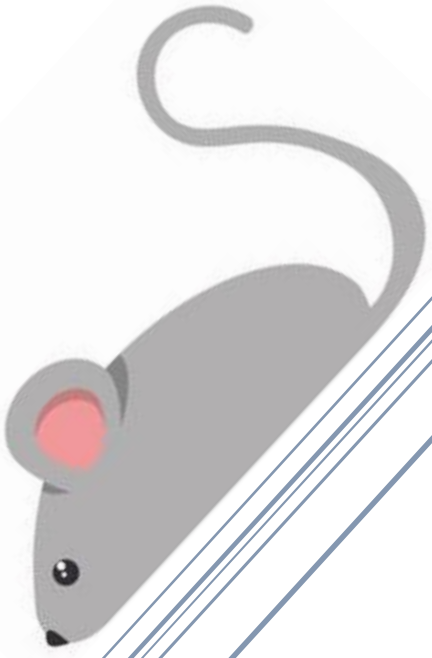
WEIGHING THE OPTIONS: ANIMAL MODELS FOR MULTIPLE SCLEROSIS

Master's Essay - MSc Biomedical Sciences



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Abstract

Multiple sclerosis (MS) is a chronic demyelinating, autoimmune disease of the central nervous system (CNS). The usage of animal models is essential for MS research. However, animals don't naturally develop MS as the disease is exclusive to humans. Therefore, many models for MS exist with varying strengths and weaknesses regarding different aspects of the disease. The aspects of the disease that need to be represented in good animal models are demyelination and remyelination of lesions, inflammation, and neurodegeneration. Toxin-based models like Cuprizone- and Lysolecithin-induced demyelination, as well as virus-induced models such as Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD), are looked into. Additionally, experimental autoimmune encephalomyelitis (EAE) models in rodents, including myelin oligodendrocyte glycoprotein (MOG)-induced EAE, T cell transfer EAE, EAE with cytokine-induced focal cortical pathology, and marmoset EAE are investigated. Additionally, the importance of considering gender in MS research is discussed, emphasizing the need for adequate representation of female animals following the relatively high prevalence of MS in females. Due to the absence of a universally perfect animal model for MS, it is crucial to carefully select a model that aligns with the specific aspects of the disease relevant to the research question at hand.

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Introduction

Multiple sclerosis (MS) is a demyelinating autoimmune neurodegenerative disease of the central nervous system (CNS) [1]. MS is the most common non-traumatic cause of disability in young adults [2]. In the Netherlands, the prevalence of MS is 1 in 700, and 75% of patients are female [3].

Many risk factors for developing MS have been identified but the direct cause of this disease is still unknown [4]. As evident by the male-female ratio of MS patients, being female increases the risk of developing MS [5]. Some other notable risk factors are Epstein-Barr virus (EBV) infection, low vitamin D, childhood obesity, and smoking [5]. Moreover, many genetic risk variants have been identified by a genome-wide association study (GWAS) [6]. Mainly variants of genes associated with peripheral immune cells and microglia are implicated [6]. Notably, MS is most common in countries with high socioeconomic status. The reason for this could be a combination of risk factors such as high latitude/less sun exposure, genetics, and hygiene in early live [5].

The presence of inflammatory lesions in the CNS is a characteristic feature of MS. These lesions can be visualized by magnetic resonance imaging (MRI) scans (fig.1a). MS is highly heterogeneous, the frequency, severity, and special orientation of lesions vary greatly between patients. This is also evident through the wide variety of symptoms patients may experience (fig.1b). Many sensory and motor symptoms can occur and cause disability, this is commonly seen in progressive MS. Paralysis and muscle weakness can lead to the need for walking aids or a wheelchair. And numbness, pain, or blindness greatly affect the sensory experiences and quality of life of patients. Moreover, cognitive symptoms like fatigue and depression are very common in all stages of MS and have a great impact on quality of life and productivity [2]. While the display of symptoms can be unpredictable for an individual, there exists a discernible pattern of overall symptom occurrence that depends on the type of MS.

