Published in final edited form as:

Pathophysiology. 2011 February ; 18(1): . doi:10.1016/j.pathophys.2010.04.004.

The Relevance of Animal Models in Multiple Sclerosis Research

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Abstract

Multiple Sclerosis (MS) is a complex disease with an unknown etiology and no effective cure, despite decades of extensive research that led to the development of several partially effective treatments. Researchers have only limited access to early and immunologically active MS tissue samples, and the modification of experimental circumstances is much more restricted in human studies compared to studies in animal models. For these reasons, animal models are needed to clarify the underlying immune-pathological mechanisms and test novel therapeutic and reparative approaches. It is not possible for a single mouse model to capture and adequately incorporate all clinical, radiological, pathological and genetic features of MS. The three most commonly studied major categories of animal models of MS include: (1) the purely autoimmune experimental autoimmune/allergic encephalomyelitis (EAE); (2) the virally induced chronic demyelinating disease models, with the main model of Theiler's Murine Encephalomyelitis Virus (TMEV) infection and (3) toxin-induced models of demyelination, including the cuprizone model and focal demyelination induced by lyso-phosphatidyl choline (lyso-lecithine). EAE has been enormously helpful over the past several decades in our overall understanding of CNS inflammation, immune surveillance and immune-mediated tissue injury. Furthermore, EAE has directly led to the development of three approved medications for treatment in multiple sclerosis, glatiramer acetate, mitoxantrone and natalizumab. On the other hand, numerous therapeutical approaches that showed promising results in EAE turned out to be either ineffective or in some cases harmful in MS. The TMEV model features a chronic progressive disease course that lasts for the entire lifespan in susceptible mice. Several features of MS, including the role and significance of axonal injury and repair, the partial independence of disability from demyelination, epitope spread from viral to myelin epitopes, the significance of remyelination have all been demonstrated in this model. TMEV based MS models also feature several MRI findings of the human disease. Toxin induced demyelination models have been mainly used to study focal demyelination and remyelination. None of the three main animal models described in this review can be considered superior; rather, they are best viewed as complementary to one another. Despite their limitations, the rational utilization and application of these models to address specific research questions will remain one of the most useful tools in studies of human demyelinating diseases.

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Keywords

multiple sclerosis; demyelination; experimental autoimmune encephalomyelitis; EAE; Theiler's murine encephalomyelitis virus; TMEV; toxic demyelination; Cuprizone; lyso-phosphatidyl choline; remyelination

1. Introduction

Multiple Sclerosis (MS) is a chronic immune-mediated demyelinating disease of the central nervous system[1]. It is the leading cause of non-traumatic disability among young adults and has great socioeconomic impact in developed countries. According to the National MS Society, approximately 400,000 people have been diagnosed with MS in the United States, with 200 new cases added every week. MS is a very heterogeneous disease from a variety of standpoints, including its clinical presentation, radiological features, immuno-pathological subtypes, response to therapy and genetic associations. A recent detailed analysis of a large series of active demyelinating lesions revealed four distinct patterns of immune-pathology [2]. The first two patterns feature well-demarcated perivascular demyelination and relative sparing of oligodendrocytes. Lesions in pattern I and II show close similarities to T cell-mediated and T cell- and antibody-mediated autoimmune demyelination. Lesions in patterns III and IV are suggestive of oligodendrogliopathy in an inflammatory background [3] (Figure 1).

To date, there is still no definitive cause and no effective cure for MS, although several genetic and environmental risk factors have been identified, and a number of partially effective preventive treatment modalities are now available to modify the disease course. Therefore, animal models of MS are needed to further explore mechanisms of disease initiation and progression and test various therapeutical and restorative approaches. Given that MS is a complex disease with an unclear etiology; a single animal model is unlikely to accurately represent all aspects of pathology and clinical features of human MS. However, the availability of three major animal models of MS enables studies of several relevant features of the human disease.

