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Animal models of multiple sclerosis: Focus on experimental autoimmune encephalomyelitis

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Abstract
Multiple sclerosis (MS) is a chronic, progressive disorder of the central nervous system (CNS) that affects more than two million people worldwide. Several animal models resemble MS pathology; the most employed are experimental autoimmune encephalomyelitis (EAE) and toxin- and/or virus-induced demyelination. In this review we will summarize our knowledge on the utility of different animal models in MS research. Although animal models cannot replicate the complexity and heterogeneity of the MS pathology, they have proved to be useful for the development of several drugs approved for treatment of MS patients. This review focuses on EAE because it represents both clinical and pathological features of MS. During the past decades, EAE has been effective in illuminating various pathological processes that occur during MS, including inflammation, CNS penetration, demyelination, axonopathy, and neuron loss mediated by immune cells.

KEYWORDS
EAE, ethidium bromide, glial cells, lysolecithin toxin and virus-induced demyelination, multiple sclerosis

1 | INTRODUCTION

Multiple sclerosis (MS) is a neuroinflammatory, demyelinating disease that is characterized by accumulation of disability. Generally, it is thought to be an autoimmune, T cell–mediated disease. However, the etiology of MS is still unclear, although many environmental factors and the interplay of multiple genes have been proposed to play a role in disease occurrence. MS is most often diagnosed between ages 20 and 40 and is more common in females than in males (now almost 3:1). Three courses of the disease are recognized: relapsing–remitting MS, primary progressive MS, and secondary progressive MS. The relapsing–remitting course initially affects most MS patients; it is most often succeeded by a secondary progressive disease course, which is characterized by a buildup of neurological deficits. The primary progressive course affects 10% to 15% of patients and is described as a progressive disease without relapses.

The two main pathological features of MS are inflammation and demyelination. In addition, activation of astrocytes and microglia, apoptosis of oligodendrocytes, and axonal loss (Bjartmar, Wujek, & Trapp, 2003; Kornek et al., 2000) are found within the affected central nervous system (CNS). A multifaceted relationship between different cell types that lead to demyelination and remyelination in the CNS during MS has been uncovered using animal models. Although the established animal models have their advantages/disadvantages, no model fully replicates the stages of MS. There are several established experimental demyelination models that, to some extent, reflect the heterogeneity of MS and are therefore seen as suitable to study MS pathogenesis. These models include immune-mediated, virus-induced, and toxin-induced models. Experimental autoimmune encephalitis (EAE) is, by far,

Significance
Multiple sclerosis (MS) is a disease that is found only in humans; however, there are several well-established animal models that resemble this disease. These models are valuable tools in MS research because the use of human tissue samples is limited. Although all of the mentioned animal models in this review have advantages/disadvantages, neither fully replicates stages during MS. Despite their limitations, a wide variety of phenomena relevant to understanding pathomechanisms of disease can be learned from these animal models. This review summarizes current knowledge about the usefulness of animal models in MS research and discusses the next generation of experimental autoimmune encephalomyelitis models.
the most exploited model for studying various aspects of autoimmunity in MS pathology. Virus-induced demyelination models support the hypothesis that some environmental factors, such as viral infections, are involved in MS and may be a trigger of the disease. Toxin-induced demyelination models are means in the evaluation of the demyelination/remyelination process in the relative absence of immune cells, even though these ways of damaging the myelin do not resemble features of the demyelination seen in MS.

Despite the limitations, a wide variety of phenomena relevant for understanding pathological events in MS and management of different therapies can be learned from animal models. In this review, we discuss several well-established experimental demyelination models that are available to study MS pathogenesis, with a special focus on EAE. We will describe the animals selected to induce EAE and address some of their genotypic and phenotypic features. The role and involvement of glial cells will also be reported. The recent advances in creating next-generation models for MS will be reviewed.

2 | TOXIN-INDUCED DEMYELINATION MODELS

There are several agents known to generate demyelination foci, using direct injections of gliotoxins in the white matter, such as ethidium bromide (EtBr) and lysolecithin, or systemically administered toxins, such as cuprizone (Woodruff & Franklin, 1999). These models are indispensable for studying remyelination processes in animals. Further, these models ensure good reproducibility and well-defined anatomical location of the demyelination area.

Calcium ionophore, antibodies against galactocerebroside, 6-aminonicotinamide, and diphtheria toxin can also be used as demyelinating agents; however, these approaches are not considered to be suitable models for gaining insights into the remyelination processes. Another drawback of these models lies in the absence of the immune activity.

2.1 | Ethidium bromide

EtBr, as an intercalating agent, is toxic for all cells with nuclei and can be used to create a focal demyelination lesion. It is assumed that EtBr intercalates with both chromosomal and mtDNA, but influences only mtDNA transcription (Kuypers, James, Enzmann, Magnuson, & Whittemore, 2013). Accordingly, EtBr injection preferentially compromises mtDNA transcription in glial cells rather than neurons and endothelial cells. To achieve demyelination, stereotactic injection of EtBr is delivered into specific white matter tracts, such as the thoracolumbar dorsal funiculus of the spinal cord or in the caudal cerebellar peduncle. Similarly, optic neuritis is generated upon EtBr injection into the subarachnoid space (Merrill, 2009). The EtBr model also has been carried out extensively in the rat as a demyelinating model to assess endogenous remyelination (Blakemore & Franklin, 2008); recently, it was used to identify white matter tracts responsible for locomotor function in mice (Kuypers et al., 2013; Salem, Assaf, Ismail, Khadrawy, & Samy, 2016). The hallmark of EtBr delivery to white matter, and thus induced lesions, is astrocyte and oligodendrocyte loss, while axons persist to be

FIGURE 1 Schematic representation of toxin-induced models of demyelination. Ethidium bromide (EtBr) induced astrocyte, oligodendrocyte, and OPC apoptosis within the first week (W1) after injection. Remyelination starts 6 weeks (W6) after EtBr delivery into the white matter of the CNS. In contrast, lysophosphatidylcholine (LPC) does not induce death of oligodendrocytes, OPC, and astrocytes, and remyelination is faster, starting 3 weeks (W3) after LPC injection. Cuprizone intoxication leads to apoptosis of oligodendrocytes. Activation of microglia starts 2 weeks (W2) after treatment with cuprizone and gradually decreases with the remyelination process. Astrocyte activation persists throughout the disease. Spontaneous remyelination occurs after cessation of cuprizone intoxication [Color figure can be viewed at wileyonlinelibrary.com]
unaffected (Blakemore, 2005). Apoptosis of oligodendrocytes occurs 3 days after the EtBr injection; afterwards, the remyelination process starts (Figure 1) (Guazzo, 2005). However, regardless of the absence of oligodendrocytes in demyelination foci, Schwann cells migrate to the lesion epicenter and remyelinate as confirmed in both rats and cats after EtBr delivery into the white matter of the spinal cord (Blakemore, 1982; Woodruff & Franklin, 1999). Studies regarding the EtBr model also report transient or persistent inflammatory response, probably because astrocyte death compromises the blood-brain barrier (BBB), leading to the infiltration of peripheral inflammatory cells (Kuypers et al., 2013). However, lymphocyte infiltration observed after a single EtBr injection was also proposed to be a consequence of the general immune activity.

The advantage of this model is that one can predict a demyelination site and determine the size of the lesion that is concentration dependent. Age is a limiting factor with regard to remyelination after EtBr injection, meaning that spontaneous myelin recovery decreases with animal age (Ibanez et al., 2004). An immunological disorder that shares some resemblance to MS, Devic’s disease, is also characterized by massive Schwann cell-mediated remyelination, as observed in EtBr-induced lesions (Ikota, Iwasaki, Kawarai, & Nakazato, 2010). In addition, EtBr injection into the hippocampus may be employed as a model to study cognition and alterations that occur in the gray matter (Goudarzvand et al., 2016), a feature that also occurs in MS.

