

# Animal experimental models for understanding and treating Multiple Sclerosis

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## ABSTRACT

MS is an inflammatory and neurodegenerative disease of unknown etiology. MS displays different clinical patterns: recurrent episodes with remission periods (“relapsing-remitting” **(RRMS)**) that can progress over the years of disease to secondary progressive form **(SPMS)**. However, 10% of patients can start from the onset of the disease with persistent progression (“primary progressive MS” **(PPMS)**) [1].

Until now, there are no specific therapeutics agents available for the progressive forms, mainly because the pathogenic mechanisms are still not clear and because researchers have limited access to human samples to develop therapeutic tools for the MS. In this context, the development of MS animal models is mandatory to clarify the pathological mechanism and to test novel therapeutics agents [2].

**Key words:** Relapsing and remitting multiple sclerosis; Progressive multiple sclerosis; EAE; Theiler's virus

**Abbreviations:** **ABH:** Biozzi antibody high (AB/H) mouse; **BBB:** Blood Brain Barrier; **C EAE:** Chronic Experimental Autoimmune Encephalomyelitis; **CIS:** Clinically Isolated Syndrome; **CNS:** Central Nervous System; **DNA:** Deoxyribonucleic acid; **EAE:** Experimental Autoimmune Encephalomyelitis; **EB:** Ethidium Bromide; **EDSS:** Human Expanded Disability Status Scale; **Ic:** Intracranial; **IFNs:** Interferons; **IL:** Interleukin; **IL-1b:** Interleukin-1beta; **LPC:** Lysophosphatidylcholine (lysolecithin); **MBP:** Myelin Basic Protein; **MHV:** Mouse Hepatitis Virus; **MOG:** Myelin Oligodendrocyte Protein; **MRI:** Magnetic Resonance Imaging; **MS:** Multiple Sclerosis; **PLP:** Proteolipid Protein; **PPMS:** Primary Progressive Multiple Sclerosis; **RNA:** Ribonucleic acid; **RR EAE:** Relapsing Remitting Experimental Autoimmune Encephalomyelitis; **RRMS:** Relapsing-Remitting Multiple Sclerosis; **SPMS:** Secondary Progressive Multiple Sclerosis; **TMEV:** Theiler's murine encephalomyelitis virus; **TNF- $\alpha$ :** Tumor necrosis factor alpha; **Treg:** T regulatory

## INTRODUCTION

MS is a chronic immune-mediated neurodegenerative disease of unknown etiology, characterized by neuroinflammation, demyelination and axonal loss, which lead to a poor transmission of the nervous impulse, and eventually loss of sensory, motor, autonomic and cognitive functions, according to the location of the CNS lesions. MS represent one of the most important cause of disability among young adults (between 20-45 years old), which generates an important socio-economic impact in developed countries [3,4]. MS is a very heterogeneous disease that displayed different clinical courses: recurrent episodes with remission periods ("relapsing-remitting" - **RRMS**) that can progress to secondary progressive form (**SPMS**); or persistent progression from the onset of the disease ("primary progressive MS" -**PPMS**).

RRMS is the most prevalent clinical course of MS and is characterized by recurrent episodes of either new or worsened symptoms. Exacerbations or relapses are followed by periods of complete remission in the early years of the disease and partial remission over the years with apparent clinical stability between relapses. Relapsing episodes are unpredictable; however, peripheral inflammation may exacerbate these events [5]. The progressive forms of MS lead to a continuous and irreversible evolution of the disease, inducing disability and decline of the quality of life either from the onset (**PPMS**) or after relapsing and remitting episodes courses, named SPMS. SPMS is diagnosed as a sustained and confirmed worsening of the symptoms after relapsing-remitting phases [6].

New findings of biological markers and advances in imaging have progressed to redefine in 2013 both the clinical course and the different MS phenotypes with the aim of improving diagnosis, prognostication, design and recruitment of clinical trials, and treatment decision-making [7].