2. Animal models of MS

Over the past several decades, a number of animal models have been developed in order to understand a variety of aspects of human MS. The main driving force for animal studies stems from the following limitations of human studies: overall limited access to human MS tissue, biopsies are rarely performed and autopsy samples are usually biased towards a chronic, burnt-out stage; experimental circumstances can't easily be modified in clinical trials and mechanistic studies addressing disease pathomechanism(s) can't readily be performed in patients.. The most commonly studied animal models of MS are (1) the purely autoimmune experimental autoimmune/allergic encephalomyelitis (EAE), (2) viral induced models, mainly Theiler's murine encephalomyelitis virus (TMEV) infection and consequential chronic demyelination and (3) toxin-induced models of demyelination, including the cuprizone model and focal demyelination induced by lyso-phosphatidylcholine (lysolecithin).

2.1. Autoimmune experimental autoimmune/allergic encephalomyelitis

EAE is the most extensively studied animal model of autoimmune disease, with over 8000 publications listed on PubMed at the time of writing this manuscript. It is an excellent model of inflammation in the brain in general, and of post-vaccinal encephalitis[4] and is most frequently used to model inflammatory aspects of MS. This model was discovered in 1930s

by Rivers et al.[5], while trying to elucidate the etiology of neurological complications that followed anti-rabies vaccinations. His work was inspired by previous findings about fatal paralyzes that occurred after anti-rabies treatment[6]. Initially, an inflammatory demyelinating disease was induced in rhesus monkeys immunized with emulsions of normal sterile rabbit brains[7]. Myelin destruction with perivascular infiltrates was found in brains and spinal cords of 6 of the 8 treated monkeys. Subsequently, EAE induction was performed by the use of adjuvants [8] added to brain emulsions, spinal cord homogenate, myelin preparations, or purified myelin proteins. Since these initial experiments, EAE has been induced in a variety of mammal species, including mice, rats, guinea pigs, rabbits, goats, sheep, marmosets and primates [4]. In 1947 Wolf et al. documented the resemblance of human demyelinating diseases and EAE[9]. Since the initial description, the name of EAE has been used interchangeably as either experimental allergic encephalomyelitis or experimental autoimmune encephalomyelitis, with the latter favored in more recent literature.

EAE can be induced by subcutaneous injection of an emulsion that contains an adjuvant and synthetic peptides derived from myelin proteins: myelin oligodendrocyte glycoprotein (MOG), myelin basic protein (MBP) or proteolipid protein (PLP); transgenic mice that develop EAE have also been reported, and EAE also can be induced by cell transfer from EAE donors to native recipients. In the classic scenario, immunization leads to activation and expansion of peripheral antigen-specific T-cells. These cells enter the CNS, encounter the specific myelin antigen and subsequently induce disease. Myelin protein-specific CD4⁺ T cells are generally considered necessary for EAE induction, as adoptive transfer of these cells from immunized into normal animals elicits EAE in 100% of animals[10]. It is generally considered that CD4⁺ Th-1 cells are the primary mediators in EAE development, and more recently, Th17 cells have also been considered as important mediators of pathology[11]. In addition, a role for autoimmune MOG specific CD8⁺ T cells responses have also been discussed [12–14]. Studies involving EAE have had an important role in identifying and delineating several aspects of the MS biology: inflammation, immune surveillance and immune-mediated tissue injury. Moreover, this experimental model has directly lead to the development of three medications approved for multiple sclerosis, glatiramer acetate, mitoxantrone and natalizumab[15] (Table 1).

A recent criticism of EAE has been the numerous therapeutical approaches that showed promising results in this mouse model that ultimately turned out to be either inefficient or in some cases harmful in human MS [16]. Therefore, the potential benefits of EAE as a predictor of therapeutic efficacy in MS will remain in question. Sriram et al suggest a more careful utilization of the EAE model in defining therapies[16]. They further proposed that EAE is more pertinent as a model of acute disseminated encephalomyelitis (ADEM) than MS, which is well aligned with the original ideas of Rivers, who initially described EAE. Recently, Nelson et al (Table 2) compared the outcome of MS related clinical trials to the outcome in EAE and TMEV models[17]. The results of this analysis implied that the viral model TMEV might be a more accurate predictor of response to therapy. However, to further validate this statement, one would need to study all, or a least most of the agents that have been tried in EAE and MS, which clearly has not been the case yet. However, it is worth mentioning that short of natalizumab, which has not been tested in TMEV, every currently used MS treatment is effective in TMEV, and the potential role of anti-glatiramer acetate antibodies in remyelination was also first described in the TMEV model [18]. However, as mentioned earlier, EAE clearly has led to the development of 3 approved therapies so far with more expected to follow.