2.2 | Lysolecithin

The toxic effect of another agent that is able to produce demyelination, lysophosphatidylcholine (lysolecithin), was first described by Hall (1972). With detergent-like agent activity, lysolecithin is able to solubilize membranes and is considered to be selective for myelin-producing cells. Therefore, lysolecithin targets the myelin, leaving other cellular components relatively unaffected, thus allowing for the recruitment of T and B cells, as well as microglia/macrophage activation at the lesion site, which have a role in clearing myelin debris and in promotion of trophic factors (Procaccini, De Rosa, Pucino, Formisano, & Matarese, 2015). Lysolecithin injection increases phospholipase A2 activity, which is restricted to activated macrophages (Trotter & Smith, 1986). Phospholipase A2 further degrades membrane lysophosphatidylcholines (Weltzien, 1979). Usually, 1% lysolecithin solution is injected into the dorsal funiculus of the spinal cord, caudal cerebellar peduncle (Woodruff & Franklin, 1999), or corpus callosum (Keough, Jensen, & Yong, 2015). Following lysolecithin injection, the formed lesion changes over the next few weeks and is capable of remyelinating completely, starting at the end of the first week after the injection (Blakemore & Franklin, 2008). The remyelination process in this model is faster compared with other toxin-induced demyelination models, mainly because oligodendrocyte progenitor cells (OPCs) are not affected (Blakemore & Franklin, 2008). Demyelinating axons are remyelinated mainly by oligodendrocytes. However, if the lesion is larger in size, Schwann cells also take part in the remyelination process. As in the EtBr model, remyelination occurs faster in young animals of different species (Shields, Gilson, Blakemore, & Franklin, 1999). Remyelination begins only after the myelin debris is removed—that is, about 7 days post-lysolecithin injection in the spinal cord of mice or young adult rats, when pale myelin sheaths begin to appear. When the lysolecithin injection is delivered into the caudal cerebellar peduncle, remyelination is rather slow compared with spinal cord injection. In these cases, remyelination starts 3 weeks after the injection, and compact myelin appears 6 weeks post demyelination (Woodruff & Franklin, 1999). Lysolecithin treatment, in contrast to EtBr, does not induce a loss of astrocytes, OPCs, or macrophages, thus facilitating faster remyelination (Figure 1). The recurrent pattern of primary demyelination that can be controlled in a spatiotemporal manner is an advantage of this model of demyelination. Lysolecithin-induced demyelination can also be performed in nonhuman primates. Namely, demyelination/remyelination processes were evaluated in adult Macaca fascicularis upon lysolecithin injection in the optic nerve or spinal cord. The remyelination occurs in the spinal cord, while it fails in the optic nerve (Lachapelle et al., 2005).

Therefore, lysolecithin proves to be a useful method to study demyelination/remyelination in the CNS. The disadvantage of the lysolecithin model of demyelination lies in the absence of the immune response that is normally observed during MS.

2.3 | Other toxins

The toxins that are able to induce demyelination, but are not widely in use, are described below.

The injection of the calcium ionophore, ionomycin, into the dorsal column of the spinal cord induces myelin vesiculation that progresses and forms confluent, large demyelination foci leading to axon degeneration in a dose-dependent manner (Smith & Hall, 1988, 1994). This type of lesion created by ionomycin injection resembles the lesion seen in the lysolecithin model of demyelination (Smith & Hall, 1988).

An antimetabolite of niacin and a well-known gliotoxin, 6-aminonicotinamide, is used to induce glial degeneration, especially in the gray matter of adult rats (Horita, Ishii, & Izumiyama, 1981; Schneider & Cervos-Navarro, 1974). Delivery of 6-aminonicotinamide into the spinal cords of rats induces formation of demyelination foci, while astrocytes and oligodendrocytes undergo degeneration. The remyelination process depends on Schwann cells, except for a few axons that are myelinated by oligodendrocytes (Blakemore, 1975). In cats, 6-aminonicotinamide injection into the spinal cord causes massive death of axons and glial cells. Surviving demyelinated axons are remyelinated by Schwann cells. However, because of extensive axonal damage, this approach is not considered to be a suitable model system for the study of cellular relationships during remyelination (Blakemore, 1978).

It was postulated that diphtheria toxin might produce a useful model for studying central demyelination (Harrison, McDonald, & Ochoa, 1972). After diphtheria toxin exposure, axons in demyelination foci show extremely thin myelin sheets, which is the main characteristic of this model. Later, it was seen that a small dose of diphtheria toxin induces peripheral demyelination in the presence of axonal degeneration (Baba, Gilliatt, Harding, & Reiners, 1984). The use of diphtheria toxin with regard to cell depletion was shown, where anatomically targeted oligodendrocyte death can also be induced by recombination in transgenic mice carrying a floxed diphtheria toxin (fragment A).
2.4 | Antibody-mediated demyelination

Galactocerebrosides are greatly enriched in myelin membranes of the CNS; they are also markers of immature oligodendrocytes (Dousset et al., 1995). Antibodies to these glycolipids were used as a specific cell-surface antigen to identify oligodendrocytes in cell culture (Raff et al., 1978). The well-established animal model of human demyelination neuritis, experimental allergic neuritis, may be induced by inoculation with galactocerebroside antibodies. No changes occur in the CNS, but several small perivascular areas of demyelination are found in the dorsal roots of the spinal cord and dorsal root ganglia (Saida, Saida, Silberberg, & Brown, 1981). However, inoculation of these antibodies with complete Freund’s adjuvant (CFA) failed to induce demyelination in guinea pigs (Khang, Chi, & Lee, 1988). Further, delivery of these antibodies into the spinal cord induces demyelination, and this approach can preserve oligodendrocytes that are able to start the remyelination process (Keirstead & Blakemore, 1997). In addition, a similar pattern of demyelination occurs after injection of these glycolipids into the caudal cerebellar peduncle, forming demyelination foci that are remyelinated by oligodendrocytes (Woodruff & Franklin, 1999). In these models, the axons are more preserved from degeneration compared with the lysolipid model. Therefore, galactocerebroside antibodies may be employed as an approach to evaluate axonal cytoskeleton changes upon demyelination. It is likely that demyelination is induced through the myelin-lysis and oligodendrocyte-lysis process because it was previously shown that these antibodies are toxic to myelin and oligodendrocytes in vitro (Raine, Johnson, Marcus, Suzuki, & Bornstein, 1981).

2.5 | Cuprizone

Cuprizone (oxalic acid bis [cyclohexyldene hydrazide]) is a well-known copper-chelating agent that is known to be toxic to myelin sheet. Cuprizone-induced demyelination is a straightforward model used to investigate brain-intrinsic inflammatory responses, together with demyelination/ remyelination processes. The hallmark of the lesions that cuprizone elicits is oligodendrocyte dysfunction. This model was regarded as a typical white matter demyelination model; however, recent studies confirm that gray matter is also afflicted (Goldberg, Clarner, Beyer, & Kipp, 2015; Khodanovich et al., 2017).

The first described experiments using cuprizone as a toxic compound were conducted by Carlton, who reported that cuprizone intoxication forms small lesions throughout the brain, followed by hydrocephalus, spongy demyelination, and astrogliosis (Carlton, 1967). Later, cuprizone intoxication was introduced as an in vivo model for chemically induced demyelination that can be controlled in a spatiotemporal manner (Ludwin, 1978). The first observation that cuprizone causes oligodendrocyte death with consequent microgliosis was presented by Blakemore (1972). He also noticed remyelinating axons in some areas of the brain. Since then, it has been reported that cuprizone is a suitable model for studying demyelination events during MS because it provides a highly reproducible system for exploring oligodendrocyte apoptosis and secondary demyelination (Matsushima & Morell, 2001). During cuprizone-induced demyelination, the apoptosis of oligodendrocytes occurs 3 days after cuprizone exposure (Hesse et al., 2010; Mason et al., 2004). Oligodendrocytes exhibit pyknotic nuclei, with enlarged mitochondria and big vacuoles, suggesting that oligodendrocytes are dying primarily because of caspase-3-dependent apoptosis (Kipp, Nyamoya, Hochstrasser, & Amor, 2017). Even though the precise mechanism of oligodendrocyte death is not fully understood, it is assumed that cuprizone causes dysfunction of mitochondrial enzymes with subsequent oxidative stress, leading to metabolic stress in oligodendrocytes (Milićević & Spasojević, 2013). Typically, the apoptosis of oligodendrocytes appears after 2 days, as a consequence of cuprizone intoxication, before demyelination is obvious (Buschmann et al., 2012). The demyelination is usually evident 3 weeks after the start of cuprizone administration and correlates with immense microgliosis, astrogliosis, and axon damage (Doan et al., 2013; Kipp et al., 2017). Six weeks after cuprizone ingestion, demyelination occurs globally, but it is most prominent in the corpus callosum and posterior cerebellar peduncles and is referred to as “acute demyelination.” Acute demyelination is followed by spontaneous remyelination, when a cuprizone diet is replaced by normal chow. The remyelination process is dependent on OPC maturation, and the reappearance of myelin in white matter is more obvious than in gray matter, where proliferation and differentiation of OPCs is prolonged in cuprizone-induced demyelination (Baxi et al., 2017). On the contrary, prolonged cuprizone intoxication (more than 12 weeks) results in a constrained spontaneous remyelination process: “chronic demyelination.” During chronic stages of the disease, apoptosis of oligodendrocytes occurs in a caspase-3–independent manner (Gudi, Ginge, Skripuletz, & Stangel, 2014). Systemic application of cuprizone generates demyelination in the corpus callosum; however, a loss of myelin is also observed in the cortex, hippocampus, and cerebellum (Baxi et al., 2017; Kipp, Clarner, Dang, Copray, & Beyer, 2009).

