced a variety of atypical symptoms in addition to tail and limb paralysis including ataxia, proprioception defects, and rolling [51, 52]. Thus, Th1 and Th17 cells appear to be critical determinants of inflammatory infiltrates as well as the inflammatory pattern.

A confounding factor in studies exploring the role of Th1 and Th17 cells in EAE is the plasticity observed in their cytokine phenotype. Th17 cells can co-express IL-17 and IFN- γ as well as their respective transcription factors ROR γ t and T-bet [53, 54]. These dual-expressing T cells have been suggested to exhibit enhanced pathogenicity in EAE [54]. There is also some evidence that Th1 cells can co-express IL-17 [55], and IL-17⁺IFN- γ ⁺ T cells have been identified in MS brains [56]. A fate-mapping EAE study using IL-17A reporter mice showed that up to two-thirds of CNS-infiltrating T cells had at some point expressed IL-17A and converted to IFN- γ producers [57]. These so-called “ex-Th17” cells down-regulated IL-17 and ROR γ t and up-regulated the IL-12 receptor in an IL-23-dependent manner.

Studies of Th1 and Th17 cells generated by exposure *in vitro* to cytokines that expand or stabilize their phenotypes suggested important functional differences in these effector T cell subsets. Whether their pathogenicity can be attributed specifically to the activity of IFN- γ or IL-17, however, has not been entirely clear. To understand the role of these cytokines, animal models that are genetically deficient in IFN- γ or IL-17 have been studied in EAE. Despite the fact that Th1 clones can induce EAE, these studies showed that IFN- γ is not required for EAE and may in fact have suppressive activity [58, 59]. However, a pilot study of IFN- γ supplementation in 18 patients with MS was halted due to increased exacerbation rates [60], suggesting that IFN- γ has more disease-enhancing than disease-suppressing activity in patients with MS.

Although IL-17 was shown to be dispensable for EAE induction [61], Th17 cells have been reported to induce more severe EAE, and models that are deficient in IL-17A or the IL-17RA receptor can lead to reduced incidence, severity, and delayed onset of EAE [62–65]. As a result of such studies, trials in patients with MS are underway to investigate the therapeutic potential of IL-17 neutralization. Initial data from a clinical trial administering anti-IL-17A neutralizing antibody to patients with RR-MS reported reduced lesion activity

and a trend towards reduced relapse rates [66], supporting the need for further studies to understand the precise mechanisms of action of these cytokines.

Because neither IFN- γ nor IL-17 is required for EAE, additional cytokines were evaluated for their role in the pathogenesis of disease. GM-CSF was identified as a critical pathogenic cytokine in EAE models as GM-CSF^{-/-} mice are resistant to EAE [67]. Specifically, T cell production of GM-CSF is required for EAE induction [68]. Recent studies showed that GM-CSF can be produced by both Th1 and Th17 cells, and that T cells producing GM-CSF can induce EAE in the absence of both IFN- γ and IL-17 [69, 70]. The exact function of GM-CSF is not known but it has been proposed to recruit inflammatory macrophages to the CNS as well as promote IL-23 production by dendritic cells. Elevated levels of GM-CSF are found in the CSF of patients with active MS [71], and clinical evaluation of the safety of a GM-CSF-neutralizing antibody in patients with MS is ongoing (see: <http://clinicaltrials.gov/show/NCT01517282>). Future studies that use genetically engineered animal models to eliminate the signaling of specific cytokines in particular cell types are needed to understand the mechanisms and effects of these different cytokines (as well as others yet to be identified), and to determine the stage at which they exert their influences on the pathology and inflammatory patterns in the CNS. Studies are also needed to determine whether distinct effector T cell subsets are more active in certain stages of disease or subsets of MS patients.