The phenotype description includes:

- Radiologically isolated syndrome (**RIS**) where incidental imaging findings suggest inflammatory demyelination in the context of absence of clinical signs or symptoms.
- Clinically Isolated Syndrome (**CIS**) is the first clinical presentation that shows characteristic of inflammatory demyelination that could be MS, but has not yet to fulfill criteria of dissemination [6].
- RRMS could be active or not active. The disease activity was defined as presence of clinical relapses and/or MRI activity (new T2 lesions, contrast-enhancing lesions).
- Progressive disease (secondary and primary) could evolve active (with or without progression) or not active (with or without progression, in the last case is called “stable disease”).

At present, there are anti-inflammatory and immunomodulatory treatments that are beneficial in CIS and RRMS, but they are ineffective in PPMS and SPMS [8,9]. This treatment provide temporary effectiveness because reduce the annualized relapse rate, but patients experience side effects, relapses or evolution to the progressive MS stages [8]. Clinical trials with anti-inflammatory therapies have proved to be inefficient in progressive forms [10]. In particular, interferons (**IFNs**) and glatiramer acetate, the most currently used anti-inflammatory agents in MS, provide good initial results in the RRMS treatment, but have proven to be ineffective for the progressive forms. IFNs act by decreasing Interleukin-1beta (**IL-1b**) production [11]. However, the effectiveness of anti-IL-1b treatments for MS has not yet been demonstrated [12]. Until now, there are no specific therapeutics agents available for progressive forms and there are no effective cure for MS, mainly because the pathogenic mechanisms are still not clear and because researchers have limited access to human samples, which include restrictions to experimental modification of them to develop therapeutic tools for the MS. In this context, the development of MS animal models is mandatory to clarify the pathological mechanism and to test novel therapeutics agents [2].

Cortical lesions have peculiar inflammatory and demyelinating hallmarks, characterized by lack of BBB disruption, differential inflammatory process, reactive microglia and neurodegeneration, suggesting different immunopathogenic mechanisms from the other MS forms [13]. However, anti-inflammatory or immunomodulatory therapies have no effect on the neurodegeneration and cognitive impairment in the progressive forms of MS [14,15]. This could be a consequence of the fact that the inflammatory environment in the progressive MS favors retention of inflammatory cells within the lesions [16,17]. Animal models of MS are useful tools for improving the knowledge of cellular and molecular mechanisms that underlie its pathology, as well as to study the interaction between the immune and the nervous system. In addition, they are potential models for testing new therapeutic drugs for MS [4].

Animal models can be divided into two groups: those which attempt to replicate the disease as accurately as possible (e.g, virus induced encephalomyelitis and different forms of Experimental

Autoimmune Encephalomyelitis (**EAE**) (reviewed in [18]) and the others that provide a reductionist approach to the disease by studying mainly demyelination and remyelination processes (e.g. ethidium bromide, lysolecithin, cuprizone) (reviewed in [19]).

In this chapter, we reviewed the use of animal's experimental model to understand the pathological mechanisms, including neuroinflammation, demyelination/remyelination, neurodegeneration and the immune compromise. Additionally, we discussed the importance of animal models to design and test specific therapeutic agents for the different forms of MS.

## Animal Models of MS

MS is a complex and heterogeneous disease. Even though, no single model represents the pathophysiological characteristic of MS, each animal model allowed studying a different aspect of the disease. MS experimental animal models represent a main character in clarifying the pathological mechanisms of the disease in order to develop therapeutic strategies. These models have been able to recapitulate several clinical features of MS and improved the knowledge of the interaction of both immune and nervous system in the disease. The fact that MS is an autoimmune disease characterized by either demyelination or neurodegeneration according to the different clinical courses requires appropriate experimental models that reflect both axonal and neuronal damage and demyelination. These models should help to elucidate the pathophysiological mechanisms as well as to develop therapeutics approaches.

Three main animal experimental models categories are the most used for understanding the MS pathophysiology: 1) Experimental allergic encephalomyelitis (**EAE**), 2) Virus models, such as: Theiler's murine encephalomyelitis virus (**TMEV**), 3) Toxin models, such as ethidium bromide (EB), lysophosphatidylcholine (lysolecithin) (**LPC**) and cuprizone.