Another criticism of the EAE model is that while MS is clearly an immune mediated disease, it lacks many features of classic autoimmunity. According to Schwartz et al[19], the

modified criteria for autoimmune diseases include 7 key factors: (1) Demonstration of an immune response to a precise autoantigen in all patients with the disease; (2) Reproduction of the pathology by administration of autoantibody or T cells into a normal animal; (3) Induction of pathology by immunizing an animal with relevant purified autoantigen; (4) Isolation or presence of autoantibody or auto-reactive T cell from the target organ; (5) Correlation of autoantibody or auto-reactive T cell with disease activity; (6) Presence of other autoimmune disorders or autoantigens associated with disease; (7) Immune absorption with purified autoantigen abrogates pathogenic autoantibody or auto-reactive T cell. While these key factors may be applicable to EAE or even to neuromyelitis optica (NMO) [20], some of these factors are not immediately applicable to MS, at least not in our current state of knowledge.

Another weakness of the autoimmune model is that in EAE lesions, CD4⁺ T-cells predominate in the perivascular infiltrates. Based on this basic feature of EAE, MS has been proposed to be a CD4⁺ T-cell mediated autoimmune disease by several investigators. However, the strict application of such a model fails to explain several features of MS [21] (Figure 2). MS lesions can be very diverse, from those that show extensive inflammation to others that feature minimal inflammation and oligodendroglial pathology [2]. Moreover, analysis of human MS lesions revealed that the predominant immune cell types are CD8⁺ T-cells and macrophages [22,23], with CD4⁺ T-cells being much less frequent [21,24]. In addition to inflammation and demyelination, both acute and chronic axon injury can be observed in some EAE lesions [25]. The mechanism of axonal damage in EAE remains to be more firmly defined, since no specific effector cell type has been identified that elicits axonal injury [26], although macrophages have been proposed to play this role. In summary, while caution is advised against the universal applicability of its derived findings to human MS (and the same can be said about every animal model), the EAE model has been used as the most frequently studied model leading to a major and critically important expansion of our knowledge about neuroinflammation and CNS centered autoimmunity. Due to the large number of teams studying the model, the vast body of data derived from the various EAE models, and as a result of the relative ease of disease induction, EAE is expected remain the most frequently studied MS model.

2.2. Virus Induced Demyelination

Epidemiological studies have put forward a hypothesis that a viral infection early in life, in the presence of a specific genetic background, may result in immune mediated attack against CNS tissue [27–31]. However, to date there is no specific virus that has been identified as a potential cause or contributor to MS. Most recently EBV infection has been linked to MS as a critical environmental susceptibility factor [32–34]. To model the contribution of viruses in human MS, some investigators utilize virus induced demyelination models.

TMEV (Theiler's murine encephalomyelitis virus) is a mouse enteric pathogen that belongs to the single-stranded RNA picornaviruses. The viruses in this family are extremely small; on scanning electron microscopy they are about the size of a ribosome. The initial observation and description of the virus that caused flaccid paralysis in mice, but not monkeys, was reported in 1934 by Max Theiler [35]. Experimentally, demyelinating disease in susceptible mouse strains is induced by intracerebral infection with TMEV. There are many strains of TMEV which are divided into 2 major subgroups. One group consists of highly virulent strains that cause fatal encephalitis. A representative of this group is the GD-VII strain. The second group is much less virulent and is currently used as a murine model of MS. The BeAn and Daniel's TMEV strains are the best-known examples for this group [36]. The disease caused by these less virulent strains is either mono- or biphasic. The monophasic disease consists of a transient meningo-encephalo-myelitis, which reaches its

peak by approximately day 7 after infection and clears in approximately 3 weeks. Every mouse strain develops this transient stage, which in general recovers without the development of a major persistent neurological disease. However, in certain susceptible mouse strains, a biphasic disease develops. Following the initial monophasic phase outlined above, a chronic demyelinating stage develops in which most of the demyelinating lesions are in the spinal cord, similarly to EAE models [37] (Figure 3). Unlike EAE, the disease is always chronic-progressive in susceptible mice.