The role of microglia in this animal model is still under debate. Activation of microglia precedes demyelination, starting after 2 weeks of intoxication. The number of microglia gradually increases over the following weeks and decreases subsequently until complete demyelination (Skripuletz, Gudi, Hackstette, & Stangel, 2011). However, the presence of activated microglia persists during the chronic phase of the disease (Lindner, Fokuhl, Linsmeier, Trebst, & Stangel, 2009; Mason et al., 2004). It is well known that microglial cells are involved in phagocytosis of disrupted myelin sheets. Also, microglial production of proinflammatory mediators increases because of cuprizone-induced oligodendrocyte death. On the other hand, microglia may take part in promoting remyelination (Praet, Guglielmetti, Berneman, Van der Linden, & Ponsaerts, 2014). Accordingly, it was observed that massive accumulation of OPCs occurs between 3 and 5 weeks of intoxication, when microglial activation is most apparent (Gudi et al., 2014). Astrocytes also increase in size and number (Gudi et al., 2014; Hiremath et al., 1998) and continue to be activated during the chronic phase of disease (Kipp et al., 2011; Lindner et al., 2009). The activation status at this stage of the disease seems to be higher compared with astrocytes observed at the acute cuprizone-induced demyelination (Gudi et al., 2014; Baxi et al., 2017).
Consequences of cuprizone-induced demyelination on functional deficits in behavioral and cognitive manner were not evaluated comprehensively. Thus far, decreased motor coordination of mice in the Rotarod test, impaired social behavior and sensorimotor function, as well as reduced anxiety have been described, although some of these deficits are withdrawn upon remyelination (Franco-Pons, Torrente, Colomina, & Vilella, 2007; Hibbits, Pannu, Wu, & Armstrong, 2009).

Cuprizone intoxication induces a variable degree of demyelination that depends on the strain, age, and gender of animals as well as the duration of exposure and dosage of this toxic agent. Indeed, it was reported that when exposed to cuprizone, Swiss mice, BALB/c mice, and Swiss Jim Lamberts (SJL) mice show demyelination, but delayed or incomplete (Ludwin, 1978; Skripuletz et al., 2008; Taylor, Gilmore, & Matsushima, 2009). Cuprizone is widely used in C57BL/6 mice, which has led to the development of a highly reproducible model of investigating demyelination/remyelination (Præt et al., 2014). Unlike mice, it was reported that Wistar rats, guinea pigs, and Syrian and Chinese hamsters do not develop areas of demyelination in the spinal cord upon cuprizone treatment (Love, 1988). However, more recent studies imply that a higher dose of cuprizone can induce demyelination in the cortex and subcortical areas in Wistar rats (Adamo et al., 2006; Basoglu, Boylu, & Kose, 2013; Silvestroff, Bartucci, Pasquini, & Franco, 2012), while in CD1 mice demyelination was not seen until 7 weeks after cuprizone administration (Yu et al., 2017). It has been documented that female SJL mice are partly resistant to demyelination and depletion of oligodendrocytes (Taylor et al., 2009). On the contrary, cuprizone administration induces similar demyelination in female and male C57BL/6 mice, with estrous cycle disruption in females (Taylor, Gilmore, Ting, & Matsushima, 2010). Therefore, use of the cuprizone model in male C57BL/6 mice is recommended (Kipp et al., 2009) because of the involvement of female hormones, which are known to be able to postpone demyelination and prompt remyelination. It was reported that the degree and pattern of demyelination does not depend on the age of the mice (Shen et al., 2008). However, oligodendrocyte turnover and recruitment of oligodendrocytes progenitor cells to the area of demyelination (Doucette, Jiao, & Nazarali, 2010), together with constant decreases in spontaneous remyelination, occurred in the old mice. The observed decline in remyelination capacity is probably due to differences in epigenetic and transcriptional control in older animals (Shen et al., 2008).

Recently, cuprizone was used in combination with actively induced EAE, resulting in inflammatory forebrain lesions (Ruth et al., 2017; Scheld et al., 2016). Therefore, this model may be an extremely valuable tool in MS research in the future.

In summary, the simplicity of the model, together with high reliability and reproducibility, makes it ideal for inducing and examining demyelination/remyelination processes (Matsushima & Morell, 2001). As in other toxin-induced demyelination, the drawback of this model is the lack of immune system involvement, which is an important component of MS pathogenesis (Table 1).
<table>
<thead>
<tr>
<th>Multiple sclerosis</th>
<th>Primate EAE</th>
<th>Rodent EAE</th>
<th>TMEV</th>
<th>Cuprizone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population prone to the disease</td>
<td>Human</td>
<td>Marmosets and rhesus monkey</td>
<td>Rats/mice (inbred strain)</td>
<td>Mice (inbred strain)</td>
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<tr>
<td>Involvement of sex</td>
<td>Female prevalence</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Induction of disease</td>
<td>Spontaneous</td>
<td>Myelin antigens/adjuvant needed</td>
<td>Myelin antigens/adjuvant needed</td>
<td>Virus</td>
</tr>
<tr>
<td>Clinical course</td>
<td>Relapsing–remitting, progressive (Bjelobaba et al., 2017)</td>
<td>Relapsing–remitting (marmoset) (T Hart et al., 2013), acute (rhesus monkey) (Kerlero de Rosbo et al., 2000)</td>
<td>Acute, chronic, relapsing–remitting (Amor et al., 2005; Lagumersindez-Denis et al., 2017; Lavmjja et al., 2008; Pitarokoili et al., 2017)</td>
<td>Acute, chronic (Mecha et al., 2013)</td>
</tr>
<tr>
<td>Involvement of B cells</td>
<td>Yes (Disanto et al., 2012)</td>
<td>Yes (T Hart et al., 2013; Jagessar et al., 2016)</td>
<td>Yes (Person et al., 2014; Delarasse et al., 2013)</td>
<td>Yes (Tsunoda et al., 2002)</td>
</tr>
<tr>
<td>Involvement of CD8⁺ cells</td>
<td>Yes (Lassmann &amp; Bradl, 2017)</td>
<td>Yes (Jagessar et al., 2016)</td>
<td>Questionable Biozzi ABH mice (Lassmann &amp; Bradl, 2017) DA rats (Constantinescu et al., 2011)</td>
<td>Yes (Johnson et al., 2014; Tsunoda et al., 2002)</td>
</tr>
<tr>
<td>White matter demyelination</td>
<td>Yes (Lassmann &amp; Bradl, 2017)</td>
<td>Yes (marmoset) (T Hart et al., 2013) Yes (rhesus monkey) (Stewart et al., 1991)</td>
<td>Strain dependent DA rats (Milecic et al., 2003; Papadopoulos et al., 2006) Biozzi ABH mice (Jackson et al., 2009)</td>
<td>Chronic form of disease (Sato et al., 2011)</td>
</tr>
<tr>
<td>Gray matter demyelination</td>
<td>Yes (Bo, 2009)</td>
<td>Yes (marmoset) (Jagessar et al., 2012) No (rhesus monkey) (Burms et al., 2016)</td>
<td>Strain dependent DA rats (Gardner et al., 2013) Biozzi ABH mice (Puentes et al., 2013)</td>
<td>Yes (Tsunoda &amp; Fujinami, 2010)</td>
</tr>
<tr>
<td>Inflammation</td>
<td>Focal (Bjelobaba et al., 2017)</td>
<td>Focal (marmoset) (Maggi et al., 2014) Mainly focal (rhesus) (T Hart et al., 2013)</td>
<td>Focal (Lassmann &amp; Bradl, 2017)</td>
<td>Multifocal (Pachner, 2011)</td>
</tr>
<tr>
<td>Axonal loss</td>
<td>Yes (Bjartmar et al., 2003; Criste et al., 2014)</td>
<td>Yes (Mancardi et al., 2001)</td>
<td>Not extensive (Kornek et al., 2000)</td>
<td>Yes (Sato et al., 2011)</td>
</tr>
</tbody>
</table>
subcortical gray matter, the hippocampus, and the basal ganglia (Johnson, Jin, Pirko, & Johnson, 2014; Pachner, 2011). Inflammation in the spinal cord is not pronounced and is localized in the anterior horns of the gray matter. During the early acute phase of TMEV infection, the white matter of the spinal cord remains unaffected (Oleszak et al., 2004). Also, it was postulated that during the acute phase, axonal damage precedes demyelination; this inside-out model of demyelination is a feature that was also observed during the chronic phase of disease (Sato, Tanaka, Hasanovic, & Tsunoda, 2011). It has been proposed that the axonal damage provokes the immune system to recruit proinflammatory mediators that generate the loss of myelin (Figure 2).