### EAE

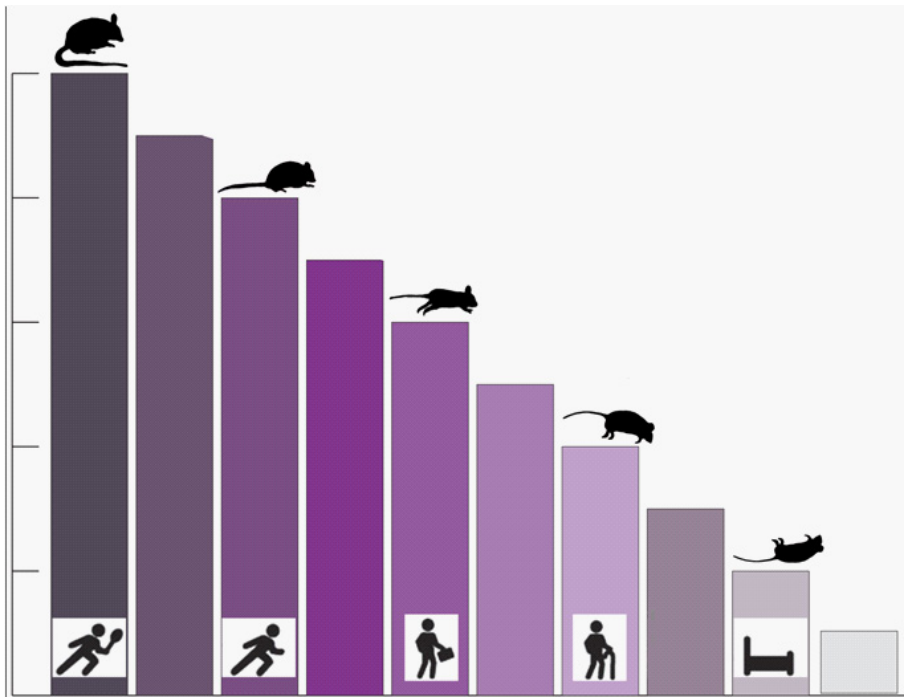
Experimental autoimmune encephalomyelitis (**EAE**) is one of the oldest models and the most extensively studied for research in MS and other autoimmune diseases of the CNS. EAE is the only model that reflects most of the pathological and clinical features of MS [20]. The EAE model was discovered in 1930 by Rivers et al [21] while investigating the neurological complications of the vaccine against rabies in monkeys. The monkeys exhibited paralysis associated to perivascular infiltrates and demyelination in the brain and spinal cord, as well as acute disseminated encephalomyelitis.

EAE is induced by injection of CNS myelin, neural or glial Cell antigens and the symptomatology includes many immune-driven inflammatory conditions [22]. EAE can be induced by immunization with CNS homogenate, proteolipid protein (**PLP**), myelin basic protein (**MBP**), or myelin oligodendrocyte protein (**MOG**) [23]. Then the model can become more complex with the addition of pertussis toxin and Freud's adjuvant to induce relapses like RRMS through the exacerbation of humoral immunity [24,25].

EAE models range from non-demyelinating monophasic clinical disease to a secondary form. According to the immunizing antigen and the strain of the used animal species the progression and the pathology vary [20]. Two clinical and pathological phenotypes of EAE were developed: relapsing remitting EAE (**RR EAE**), and chronic EAE (**C EAE**), that try to reflect aspects of RRMS and PPMS respectively [24,26].

Even though this model is more frequently used in rats and mice, it can also be developed in rabbit, primates and marmoset, sheep, guinea pigs and goats [4,24]. Additionally, this model is the only animal model that has a clinical score reflecting disease progression, such as human Expanded Disability Status Scale (**EDSS**) scale in MS patients (Figure.1). At disease onset rodents experiment tail weakness (score=1). Then, the disease progress to hind limb, with gait impairment, paresis and slow righting reflex (score=2), complete paralysis of hind limbs (score=3) and involvement of fore limbs with paraparesis (score=4) and eventually death (score= 5).

**Figure 1:** Schematic diagram showing that EAE is the only animal model that exhibits a clinical score similar to the disease progression as it was described by the human Expanded Disability



Status Scale (**EDSS**) scale in MS patients. Rodents experiment tail weakness (score=1), hind limb, with gait impairment, paresis and slow righting reflex (score=2), complete paralysis of hind limbs (score=3) and involvement of fore limbs with paraparesis (score=4) and eventually death (score= 5).