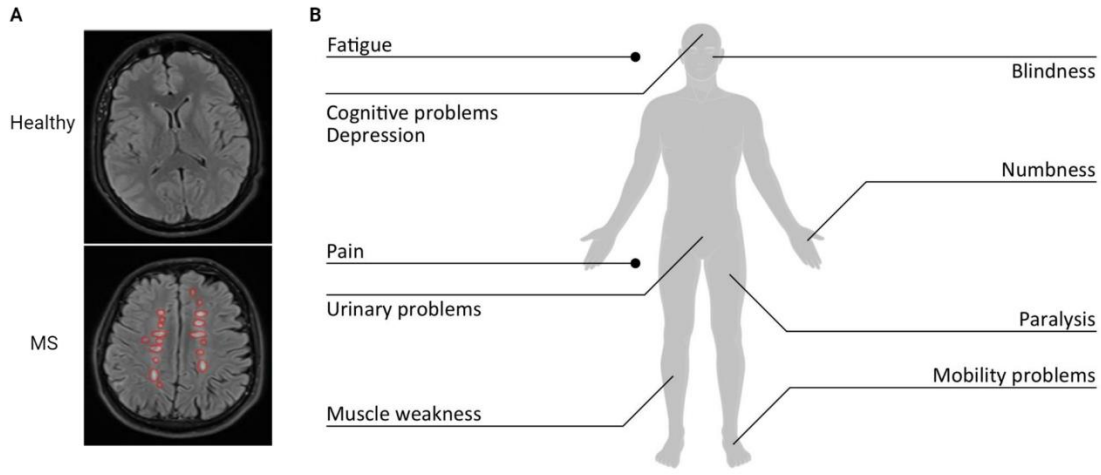

Figure 1: Magnetic resonance imaging (MRI) of multiple sclerosis (MS) and common symptoms of MS.
(A) Magnetic resonance imaging (MRI) of a healthy person and a person with multiple sclerosis (MS) experiencing active lesions. The lesions, seen as white spots on the MRI are circled red (Image adapted from fig.1 of Macin et al. (2022)). (B) The symptoms of MS are extremely heterogeneous among patients. Some patients will experience many of the common symptoms shown here over the disease course. Any part of the body and cognitive performance can be affected by MS. (Created with BioRender.com)

Types of multiple sclerosis

MS can be divided into 2 types, relapsing-remitting MS (RRMS) and progressive MS (PMS) [8]. Most patients (85%) first present with relapses of neurological symptoms that may resolve without intervention [3]. Over time, the symptoms occur in a relapsing-remitting pattern (fig 2) [8]. Furthermore, most RRMS patients will develop secondary progressive MS (SPMS) within a median of 19 years after disease onset [9]. Apart from the inflammatory lesions prominent in RRMS, MS causes underlying neurodegeneration in all stages of the disease (fig 2) [10,11]. In SPMS, this neurodegeneration becomes more prominent and causes disease progression with no remission [10,11]. At this point, relapses also become less common and less severe (fig 2) [9]. Additionally, a small portion (12%) of MS patients will start with primary progressive MS (PPMS) [3].

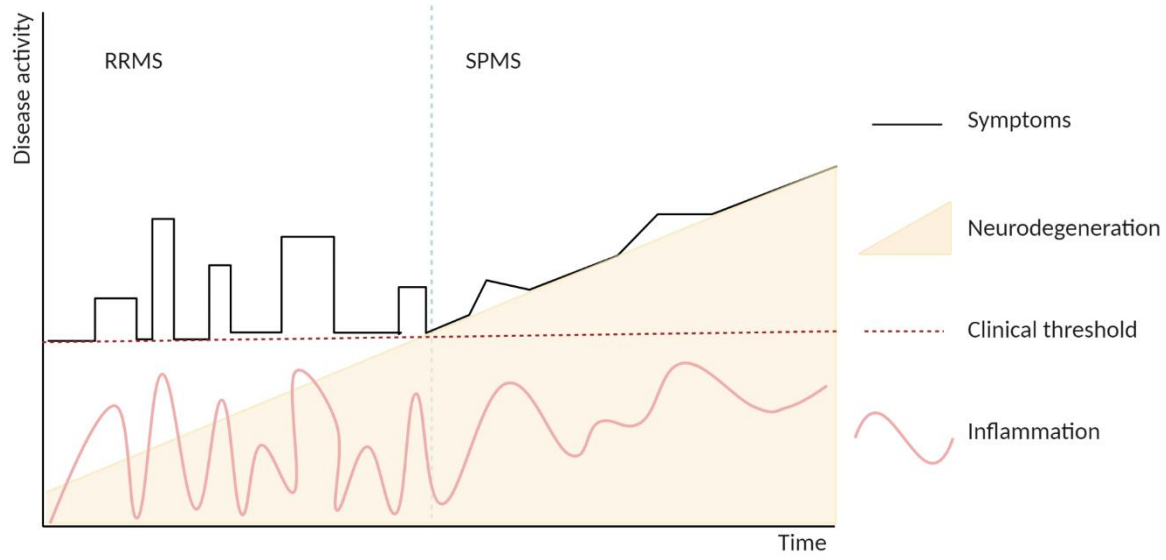

Figure 2: Disease course of multiple sclerosis.
The symptoms of multiple sclerosis (MS) change over time. During relapsing-remitting MS (RRMS), bouts of symptoms come and go in an unpredictable pattern. These relapses correspond with inflammation in the brain. RRMS can progress to secondary progressive MS (SPMS). During SPMS, relapses, and inflammation decrease, but symptoms steadily increase with no remission. Underlying neurodegeneration that goes unnoticed during RRMS, is the driver behind disease progression in SPMS. Figure created with Biorender.com and inspired by Stys et al. (2012).