The chronic phase, which usually appears at about 30 days post infection, is outlined by an inflammatory demyelination, together with functional deficits, such as ataxia and spastic paralysis (Miller et al., 1997; Procaccini et al., 2015). The pathological features at the onset of the chronic phase include massive inflammation of the white matter comprising primarily T cells and monocytes/macrophages, together with microglial proliferation predominantly in the brain stem and thalamus, and perivascular inflammation in the spinal cord white matter (Procaccini et al., 2015). During this phase of the disease, the highest level of viral antigens has been demonstrated in astrocytes, microglia/macrophages, and oligodendrocytes, but not in neurons. Histologically, demyelination is characterized by an obvious loss of myelin and vacuolization of the white matter, together with the appearance of phagocytic macrophages inside the lesions. Later, during the progressive stages of the chronic phase of disease, within the areas of demyelination, axonal swellings can be found (McGavern, Murray, & Rodriguez, 1999; McGavern et al., 2000). Further, the observed secondary autoimmune reactivity in the chronic phase of the disease does not appear to be due to T cell–specific molecular mimicry, due to the epitope spreading, where autoreactive T cells are triggered by constant release of endogenous epitopes of myelin (Miller et al., 1997).

The similarities between this model and MS can be seen, although the relapses are lacking in TMEV. Also, viral infections are linked to the initiation and progression of MS (Bjelobaba, Savic, & Lavrnja, 2016), and the autoimmune response upon TMEV infection that leads to axonal damage shows a resemblance to MS. The pitfall of this model is that experiments are time-consuming, and demyelination and remyelination occur simultaneously (Table 1).

4 | EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

EAE is one of the most studied animal models of MS and imitates various histopathological features and immunological facets of MS. There are different types of EAE, each displaying some aspects of MS (Table 1).

Therefore, it was suggested that EAE is a useful model of MS, if used wisely (Baker & Amor, 2014). A detailed critical evaluation of EAE as an MS model has been reviewed elsewhere (Baker, Gerritsen, Rundle, & Amor, 2011; Behan & Chaudhuri, 2014). Generally, besides transgenic mice that can spontaneously develop the disease, all animal strains used in EAE models have to be immunized with self-antigen to develop the disease (Croxford, Kurschus, & Waisman, 2011). As a consequence, depending on animal strain, age, and gender, an acute and/or chronic-relapsing inflammatory demyelinating autoimmune disease is acquired. EAE can be induced through two different approaches: active immunization with myelin peptides, or passively or adoptively transferred encephalitogenic T cells (Stromnes & Goverman, 2006a, 2006b).

4.1 | Active EAE

In actively induced EAE, susceptible strains of rodents (e.g., mice, rats, guinea pigs) or nonhuman primates are subcutaneously immunized
with a myelin-related antigen or peptide emulsified in CFA, a mineral oil–based adjuvant supplemented with heat-inactivated mycobacteria (Linker & Lee, 2009). The ratio of antigen and adjuvant is of crucial importance for the EAE development. CFA is the most frequently used adjuvant for EAE induction. While CFA promotes Th1 immune response, an adjuvant lacking Mycobacterium tuberculosis (denoted as incomplete Freund’s adjuvant [IFA]) induces a Th2-dominated response. In addition to enhancing the peripheral immune response, an advantage of CFA is that it increases BBB permeability, without obvious activation of microglia and astrocytes (Rabchevsky, Degos, & Dreyfus, 1999). The main disadvantage of Freund’s adjuvant is that it can cause granulomas at the inoculation site and lesions.

To generate a disease, mice require additional injections of pertussis toxin given on the day of immunization and 48 hr after (Stromnes & Goverman, 2006a). Pertussis toxin is a significant virulence factor of Bordetella pertussis, and the mechanisms of enabling EAE induction by pertussis toxin are complex. Although the precise effect of pertussis toxin in EAE is unknown, it is thought to facilitate immune cell entry to the CNS, as well as to promote proliferation and cytokine production by T cells and break T cell tolerance (Waldner, Collins, & Kuchroo, 2000).

Immunization leads to the priming of myelin-specific T cells in the secondary lymphoid organs. Activated T cells undergo maturation and clonal expansion, thus forming a large pool of myelin-specific CD4⁺ T cells in the periphery. Later, they differentiate into effector cells and become able to egress the secondary lymphoid organs through the efferent lymphatic vessels and to pass into the blood circulation. The entry of those cells to the CNS is accomplished upon an expression of adhesion molecules, cytokines, and chemokines, together with their receptors. All of this leads to disintegration of BBB (Engelhardt, 2006). In the perivascular space or within CNS, effector T cells become reactivated after they recognize antigens on antigen-presenting cells, such as microglia/macrophages and/or astrocytes, which amplify inflammatory response through continuous activation of those cells and constant recruitment of other cells. These events are followed by secretion of proinflammatory mediators attracting huge numbers of effector T cells and mononuclear cells in the CNS, resulting in tissue damage and demyelination (Figure 2) (McQuilter & Bernard, 2007). Functional deficit, such as tail and hind limb paralysis, occurs. T regulatory cells (Tregs) are also recruited in the CNS to regulate the immune response. During the later stages of the disease, the higher number of Tregs leads to EAE resolution (Kohm, Carpenter, Anger, & Miller, 2002). Furthermore, it has been reported that high levels of cytokines, such as IFN-γ, as well as NO, may have a regulatory role by suppressing T cell responses in the later stages of EAE disease (Arellano, Ottum, Reyes, Burgos, & Naves, 2015).

4.2 | Passive EAE

Another way to induce EAE is accomplished with the adoptive transfer by inoculating naïve syngeneic mice with activated, myelin antigen-specific T cells, denominated as “passive EAE” (Racce, 2001). The first verification that injection of lymph node cells from previously sensitized rats into naïve rats may develop EAE was performed by Paterson (1960). Twenty years later, the protocol for expansion of antigen-specific T cells was established, and passive EAE with myelin basic protein (MBP)-specific T cells was induced (Ben-Nun, Wekerle, & Cohen, 1981).

Passive EAE proves to be valuable in evaluating the central role of CD4⁺ T cells during the disease. This model is also useful for studying the effector phase of the disease in the absence of adjuvant. EAE develops faster and more homogeneously, permitting the study of the effector phase independently from the induction phase, which has questionable relevance to MS (Wekerle, Kojima, Lannes-Vieira, Lassmann, & Linington, 1994). It is possible to manipulate the T cells in vitro with a variety of inflammatory molecules prior to transfer, which allows studying the influence of different subtypes of T cells involved in the disease. Passive EAE can also be used to look at the migration of T cells into the CNS more closely (McCarthy, Richards, & Miller, 2012). This model was important for finding proper anti-inflammatory therapies (Gold, Linington, & Lassmann, 2006). The disadvantage of passive EAE is that the myelin antigen–specific T cells transferred to donor animals may not have the encephalitogenic capacity in vivo, meaning that adoptive transfer of T cells may be unable to induce the loss of myelin.

4.3 | Histopathology of EAE

Regardless of the animal used to induce EAE, the ongoing disease is generally associated with encephalitogenic lymphocyte-mediated demyelination. The initial period of EAE is characterized by perivascular infiltration seen in the white matter of the CNS. During EAE, the inflammatory response is accompanied by activation of microglia and astrocytes, demyelination, and axonal loss, mainly seen at the peak of the disease. The degree of damaged tissue correlates with the functional deficit seen after the onset of EAE. The most pronounced changes are observed in the spinal cord, while only sparse inflammation is seen in the brain (Schmitt, Straziele, & Gherisi-Egbe, 2012). It is of importance to note that the disease phenotype and histopathological changes in the CNS vary, depending on the species and strain of animals used.

Microglia cells are a component of the innate immune system in the CNS, and they serve to defend it from signs of danger and promote repair. Microglia cells are involved in the progression of MS/EAE, since they may serve as antigen-presenting cells to encephalitogenic T cells in the CNS. Resting microglia gradually change their phenotype in response to inflammation and demyelination and start to resemble classical activated macrophages (Kreutzberg, 1996). Macrophages from the periphery are also found to promote EAE disease (Trifunovic et al., 2015). These two populations of cells are functionally distinct; however, it is postulated that both macrophages and microglia have a role in EAE pathogenesis (Duffy, Lees, & Moalem-Taylor, 2014). Upon activation, microglia dynamically rearrange a plethora of surface adhesion molecules and receptors. Furthermore, cellular rearrangement of the microglia cytoskeleton is observed subsequent to inflammation (Bozic, Savic, Jovanovic, et al., 2015; Bozic, Savic, Laketa, et al., 2015). In
addition, activated microglia have the ability to clear the damaged tissue by phagocytosis (Sierra, Abiega, Shahraz, & Neumann, 2013), a feature needed for proper remyelination (Lampron et al., 2015). Activated microglia display a proinflammatory phenotype that leads to a "vicious cycle of inflammation" (Kettenmann, Hanisch, Noda, & Verkhratsky, 2011), which is characterized by production of potentially neurotoxic substances, such as proinflammatory cytokines and oxygen/nitrogen radicals (Bozic, Savic, Stevanovic, et al., 2015; Hanisch, 2013). Activated microglia may induce reactive astrocytes to be neurotoxic (Lidelow et al., 2017). It has been proposed that these astrocytes exist in neuroinflammatory conditions in two differential states of activation (Lidelow & Barres, 2017). Specifically, proinflammatory A1 astrocytes damage the synapses and inhibit OPCs, while A2 reactive astrocytes promote survival of neurons and tissue repair (Lidelow et al., 2017).