The TMEV model can be considered attractive for two major reasons: (1) its virus-induced pathology has clear similarities to human MS [28,38,39]; (2) it models an autoimmune response triggered by viral infection in the CNS [40]. Epitope spreading from virus to self epitopes has been observed in the TMEV model [41,42]. Pathological features of virus-induced demyelinating disease are in general mediated by the immune system and not by direct viral cytopathy, and the clinical presentation is very similar to that observed in chronic progressive MS [43]. There are potential advantages of using TMEV-induced demyelinating disease: (1) chronic disease course that lasts for the entire lifespan of the studied rodents; (2) pathological abnormalities are limited to the CNS; (3) demyelination and axonal injury in spinal cord of some mouse strains are extensive [40]; (4) certain mouse strains that demyelinate feature a reparative phenotype [44] (5) several viruses are proven to cause demyelination in humans (JC virus, rubella, measles and HTLV-1) (although in these cases, epitope spread towards autoimmune epitopes has not been conclusively demonstrated) and (6) epidemiologically, viral infections were associated with clinical exacerbations of MS [45,46] but are unlikely to be the direct etiological factors of demyelination in human MS.

Susceptibility to TMEV induced demyelinating disease is related to major histocompatibility complex (MHC) class I molecules. Mice with the H-2^{b,d,k} MHC haplotypes are resistant to chronic TMEV infection [47]. These mouse strains only develop the above detailed monophasic disease, without demyelination and/or viral persistence. In contrast, mice with the H-2^{q,r,s,v,f,p} MHC haplotypes are susceptible to persistent virus infection. In all mice, intracerebral injection of TMEV causes acute non-lethal encephalitis, with the highest viral antigen load in the midbrain, thalamus, hypothalamus, brain stem and spinal cord gray matter [43]. In most animals, the early phase of the disease is largely asymptomatic. In susceptible mice, the host immune system usually clears the virus from the brain but not from the spinal cord, which results in chronic demyelination with viral persistence in oligodendrocytes [48] and macrophages [49] of the spinal cord white matter. Epitope spread towards myelin epitopes was also observed in this chronic stage.

The appearance of inflammatory cells in close anatomical and temporal correlation with myelin destruction is also prevalent in TMEV infection. Immune-mediated destruction of myelin was suggested after experiments in which demyelination did not occur in immunosuppressed mice [50]. In TMEV induced demyelinating lesions, electron microscopy reveals numerous macrophages with engulfed myelin [43]. In the classic demyelinating SJL/J strain, demyelinating lesions are evident as early as 3 weeks post infection. Demyelination progresses and reaches a plateau at around 100 days post-infection. At that time, infected mice begin to present with neurological symptoms, including hind limb weakness, spasticity and gait abnormalities. After this time, spinal cord atrophy continues to progress which is explained at least in part by loss of medium and large diameter axons [51]. In TMEV susceptible mice, complete hind limb paralysis and bladder incontinence occurs 6–9 months following infection. SJL mice are a typical example of TMEV susceptible mice, which makes them ideal for testing therapeutic agents for their efficiency in promoting myelin repair or improvement of neurological function. Furthermore, genetically engineered mice that are deficient for specific regulatory proteins or immune components are valuable tools in the study of the disease process. For example, mice with targeted mutations in CD8, CD4

and β 2-microglobulin have been developed and experiments in these immune cell deficient mice have provided evidence that demyelination and axonal damage with neurologic deficits are functionally separable. MHC class I specific CD8+ T cells have been demonstrated to have a role in the progression of neurological deficits after demyelination has occurred [52,53].