Many effective therapies are available for RRMS, these decrease the frequency and severity of relapses (table S1) [1]. While effective, these treatments do not stop disease progression. Moreover, current treatment options are exclusively immunomodulatory or immunosuppressive and are not very effective in PMS (table S1). It is thought that the reason for this is that the mechanism driving progression in PMS is not inflammatory, but neurodegenerative [8,11]. Extensive research is ongoing to deepen our understanding of MS and develop effective treatment approaches.

Many research questions regarding MS require an animal model for MS. However, MS is a uniquely human disease not seen in other animals [13,14]. The selection of animal models currently used for MS research is effective at mimicking aspects of the disease [13,14]. However, none of these models show all aspects of MS. Therefore, we aim to investigate the existing animal models used for MS research and evaluate their respective strengths and weaknesses.

Multiple sclerosis and animal models

To determine what makes a good animal model for MS, a deeper understanding of the disease is necessary. One of the most important hallmarks of MS is demyelination, which occurs in focal lesions. Practically all MS animal models feature demyelination in some way.

Demyelination and remyelination

In MS, demyelination takes place in focal inflammatory lesions [4]. A demyelinated axon can't function properly anymore because myelin enables fast and efficient signal transduction (saltatory signal transduction) through the axon, by forming the nodes of Ranvier [15,16]. The myelin sheath is made and maintained by oligodendrocytes and directly supports the axon with energy, chronically demyelinated axons degenerate [16]. After the inflammation is resolved, some lesions heal through remyelination [4]. However, remyelination failure is a prominent issue in MS, and therefore a promising treatment target [17]. In animal models for remyelination, oligodendrocyte progenitor cells (OPCs) are recruited to demyelinated areas, then they differentiate into myelinating oligodendrocytes and remyelinate the axons [18]. However, it is unclear if the same happens in humans. There is a possibility that not just OPCs, but surviving oligodendrocytes are most responsible for remyelination in MS [18]. Further research is necessary to find treatments focusing on remyelination.

Lesions and inflammation

MS lesions can be categorized into 3 main types, active lesions, mixed active/inactive lesions, and inactive lesions [19]. These categories correspond to the pattern of immune cells that are present or absent in the lesions. The most important immune cells in MS pathology are microglia, macrophages, monocytes, T cells, and B cells. Microglia surveil their microenvironment in the CNS and will remove pathogens and debris. However, overactive microglia may contribute to demyelination in lesions. Microglia and macrophages behave very similarly in MS lesions and are prominent in the borders of mixed active/inactive lesions [19].

Normally, the CNS is immune privileged, as no peripheral immune cells can enter through the blood-brain barrier (BBB). However, the integrity of the BBB is compromised in MS. This enables peripheral immune cells like T and B cells to infiltrate into the CNS [20]. T cells can be cytotoxic CD8+ T cells or CD4+ helper T cells. Cytotoxic CD8+ T cells sensitive to myelin self-antigens are implicated in the autoimmune component of MS [19]. Moreover, B cells work

closely with T cells when forming an immune reaction and secrete antibodies. Notably, B cells are a target of infection for EBV, and B cells can perform as antigen-presenting cells (APCs).

Neurodegeneration

Another important hallmark of MS is neurodegeneration. What exactly causes this underlying neurodegeneration is not fully understood, but some of the following factors may play a role. Firstly, oligodendrocytes support axons through trophic factors released by the myelin sheath [16]. Chronically demyelinated axons are not sufficiently supported, which leads to the degeneration of neurons [11,15]. Additionally, ongoing inflammation and active microglia can cause neurodegeneration [11]. Moreover, it is thought that soluble factors of unknown nature produced by B cells may cause neurodegeneration in MS [11]. Furthermore, an unbalance between repair and damage might cause neurodegeneration, this balance might be maintained in younger individuals but fails with older age [11]. PMS is most often seen in relatively aged individuals or after a duration of RRMS. The tissue fails to maintain homeostasis as oxidative stress has built up and mitochondrial damage causes cell death of neurons or senescence of beneficial cells like OPCs [11,15].