In general, astrocytes are cells of the innate immune system that preserve homeostasis within the CNS. They are considered nontraditional antigen-presenting cells; however, astrocytes are capable of modulating T cell and microglial immune response within the CNS (Bjellobaba et al., 2017; Chastain, Duncan, Rodgers, & Miller, 2011). Astrocytes serve to maintain the BBB in normal conditions, but in response to inflammation they produce cytokines, which allow T cell migration through the damaged BBB. Previously, it was concluded that astrocytes become reactive even before infiltration of immune cells in the CNS (Eng, D’Amelio, & Smith, 1989). At the onset of clinical deficits in EAE animals, astrocytes start to proliferate, while elongated fibrous branches border inflammatory infiltrates (Lavrjna et al., 2015). During the inflammatory phase of the disease, astrocytes undergo massive proliferation and extensive hypertrophy of cell bodies with enlarged intertwining processes, forming the scar border (Lavrjna et al., 2012). Scar formation is a physical border composed of reactive astrocytes that demark and divide an injured area from the normal-appearing tissue, resulting in extracellular matrix composition changes, which prevent remyelination (Miljkovic, Timotijevic, & Mostarica Stojkovic, 2011). Hypertrophied astrocytes persist in the later stages of the disease in both white and gray matter of the CNS. The permissive or inhibitory environment affects the outcome of interaction between glial cells. Namely, in the inflammatory milieu, astrocytes express chemokines that induce migration of OPCs toward the lesion sites; however, a glial scar can be an obstacle for remyelination (Nair, Frederick, & Miller, 2008). Further, astrocytes secrete different growth factors, FGF2 and PDGF among others, which promote OPC proliferation. On the other hand, these factors can inhibit differentiation of OPCs into mature oligodendrocytes (Nash, Ioannidou, & Barnett, 2011).

Demyelination is a process that results in damage of oligodendrocytes and a loss of myelin. It may occur during EAE in some strains of susceptible animals and is followed by spontaneous remyelination (Constantinescu, Farooqi, O’Brien, & Gran, 2011). The exact mechanism of remyelination is still unknown.

Understanding the multifaceted interaction between astrocytes, microglia, and oligodendrocytes, which are able to promote direct or overlapping effects that lead to protective or detrimental pathways in cells, will broaden our knowledge of demyelination/remyelination in EAE and ultimately in MS.

5 | SELECTION OF ANIMALS IN EAE

The choice of animals used for the experiments has a profound influence on disease susceptibility, severity, and course of EAE. The first choice of animals for studying MS was an EAE model generated in rats. Indeed, several rat strains, including Lewis, Dark Agouti (DA), and Brown Norway (BN), show a high reproducibility rate in functional and histopathological features (Milicevic et al., 2003; Staykova, Paridaen, Cowden, & Willenborg, 2005; Swanborg, 1995). Below, we give a brief overview of animal species and strains used for EAE experiments and specific characteristics of these animal models.

5.1 | Primate model of MS

Considering genetic and immunological resemblance to humans, nonhuman primates provide advantageous models of MS. The most employed nonhuman primates in MS research are common marmosets and rhesus monkeys.

The first recognized case of EAE in a primate model was seen in the rhesus monkey (Macaca mulatta), induced by repeated immunization, although without adjuvant (Rivers & Schwentker, 1935). Three Macaca species were challenged with MBP or whole-brain homogenate to generate EAE. M. mulatta is more susceptible than M. fascicularis, while Macaca nemestrina is regarded to be resistant (Wolfe-Coote, 2005). Upon immunization, M. fascicularis usually develops an acute course, although an MS-like chronic disease pattern can be also found (Kerlero de Rosbo et al., 2000). The histological feature of this nonhuman primate model is the presence of inflammation and large demyelinating lesions, leading to severe damage of white matter, followed by neurodegeneration. Those changes do not affect the spinal cord (Ravkina, Rogova, & Lazarenko, 1978; Stewart, Alvord, Hruby, Hall, & Paty, 1991). The gray matter demyelinating lesions are not present in the rhesus EAE model; thus, it is not suitable for MS research (Burn et al., 2016). Overall, it seems that white matter lesions in this model are due to inflammatory necrosis rather than selective demyelination. In this model of EAE, the observed histological changes bear a resemblance to the acute fulminant forms of MS rather than the more common chronic forms of MS.

Common marmosets (Callithrix jacchus) are small-bodied Neotropical primates, easy to maintain in specialized animal husbandries. They are very fertile and usually give birth to several fraternal siblings per year. The common marmoset is highly susceptible to EAE. The first EAE in the marmoset was induced with human myelin emulsified with CFA and B. pertussis (Massacesi et al., 1995). However, this model has little similarity with MS because massive inflammation and selective demyelination occur as in an acute form of neuroinflammation (Kap, Laman, & ‘t Hart, 2010; Maggi et al., 2014).

Active immunization of these marmosets using MBP mixed with CFA induces a mild form of the disease, while myelin oligodendrocyte glycoprotein (MOG)-induced EAE produces inflammatory and demyelinating disease with a chronic relapsing–remitting course (Genain & Hauser, 1997; Uccelli, Giunti, Capello, Roccagagliata, & Mancardi, 2003). It was demonstrated by Jagessar et al. that marmosets can be sensitized with recombinant human MOG or MOG24-36 in CFA to
develop EAE, while a severe form of EAE in marmosets can be induced using MOG_{34-58} in IFA—that is, without microbial antigens for innate immune activation (Jagessar et al., 2010). Histopathological examination of samples from the chronic phase of the disease reveals inflammatory demyelinating lesions and widespread astrogliosis and axonal loss (Mancardi et al., 2001). The area of demyelination varies in size, number, and location, but with very precise correlation between clinical and pathological features (Genain & Hauser, 2001; Jagessar et al., 2012). The marmoset EAE makes a good model for studying MS because of the resemblance of immune systems (t Hart et al., 2013), together with the possibility of exploring T cell-adoptive transfer and passive transfer of antibodies to genetically distinct siblings (Genain & Hauser, 2001).

These studies can be done easily because these marmosets are naturally occurring bone marrow chimeras. This state occurs because they share a placental bloodstream between fraternal siblings during pregnancy (Haig, 1999). The natural chimerism provides greater immunological similarity between nonidentical siblings rather than with siblings from other births and induces permanent tolerance (Hamawy & Knolle, 1999). This makes fraternal siblings equally allotolerant, allowing adoptive transfer toward a fraternal sibling’s alloantigen in each marmoset (Haig, 1999). This leads to functional immune tolerance, making it possible to perform T cell transfer studies between fraternal siblings of a twin because they are immunologically compatible (Mansfield, 2003). Both rhesus monkey and common marmosets are equally prone to EAE induction; however, these models require different adjuvants for EAE induction with divergent disease progression (Maggi et al., 2014). Although rhesus monkeys are evolutionary closer to the humans than marmosets, rhesus monkeys display only acute EAE (Kerlero de Rosbo et al., 2000), while marmosets develop relapsing-remitting form of EAE (t Hart et al., 2013).

### 5.2 Rats in EAE

The Lewis inbred rat strain has been used for EAE studies since the 1960s (Swanborg, 1995). This strain is exquisitely susceptible to EAE, induced by guinea pig myelin antigens (Swanborg, 2001), which have been shown to be potent encephalitogens compared with rat myelin antigens (Gould, Stepaniak, & Swanborg, 1994). In general, among different autoantigens, myelin proteins such as MBP and MOG have proved to be stronger encephalitogenic epitopes than proteolipid protein (PLP), which fails to induce EAE in Lewis rats (Stepaniak, Wolf, Sun, & Swanborg, 1997). In addition, MBP obtained from human, rabbit, or bovine subjects have shown to be inadequate for EAE induction (Mannie, Swanborg, & Stepaniak, 2009). There is ample evidence that after immunization with CNS antigens mixed with CFA, Lewis rats develop an acute monophasic form of the disease (Shin, Ahn, & Matsushita, 2012). In this strain, after spontaneous recovery, EAE cannot be induced again (Willenborg, 1981). Because this strain is syngeneic, passive EAE can be induced to define a role of T cells (Gould et al., 1994). Further, there are no sex differences in inducing a disease.

