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Review

Animal models of Multiple Sclerosis

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ABSTRACT

Multiple Sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) which involves a complex interaction between immune system and neural cells. Animal modeling has been critical for addressing MS pathogenesis. The three most characterized animal models of MS are (1) the experimental autoimmune/allergic encephalomyelitis (EAE); (2) the virally-induced chronic demyelinating disease, known as Theiler's murine encephalomyelitis virus (TMEV) infection and (3) the toxin-induced demyelination. All these models, in a complementary way, have allowed to reach a good knowledge of the pathogenesis of MS. Specifically, EAE is the model which better reflects the autoimmune pathogenesis of MS and is extremely useful to study potential experimental treatments. Furthermore, both TMEV and toxin-induced demyelination models are suitable for characterizing the role of the axonal injury/repair and the remyelination process in MS. In conclusion, animal models, despite their limitations, remain the most useful instrument for implementing the study of MS.

1. Introduction

Multiple Sclerosis (MS) is a chronic immune-mediated demyelinating disease of the central nervous system (CNS) (Hafler et al., 2005). It represents the leading cause of non-traumatic disability among young adults and has a great socio-economic impact in developed countries. MS is a very heterogeneous disease, indeed its clinical signs and symptoms are very variable and depend on the parts of the affected CNS (brain and spinal cord), including motor, sensory, autonomic and cognitive disabilities (Noseworthy et al., 2000). It can run at least three clinical courses: the relapsing–remitting (RR), which is the most frequent (85%) and it is characterized by exacerbations and subsequent periods of clinical stability; secondary progressive (SP) and the primary-progressive (PP) subtype (Noseworthy et al., 2000). CNS tissue from Multiple Sclerosis patients shows discrete lesions with inflammatory infiltrates, demyelination, astrogliosis and early axonal damage. MS is widely considered an autoimmune demyelinating disease, where an autoimmune reaction by myelin-specific $CD4^+$ T helper 1 (Th1) and Th17 cells, which initiate the neuropathology, has been described (Hafler et al., 2005; Sospedra and Martin, 2005). A specific cause for the pathogenesis of MS has not been identified so far, although several genetic and environmental risk factors have been suggested to play a central role. In this context, animal models of MS had allowed to explore mechanisms of disease initiation and progression and test several novel therapeutical approaches for the disease.

2. Positive and negative aspects of MS animal models

Since MS is a complex disease, there is no a single animal model that can capture the entire spectrum of heterogeneity of human MS and its variety in clinical and radiological presentation. However, over the last few years, animal models have been used to study the pathogenic mechanisms of MS. The main positive aspect is that they can surely serve as a testing tool to study disease development and for novel therapeutic approaches. In addition, they are a relatively convenient source of tissue from the CNS, which is the main target of MS, in contrast to human tissues, biopsies, or autopsy samples which are rarely performed. Several researchers have recently raised

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the question whether these animal models could really represent a good model for MS since they do not perfectly reflect all the aspects of the human disease. In particular, disease initiation is usually highly artificial in the animal models (induced by active immunization with an auto-antigen). Also the time-frame of the clinical symptoms onset is different between humans and mice. In humans, physiological processes underlying the disease are undetected for years before the onset of clinical manifestations, while symptoms in the animal models can be detected within weeks or even days after induction of the disease. Moreover the treatment in these therapy studies started very early in the course of the induced autoimmune disease, whereas any therapy for humans is administered in a late phase of the disease. More importantly, most of the experimental studies use inbred strains mice with genetic homogeneity and often these mice may have accumulated genetic irregularities that are very difficult to find in human population. Although it has become clear that rodent and human immune systems have profound differences (as they are evolutionarily distant), they share some essential principles and, in this context, the availability of three major animal models of MS allowed the understanding of relevant features of the human MS. The most commonly studied animal models of MS are (1) the experimental autoimmune/allergic encephalomyelitis (EAE); (2) viral induced models, mainly Theiler's murine encephalomyelitis virus (TMEV) infection and consequential chronic demyelination and (3) toxin-induced models of demyelination, such as the cuprizone and the lyso-phosphatidylcholine (lysolecithin) models.