Start of multiple sclerosis and involvement of Epstein-Barr virus

Both inflammation and neurodegeneration remain undeniably important in MS. However, it is unclear if one proceeds the other [12]. Classically, it is thought that MS starts with a peripheral autoimmune component that in turn causes demyelinating lesions. However, an alternative theory states that initial damage to the CNS causes the immune system to react and clear the debris. Arguments can be made for both of these theories, which are known as outside-in and inside-out [12]. Currently, there are no animal models modeled after the inside-out (neurodegeneration first) hypothesis. But some animal models, like Experimental autoimmune encephalomyelitis (EAE) are made in accordance with the outside-in hypothesis.

Additionally, the role of EBV infection in MS shouldn't be overlooked. Universally every MS patient is seropositive for EBV [21]. And people uninfected with EBV are protected from developing MS [21]. EBV typically infects B cells, and the recent success of anti-CD20 therapy, which depletes B cells, suggests this involvement in disease progression [22].

Animal models for multiple sclerosis

Propper MS research needs to account for all the diverse aspects of the disease. Pathological interactions between neurological and immunological mechanisms are currently impossible to replicate *in vitro* [23]. Therefore, when it comes to investigating the development of new treatments *in vivo*, human experiments are constrained to ethically controlled clinical studies, making animal models the preferred option [23]. While the scientific community strives to minimize the use of laboratory animals, they remain an absolute necessity [23].

Furthermore, an ideal MS animal model should encompass all the aforementioned aspects of the disease. Moreover, the likeness of the immune system and CNS should be close enough to humans. Hence, mammals are best suited and will be further explored [23]. Yet, many neurological issues are exclusively human. Therefore, research into neurodegenerative diseases often needs to be done in transgenic models. E.g. the genes of mice need to be altered to enable a murine approximation of human diseases. An example of this is the J20 Alzheimer's disease mouse model that overexpresses the amyloid precursor protein [24]. An approximation like this can't so easily be made for MS as it is not a completely genetic disease. Many genes are involved in MS and the involvement of the genes known is not fully understood. Some models that are made to study single MS genes exist. These give insight into the role of these genes but don't effectively simulate MS. Additionally, genes that induce cell death can be programmed oligodendrocyte lineage cells to study the effect of their absence [16]. E.g. inducing the expression of the "suicide" gene diphtheria toxin subunit A in adult oligodendrocytes causes loss of these cells and consequent demyelination [25]. Perhaps in the future, a selection of genes could be manipulated to simulate MS in an animal model. However, there are many other varieties of MS animal models (Table.1) [13].

Toxin-based models

Arguably the simplest animal models for MS are toxin-based demyelination models. Here, a toxin is introduced to initiate demyelination. Whereafter healing and remyelination take place [13].

Cuprizone

The cuprizone model causes demyelination as long as cuprizone is ingested through a diet. Cuprizone is a copper chelator that causes oligodendrocyte degeneration, which then causes

demyelination [26]. The mechanism causing oligodendrocyte death is speculated to be one of the following. Firstly, cuprizone might cause copper dyshomeostasis through its copper chelator properties. Copper is essential for cellular respiration and oligodendrocytes might be most sensitive to this dysregulation. Alternatively, cuprizone might form a toxic cuprizone-copper complex directly toxic to oligodendrocytes [26].

5 weeks of the cuprizone diet is sufficient to complete demyelination. However, cuprizone can be continued for up to 12 weeks for prolonged demyelination. Remyelination takes a few weeks after cuprizone feeding has been stopped. This aspect of cuprizone makes it versatile to study the effect of time with demyelination. While both gray and white matter are affected, some areas in the brain are more affected by cuprizone than others. E.g. the corpus callosum is often entirely demyelinated while the cingulum is less affected [26].

The predictable and controlled nature of the cuprizone mouse model can give insights into the effects of oligodendrocyte degeneration and the effects of chronic demyelination [16,27]. Moreover, the predictable remyelination period facilitates a detailed examination of the remyelination process [27]. However, it fails to account for the inflammation and immune aspect of MS [27]. Even though microglia become activated in the cuprizone model, their activation is non-inflammatory and serves solely to phagocytose the damaged myelin from the degenerated oligodendrocytes [26]. Additionally, the spatial effect of cuprizone can't be altered to include or exclude certain parts of the brain [16,26,27].

Lysolecithin

In contrast to cuprizone, lysolecithin injections offer both great spatial and temporal control over demyelination and remyelination. Lysolecithin acts fast to cause demyelination and rapid remyelination is seen thereafter. Through its lipid-disrupting detergent properties, it disrupts membranes and induces demyelination [28]. The non-specific nature of lysolecithin causes membranes of non-target cell types to be affected too. Astrocytes are often killed by this, which then causes calcium accumulation, which causes the degeneration of axons [28]. These properties of the lysolecithin model make it unsuitable for research into neurodegeneration in MS [16]. Moreover, lysolecithin completely misses the immune aspect of MS. While inflammation and microglial activation is seen after lysolecithin injections, this reaction is secondary to demyelination and does not contribute to it [28].