Pioneering work by Levine and Wenk, using guinea pig MBP mixed with CFA, additionally injected with pertussis toxin, induced hyperacute EAE characterized by massive inflammation that correlated with clinical signs (Levine & Wenk, 1964, 1965). Later, pertussis toxin was excluded from adjuvants used to afflict animals. The rat or guinea pig MBP/CFA-induced EAE develops as an acute widespread encephalomyelitis, manifested by massive inflammation and the presence of a few demyelinating foci, especially at the dorsal root entry zone (Pender, Tabi, Nguyen, & McComb, 1995; Shin et al., 2012). However, it has been argued that the observed demyelination is probably due to perivascular cuffing leading to edema, rather than an actual loss of myelin (Batoulis, Addicks, & Kuerten, 2010). Although demyelination is not a prominent feature after active induction with myelin antigens, it was reported that induction with rat MOG (aa1–125) leads to severe loss of myelin, together with axonal damage (Adelmann et al., 1995). On the contrary, sensitization with rat MOG in different doses of IFA fails to induce EAE in Lewis rats; however, when additionally injected with proinflammatory cytokines (Kerschensteiner et al., 2004) or growth factor, such as VEGF (Sasaki, Lankford, Brown, Ruddle, & Kocsis, 2010), rats displayed a highly reproducible inflammatory demyelination, even in gray matter, where cortical EAE lesions were observed (Merker, Ernsting, Kerschensteiner, Bruck, & Stadelmann, 2006; Storch et al., 2006). Further, PLP/CFA induces inflammatory demyelination, predominately in the spinal cord, while demyelination at dorsal root entry is absent (Chalk, McCombe, Smith, & Pender, 1994). Severe demyelination with inflammation throughout the CNS, with minimal functional outcomes, was seen in passive EAE, with adoptive transfer of S100β-specific T cells (Kojima et al., 1994). Also, chronic-relapsing EAE can be induced in Lewis rats using guinea pig spinal cord homogenate and additional treatment with cyclosporine A (Tanuma, Shin, Kogure, & Matsumoto, 1999). A detailed overview of inducing EAE in Lewis rats has been extensively reviewed elsewhere (Pitarokoili, Ambrosius, & Gold, 2017).

Another highly EAE susceptible strain is the DA rat. DA rats have distinctive immunological and genetic features, including elevated basal levels of IL-2 (Vukmanovic, Mostarica Stojkovic, & Lukic, 1989), IFN-γ (Arsov et al., 1995), and TNF-α (Lukic, Stosic-Grujicic, & Shahnin, 1998), making them prone to various autoimmune-mediated diseases, including adjuvant arthritis (Cannon, Woods, Clayton, & Griffiths, 1993) and multiple low-dose streptozotocin-induced diabetes mellitus (Stosic-Grujicic et al., 2001).

As observed in Lewis rats, DA rats generate acute monophasic EAE when afflicted with MBP or MBP peptides emulsified in CFA. However, there is a difference in the encephalitogenic potential of MBP epitopes between these two strains. Namely, a severe form of EAE developed upon immunization with MBP_{63-81} in DA rats, while the disease was completely absent in Lewis rats. In addition, MBP_{87-99} failed to induce disease in DA rats but generated acute monophasic disease in Lewis rats (Stepaniak et al., 1997). Acute monophasic disease can be induced using whole spinal cord homogenate combined with CFA (Lavmjō et al., 2008, 2012), where the severity of disease is dependent on the amount of additionally administered M. tuberculosis (Milicevic et al., 2003). It was also reported that in DA rats, the homologous, rather than heterologous, tissue is a more efficient encephalitogen (Stosic-Grujicic, Ramic, Bumbasirevic, Harhaji, & Mostarica-Stojkovic, 2004). Furthermore, DA rats can develop disease by using a single injection of spinal cord tissue,
without any adjuvant. In addition, upon recovery from clinical symptoms, EAE disease can be reinduced by immunization using spinal cord tissue and CFA (Stosic-Grujicic et al., 2004).

The differences in the onset and progression of disease are observed between male and female DA rats. Recently, it was reported that male rats develop a more severe disease, although they display lower disease incidence compared with female rats (Nacka-Aleksić et al., 2015). Also, the mortality rate was higher in male (Stojkov et al., 2008) than in female DA rats (Lavrňa et al., 2009) after immunization with whole spinal cord emulsified with CFA. Furthermore, females develop neuromyelitis optica, while demyelination of the optic nerve does not occur in male DA rats (Storch et al., 1998b).

DA rats afflicted with syngeneic spinal cord with IFA developed a chronic–relapsing EAE model (Lorentzien et al., 1995). Similarly, relapsing–remitting EAE can be induced with MOG(aa1–125) mixed with CFA (Weissert et al., 1998, 2001). Adoptive transfer experiments in DA rats served to elucidate a basic role of T cells and antibody-mediated demyelination. DA rats induce a severe form of EAE in adoptive transfer experiments with MOG-specific T cells, while Lewis rats develop mild disease with massive inflammation (Kojima et al., 1994; Storch et al., 1998a). The progression of EAE in these models is controlled by T cell activity. Historical observation proposed that this model is characterized by inflammation rather than demyelination (for details, see Figure 2).

In general, regardless of the antigen used for immunization, DA rats display perivascular and subpial inflammation, mediated by T cells and macrophage/microglia (Lavrňa et al., 2009; Milicevic et al., 2003; Storch et al., 1998b; Trifunovic et al., 2015). Those events are associated with the demyelination process, together with neurodegeneration, mainly seen in the lumbosacral region of the spinal cord (Lavrňa et al., 2012; Milicevic et al., 2003; Papadopoulos, Pham-Dinh, & Reynolds, 2006). Around areas of demyelination, reactive astrocytes forming glial scar can be found (Lavrňa et al., 2012, 2015). In addition, there is an accumulation of T cells and neutrophils in cerebrospinal fluid, suggesting that in EAE, choroid plexus is a site of entrance for immune cells (Schnitt et al., 2012). Recently, immunization using MOG/IFA with additionally administered cytokines led to the development of widespread cortical demyelination in both hemispheres (Gardner et al., 2013; Ucal et al., 2017).

The spontaneous recovery from disease in Lewis and DA rats probably occurs because of activation-induced cell death in the target tissue (Lučić, Mensah-Brown, Galadari, & Shahin, 2001). It appears that remission occurs through elimination of proinflammatory cells. The other factors that contribute to the recovery of the disease include activation of Tregs, which produce anti-inflammatory mediators and consequently defeat encephalitogenic T cells (Castelo-Branco et al., 2014; Constantinescu et al., 2011). Further, differentiation of M1 macrophages (found to be predominantly present at the onset of the disease) into M2 macrophages has a neuroprotective effect (Shin et al., 2012). Although it has been reported that natural killer cells can increase or decrease the severity of the disease, a recent report points to the regulatory role of these cells leading to remission of EAE and MS (Chanvillard, Jacolik, Infante-Duarte, & Nayak, 2013).

The BN rat has been traditionally considered an EAE-resistant strain because of the lack of the disease upon immunization with guinea pig CNS homogenate combined with CFA. The observed resistance is linked to its specific genetic background, related to both Major histocompatibility complex (MHC) and non-MHC regions of the genome (Levine & Sowinski, 1975). However, the innate immune response to myelin antigens with carbonyl iron results in EAE development (Levine & Sowinski, 1975; Staykova, Linares, Fordham, Paridaen, & Willenborg, 2008). Similarly, BN rats are susceptible to antibody-mediated diseases, where MOG/CFA-induced immunization results in massive inflammation and demyelination in the spinal cord (Meyer et al., 2001; Stefferl, Linnington, Holsboer, & Reul, 1999), as well as an extensive demyelination of the optic nerve (Fairless et al., 2012). These studies suggest that the haplotype controls MOG-induced immune response and point to the B cell–mediated autoantibody response as an important player in the degree of susceptibility to develop a disease (Steffel, Brehm, et al., 1999). Besides the obvious involvement of Th2-type immune response in proneness to EAE, a number of non–MHC-regulated mechanisms have been proposed as factors contributing to the development of EAE. In general, robust HPA axis response (Steffel, Linnington, et al., 1999), endogenous corticosterone level (Peers, Duncan, Flower, & Bolton, 1995), increased TGF-β production (Fournié et al., 2001), and a defect in the CD8 cell compartment (Cautain et al., 2001) modulate the susceptibility to EAE in BN rats. Overall, this strain is suitable to investigate Devic’s disease after MOG/CFA immunization (Gold et al., 2006).