EAE models highlight the role of regulatory CD4⁺ T cells

Early studies using a MBP-specific TCR transgenic model on the Rag^{-/-} background revealed that spontaneous EAE in these mice could be prevented by adoptive transfer of non-transgenic CD4⁺ T cells [33, 72]. CD4⁺CD25⁺ T cells (Tregs) were later identified as an important suppressive subset in EAE, as adoptive transfer of this T cell subset reduced disease severity [73]. Administration of anti-CD25 antibody during EAE also ablated Treg-mediated protection [74, 75]. The generation of Foxp3-GFP reporter mice facilitated detailed studies of Treg activity. Use of these mice showed that the population of Tregs in the CNS is initially small but rapidly expands during EAE, and the majority of Tregs in the CNS of EAE mice were found to be antigen specific. The observation that the Treg population peaks at the recovery phase of disease [76–78] provides a rationale for current attempts to harness Treg activity in the treatment of ongoing autoimmune diseases [79]. Additional support for this approach came from studies that demonstrated impaired function of Tregs in patients with MS. Compared to healthy controls, Tregs isolated from peripheral blood and CSF of patients with MS have significantly reduced suppressive function [80–82]. Tregs from patients with MS also exhibited a greater propensity for IFN- γ expression compared to healthy controls [83]. Recently, *CD25*, *CD127*, and *CD58*, all of which contribute to Treg function, have been identified as risk alleles for susceptibility to MS, further suggesting an intrinsic Treg defect [84–87]. Treating MS patients with IFN- β appears to restore suppressive function to Tregs [83, 88, 89]. Thus, the discovery that enhanced Treg activity can ameliorate EAE, as well as studies of Treg activity using reporter mice, have provided insight into current therapies and led to new therapeutic strategies for targeting pathways that enhance Treg function. However, a note of caution has also emerged from studies using EAE models. Tregs isolated from the CNS during the peak of disease were able to suppress most peripheral effector T cells but failed to inhibit myelin-specific effector T cells isolated from the CNS. This observation suggests that effector T cells in the inflamed CNS may be resistant to suppression by Tregs [77].

EAE models reveal a role for B cells in CNS autoimmunity

While T cells are believed to be the main cell type that initiates CNS inflammation, observations in several animal models as well as patients with MS have indicated important roles for B cells in the pathogenesis of MS and EAE. Early studies demonstrated increased

intrathecal production of immunoglobulins (Ig) in the CSF of most patients with MS [90], and the identification of clonotypic B cells in MS lesions suggests an ongoing humoral immune response within the CNS [91–94].

Both B cell deficient ($\mu\text{MT}^{-/-}$) mice and anti-CD20-mediated depletion have been used to investigate the role of B cells in EAE. Results varied depending on the mouse strain and the antigen used. For example, B10.PL $\mu\text{MT}^{-/-}$ and wild-type mice exhibit similar susceptibility to MBP-peptide induced EAE [95]; however, EAE was induced by immunization with recombinant human MOG protein only in wild-type and not in $\mu\text{MT}^{-/-}$ C57BL/6 mice [96, 97]. The lack of EAE induction by human MOG in $\mu\text{MT}^{-/-}$ mice was attributed to a requirement for antibodies elicited by immunization with human MOG protein. EAE was not dependent on B cells when rat recombinant MOG was used to induce disease, highlighting the potential for differences in immune responses to distinct immunogens to determine susceptibility to EAE [96]. Depletion of B cells by in vivo treatment of C57BL/6 mice with anti-CD20 also revealed disparate roles for B cells when recombinant rodent MOG protein versus MOG peptide were used as immunogens [98]. In EAE induced by recombinant protein, B cells enhanced EAE by promoting differentiation of proinflammatory MOG-specific Th1 and Th17 cells, presumably by serving as antigen-presenting cells (APCs). In contrast, anti-CD20 treatment exacerbated EAE induced by MOG peptide, implicating a protective role for B cells [98]. A regulatory subset of B cells has been identified in an EAE model in which IL-10 producing B cells promoted recovery from EAE [99], providing a potential explanation for differences in EAE incidence in B cell deficient mice. In a separate anti-CD20 antibody-mediated B cell depletion study, regulatory B cells appeared to have a stronger suppressive effect during the priming phase of EAE induction, while pathogenic B cells appeared to dominate after onset of EAE [100].

Support for a pathogenic role of B cells in EAE has emerged from the combination of two genetically-engineered mouse models. Mice expressing a transgenic TCR specific for MOG in C57BL/6 mice develop a very low incidence of spontaneous EAE (although 47% develop spontaneous optic neuritis) [35], and C57BL/6 mice in which a heavy chain from a MOG-specific antibody was knocked into the Ig locus do not develop clinical disease. When these mice were bred together, however, the incidence of spontaneous EAE significantly increased. B cells were shown to enhance MOG-specific T cell activation in this model [101, 102]. Interestingly, inflammation primarily targeted the optic nerve and spinal cord in these mice, suggesting that this may serve as a model of optico-spinal MS. In a separate model of spontaneous relapsing-remitting EAE (RR-EAE) in SJL/J mice expressing a transgenic MOG-specific TCR, relapses alternated between targeting cerebellum, brainstem, spinal cord, and optic nerve tissue [103]. In these RR-EAE mice, B cells that secreted anti-MOG antibodies were expanded from the endogenous repertoire, with evidence of ongoing germinal center reactions in the cervical lymph nodes [103, 104]. B cell depletion suppressed RR-EAE in these mice, providing support for the pathogenic role of B cells.