Virus-induced models: Theiler's murine encephalomyelitis virus

Theiler's murine encephalomyelitis virus (TMEV) can be used as an MS model. Infection with this virus will cause immune-mediated attacks against the CNS in susceptible mice [13] Uniquely, certain viruses can be used as MS models. E.g. mouse hepatitis virus, Semliki Forest virus, and TMEV [29,30]. No human virus parallel to these CNS infections exists. TMEV-induced demyelinating disease (TMEV-IDD) is an MS-like neurological disease created through the injection of the virus into the CNS of susceptible mice. [29]. Normally, this virus infects the gastrointestinal tract. TMEV-IDD occurs in 2 phases, fist an acute phase weeks after injection, then a chronic phase starting 1 month after injection. During the acute phase, the virus infects neurons and spreads down the spinal cord. The spinal cord is most affected by TMEV and mainly virus-specific T cells are recruited. During the chronic phase, mostly astrocytes host the virus as they resist virus-induced apoptosis. Like in PMS, the BBB is intact in the chronic phase of TMEV-IDD [29].

Some interesting parallels between TMEV-IDD and MS exist, sex differences like those seen in human MS are also seen in TMEV, depending on the mouse strain. Mechanisms like molecular mimicry and bystander effect cause autoantigens to be recognized by T cells in the chronic phase of TMEV-IDD. These mechanisms might play a role in MS according to the hypothesis that EBV triggers MS. Nevertheless, MS is not known to be directly caused by a virus like TMEV-IDD. These parallels make this model an interesting option for the right research questions related to viral aspects of MS [29]. However, it should be carefully minded that the viral disease shown here is not the same as MS or any other human condition.

Experimental autoimmune encephalomyelitis models

The most common animal model for MS research is EAE in rodents. In EAE, e.g. mice are artificially immunized with self-antigens from the CNS [30]. This causes autoimmunity in susceptible mice that resembles the neuroinflammation seen in MS There are many variations of the EAE model, varying in the method of immunization and antigen of choice [13,30]. Some notable variations of EAE are discussed below.

Rodent myelin oligodendrocyte glycoprotein (MOG) induced EAE

One of the common self-antigens used for EAE is myelin oligodendrocyte glycoprotein (MOG) to create MOG-EAE. A peptide of MOG (e.g. MOG35–55) is injected with a substance (e.g.

complete Freund's adjuvant) to activate the immune response. Complete Freund's adjuvant promotes antigen presentation in MHC Class II and subsequent activation of CD4+ T cells [30]. This process alone does not cause an autoimmune reaction. Only after treatment with pertussis toxin the auto-immunization against MOG is complete and the MOG-EAE model is created [30]. While CD4+ T cells have a well-documented role in this model, CD8+ T cells, B cells, macrophages, and monocytes, also play a role. E.g. B cells secrete MOG-specific antibodies [13]. This model reliably causes inflammatory demyelinating lesions characterized by CD4+ T cell infiltration. These lesions mostly take place in the spinal cord and little in the brain, unlike MS [30]. Moreover, this model only features one phase of inflammatory demyelinating lesions, but no relapses like in MS. The primary axonal injury caused by the immune-mediated attack causes secondary demyelination, primary demyelination, as seen in MS, is rare [30]. Variations of EAE using different antigens (e.g. myelin basic protein (MBP), proteolipid protein (PLP)) for sensitization can also cause chronic EAE with some relapses and remissions [13]. MOG-EAE is a reliable tool to investigate the immune response against the myelin component MOG [30]. While this is certainly a relevant part of (RR)MS pathology, this artificially initiated immune response can't provide answers to the etiology of MS.

Rodent T cell transfer induced EAE

Since EAE is T cell-driven, intravenous transfer of T cells that were sensitized to brain tissue achieves T cell transfer EAE in naïve animals. T cells can be sensitized *in vivo* (e.g. MOG-EAE) or *in vitro* and then cultured and expanded. This way, T-cell lines that target myelin are readily available to initiate a consistent immune response in naïve animals. This EAE model functions almost the same as the aforementioned. Although, the immune system of the recipient animal is not used to create to initiate the immune response. This consistency is an advantage since it's a very controlled setup. However, it is also a disadvantage because the model is restricted to the T cell-mediated immune response [30]. Despite the importance of B cells in MS, their involvement is completely missing in this model [31]. Therefore, this type of EAE is not very suitable to study MS pathology as a whole but may give insights into the dynamics of T cell-mediated inflammation and infiltration into the CNS.