5.3 | Mice in EAE

Although immunization of susceptible rats does not need pertussis for amplification of the immune response, mice are used more often in EAE experiments. Mice are cheaper to maintain and are easily genetically manipulated (Miller & Karpus, 2007); transgenic and gene knockout models are widely used (Croxford et al., 2011).

For active EAE disease development, mice need to be induced with CFA and pertussis toxin. Mice differ in susceptibility to specific antigens. Specifically, the SJL strain of mice is susceptible to PLP, MOG, and MBP immunization and often exhibits a relapsing–remitting form of the disease (Kono et al., 1988; Miller & Karpus, 2007). Therefore, this strain is useful in studying T cell–mediated demyelination, together with evaluation of resident macrophage-driven damage that controls the later stages of chronic disease. Immunization of SJL mice with MBP or its peptide induces a relapsing form of the disease but requires administration of pertussis toxin at the time of immunization, and again 48 hr later (Miller, Karpus, & Davidson, 2007; Zamvil et al., 1985). Young male SJL mice immunized with PLP are relatively resistant to EAE, whereas older male and female SJL mice are more susceptible and develop demyelination lesions (Rasmussen et al., 2007). MOG is expressed at relatively low levels on the surface of myelin sheaths; however, it proves to be encephalitogenic in C57BL/6 mice (Delerasse, Smith, Baker, & Amor, 2013). Young C57BL/6 mice develop an acute monophasic form of EAE, whereas middle-aged males developed severe chronic EAE (Ditamo, Degano, Maccio, Pistoresi-Palencia, & Roth, 2005; Matejuk, Hopke, Vandenbergk, Hum, & Offner, 2005). Administration of pertussis is required for disease induction with
MOG_{35-55}/CFA in C57BL/6 mice, which develop a self-limited monophasic disease (Bittner, Afzali, Wiendl, & Meuth, 2014; Schreiner, Heppner, & Becher, 2009). Similarly, PL/J mice develop an acute monophasic disease course (Schreiner et al., 2009; Terry, Ifergancigil, & Miller, 2016). Alternatively, the C57BL/6 strain of mice is often induced with MOG peptide plus adjuvant to develop a chronic form of EAE (Bernard et al., 1997). In addition, the pathogenic B cell epitope of MOG_{113-127} induces EAE, suggesting that this strain may be useful for immunological studies (Delarasse et al., 2013). Previously, it was reported that C57BL/6 mice immunized with MOG_{35-55} develops cortical and callosal lesions (Mangiardi et al., 2011). However, in MOG_{35-55} or rMOG_{1-125}-immunized C57BL/6 mice, cortical demyelination lesions are not a regular feature of EAE (Lagumersindez Denis et al., 2017). In general, EAE induction in C57BL/6 mice displays heterogeneity in disease induction and progression. Namely, the ongoing disease is dependent on the amount of M. tuberculosis in the CFA (Terry et al., 2016) and pertussis toxin as well (Croxford et al., 2011). Thus, the more reliable and reproducible mouse model for EAE induction is in SJL/J mice (Terry et al., 2016), where the disease can be induced with some myelin antigens (PLP) without pertussis toxin (Miller et al., 2007).

Passive EAE can also be induced in SJL or C57BL/6 mice. It is accomplished by an adoptive transfer of myelin-activated lymphocytes from mice immunized with these encephalitogenic myelin antigens. Although EAE induced by adoptive transfer results in a severe form of the disease, the clinical and histological findings in passive EAE resemble those seen after active EAE. However, T cells from SJL/J mice are more encephalitogenic than from C57BL/6. In this manner, only by using a large number of activated and Th1-polarized MOG-specific T cells can passive EAE be induced in C57BL/6 mice (Croxford et al., 2011).

### 5.4 Mouse model of chronic EAE

Guido Biozzi reported a mouse strain that developed high titers of antibodies when exposed to specific antigens. Therefore, this animal strain was found to be suitable for inducing EAE. Indeed, the Biozzi ABH strain was highly susceptible to development of EAE. Following immunization with myelin antigens, Biozzi ABH mice generate relapsing–remitting EAE, which, in later stages, results in a steady progression of the disease and may be used for studying both relapsing–remitting and secondary progressive MS (Al-Izki, Pryce, Jackson, Giovannoni, & Baker, 2011; Hampton et al., 2008). Biozzi ABH mice exert a robust antibody response and widespread demyelination in response to antigens, making this strain a good model for MS research (Al-Izki et al., 2012).

A variety of myelin epitopes have been shown to be encephalitogenic in this mouse strain (Amor, Smith, Hart, & Baker, 2005). In this disease, there is a lack of protective autoimmunity because this strain is unaffected by innate lymphoid cell involvement (Al-Izki et al., 2011). Age is a limiting factor in EAE development—it appears that young mice are resistant, while aged mice are more susceptible to EAE induction (Peferoen et al., 2016). This can be explained by the age-related decline in antigen-presenting cell function and the presence of myeloid-derived suppressor cells in older mice. Biozzi ABH mice display the pathological hallmarks of MS, such as inflammatory-mediated demyelination, together with neurodegeneration (Hampton et al., 2008; Jackson, Lee, Nikodemova, Fabry, & Duncan, 2009; Peferoen et al., 2016). During relapses, these mice undergo excessive demyelination, where demyelination areas are filled with T cells, macrophages, and immunoglobulin depositions (Amor et al., 2005; Baker et al., 1990; Kipp et al., 2017). It is interesting that autoimmune tolerance can eliminate relapses, but permanent neurological deficits are accumulated slowly (Pryce et al., 2005). Areas of demyelination contain OPCs (Hampton et al., 2008), and it has been suggested that early tolerance is also associated with increased remyelination (Hampton et al., 2013). The pitfall of this model is that the observed chronic disease is not clinically progressive, although there is an accumulation of neurological deficits over time. Clinical neurological disease, accompanied by axonal loss and demyelination of gray matter, can also be induced in Biozzi ABH mice immunized with neurofilament-L and CFA (Huizinga et al., 2007a; Puentes et al., 2013). It appears that this disease is mediated by CD4⁺ T cells directed against neurofilament-L and that it fails to reproduce the chronic phase of disease. Overall, it seems that neurodegeneration occurs as a consequence of interaction between autoreactive T cells and neurons (Huizinga et al., 2007b; Peferoen et al., 2016; Puentes et al., 2013).

Understanding the strain differences in cellular and molecular mechanisms in this experimental autoimmune model will provide a better insight for the complex clinical, immunological, and genetic diversity associated with MS.

### 5.5 Genotypic characteristics of animals used in EAE research and EAE phenotype

Genetic predisposition to MS is traditionally associated with major histocompatibility complex or human leukocyte antigen (MHC and HLA, respectively) locus on chromosome 6. Some class II HLA alleles (e.g., HLA-DRB1*15: 01, HLA-DRB1*13: 03, HLA-DRB1*03: 01, HLA-DRB1*08: 01, HLA-DRB1*03: 02) are considered to carry significant risk, whereas some class I alleles (e.g., HLA-A*02: 01, HLA-B*44: 02, HLA-B*38: 01, HLAB*55: 01) are considered protective (de Jong et al., 2002; Marrosu et al., 2001; Moutsianas et al., 2015; Silva et al., 2007). Genome-wide associated studies also have implicated over 100 different, non-MHC genes in MS susceptibility (De Jager et al., 2009; Hafler et al., 2007).

The same concept is true regarding susceptibility of different strains of rats and mice to EAE. The identification of loci involved in autoimmunity in laboratory animals was done by comparing inbred strains susceptible to EAE and strains in which EAE could not be induced, with congenic strains of animals. Both genetic and epigenetic interactions were shown to contribute to the EAE phenotype in mice (Sobel, 2000; Sobel, Tuohy, & Lees, 1991).