The efficacy of Rituximab, an anti-CD20 antibody, in reducing inflammatory lesions and clinical relapses in patients with RR-MS provides further support for a pathogenic role of B cells in MS [105]. Anti-CD20 did not deplete plasma cells, suggesting a pathogenic function of B cells aside from antibody production. Multiple roles for B cells in promoting T cell function and activation have been suggested, including antigen presentation and inflammatory cytokine production. However, while many models clearly implicate a role for B cells in affecting T cell activation, most do not distinguish their function as antigen presenting cells versus production of immune mediators in influencing disease. Recent work showed that splenic B cells from mice with EAE produce abundant IL-6 and that specific depletion of IL-6-producing B cells reduces the severity of EAE [106]. Accordingly, B cells from patients with RR-MS have been shown to secrete higher levels of pro-inflammatory

cytokines such as IL-6, TNF- α and lymphotoxin [106–108]. Overall, the balance between regulatory and pathogenic B cells appears to modulate the function of T cells and, consequently, influence the initiation and progression of inflammation in MS and EAE.

EAE models reveal a role for the microbiome in disease induction

Studies of the first CD4⁺ myelin-specific TCR transgenic model suggested that the microbiome may be an important component in determining susceptibility to CNS autoimmune disease. The incidence of spontaneous disease in these mice increased as the degree of microbial exposure in the environment increased [32, 109]. This finding provided strong support for the hypothesis that microbes may be one of the environmental factors that increase susceptibility to MS. This notion has been further developed in recent studies examining the role of the microbiome in conferring susceptibility to EAE. Both germ-free mice as well as antibiotic-treated mice have been used to investigate the effects of gut microflora on EAE development. While studies in germ-free mice revealed varying incidence of MOG-induced EAE [110, 111], in a TCR transgenic model of spontaneous RR-EAE, germ-free mice were resistant to EAE and exhibited decreased Th17 and B cell responses [112]. The authors reported that reconstitution of commensal gut flora was required for EAE to develop spontaneously. Germ-free mice are known to have a severely compromised immune system (both mucosal and systemic) with poorly structured lymphatic tissues and reduced serum IgG. These defects are rescued upon reconstitution with commensal bacteria [113], and this likely contributed to the results described by Berer *et al.* that indicated that reconstitution with commensal bacteria facilitated activation of CD4⁺ T cells and promoted recruitment and activation of myelin-specific B cells [112]. Other studies have shown that colonization of germ-free mice with segmented filamentous bacteria induces a Th17 response and restores susceptibility to EAE [110]. In models in which wild-type mice were treated with oral antibiotics to reduce the gut bacterial burden prior to induction of EAE by immunization with PLP or MOG, antibiotic-treated mice exhibited reduced pro-inflammatory responses and decreased overall EAE severity with elevated IL-10 production by regulatory T and B cells [114, 115]. Additional studies have shown that certain types of microbes can be protective against EAE, typically by promoting Treg induction [116, 117]. Overall, these studies suggest that the gut microbiota may influence the induction and severity of EAE by generating a gut microenvironment that facilitates T cell activation, and by altering the balance of effector and regulatory T and B cells. Translation of these findings to MS may lead to new strategies to prevent an autoimmune-prone environment in genetically susceptible individuals.

Models of different clinical manifestations of MS

EAE models of RR-MS provide evidence for epitope spreading

EAE in SJL mice represents a model of RR-MS because the clinical signs are manifested in a relapsing-remitting pattern. Mechanistic studies have provided one explanation for this clinical course. Analysis of the specificity of T cells infiltrating the CNS over time demonstrated the phenomenon of epitope spreading in which some naïve T cells that are non-specifically recruited to the CNS by inflammatory signals are activated by encounter with myelin epitopes generated during tissue damage that are distinct from the epitope targeted by the T cells that initially induced disease [118–120]. Activation of these T cells specific for different myelin epitopes trigger new waves of autoimmune T cell responses that promulgate the disease. Thus, epitope spreading may explain how recurring autoimmune responses in RR-MS are propagated over time [121]. Importantly, it has been shown that initially tolerizing T cell responses to epitopes expected to occur later in the immune response can prevent relapses [122]. Other animal models have also suggested that epitope spreading contributes to CNS autoimmunity. Infection of mice with the demyelinating virus

Theiler's murine encephalomyelitis virus (TMEV) leads to infiltration of virus-specific T cells that facilitate tissue damage as they eliminate virally infected cells. Myelin epitopes are presented by APCs during TMEV infection [123], and T cells specific for myelin epitopes expand in the CNS later in infection and promulgate an autoimmune response [124]. The notion of epitope spreading provides a satisfying explanation for the chronicity of the autoimmune response in the CNS; however, others have reported that the dominant T cell response remains directed toward the priming epitope throughout relapses [125], and that relapses can be prevented by depleting the transferred T cells that initiated disease but not host cells that would contribute to epitope spreading [126].