3. Experimental autoimmune encephalomyelitis (EAE)

MS is a chronic, immune-mediated, inflammatory disorder of the CNS (Frohman et al., 2006). The most-studied animal model of MS is the experimental autoimmune encephalomyelitis (EAE), in which autoimmunity to CNS components is induced in susceptible mice through immunization with self-antigens derived from basic myelin protein. Rivers et al. (1933) firstly described, in monkeys immunized with rabbit brain extracts, paralyisis associated to perivascular infiltrates and demyelination in the brain and spinal cord, as acute disseminated encephalomyelitis, later called experimental autoimmune encephalomyelitis (EAE). Freund's adjuvant (CFA) (Freund and McDermott, 1942) and pertussis toxin (PT) (Munoz et al., 1984), were later added to potentiate the humoral immune response and to induce oscillatory symptoms typical of the relapsing–remitting disease (Kabat et al., 1947), similar to that found in MS patients. Experiments were also performed in other animal species such as guinea pigs (Freund et al., 1947), monkeys (Kabat et al., 1947; Morgan, 1947); however mice (Olitzky and Yager, 1949) and rats (Lipton and Freund, 1952) resulted the best model to evaluate acute monophasic, relapsing–remitting and chronic progressive EAE through immunogenetic, histopathological and therapeutic studies. EAE in mice is characterized by an ascending paralysis beginning at the tail (Batoulis et al., 2011), followed by limb and forelimb paralysis, assessed by using a 5-points scale (McRae et al., 1992; Rangachari et al., 2012; Berard et al., 2010). EAE may be induced in mice with different genetic backgrounds, such as SJL/J, C57BL/6 and NOD, through either active immunization with protein or peptide, or by passive transfer of encephalitogenic T cells. In all cases, the relevant immunogen is derived from self-CNS proteins such as myelin basic protein (MBP), proteolipid protein (PLP) or myelin oligodendrocyte glycoprotein (MOG). Immunization of SJL/J mice with the immunodominant epitope of PLP ($PLP_{139-151}$) induces a relapsing–remitting (RR) disease course (Tuohy et al., 1989), while disease induced by the immunodominant MOG_{35-55} peptide in C57BL6/J mice is of chronic nature (Tompkins et al., 2002).

More recently a variety of additional antigens have been supposed to be involved in autoimmune reaction in MS and EAE. Some of them are myelin constituents, such as neurofascin NF 155 (Mathey et al., 2007), others are expressed on myelin and axons, such as contactin-2/transient axonal glycoprotein-1 (TAG-1) (Derfuss et al., 2009) and some other are entirely non-myelin antigens, such as the neuronal membrane protein neurofascin NF 186 (Mathey et al., 2007), the neuronal cytoskeletal protein

Table 1
Characteristics of the different mouse models of multiple sclerosis.

Model of MS	Mechanism	Application	Involved cells	Translational value	Main references
Relapsing–remitting EAE in SJL/J mice	Immunization of SJL/J mice with $PLP_{139-151}$	Study of neuroinflmmation and immune system activation	CD8, CD4, Th17, monocytes, macrophages, B cells, Treg cells	Relapsing–remitting MS, study of the relapse rate, testing therapeutical agents	Zamvil et al., 1985; McRae et al., 1995; Whitham et al., 1991; Miyagawa et al., 2010; Adlard et al., 1999
Chronic EAE in C57BL/6J mice	Immunization of C57BL/6J mice with MOG_{35-55}	Study of neuroinflmmation and immune system activation	CD8, CD4, Th17, monocytes, macrophages, B cells, Treg cells	Primary progressive MS, secondary progressive MS, testing therapeutical agents	Mendel et al., 1995; Berard et al., 2010; Hjelmstrom et al., 1998; Bullard et al., 2007; Koh et al., 1992; Baron et al., 1993
EAE in transgenic mice	T cell clone (2D2) expressing Vα and Vβ chains reacting specifically to MOG_{35-55}, or B cell heavy chain knock-in mouse strain (IgH MOG)	Study of neuroinflmmation and immune system activation	CD8, CD4, Th17, monocytes, macrophages, B cells, Treg cells	*In vitro* study of immune cell activation and function	Bettelli et al., 2003; Litzenburger et al., 1998; Jäger et al., 2009; Encinas et al., 1999; Anderson et al., 2012
Theiler's murine encephalomyelitis virus (TMEV)	Infection with picornavirus, such as Theiler's murine encephalomyelitis virus (TMEV)	Study of axonal damage and inflammatory-induced demyelination	Macrophage/microglia, oligodendrocyte, astrocytes and CD4, CD8	Primary progressive MS, study of brain, brainstem and spinal cord lesions, study of new therapeutic approaches targeting adhesion molecules, axonal degeneration	Tsunoda and Fujinami, 2010; Libbey and Fujinami, 2003; Owens, 2006; Tsunoda et al., 1996; Tsunoda et al., 2003
Cuprizone-induced MS	Feeding C57BL/6 mice with 0.2% cuprizone for 6 weeks	Study of the de- and re-myelination processes	Oligodendrocytes, astrocytes, microglia	Therapeutical trials designed to repress demyelination or accelerate remyelination	Matsushima and Morell, 2001; Blakemore and Franklin, 2008; Lucchinetti et al., 2000
Lysolecithin-induced MS	Lysolecithin injection in SJL/J mice	Study of the de- and re-myelination processes	Oligodendrocytes, astrocytes, microglia	Therapeutical trials designed to repress demyelination or accelerate remyelination	Jeffery and Blakemore, 1995; Shields et al., 1999; Bieber et al., 2003

neurofilament-M (Krishnamoorthy et al., 2009) and the astrocyte-typical Ca2+-binding protein S100b.