Backcrossing studies in rats also revealed that both MHC and non-MHC genes determine the susceptibility to EAE (Lorentzen et al., 1997). Further, the DA MHC haplotype (RT1.av1) was associated with relapses and prolonged inflammatory response, while non-MHC DA genes intensify inflammation. Congenic strains of the susceptible DA
strain and resistant Piebald Virol Glaxo strain or ACI strain (which all share MHC RT1.av1 haplotype) enabled identification of various non-MHC loci involved in EAE susceptibility. Multiple loci on chromosomes 10, 12, 13, and 18 were the first to be identified (Dahlman et al., 1999). It was later established that the locus termed Eae19 on chromosome 15 (Sheng et al., 2005); Eae20, Eae21, and Eae22 on chromosome 4 (Jagodic et al., 2005); as well as Eae23 on chromosome 17 and Eae24-Eae27 on chromosome 4 (Marta et al., 2010) all contribute to specific features of the disease. For example, the Eae23 locus contains the Zeb1 gene, which is a known repressor of IL-2, while anti-MOG antibody levels for IgG1 and IgG2b are linked to Eae24 and Eae26. The same group also established that parent-of-origin effects, which play a role in MS (Ruhrmann, Stridh, Kular, & Jagodic, 2015), also influence EAE (Jagodic et al., 2005; Stridh et al., 2014). Surprisingly, it was established that a substantial percentage of disease-predisposing loci (up to 54%) depend on parental transmission. Backcrossing experiments also confirmed that imprinting of the Y chromosome of the susceptible strain contributes to disease proneness, while paternal imprinting of an atypical notch ligand, Dlk1 on chromosome 6, adds to disease severity (Stridh et al., 2014). Epigenetic factors, often referred to as missing heritability, were (almost two decades ago) proposed to influence EAE occurrence, severity, neurological score, and CNS lesion distribution in mice (Sobel, 2000). The specific genes involved in epistatic interactions that regulate the EAE phenotype are just beginning to emerge.

6 | NEXT-GENERATION ANIMAL MODELS OF MS

While EAE is often disputed as a true model for MS, it is, up to now, the only model that can reproduce at least some aspects of this human disease. It is thus not surprising that all novel models are actually using EAE as a foundation. With the expansion of genetic modifications in rodents, it became possible to generate various transgene animals that are used to evaluate the contribution of specific genes to the development and resolution of EAE. Various knockout and, with the development of Cre-lox technology, conditional knockout mice have been used in EAE research. Because C57BL/6J mice are the preferred background for genetic manipulations, this promoted the development of the EAE model in this strain. Listing even some of the gene deletions employed in the EAE model would be exhaustive and beyond the scope of this review. In this section, we will give an overview of other genetic manipulations that have been developed to improve EAE as a disease model for MS.

6.1 | Genetic manipulations in spontaneous EAE models

In 1993, Goverman et al. first reported that manipulation of the MBP-specific T cell receptor in B10.PL mice of H-2d haplotype resulted in spontaneous EAE, albeit only in animals that were not housed in sterile conditions. The onset of disease occurred at different ages, and the actual frequency of spontaneous illness was probably below 50%. In transgenic animals housed in both sterile and nonsterile conditions, administration of pertussis toxin alone could induce a disease similar to EAE induced in nontransgenic animals subjected to immunization with MBP and pertussis toxin emulsion (Goverman et al., 1993). Another research group crossed animals with MBP-T cell-specific receptor with RAG-1−deficient mice to obtain a line of mice that expressed modified TCR in CD4 cells only. These animals developed EAE within 12 months (Lafaille, Nagashima, Katsuki, & Tonegawa, 1994). Later, SJL/J mice with PLP-specific TCR also displayed inflammatory foci in the CNS and EAE (Waldner, Whitters, Sobel, & Kuchroo, 2000), while MOG-specific TCR resulted in spontaneous optic neuritis in 30% of C57BL/6 mice; EAE develops in 4% to 15% of animals (Bettelli et al., 2003). These mice were termed 2D2 and they are the most frequently used model of spontaneous EAE.

Although CD8 T cells have a distinct role in MS pathology, introduction of MBP-specific receptors in mouse CD8 TCR did not result in spontaneous disease (Perchellet, Stromnes, Pang, & Goverman, 2004); however, a viral infection could trigger autoimmunity via activation of CD8 cells that express receptors for both viral antigens and MBP (Ji, Perchellet, & Goverman, 2010). In pursuit of a better model that would represent CD8 T cell involvement in CNS autoimmunity, CD8 TCR was made specific for ovalbumin, while ovalbumin itself served as a self-antigen, expressed under the MBP promoter in oligodendrocytes. In these animals, spontaneous and severe EAE was observed early, from 12 to 19 days of life (Na et al., 2008). Similarly, Saxena et al. expressed influenza hemagglutinin in oligodendrocytes; although these animals did not show spontaneous illness, the transfer of preactivated hemagglutinin-specific CD8 T cells could elicit EAE (Saxena et al., 2008). More recently, CD8 TCR was modified for GFAP reactivity, which led to spontaneous relapsing–remitting EAE (Sasaki et al., 2014).

Increasing interest in B cells for CNS autoimmunity led to a generation of transgenic mice with manipulated B cell receptors (BCRs). Double transgenics carrying MOG-specific CD4 T cells (2D2) and a heavy chain of MOG-binding immunoglobulin were the first models that aimed to evaluate the involvement of B cells in spontaneous EAE (Bettelli, Baeten, Jager, Sobel, & Kuchroo, 2006; Krishnamoorthy, Lassmann, Wekerle, & Holz, 2006). Both groups concluded that the interaction between T and B cells drives their own activation.

Transgenic mice that carry human genes involved in antigen processing and presentation were constructed to serve as tools for validating the encephalitogenic potential of human T cells. The first transgenic mouse model expressing human MBP 84–102 TCR and HLA DR15 was described by Madsen et al. (1999). In 4% of these animals, spontaneous EAE occurred; however, in a RAG-2−deficient background, all of the mice eventually showed the symptoms of spontaneous disease. Later, a human PLP 45–53 CD8 TCR mouse was developed. A double transgene carrying PLP45–53 TCR and a human HLA-A3 showed spontaneous MS-like disease. Further, the presence of HLA-A2 suppressed the disease (Friese et al., 2008), thus supporting the findings of a study that found an increased presence of HLA-A3 in MS patients, and suggested a protective role for HLA-A2 (Fogdell-Hahn, Ligers, Gronning, Hillert, & Olerup, 2000).

The development of new humanized models could include other proteins involved in MS pathogenesis; however, introducing multiple genes is technically demanding and increases the model’s complexity.
6.2 | Manipulation of sex chromosomes in EAE research

Given the pronounced sex bias of MS, a necessity to compare males and females in EAE emerged. However, it is suspected that gonadal steroids are not the only factor contributing to the female preponderance in MS and other autoimmune disorders. While the use of both males and females in EAE research (and other research as well) is a good practice (Clayton & Collins, 2014), another useful model was developed. Genetic manipulation that moves the Sry gene from the Y chromosome to the autosomal yields mice in which gonadal sex does not correlate with sex chromosome, thus allowing investigation of sex chromosome effects without the influence of gonadal steroids. Using this model, Voskuil's group was able to show that XX sex complement contributes to the susceptibility of EAE (Smith-Bouvier et al., 2008). On the other hand, the same group showed that XY chromosome complement in SJL (either gonadal males or females) displayed a more severe disease with greater neurodegeneration in the CNS (Du et al., 2014). These results grant further studies of sex complement influences on gender differences in MS course and prevalence.

6.3 | Bioluminescence imaging in EAE research

The expression of firefly luciferase under promoters of different genes enabled monitoring of the processes that involve the target molecule in living mice. This model was proposed to be a functional and valuable tool for monitoring whole-body in vivo inflammation in various disease models including EAE (Hayashi et al., 2015; Li et al., 2008; Yang et al., 2013). Moreover, bioluminescence imaging was used to track the transplanted human embryonic stem cell-derived oligodendrocyte progenitors and neural stem cells during EAE (Ayzenberg et al., 2015; Steffel, Brehm, et al., 1999). By expressing firefly luciferase under the IL-6 promoter, Hayashi et al. were able to show bioluminescence in the spinal cord and brain in the EAE mice (Hayashi et al., 2015). Similarly, mice with luciferase firefly expressed under a c-Rel promoter showed bioluminescence in the spinal cord even before the onset of EAE, indicating that reporter animals could be a valuable tool for monitoring early events in EAE development (Yang et al., 2013). In concordance with this, recording of bioluminescence early after EAE induction in GFAP-luc animals was suggested to be a predictor of disease severity (Luo, Ho, Steinman, & Wyss-Coray, 2008).

Although bioluminescence imaging in EAE was recently proposed to have its shortcomings (Ayzenberg et al., 2015), it may be a valuable tool in animal model research of MS, especially when validated by other tools like magnetic resonance imaging and histology (Guglielmetti et al., 2014).

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

I.L. and I.B. designed the scope of the paper and drafted the manuscript. V.B.K. and S.P. provided critical revision of the manuscript. All authors take responsibility for the accuracy and integrity of the manuscript. Conceptualization, I.L.; Methodology, I.L. and I.B.; Investigation, I.B., V.B.K., S.P. and I.L.; Formal analysis, I.L., I.B., S.P. and V.B.; K.; Writing - Original Draft, I.L. and I.B.; Writing - Review and Editing, I.B., V.B.K., S.P. and I.L.

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