## Toxin models

The toxin models are based in either focal or systemic administration of demyelinated agents. These models provide a more reductionist approach that allows studying specific aspects of the disease, such as, demyelination and remyelination. Among these agents we can named ethidium bromide (**EB**), lysolecithin (**LPC**), cuprizone. Some of these agents are delivered with the aid of specific equipment, such as stereotaxic frame. In contrast, cuprizone demyelination are induced by oral administration of the substance. The election of the different demyelination methods should be chosen based on the scientific hypothesis.

**Ethidium bromide and lysolecithin:** The most commonly used focal demyelinated agents are EB and LPC. Both toxins induced demyelination in the injected area by breaking down the myelin sheaths. Lysolecithin is an activator of phospholipase A2, which induce demyelination due to damage in the myelin sheaths with no axonal damage and no immune mediation [19,28]. EB has the ability to intercalate between the DNA chains; therefore, its effect is not specific to oligodendrocytes [29,30]. Both toxins can be delivered in several regions of the brain: such as, striatum, spinal cord, caudal cerebellar peduncles, cerebellum, corpus callosum [20,31]. After a period of time, both agents allow the remyelination process, which restores myelin sheaths. Both EB and PLC induced peripheral leucocyte recruitment, such as: CD4+/CD8+ T cells and neutrophils at early time points and activated CD11b+ macrophages at later time points [32]. The main difference between the two toxins is that EB, besides demyelination, induced astrocyte depletion, which facilitates the remyelination process [33]. Therefore, this toxin models are suitable to study the biology of the demyelination and remyelination process [19].

**Cuprizone:** Cuprizone, biscyclohexanone-oxaldihydrazone, is a copper chelating reagent that causes oligodendroglial cell death and therefore demyelination, along with astrocytes and microglia activation [34]. The first experiment using cuprizone was performed by Carlton [35]. Feeding of cuprizone for 4-6 weeks induced oligodendrocytes damage followed by microglia and astroglia activation [20]. The mechanism of cuprizone-induced oligodendroglia death is disrupting the energy metabolism in the mitochondria of the cells [36]. The cuprizone is orally administrated and induced demyelination in certain areas of the white matter in the brain, such as, corpus callosum. After 6 weeks of administration, cuprizone induce total demyelination of the corpus callosum, cerebellar peduncles, anterior commissure and internal capsule. Acute demyelination is followed by spontaneous remyelination after several weeks [36]. Long-term administration of cuprizone induced chronic demyelination, in which the remyelination process is impaired [36]. These models were very useful in understanding both remyelination and oligodendrocyte death.

## Virus models

Viral infections can also induce demyelination in mice CNS and the most studied are the Theiler's murine encephalomyelitisvirus (**TMEV**) and certain strains of the coronavirus, such mousehepatitis virus (**MHV**) and adenovirus.

**Theiler's murine encephalomyelitis virus model:** TMEV is a non-enveloped, single stranded RNA picornavirus and represents one of the neurotropic viral infection models for MS [37,38]. The first description was described by Theiler (1934), demonstrating paralysis in mice. TMEV model generates an autoimmune response triggered by viral infection in the CNS, brain and spinal cord [39]. The main characteristics of TMEV as MS experimental model are: 1) its pathology is very similar to MS, 2) it models demyelination via an autoimmune response in the CNS, different from a viral cytopathology in targets cells [4]. There are two main groups of TMEV, one is highly virulent that cause mortal encephalitis and the other one is less virulent and it is used as experimental model of MS [4,40]. Experimental demyelination is induced by intracerebral infection with TMEV in mice CNS [4]. Unlike EAE, TMEV can induce inflammatory demyelinating disease only in mice and it represents the chronic-progressive phase of demyelination in mice, which has clear similarities to human MS [41-43]. The main difference related to EAE is that TMEV infection induced axonal damage that precedes demyelination [37], according to the inside-out model. Additionally, the damaged axons are distributed in the same area where the inflammatory process occurs during the chronic phase, suggesting that axonal degeneration triggers recruitment of T cells and macrophages into the CNS, leading to subsequent loss of myelin [24].