Other relapsing/remitting EAE models have also been described. C57BL/6 mice can develop a relapsing-remitting course when disease is induced with low doses of MOG 35–55 [127], or when the saponin extract Quil A is used as an adjuvant [128] in place of complete Freund's adjuvant. The mechanisms responsible for relapses in these models have not been identified.

EAE models of SP-MS may be useful for development of therapies

A change in pathogenic mechanisms is hypothesized to occur when patients with MS transition from RR- to SP-MS. As discussed above, inflammation in the CNS (at least as detected by imaging techniques) decreases while brain atrophy and axonal loss steadily increase. Accordingly, patients with SP-MS do not respond to immunosuppressive reagents [20]. Thus, new therapies that focus on neuroprotective strategies may be needed.

A few EAE models exist that exemplify some characteristics of SP-MS. Biozzi ABH mice initially develop relapsing-remitting disease which then transitions to a steadily progressive disease course [129]. In addition, NOD mice induced for EAE demonstrate a partial recovery after the initial acute phase that is followed by a chronic progressive disease course [130]. Significant axonal and neuronal loss are found in both NOD and Biozzi mice with chronic progressive EAE, similar to patients with SP-MS. As in patients with SP-MS, immunosuppressive strategies fail to ameliorate secondary progressive EAE (SP-EAE) in these models. For example, immunosuppression by FTY720 ameliorates disease in the RR but not in the SP phase of Biozzi mice [131]. Additionally, an immunosuppressive strategy of CD4⁺ T cell depletion combined with an agent designed to promote tolerance to CNS antigens was able to halt relapses, but not subsequent progression of disability in the Biozzi relapsing-progressive model of EAE [132]. Importantly, neuroprotective therapeutic approaches have offered some promise in SP-EAE models. Axon loss and clinical progression were reduced in NOD mice with progressive EAE following treatment with the neuroprotective agent ABS-75, despite demonstrating no effect in traditional relapsing and monophasic EAE models [130]. In addition, the Toll-like receptor 2 (TLR2) and poly(ADP-ribose) polymerase 1 (PARP-1) pathway has been identified as potential new therapeutic targets using this same progressive EAE model in NOD mice [133]. 15 α -hydroxycholestine (an activator of the PARP-1 pathway) is increased in patients with SP-MS and NOD mice with SP-EAE, and the PARP-1 inhibitor 5-aminoiso-quinolinone (AIQ) inhibited clinical signs, axonal loss and demyelination in NOD mice with SP-EAE. Whether therapeutic strategies identified in these progressive EAE models are efficacious in patients with SP-MS remains to be seen.

Atypical and classic EAE models represent different inflammatory patterns seen in patients with MS

As discussed above, most patients with MS develop lesions in the brain with or without accompanying spinal cord lesions. Only a subset of patients exhibit a form of MS in which lesions are found primarily in the spinal cord and optic nerves with relative sparing of the

brain. Most mouse EAE models also exhibit an inflammatory pattern in which parenchymal lesions are found predominantly in the spinal cord and optic nerve with inflammation in the brain primarily restricted to the meninges. This distribution of lesions that causes primarily spinal cord injury accounts for the major clinical signs of ascending flaccid paralysis associated with classic EAE. There are a few EAE models, however, in which mice develop parenchymal inflammation in the brain (with or without accompanying spinal cord inflammation). Mice with brain inflammation exhibit clinical signs that include leaning, rolling, and ataxia, referred to as “atypical EAE”. Studies of both atypical and classic EAE models have identified some mechanisms that lead to inflammation in the brain versus the spinal cord, and may help illuminate mechanisms underlying the varying lesion localization patterns among patients with MS.