3.1. Relapsing–remitting EAE (RR-EAE) in SJL/J mouse model

Zamvil et al. (1985) were able to induce EAE in SJL/J mice by using both MBP-derived peptides and MBP-reactive T cell clones (Table 1). Initial studies suggested that only MBP was able to induce EAE. However, further studies demonstrated that SJL/J mice could be immunized, for the induction of EAE, by synthetic myelin $PLP_{139–151}$ which was identified as an immunodominant epitope of myelin without omology with MBP (McRae et al., 1995; Whitham et al., 1991). Indeed, the majority of $PLP_{139–151}$ immunized mice showed several relapses and remissions after an initial attack. Moreover, RR-EAE could also be induced through the passive transfer of PLP-reactive T cell lines. Classic models of adoptive transfer involve the immunization of donor mice with PLP-derived peptides, isolation of peripheral lymphoid cells after 7/10 days of culture, *in vitro* restimulation and subsequent transfer into naïve recipients. Despite these models were useful to demonstrate the central role of $CD4^+$ T in the pathogenesis of EAE, there are several limitations for their use in the research field. Indeed, it is difficult to isolate, from peripheral lymph nodes, T cells directed against a specific antigenic epitope. Further, the encephalitogenic capacity of transferred T cells not necessarily reflects the *in vivo* condition in donor animals. The decreased capability of knockout T cells to transfer the disease could, for example, reflect defective antigen presentation in the donor animals, rather than alteration in T cell functions from lesions. This aspect, combined with the pleiotropic nature of immune gene expression, makes it difficult to use the adoptive transfer model to define the contributions of individual genes to encephalitogenic T cell function. The development of T cell receptor (TCR) transgenic mouse models in over the past 20 years has greatly helped the study of antigen-specific T cell responses. T cells from the resulting transgenic animals escape Rag1- or Rag2-mediated recombination of their TCR loci and express transgenic TCRs at a very high frequency. T cells from TCR transgenic mice can be $>95\%$ specific for a defined epitope that may be either ectopic or self-derived (Miyagawa et al., 2010). MBP specific TCR transgenic mice were the first to be generated, and were shown to develop spontaneous paralytic disease on the PL/J and B10.PL backgrounds (Adlard et al., 1999). Consequently also TCR transgenic mouse lines on the SJL/J background (5B6 and 4E3) that are specific for $PLP_{139/151}$ have been generated to study the pathogenic mechanism of EAE.

3.2. Chronic EAE model in C57BL/6J mouse model

While several peptides could induce T cell responses upon *ex vivo* re-stimulation, only $MOG_{35–55}$ was able to induce directly CNS autoimmunity, and concomitant administration of pertussis toxin (PT) was able to enhance disease onset and severity (Table 1). Differently from PLP, $MOG_{35–55}$ was able to induce a chronic form of the disease that did not remit (Mendel et al., 1995). However, Berard et al. (2010) have reported that while a relatively high dose of peptide and adjuvant induces chronic non-remitting EAE in B6 mice, lower doses of peptide/adjuvant could promote a relapsing–remitting disease course, thus suggesting that the peptide dosage is essential for the type of disease developed upon $MOG_{35–55}$ immunization. $MOG_{35–55}$ EAE has also allowed to obtain a growing number of evidence on the role of immune system in the pathogenesis of MS, elucidating the contribution of B cells (Hjelmstrom et al., 1998), monocytes (Bullard et al., 2007), $CD8^+$ Tcells (Koh et al., 1992) and $CD4^+$ T cells (Baron et al., 1993) in the pathogenesis of EAE and MS.