There is no consensus about the exact mechanism of demyelination in the TMEV model. Therefore, several mechanisms are proposed with a possibility that various combinations of these mechanisms could be responsible for myelin damage throughout the course of the disease[43]. The proposed mechanisms include: (1) direct viral cytopathic effects on oligodendrocytes, (2) TMEV-specific, autoimmune destruction of infected oligodendrocytes, (3) bystander demyelination due to toxic metabolites from activated macrophages, (4) epitope spreading and (5) molecular mimicry[54,55], and (6) “inside out” demyelination: axonal damage first, resulting in demyelination as a secondary consequence [56].

With the advent of small animal MRI and MRS techniques, a growing number of investigators are trying to draw parallels between human MS and its rodent models. It is worth noting that several features of MS have been successfully captured in the TMEV model. These include: (1) the presence of brain, brainstem and spinal cord lesions in transgenic mice[57]; (2) T2 hyperintense spinal cord lesions in the classic TMEV model in SJL mice[58]; (3) T1 hypointensities or T1 black holes in the cerebrum[59]; (4) deep gray matter T2 hypointensity, which similarly to the human disease is best detectable in the thalamus and correlates strongly with disability and atrophy[60]; (5) brain and spinal cord atrophy, which correlates with disability; and (6) hemorrhagic demyelination in certain strains, with specific induction methods[61].

2.3. Toxin Induced Demyelination

The two most widely used toxins to induce demyelination in animal models of MS are lysolecithin and cuprizone. Lysolecithin is an activator of phospholipase A₂ and cuprizone is a copper chelator. Compared to EAE and virus induced demyelinating syndrome, toxin induced demyelination models do not attempt to mimic MS as a disease, but are mainly established as systems to study the process of focal demyelination and remyelination[62].

Injection of 2 microliters of 1% lysolecithin into the spinal cord is a well-established method for rapid induction of focal areas of demyelination. Usually the dorsal or ventrolateral funiculi of the spinal cord at the thoracolumbar level are used as an injection site. To date, lysolecithin injections into the spinal cord have been described in several mammals, including cat, rabbit, rat and a mouse[63]. More than 30 years ago, it was proposed that demyelination occurs due to primary toxic effects of detergent on myelin sheaths, rather than secondary effects on oligodendrocytes[64]. Demyelination is not immune-mediated and is evident even in immune-deficient mice. In the acute phase immediately following the lysolecithin injection, lesion sites are often infiltrated with T cells, B cells and macrophages. This short-lived infiltration is proposed to have a beneficial role in CNS repair[65]. However, chronic inflammation in lesions is minimal and complete remyelination occurs in 5–6 weeks. If young animals are used, lysolecithin lesions show rapid repair. Conversely, repair in older animals is much slower[66].

Cuprizone as a toxin induces myelin damage and precise features of the model vary depending on the utilized animal species. When exposed to cuprizone, mice, rats and guinea pigs develop spongiform encephalopathy [67,68]. However, unlike mice, rats do not develop clear demyelination in the spinal cord[69]. Therefore, specific features of cuprizone-induced disease depend on the animal being used, cuprizone dose and age of application. In the basic

model, addition of 0.2% of cuprizone to the diet in young adult mice results in demyelination of several white matter structures in the brain, including cerebellar peduncles, corpus callosum, internal capsule, anterior commissure and thalamic white matter[70]. Specific targets are mature oligodendrocytes, which fail to fulfill the extensive metabolic demand and eventually undergo apoptosis. Other cell types in the CNS are not affected. The main reason for metabolic failure is copper deficiency due to the copper chelation properties of cuprizone. Why oligodendrocytes are particularly susceptible to the effects of cuprizone remains unclear, considering that demyelination is not chronic and axons are still preserved. After cuprizone is removed from the diet, extensive remyelination is evident within 3–4 weeks. However, if dietary cuprizone is continued, oligodendrocytes are completely depleted and subsequent demyelination becomes persistent[71]. In conclusion, the cuprizone model is reproducible and appears to be suitable for therapeutical trials designed to repress demyelination or accelerate remyelination in multiple sclerosis[71].