Both IFN- γ and IL-17 appear to play important roles in differentially regulating inflammatory responses in the brain and spinal cord. Mice that are genetically deficient in IFN- γ or IFN- γ R exhibit a high incidence of atypical EAE [59, 134–136]. This change in clinical manifestation reflects an increase in the localization of inflammatory cells in the brain, suggesting that IFN- γ may in fact inhibit inflammation in the brain during CNS autoimmunity. Interestingly, some studies have shown that IFN- γ signaling enhances spinal cord inflammation, indicating that this cytokine may exert opposite effects in the brain compared to the spinal cord [134]. The inflammatory infiltrate observed in mice genetically deficient in IFN- γ signaling is often characterized by a high number of neutrophils [135, 137, 138]. Neutrophils are not a feature in the CNS pathology of patients with MS; however, neutrophils are often the first-responders during inflammation and usually disappear within 24–48 hours of tissue infiltration. Therefore, neutrophils may contribute to disease during MS onset or relapse without being detected in pathology samples. As mentioned above, a pilot study of IFN- γ supplementation in 18 patients with MS was halted due to increased exacerbation rates [60]. It is possible that this worsening of disease activity may have been due to enhancement of spinal cord lesions, as EAE studies would predict.

Other models of atypical EAE have been described that do not depend on IFN- γ deficiency. For example, certain H-2^k mouse strains generate PLP-specific T cells that preferentially induce lesions in the brainstem and cerebellum [139]. This was hypothesized to reflect either the ability of these particular peptide/MHC ligands to elicit T cells whose cytokine production promoted brain inflammation or differential processing of myelin antigen in different CNS compartments. Later studies using a different myelin antigen revealed that the priming epitope influences the effector cytokines produced by T cells, and that these cytokines contribute to lesion localization [51]. Th17 cells in particular were implicated as important promoters of brain inflammation. Our studies in C3HeB/Fej mice showed that EAE was strongly affected by the abundance of Th1 and Th17 cells within the T cell population infiltrating the CNS. We found that inflammation in the spinal cord was induced by transferring T cells with a wide range of Th17:Th1 ratios; however, inflammation in the brain was induced only when the Th17:Th1 ratio was ≥ 1 [51]. Blockade of IL-17 signaling via neutralization of IL-17 activity [51] ameliorated brain but not spinal cord inflammation in this atypical EAE model. Additionally, atypical EAE that developed in C57Bl/6 mice following adoptive transfer of IFN- γ ^{-/-} T cells converted to classic EAE in IL-17R^{-/-} recipients [135]. These findings demonstrate that IL-17 signaling strongly promotes inflammation in the brain, but does not seem to be required for inflammation in the spinal cord. Importantly, these studies indicate that the brain and spinal cord are very distinct microenvironments that exhibit disparate responses to infiltrating myelin-specific T cells. Future studies are needed to determine whether preferential localization of lesions in the brain versus spinal cord of patients with MS correlates with expression of IFN- γ , IL-17, or other inflammatory mediators.

Some animal models exhibit lesions that reflect differences in lesion pathology seen in patients with MS

The pathological features of classic EAE models are most reminiscent of patterns I and II lesions in MS [6], supporting the idea that pattern I and II lesions may result primarily from the activity of T cells and macrophages. As pattern II MS lesions are also distinguished by immunoglobulin and activated complement deposits, B cells and complement likely play a pathogenic role in patients with this pattern of lesions. Similarly, B cells appear to contribute to the pathogenesis in some, but not all, EAE models (see above). Unlike pattern I and II lesions, pattern III and IV lesions resemble a primary oligodendrocyte dystrophy, demonstrating a high degree of oligodendrocyte cell death with resulting demyelination. This type of primary oligodendrocyte damage is seen more in cuprizone-induced demyelination models [140, 141] or virus-induced demyelination in mice [142], than in commonly used EAE models. However, similarities to pattern III and IV pathology were reported in a CD8⁺ T cell-mediated EAE model [143], as will be discussed further below. Future studies should determine whether these different models will be able to better predict therapeutic response by patients with corresponding pathological patterns, or aid in our understanding of various pathogenic mechanisms contributing to varied lesion patterns.

Modeling CD8⁺ T cell activity in CNS autoimmune disease

CD8⁺ T cells are implicated in the pathogenesis of MS

Substantial evidence from patients with MS indicates that CD8⁺ T cells play a significant role in the pathogenesis of the disease [144–146]. CD8⁺ T cells are more abundant than CD4⁺ T cells in acute and chronic CNS lesions in MS [147, 148], and CD8⁺ but not CD4⁺ T-cell clones persist in both the CNS and blood of patients with MS [3, 149–152]. An enrichment of neuroantigen-specific CD8⁺ but not CD4⁺ T cells in the CNS of patients with MS relative to healthy controls has been reported [153, 154], and the observation that the extent of axonal damage correlates with the number of CD8⁺ T cells and macrophages suggests that these cells could have pathogenic activity [155, 156]. Importantly, immunotherapies specifically targeting CD4⁺ T cells failed to show significant clinical benefit in MS whereas therapies that affect all leukocytes can improve disease [157]. One of the most challenging aspects of developing animal models of MS; however, has been to generate models in which the role of CD8⁺ T cells can be studied.