Therefore, TMEV model represents a combination of mechanisms as those responsible for myelin damage through “inside out” demyelination (axonal damage first, leading to demyelination as a secondary consequence), macrophage-mediated demyelination, molecular mimicry and immune response [4,24].

**Mouse hepatitis virus:** MHV encephalitis induces inflammatory demyelination with no influence of viral particles after intracranial (**ic**) administration [44,45]. Viral demyelination are associated with cluster of activated microglia/macrophages, CD4+ T cells, CD8+ T cells, natural killer (**NK**) cells, B cells [46,47]. However, after MHV ic injection viral replication can be observed in oligodendrocytes, astrocytes and microglia [40,48]. MHV model represent a useful model of demyelination for studying the disease and evaluating therapeutic approaches to protect cells of the oligodendrocyte lineage and promote remyelination [46,47].

**Adenovirus:** The use of non-replicative adenovirus expressing pro-inflammatory cytokines has been developed during the last few years. In particular, the injection of an adenovirus expressing the pro-inflammatory cytokine interleukin 1beta (**IL-1**) in the striatum was demonstrated to induced neutrophils recruitment, blood brain barrier breakdown, demyelination and subsequent remyelination, microglia and astroglia activation with no neurodegeneration [49]. Therefore, the long-term expression of IL-1 mediates the activation of the immune system and it is not related to a toxic effect by the virus on the targets cells. This simple model allows us to study demyelination and remyelination independently of the autoimmune and adaptive immune components [50]. Re-exposure to IL-1 when the first inflammatory response was still unresolved generated a lesion with decreased neuroinflammation, demyelination, axonal injury and glial response [51].



However, a second injection of IL-1 when the first lesion was resolved could not be differentiated from the first event. Therefore, the response to a second inflammatory stimulus varies depending on whether the initial lesion is still active or has been resolved [51]. Additionally, peripheral pro-inflammatory stimulation aggravated the ongoing central lesion, including axonal degeneration, independently of the blood–brain barrier (BBB) integrity [52]. This model allows studying the role of specific molecules and cells from the innate immune system, in the relationship between central and peripheral communication, and on relapsing episodes of demyelinating lesions.

## The use of animal models in the different MS forms

### Animal models of RRMS

RR EAE model, that presents a course of exacerbations and remissions as occurs in human MS, was induced by Touhy et al [53] through immunization of genetically modified mice (SJL/J mice) with the immunodominant epitope of PLP (PLP<sub>139-151</sub>). In addition, pathophysiology RR EAE can be induced by passive transfer of PLP reactive cell lines that involve the immunization of donor mice with PLP derived peptides, isolation of peripheral lymphoid cells after 7/10 days of culture, *in vitro* re-stimulation and later transfer into native recipients. This method allow for *in vitro* manipulation of the encephalitogenic T cell population and disease with homogenous population of antigen specific T cells [23]. Additionally, MBP derived peptides and MBP reactive T cell clones was also used to induce RR EAE [54]. The stimulation either with MBP or PLP leads to activation and expansion of peripheral antigen specific CD 4 and Th 17 cells which enter the CNS, attack specific myelin antigen and in turn induce disease [55]. CD 4, CD 8, Th 17, monocytes, macrophages, B cells, Treg cells have been involved in the pathophysiology of this model, which is most frequently used to study neuroinflammation and immune system activation [4,23].

### Animal models of Progressive

The main challenge in MS therapeutics has been the difficulty in replicating the chronicity and injury pattern of SPMS and PPMS, which is rather different to RRMS [2]. Cortical demyelination is considered a key feature for PPMS and SPMS patients, but is sparse in RRMS [56]. Neurodegenerative cortical lesions characterized the PPMS and SPMS in patients, and contribute to the clinical outcome of the disease, such as cognitive impairment [57]. The mechanisms involved in cortical demyelination and neurodegeneration are still unknown. An animal experimental model specific for the progressive forms should include chronic demyelination, neurodegeneration, autoimmune inflammatory process and glial activation [2,4].

In order to develop a model for the progressive forms, a variant of EAE, C EAE model, was induced in C57BL/6J mouse. In this model, myelin oligodendrocyte protein (MOG<sub>35/55</sub>) induces a chronic form of the disease that did not remit. Myelin Oligodendrocyte protein (MOG) induces CNS autoimmunity and increase disease onset and severity with adding the use of pertussis toxin [58]. This model allowed studying the function of CD8 and CD4, B cells and monocytes in the pathogenesis of the disease [24].