Conclusions

There is no single animal model that can capture the entire spectrum of heterogeneity of human MS and its variety in clinical and radiological presentation. However, over the last several decades, useful and relevant animal models have been developed that represent selected aspects of the human disease. Depending on the specific research question, the rational selection of appropriate animal models is likely to yield outcomes that will result in translatable findings applicable to MS. Despite the clearly existing limitations, basic science MS research will continue to rely on these models for new drug development and for understanding the ramifications and diversity of pathomechanism in MS.

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Figure 1.

Summary of pathogenetic mechanisms involved in the formation of acute multiple sclerosis lesions. All active lesions occurred on a background of an inflammatory process, composed mainly of T lymphocytes and macrophages. Despite the similarities in the inflammatory reaction, the lesions segregate into four patterns of demyelination based on plaque geography, extent and pattern of oligodendrocyte pathology, evidence for immunoglobulin deposition and complement activation, and pattern of myelin protein loss. The four patterns are:

Pattern I: Macrophage-associated demyelination

Pattern II: Antibody/complement-associated demyelination

Pattern III: Distal dying-back oligodendrogliopathy

Pattern IV: Primary oligodendrocyte degeneration

Figure adopted and modified with permission from[3]

Figure 2.

Pathogenetic mechanisms proposed to be involved in the induction and propagation of MS, highlighting the differences between experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (MS). The text in red indicates observations that contradict the propositions based on a pure Th-1 cell mediated inflammatory disease. Abbreviations: CNS, central nervous system; CSF, cerebrospinal fluid; DC, dendritic cell; IFN- γ , interferon- γ ; IL-12, interleukin-12; IL-12R, IL-12 receptor; TNF- α , tumor necrosis factor- α .

Figure 3.

Time course and development of demyelination in the spinal cord of TMEV infected SJL/J mice. **(A)** Following TMEV infection of susceptible SJL/J mice at 45 d.p.i. (closed circles) to 92–100 d.p.i. (closed triangles), demyelination progresses from 3 to 11%. The 14% of spinal cord white matter showing demyelination at 195–220 d.p.i. (open squares) was not statistically different from the demyelination observed at 92–100 d.p.i. The black line denotes the mean for the group. Each point represents the cumulative demyelination score for a single animal. Representative examples of thoracic spinal cord sections **(B, C and D)** are shown for an uninfected **(B)** – low resolution overview, **(E)** – high resolution white matter segment), a 90-day infected **(C)** – low resolution, **(F)** – high resolution demyelinated white matter area) and a 195-day-infected **(D)** – low resolution, **(G)** – high resolution) SJL/J mouse. The lesion size became progressively larger between 45 d.p.i. **(C)** and 195 d.p.i. **(D)**; however, the lesion size at 92–100 and 195–220 d.p.i. was comparable. Adopted and extended from [51]

Table 1

Therapeutics approved for preventive treatment of MS that have been developed in the EAE model. The table also illustrates the lengthy process leading to the FDA approval of these therapeutic modalities. Modified with permission from [15]

Treatment	“Proof of concept” in EAE	FDA approval	Approved Indication
Glatiramer Acetate	1971	1996	RR-MS
Mitoxantrone	1987	2000	Secondary progressive MS, worsening RR-MS
Natalizumab	1992	2004 Withdrawn in 2005 Reinstated in 2006	RR-MS

Table 2

Comparison of treatment outcome in EAE, TMEV and MS. Note the overall better overlap between the outcome of the above treatment trial in TMEV and MS versus EAE and MS. Modified with permission from [17]

Treatment	Outcome in EAE	Outcome in TMEV	Outcome in MS
Beta interferon	Effective	Effective	Effective
Gamma interferon	Effective	Effective	Exacerbated
Linomide	Effective	Ineffective	Ineffective/toxic
Oral myelin	Effective	Ineffective	Ineffective/ongoing trials
Anti-TNF-alpha	Effective	Exacerbated	Exacerbated
IVIG	Effective	Effective	Effective
Anti-CD4	Effective	Exacerbated	Ineffective
Anti-CD8	Ineffective	Effective	Unknown
Cyclophosphamide	Effective	Effective, partial	Effective, partial
Cyclosporin	Effective	Effective, partial	Effective, partial
Minocycline	Effective	Effective	Effective