Initial studies of CD8⁺ T cell activity in EAE models implicated a regulatory function [158–160]. Different subsets of regulatory CD8⁺ T cells have since been described in EAE [161–163]. In contrast, several animal models using CD8⁺ T cells specific for either myelin antigens [143, 164–167] or neo-self antigens expressed in the CNS [168, 169] demonstrated a pathogenic role for CD8⁺ T cells in CNS autoimmunity. Collectively, these models reveal a diverse potential for CD8⁺ T cells to contribute to the pathogenesis of MS.

EAE models provide evidence for a regulatory role of CD8⁺ T cells

CD8⁺ T cells were first investigated in EAE by inducing disease in mice either treated with CD8-depleting antibody or in mice genetically deficient in the expression of CD8. Many studies reported more relapses, exacerbated clinical signs and increased susceptibility to re-induction of EAE, suggesting that CD8⁺ T cells exert a regulatory function in ameliorating CNS autoimmunity initiated by CD4⁺ T cells [158–160], although one report implicated a pathogenic function [170]. Several types of regulatory CD8⁺ T cells have since been described in EAE, some of which do not depend on specificity for the relevant auto-antigen. CD8⁺ T cells specific for the MHC class Ib molecule Qa-1 complexed to peptides generated by most activated CD4⁺ T cells regardless of antigen specificity have been identified that suppress autoimmune responses, including EAE [171, 172]. CD8⁺CD28^{-/-} polyclonal T

cells have also been shown to exert a suppressive function in EAE by interacting with APCs and suppressing costimulatory molecule expression [162]. Immunoregulatory activity has also been attributed to some myelin-specific CD8⁺ T cells. One group reported that adoptive transfer of MOG_{35–55}-specific CD8⁺ T cells isolated from C57BL/6 mice did not induce EAE and instead mediated a protective effect when transferred into animals at the peak of EAE induced by CD4⁺ T cells [173].

EAE models reveal pathogenic activity of myelin-specific CD8⁺ T cells

In contrast to the regulatory role for CD8⁺ T cells described above, most studies of myelin-specific CD8⁺ T cells demonstrated pathogenic activity [143, 164–166]. Interestingly, adoptive transfer of CD8⁺ T cell clones specific for MBP_{79–87} associated with the H-2K^k MHC class I exhibited some pathological features seen in a subset of patients with MS that are not typically seen in CD4⁺ T cell-mediated models [143]. Lesions were observed only in the brain in these animals, and were most common within the white matter of the cerebellum. However, widely scattered, focal involvement of gray and white matter in the brain stem, midbrain, and cerebral cortex was also seen. The lesions seen in this CD8-mediated EAE model demonstrated significant oligodendrocyte cell death (both apoptotic and necrotic) with perivascular cuffing and tissue damage consistent with focal cytotoxic or ischemic cell injury [143], resembling aspects of Patterns III and IV lesions seen in patients with MS [6].

The MBP_{79–87}-specific CD8⁺ T cell model was further developed by generating mice expressing transgenic TCRs specific for this epitope [174]. Two models expressing distinct TCRs revealed different fates of CD8⁺ T cells expressing MBP_{79–87}-specific TCRs. T cells expressing one MBP_{79–87}-specific TCR were subjected to tolerance mechanisms that prevented them from contributing to autoimmunity. T cells expressing the other MBP_{79–87}-specific TCR, however, were not subjected to tolerance and these T cells populated the periphery in a seemingly naïve state [174]. Autoimmunity could be induced in these mice by infection with viruses encoding MBP [167]. Strikingly, disease could also be induced in these mice by infection with either wild-type vaccinia or adenovirus that did not express MBP [167]. Mechanistic studies revealed that wild-type viral infection induced disease in the CD8⁺ TCR transgenic mice by activating the small subset of T cells that express two TCRs, one specific for the virus and one for MBP_{79–87}. This model provides an intriguing explanation for how a common viral infection could serve as a trigger for autoimmunity by demonstrating that activation of dual TCR-expressing CD8⁺ T cells capable of recognizing both MBP and viral antigens (presumably a rare event in humans) can induce autoimmune disease [167]. The pathogenic potential of myelin-specific CD8⁺ T cells has also been demonstrated in humanized mice in which a human MHC class I molecule and a human PLP-specific TCR are co-expressed [166].