EAE has contributed to the study of MS pathophysiology and the development of therapeutic agents, however, few EAE papers analyses cortical lesions demonstrating that this model has some limitations in studying the progressive forms of MS and in. However, chronic relapsing EAE in Biozzi antibody high mouse (**ABH**) mouse developed key features of secondary progressive disease, with progressive disability, demyelination, axonal damage, neurodegeneration and gliosis, with no cortical lesions [59,60]. EAE animal models also developed cortical demyelination. The presence of cortical lesions were described in EAE in marmoset, mice, LEWIS rats immunized with a cocktail of cytokines and in animals with EAE stereotactically injected with TNF- $\alpha$  in the cortex [61-63].

The TMEV model exhibits some of hallmarks that characterized the features of the progressive forms of MS. These features last for the entire life of the mice and shares several MRI findings of the human diseases [4]. Additionally, TMEV induce an extensive demyelination, neuroinflammation and axonal injury [4,24,42].

Even though, several animal models were described as representative of the progressive forms of the disease, no results have been demonstrated yet [59]. The main problem is the impossibility of replicating the chronicity and injury pattern of SPMS and PPMS, which are rather different to RRMS. Furthermore, most of the models do not reflect the irreversibility that characterized the MS progressive forms [2]. Animal experimental models that conclusively demonstrate chronic demyelination, neurodegeneration, autoimmune inflammatory process and glial activation, as occur in the progressive MS are required.

In summary, there are no an animal experimental models that reproduce the unique characteristics of the human progressive MS, but some aspects can be represented in several animal models. Therefore, the development of experimental animal models that models specific aspect of the disease, will improve the discovery of therapeutic specific agents for the progressive forms of MS.

## Contribution to Understand the Pathophysiological Mechanisms

EAE model reflects de range of the pathological and clinical features of MS. This model has been useful to elucidate mainly: distinct pathogenic T-cell subsets, regulatory T-cell maintenance of self-tolerance, discover multifaceted T-cell populations and epitope spreading [23]. Although that this model has contributed to the development of medications in MS patients, it has limitations, because:

- Poor contribution to study the pathophysiology of remyelination and MS progression, role of CD8 T cells, B cells and cerebral-cerebellar cortex [24].
- EAE is more applicable as a model of ADEM (acute disseminated encephalomyelitis). There are many differences between EAE and MS [4,26,64,65].

- EAE lesions showed perivascular CD4 infiltrates, without CD8, macrophages and B cells as MS lesions. Additionally, MS lesions has shown heterogeneity in neuroinflammation: from extensive to less inflammation with neurodegeneration and oligodendroglial pathology [66], which are not observed in EAE lesions.
- Most of the potential therapeutic targets described in EAE (e.g. IL 23, TNF, IFN, IL12, were proven to be unsuccessful in MS human trials [26].
- Mechanism of both acute and chronic axonal damage remains to be firmly elucidated in EAE model [4].

## The Use of Animals Models in Developing Therapeutics Agents

### Acute EAE studies

Even though, not all therapeutic strategies for MS have been developed in EAE, acute EAE studies have led to the development of three approved drugs for RRMS:

- Glatiramer acetate: a copolymer of four amino acids that has shown efficacy in the treatment of RRMS decreasing annualized relapse rate about 30% [67]. This drug is widely used at present.
- Mitoxantrone is a synthetic anthracenedione that inhibits topoisomerase II, interfering with DNA repair, causing generalized immunosuppression, and decreased secretion of pro-inflammatory cytokines. Mitoxantrone was approved for SPMS but, it was describe to worse the symptoms of RRMS [68,69]. However, currently it is not used because it causes severe adverse effects.
- Natalizumab: is a monoclonal antibody against the  $\alpha 4\beta 1$  integrin which inhibits leukocyte migration across the blood brain barrier, which has shown higher efficacy to decrease annualized relapse in 68% [70]. Due to its high efficiency it has revolutionized the management of MS. Fingolimod and dimethylfumarate are others approved therapies have shown some efficacy in ameliorating EAE symptoms [71-74,75]. On the other hand, an EAE-based study explains why interferon  $\beta$  (interferon  $\beta$ -1a and interferon  $\beta$ -1b), the most widely used treatment in MS, does not work in one-third of MS patients [76] (Table 1).