Rodent EAE with cytokine-induced focal cortical pathology

Most EAE models emulate RRMS, but a variation on EAE with cytokine-induced focal cortical pathology strives to emulate PMS [32]. This is commonly performed in susceptible rat strains,

where EAE is first initiated. Thereafter, lentiviral vector injections make cells produce TNF and IFNγ at levels like those seen in post-mortem SPMS. Compared to regular MOG-EAE, these cytokines were able to produce greater cortical demyelination and neuronal loss than regular MOG-EAE [33]. In this model, cortical microglia with a PMS-like inflammatory phenotype were seen [33]. Moreover, pre-synaptic phagocytosis by microglia was observed [33]. It was also found that B cell numbers rise over time in this model [33]. These pathological signs typically seen in PMS displayed in this model reconfirm the involvement of pro-inflammatory cytokines in the CSF of PMS patients [34]. However, it should be recognized that the complex pathological mechanisms in PMS can't be simplified to solely focusing on TNF and IFNγ. Nevertheless, this recently developed model shows promising evolution in the EAE model collection to highlight the meningeal inflammation seen in PMS [33].

Marmoset EAE

While many insights have been gained from rodent EAE models, non-human primates could serve as a vital bridge between these animal models and humans. The marmoset (*Callithrix jacchus*) EAE model approximated human MS more closely than rodent EAE models for several reasons [35,36]. Firstly, the marmoset population is outbred, unlike genetically identical lab mice and rat strains. Genetic variety in this model is more similar to the genetics of the human population. Furthermore, the CNS and immune systems of marmosets are more similar to that of humans. Findings regarding the immune responses and the CNS in this model might be better translatable to human trials. And most importantly, the suspected effect of EBV infection in MS is considered in the marmoset EAE model. Marmosets are infected with lymphocryptovirus (LCV), this is the marmoset equivalent of human EBV. Like EBV in humans, LCV infects and resides in B cells. This infection alters the antigen presentation behavior of the B cells to allow for antigen presentation of self-antigens [35]. The consequent interactions between the LCV-infected B cells and T cells create an autoimmune reaction resembling MS [36]. Moreover, this model features both WM and GM oxidative stress and mitochondrial defects as seen in MS [35]. While this marmoset EAE is very promising, the logistical and ethical concerns prevent the widespread use of this model [37]. As non-human primates, these animals are intelligent, social, and live relatively long lives (+-12 years) [37]. These aspects restrict the availability of models like this, making them suitable only for a select few research questions. These questions typically explore the bridge between rodents and humans or the involvement

of EBV injection in MS. The close similarities between human MS and marmoset EAE may provide confounding insights into MS pathology. Moreover, the role of EBV and perhaps the mechanism driving disease progression through EBV could be further studied through this model.

Table 1. Summary of MS animal models.

Model of MS	**Mechanism**	**Cells involved**	**Aspect of MS**
Cuprizone	Toxin-induced oligodendrocyte death through diet	Oligodendrocytes, microglia, astrocytes	Demyelination, remyelination
Lysolecithin	Myelin and membrane damage through injection	Oligodendrocytes, microglia, astrocytes	Demyelination, remyelination
TMEV-IDD	Infection with Theiler's murine encephalomyelitis virus	CD4+ T cells, CD8+ T cells, macrophages, microglia, astrocytes, oligodendrocytes	Virus-induced demyelination, inflammation
MOG-EAE	Immunization against MOG35–55	CD4+ T cells, CD8+ T cells, B cells, macrophages, monocytes	Immune response against myelin, inflammation
T cell transfer EAE	Transfer of immunized T cells to naive recipients	CD4+ T cells, CD8+ T cells, macrophages, monocytes	T cell-mediated immune response against myelin, CNS infiltration
EAE with cytokine-induced focal cortical pathology	Immunization against MOG35-55, periodical injections with TNF and IFNγ	CD4+ T cells, CD8+ T cells, B cells, macrophages, monocytes, microglia	Immune response against myelin, chronic inflammation
Marmoset EAE	Natural infection with an EBV equivalent followed by immunization against MOG35-55.	CD4+ T cells, CD8+ T cells, B cells, macrophages, monocytes, microglia	Involvement of EBV in MS, immune response against myelin, inflammation

Inspired form: Procaccini et al. (2015)

Gender in multiple sclerosis research

While the type of MS animal model used needs to be carefully considered to fit the research question at hand, the gender of said animals may be of importance too. In the past, men and male animal models were preferred as research subjects for technical reasons. Women and female animal models were not taken into consideration because of hormone cycles. Over time, the discrepancy in female representation in research is lessening [38]. However, in neuroscience and biomedical research, the underrepresentation of female animal models is still an ongoing issue. This discrepancy is most prominent in neuroscience, where male animal studies are 5 times as common as female animal studies [39]. More recently, a meta-analysis of remyelination-promoting therapies in MS animal models found that most studies were performed with male animals [17]. Only about 27% of the 88 studies analyzed were done with female animals. This is in sharp contrast to the high prevalence of MS in women compared to men. The sex differences seen in human MS may not always be identical to those observed in animal models. However, it is noteworthy that female mice in EAE exhibit earlier disease onset and stronger Th17 immune responses compared to male mice [40].