Animal models have also been developed in which mice expressing a transgenic neo-self antigen in oligodendrocytes are bred to mice expressing a transgenic TCR specific for the same neo-self antigen [168, 169, 175]. Interestingly, spontaneous disease was triggered in double-transgenic mice that co-expressed the neo-self antigen with a MHC class I-restricted, but not MHC class II-restricted, TCR specific for the neo-self antigen [168, 175]. The reason for the difference in susceptibility to spontaneous disease conferred by these TCRs is not clear, and may be due to varying affinities of the TCRs used in the study; however, this model suggests that naïve CD8⁺ T cells can initiate CNS autoimmunity. In a separate model, adoptive transfer of activated CD8⁺ T cells into mice expressing a distinct neo-self antigen in oligodendrocytes resulted in lesions with focal loss of oligodendrocytes, demyelination, and microglia activation [169].

New animal models reveal interplay between myelin-specific CD4⁺ and CD8⁺ cells in EAE

Both CD8⁺ and CD4⁺ T cells are found in the CNS of patients with MS, and it is likely that both T cell subsets contribute to the pathogenesis of the disease. Interplay between CD8⁺ and CD4⁺ myelin-specific T cells was first reported in the animal model using humanized mice expressing the MHC class-restricted transgenic TCR specific for PLP [166]. A low incidence of spontaneous disease was seen in this model, although mild disease could be induced by immunization with PLP_{45–53} in CFA. Importantly, determinant spreading from the initial CD8⁺ PLP-specific T cell response to a separate CD4⁺ T cell response specific for a MHC class II-restricted epitope of MOG was observed in these mice. As the disease initiated by the CD8⁺ TCR transgenic T cells did not progress in mice on a Rag2^{−/−} background, these results suggest that additional contributions from MHC class II-restricted CD4⁺ T cells are required in some cases for disease progression [166].

Our recent studies demonstrate CD4⁺/CD8⁺ T cell interplay in the opposite direction: CD4⁺ MOG-specific T cell-initiated EAE led to determinant spreading to CD8⁺ MBP-specific T cells whose epitope was cross-presented by Tip-DCs in the inflamed CNS [176]. Importantly, the MHC class I-restricted epitope of MBP was also presented by oligodendrocytes as well as by Tip-DCs. This model raises the intriguing possibility that newly activated CD8⁺ T cells might exert a regulatory function by lysing the Tip-DCs in the CNS that present antigen to both MHC class I- and II-restricted T cells, and also contribute to tissue damage by lysing oligodendrocytes. Thus, we speculate that the same myelin-specific CD8⁺ T cells could exert both pathogenic and immunoregulatory activities in the CNS. However, the potential pathogenic and immunoregulatory role of CD8⁺ T cells in the disease course in MS patients remains to be seen.

Concluding remarks

EAE models have been invaluable tools in helping us understand the different ways various immune cells could contribute to MS. We have discussed EAE models that illustrate the regulatory potential of CD4⁺ T cells, B cells, and CD8⁺ T cells as well as their pathogenic capability in inducing inflammation and demyelination in the CNS (Figure 1). As our understanding of CNS inflammation grows, the importance of developing EAE models that allow us to study the interaction between these various immune cell types in CNS autoimmunity is clear.

The vast number of animal models developed for MS has garnered both criticism and praise in their actual ability to translate to advances in treatment for patients with MS, resulting in both great successes and disappointing failures. By attempting to replicate various heterogeneous aspects of MS in specific animal models, we may be able to improve the ability of such models to develop and predict treatments targeted for specific aspects of this heterogeneous disease.

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Future Challenges and Outstanding Questions

How well are the key features of SP-MS recapitulated in the current animal models? Additional models will likely be needed to determine the role of inflammation, define mechanisms underlying continuous tissue damage and to design treatment strategies for patients with SP-MS.

Is the location of inflammatory lesions within the CNS of patients with MS defined by the relative abundance of T cells producing IFN- γ and IL-17? Animal models suggest that differential effects of IFN- γ and IL-17 signaling in the brain versus the spinal cord determines where inflammatory lesions localize, suggesting heterogeneity in the responses of resident cells in the brain and spinal cord. It remains to be determined whether these findings translate to humans and/or whether there are other inflammatory mediators that specify sites of lesion localization.