**Table 1:** Summary of the animal models used for developing and testing MS drugs.

Animal Model	Animal	Involved CNS area	Pathology	Tested and used in MS patients Therapeutics Agents
RRMS				
Acute EAE	Rat, mouserabbit, primates, marmoset, ship, guinea pigs and goats	Brain, spinal cord	Demyelination, CD4Th1 , Th17	<i>Glatirameracetate, mitoxantrone natalizumab</i> *(69), fingolimod(77-78), beta-interferon (79-80) Teriflunomide (81), alemtuzumab (82), dimethylfumarate(83) Fingolimod (90), Dimethylfumarate (92), beta –interferón (91)
THV	Mouse	Brain, spinal cord	axonal damage, demyelination	None
MHV	Mouse	Brain, spinal cord	Demyelination, axonal damage, CD8+	None
Adenovirus	Rat	Focal	Demyelination, remyelination, neutrophils BBB breakdown, gliosis	None
EB	Rat, mouse	Focal	Demyelination, remyelination, Macrophages, astrocyte depletion	beta-interferon (93-94)
LPC	Rat, mouse	Focal	Demyelination, remyelination, Macrophages, CD8/CD4 T cells	Fingolimod (95), glatirameracetate (96)
Cuprizone	Rat, mouse	White matter	Demyelination, remyelination, no peripheral recruitment	Fingolimod (97), glatiramer acetate (98), dimethylfumarate (99)
<b>Progressive MS</b>				
Chronic EAE	Biozzi ABH mouse, Lew Rat, marmoset	Brain, spinal cord, cortex	Demyelination, axonal and neuronal loss, gliosis. Cortical demyelination	Fingolimod (84), glatiramer acetate (85-86), dimethylfumarate (75, 87), beta-interferon (88-89)
THV	Mouse	Brain, spinal cord	Demyelination, axonal injury, gliosis	None

\*Glatiramer acetate, mitoxantrone, and natalizumab were developed in EAE model.

Additionally, other drugs used in RRMS therapy have been test in the acute EAE model, such as fingolimod [77,78], beta interferon [79,80], teriflunomide [81], alemtuzumab [82] and dimethylfumarate [83]. Notwithstanding that the EAE approaches showed promising results; sometimes it has been proven to be ineffective or harmful in MS. On the other hand, some authors stated that EAE model was not useful in the development of therapies for relapsing remitting and progressive forms of MS [4,69].

**Chronic EAE:** The chronic model of EAE were used to test several therapeutics agents, e.g. fingolimod [84], glatiramer acetate [85,86], dimethylfumarate [75,87], beta interferon [88,89]. (Table 1).This model has been used only or testing drugs, because it was not successful in developing new MS.

## Virus models

THV model has been useful in testing therapeutic drugs such as: fingolimod [90], beta-interferon [91] y dimethylfumarate [92]. No therapeutics agents were tested in either MHV or adenovirus model (Table 1).

## Toxin models

The EB demyelinating model were used for testing: beta interferon [93,94], in LPC: fingolimod [95], glatiramer acetate [96] and the cuprizone model for: fingolimod [97], glatiramer acetate [98], dimethylfumarate [99] (Table 1).

## CONCLUSIONS

Multiple sclerosis is an inflammatory neurodegenerative demyelinating disease of the human CNS. It involves a complex interaction between the immune and the nervous system. Animal modeling has been critical for addressing MS pathogenesis. However, a translatable animal model is still a debt in the field. It is important to note that every form of the disease present different pathological forms: Relapsing-remitting: inflammation, demyelination and the progressive forms: neurodegeneration. In particular, no animal model for the progressive forms of MS is available nowadays.

Some MS treatments were designed with the help of animal model research, however, the seeking for specific animal models should be continued in order to develop both the knowledge and the cure for the disease.

The progression in the knowledge and cure of the disease should be the results of the data coming from different approaches, especially those concerning the interaction between the immune system and the CNS.

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