Discussion & conclusion

How to choose an animal model

The abundance of diverse animal models available to study MS may complicate the design of research. However, this highly heterogeneous disease requires diverse perspectives. The existence of many animal models reflects the diverse aspects of MS as well as currently possible. Although, this reflection is based on assumptions about MS, e.g., the outside-in mediated perspective of EAE [12]. This insight and careful selection of the type of animal model is crucial to properly perform research that is translatable to human patients. The following strengths and weaknesses should be considered when choosing between animal models.

Cuprizone and lysolecithin are valuable models to understand (toxin-induced) demyelination and (healthy) remyelination. They can give insight into factors that cause remyelination failure, which helps identify therapeutic targets to promote remyelination [26,28]. Additionally, compounds suspected to promote remyelination can be studied and verified. Remyelination failure is a major problem in PMS and therapeutics in that area are highly sought after [17]. However, the connection of these models to MS begins and ends with remyelination [26,28]. All other aspects of MS, such as autoimmunity and neurodegeneration, are not represented in these models [26,28].

TMEV-IDD has some major similarities to MS. The infiltration of the immune system into the (normally immune privileged) CNS through the BBB, inflammation in the CNS, and demyelination are important hallmarks in this model and MS [13,29]. In chronic TMEV, autoantigens of the CNS can get recognized by T cells through mechanisms (molecular mimicry and bystander effect) that may play a role in the initiation of autoimmunity in MS [29]. While no human equivalent to TMEV exists, the role of EBV in MS does indicate the viral aspect of the disease [29]. EBV, which infects B cells, does not infect cells of the CNS as TMEV does [1,29]. The complex nature of potential similarities between MS and TMEV-IDD, coupled with a limited understanding of certain aspects of MS, raises doubts about the applicability of this model in extrapolating shared mechanisms between the two.

Rodent EAE enables the induction of controlled autoimmune-mediated demyelination [30]. An abundance of variations of EAE is available to provide tools to investigate the immune response against myelin [30]. This model is widely used to research therapeutics to decrease this

immune response and decrease the subsequent demyelination [13]. These practices have led to the development of numerous successful immunomodulatory and immunosuppressive therapeutics for the treatment of RRMS [1]. However, some important differences should be minded when extrapolating findings to MS. EAE is T cell-mediated, while B cells are also important in MS [31]. There is currently no B cell-mediated EAE variation. Moreover, MS is not caused by immunization to a CNS self-antigen [30]. It is under speculation whether MS starts inside the CNS or outside the CNS in the periphery [12]. EAE simplifies the induction of disease and thereby relies on the outside-in hypothesis of MS. Additionally, EAE models often exhibit limitations in the representation of PMS [32]. However, a variation of EAE with cytokine-induced focal cortical pathology aims to emulate PMS and successfully replicates certain pathological features observed in PMS [33]. This highlights the remarkable versatility of EAE variations, rendering it a valuable model for MS research.

The disparity between rodent EAE models and human MS remains substantial. Compounds found successful in rodent EAE often fail in clinical trials [30]. To bridge this gap, an alternative animal model may be needed. Non-human primates like the common marmoset are much more closely related to humans than rodents. Additionally, marmoset EAE incorporates the suspected role of EBV infection in MS [35]. LCV-infected B cells play an important role in marmoset EAE [35]. This process is likely closely related to the processes of EBV in MS [36]. The recent success of anti-B cell therapy highlights the importance of this aspect for potential treatment strategies [22]. Therefore, this model provides a unique perspective on this aspect of the disease. However, logistical, and financial hurdles will likely prevent common usage of this model. For example, due to financial and time constraints, it is not feasible to raise marmosets to old age [37]. Marmoset EAE with young individuals mimics RRMS, PMS is more common in aged people. Given the opportunity and sufficient time, this model may even evolve into something like SPMS. Further research into these options is needed to address the current shortage of models available for PMS.

Female representation in animal models

Apart from selecting the right type of model for a particular research question, the gender of the animal should be brought into consideration. MS affects men and women differently. The most striking difference between men and women regarding MS is the male-female ratio [1]. For every 1 man, at least 3 women get diagnosed with MS [3]. Women also have an earlier

disease onset and more relapses than men. But men usually progress faster and have worse disease outcomes than women. Differences in sex hormones may be the most important factor for these sex differences, this is thoroughly explored in the work of Ysrraelit & Correale (2019).