Can pathogenic mechanisms underlying the different structures of lesions in patients with MS be identified using animal models? It is not known whether the heterogeneity in lesion structure among patients reflects different pathogenic mechanisms. More animal models that generate different types of lesions, such as the CD8+ T cell-mediated model of EAE, are needed to address this question. Understanding the mechanisms underlying pathologic differences may lead to tailored therapeutic treatments for individual patients.

How does the interplay between CD4+ and CD8+ T cells contribute to the pathogenesis of MS? In animal models, CD8+ T cells appear to play both pathogenic and regulatory roles. It is not known if these activities reflect distinct subsets of CD8+ T cells, or how the activity of CD4+ and CD8+ T cells influence each other and resident CNS cells during the pathogenesis of MS. It is important to determine if the interplay between CD4+ and CD8+ T cells changes during the course of the disease, and whether this dynamic contributes to the transition from RR-MS to SP-MS.

Highlights

Multiple animal models are needed to model the heterogeneous features of MS

Individual EAE models replicate specific features of disease

Collectively, EAE models link CD4 T, CD8 T and B cells to disease pathogenesis

Additional models are needed for primary and secondary progressive MS

Figure 1. Model of CD4⁺ T cell-initiated CNS autoimmunity

The sequential steps proposed for the pathogenesis of CD4⁺ T cell-initiated disease are indicated by numbers. CD8⁺ T cell initiated autoimmunity may occur, but is not included in this schematic. (a) Genetic and environmental factors both promote myelin-specific CD4⁺ T cell activation and influence the type and efficacy of the corresponding immunoregulatory response mediated by regulatory (reg) CD4⁺ and CD8⁺ T cells and B cells. (b) Activated CD4⁺ T cells enter the CNS and are re-activated by resident APCs, triggering production of inflammatory mediators. (c) These mediators promote (i) localized inflammation of the blood brain barrier (BBB) that facilitates recruitment of naïve CD4⁺ and CD8⁺ T cells, B cells, and monocytes to the CNS, and (ii) may directly damage myelin and/or

oligodendrocytes. (c) Determinant spreading occurs as APCs presenting epitopes derived from myelin debris activate newly recruited T cells with different myelin specificities. (d) Newly activated CD8⁺ T cells may gain the ability to lyse both (a) APCs presenting myelin antigen and (e) oligodendrocytes. Dashed lines indicate pathways not yet verified with experimental evidence.

Table 1

Different animal models replicate various aspects of MS

	<u>Clinical Course</u>		
	Model System	Strain:Antigen	References
Relapsing-Remitting	Wild-type mice	SJL/J: PLP ₁₃₁₋₁₅₁	[102]
	Transgenic TCR ^{MOG}	SJL/J: MOG ₉₂₋₁₀₆ (spontaneous)	[88]
	Wild-type mice, adjuvant specific	C57BL/6:MOG ₃₅₋₅₅ (adjuvant Quil A)	[112]
	Wild-type mice, antigen/adjuvant dose specific	C57BL/6: MOG ₃₅₋₅₅ (low dose)	[111]
Secondary Progressive	Wild-type mice	Biozzi ABH: spinal cord homogenate	[113]
	Wild-type mice	NOD: MOG ₃₅₋₅₅	[114]
	<u>Lesion Localization</u>		
	Model System	Strain:Antigen	References
Spinal Cord	Most EAE models		
Opticospinal	Transgenic TCR ^{MOG} x IgH ^{MOG}	C57BL/6: MOG (spontaneous)	[86,87]
Brain (+/- Spinal Cord)	IFN γ or IFN γ R deficiency	Multiple	[44,118-120]
	Wild-type mice	CBA/J: PLP ₁₉₀₋₂₀₉	[123]
	Wild-type mice	C3H/HeJ: PLP ₁₉₀₋₂₀₉	[123]
	Wild-type mice	C3HeB/FeJ: MOG ₉₇₋₁₁₄	[36]
	Transgenic TCR ^{MOG}	SJL/J: MOG ₉₂₋₁₀₆ (spontaneous)	[88]
	Wild-type mice (CD8 T cell clones)	C3H/FeJ: MBP ₇₉₋₈₇	[127]
	<u>Pathological Pattern</u>		
	Model System	Strain:Antigen	References
Pattern I/II	Most CD4-mediated EAE Models		[4]
Pattern III/ IV	Wild-type mice (CD8 T cell clones)	C3H/FeJ: MBP ₇₉₋₈₇	[127]
	Cuprizone-induced demyelination	C57BL/6, Swiss Webster	[124,125]
	TMEV-induced demyelination	SJL/J	[126]