The hormonal differences between men and women vary over time. Both go through puberty and women may experience pregnancy and menopause. Interestingly, the mentioned gender biases in MS aren't seen before puberty or after menopause. This indicates a major role of (female) sex hormones in the prevalence of MS. There is also an increased risk of MS correlated to the age of first menstruation (menarche). Younger age at menarche increases the risk of MS, and each year it is delayed risk decreases by 13% [41].

Pregnancy has a protective effect on MS patients with a 70% decrease in relapses compared to pre-pregnancy levels. Hormonal changes occur, like increases in progesterone and estrogen. Immune tolerance is built to protect the fetus. Therefore, a shift to the Th-2-like anti-inflammatory response is made. This immune tolerance may be beneficial for autoimmunity and lead to a decrease in relapses. However, after childbirth, normal immunity is restored rapidly. The chance of relapses increases to up to 3 times the rate before pregnancy. Hormone levels of progesterone and estrogen return to normal and an immune shift towards Th-1 pro-inflammatory responses happens [41].

Taken together, (female) sex hormones play an important role, not only in the risk for MS but also in disease progression and the frequency and severity of relapses [41]. The complex interaction between hormones and the immune system is an important factor to consider for MS research and the development of treatments. Therefore, the inclusion of (a majority) female animal models should be the norm for MS research.

The need for (good) animal models

Currently, there is no treatment aimed at slowing neurodegeneration, no treatment that completely stops disease progression, and no treatment that can reverse damage after it has accumulated. Moreover, only one treatment option exists for PMS [22]. Thus, the current assortment of treatment options is severely lacking (Table S1). The research conducted with animal models plays an essential role in expanding this assortment [23]. The aspects that can be considered when using an animal model (e.g., CNS, immune system, gender) presently have

no suitable *in vitro* alternatives [23]. This is unlikely to change soon. Therefore, it is essential to protect the validity and efficacy of laws that facilitate safe and humane research with animal models.

At present, all treatments for MS are based on immunomodulatory or immunosuppressive strategies (Table S1) [1]. These treatments wouldn't exist without the help of animal models. However, the development of these treatments is guided by the immune reactions seen in the models [30]. The most common immunological MS model is rodent EAE, which mainly sees the involvement of T cells (Table 1) [13]. A misguided focus on T cells in MS research may lead to the oversight of the importance of e.g. B cells. Treatments aimed towards T cells may show promising results in EAE but fail in MS. Hence, the immune aspect of MS is imperfectly modeled by the currently available models. Another aspect of MS severely lacking representation in animal models is neurodegeneration. This aspect, which is prominent in PMS, is somewhat represented through recent developments in EAE variations [33]. Moreover, the aspect of EBV infection in MS is very underrepresented in animal model usage. The only animal model which includes this aspect is marmoset EAE [35]. As discussed, none of the models encompass all aspects of MS or achieve a flawless replication of any single aspect. We may encounter a juncture where the disparities between existing models and MS become too great. Thus, it is necessary to continue developing improved animal models for the advancement of MS research.

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Appendix

Table S1

Therapeutic compound	**Mechanism**	**Efficacy**
IFN-beta 1a and 1b	Immunomodulatory, pleiotropic immune effects	Moderate
Glatiramer acetate	Immunomodulatory, pleiotropic immune effects	Moderate
Dimethyl fumarate	Pleotropic, NRF2 activation, downregulation of NF-κB	Moderate/High
Teriflunomide	Dihydro-orotate dehydrogenase inhibitor (Reduced de novo pyrimidine synthesis), anti-proliferative	Moderate
Fingolimod	Selective sphingosine 1-phosphate modulator, prevents egress of lymphocytes from	High

	lymph nodes	
Natalizumab	Anti-VLA4, selective adhesion molecule inhibitor	Very high
Ocrelizumab	Anti-CD20, B-cell depleter	Very high *
Alemtuzumab	Anti-CD52, non-selective immune depleter	Very high
Cladribine	Deoxyadenosine (purine) analogue, adenosine deaminase inhibitor, selective T- and B-cell depletion	High
Mitoxantrone	Immune depleter (topoisomerase inhibitor)	Very high
Autologous hematopoietic stem cell transplantation	Autologous stem cell transplantation	Very high

Table S1 – Overview of current MS treatments. * Ocreluzimab is not only effective in RRMS, but also approved for the treatment of PMS. All other treatments are only effective in RRMS. Adapted from Table 3 of the work of Dobson & Giovannoni (2019).