3.3. Transgenic mice

Bettelli et al. (2003) firstly generated a class II-restricted TCR transgenic model to study $MOG_{35–55}$-induced-EAE by using a C57BL/6J background (Table 1). An epitope-reactive T cell clone (2D2) was obtained which expressed Vα and Vβ chains reacting specifically to $MOG_{35–55}$. These 2D2 mice also showed a more severe EAE than non-transgenic littermate with a high frequency to develop spontaneous EAE. Another transgenic mouse model used to study EAE was a B cell heavy chain knock-in mouse strain (IgH MOG; also known as TH), that contains a high frequency of anti-MOG antibody-secreting B cells in the peripheral repertoire. Upon active immunization with recombinant MOG, IgH MOG animals develop EAE, with typical inflammatory lesions, at a higher frequency than their non-transgenic littermates (Litzenburger et al., 1998) (Table 1); however, the mice did not develop spontaneous EAE, emphasizing the necessity for myelin specific T cell in order to progress the disease. However these last EAE models showed some disadvantages such as the clonal heterogeneity of the T cell populations and the generation after immunization of several T helper cell lineages *in vivo*, without the possibility to delineate a specific T cell subset involved.

More recently, Jäger et al. (2009) developed a model that allowed the generation of different T helper subsets to induce EAE. In this model, 2D2 T cells were stimulated *in vitro*, before their transfer, in the absence of antigen-presenting cells (APCs). Specifically, once isolated, $CD4^+$ T cells were stimulated *in vitro* using antibodies against CD3 and CD28, cultured with various combinations of cytokines and blocking antibodies to generate Th1, Th2, Th17 and Th9 subsets that were subsequently transferred to naïve host animals. These last experiments further allowed to delineate the central role of Th1/Th17 subsets in the pathogenesis of disease. Recent evidence has indicated the non-obese diabetic (NOD) strain mice, which spontaneously develop diabetes, are also susceptible to EAE upon active immunization with $MOG_{35–55}$. More specifically, these animals display a RR disease course that is followed by a chronic non-remitting stage (Encinas et al., 1999), much more similar to the disease course observed in MS. Moreover, Anderson and colleagues created a $CD4^+$ TCR transgenic model for $MOG_{35–55}$-driven EAE on the NOD background (1C6), which displayed both $CD4^+$ and $CD8^+$ $MOG_{35–55}$-reactive T cells. 1C6 $CD8^+$ T cells alone can induce optic neuritis and mild EAE with delayed onset; however, 1C6 $CD4^+$ T cells alone were able to induce severe EAE and predominate in lesions when both cell types are present, thus supporting the central role of $CD4^+$ T cells in the pathogenesis of disease (Anderson et al., 2012).

3.4. Immune system and pathogenesis of EAE

Substantial evidence from MS subjects indicates that $CD8^+$ T cells play a key role in the pathogenesis of the disease (Friese and Fugger, 2009; Goverman, 2009; Mars et al., 2011). $CD8^+$ T cells have been found more represent than $CD4^+$ T cells in acute and chronic CNS lesions of MS patients (Woodroofe et al., 1986; Hauser et al., 1986; Monteiro et al., 1995; Jacobsen et al., 2002; Junker et al., 2007). Importantly, immunotherapies specifically targeting $CD4^+$ T cells failed to show significant clinical benefit in MS course, whereas therapies that affect all leukocytes can improve disease progression (Coles et al., 2006). This latter observation led to the development of animal models of MS, by using MOG-immunized mice, to study the role of $CD8^+$ T cells in the pathogenesis of disease. Specifically, Ford and Evavold (2005) identified $MOG_{37–46}$, as a minimal peptide, able to induce specific $CD8^+$ T cell responses in B6 mice.

MOG-immunized mice on the C57BL/6 background also contributed for the identification of $CD4^+$ T cells that is nowadays considered as a major effector cell in EAE, providing an explanation for the strong correlation of MS susceptibility to particular MHC class II alleles. IFN-γ-expressing Th1 cells were initially considered to be the effector $CD4^+$ T cell subset that induced EAE (Sospedra and Martin, 2005). Indeed, adoptive transfer of Th1 clones in mice deficient in T-bet (a transcription factor required for Th1 cell differentiation) is resistant to EAE induction (Baron et al., 1993; Segal and Shevach, 1996; Bettelli et al., 2004). More recently, the finding that interleukin 23 (IL-23) was required for EAE development (Becher et al., 2002) led to the identification of the IL-23-dependent Th17 subset. It is now well established the role of both IFN-γ-producing and IL-17-producing T cells in the pathogenesis of MS and EAE; indeed, both cells have been identified in the CNS and CSF of MS subjects (Traugott and Lebon, 1988; Link et al., 1992; Kebir et al., 2007; Lock et al., 2002; Abromson-Leeman et al., 2009; Peters et al., 2011). Recent studies have identified different types of inflammatory infiltrates in CNS. Th1 cells have been shown to correlate with a predominantly monocytic CNS infiltrate, while Th17 cells were associated with a higher proportion of neutrophils in the CNS infiltrate (Kroenke et al., 2008). Additionally, the clinical symptoms of Th1- and Th17-mediated EAE were found to be different: Th1 cells induced classic EAE, whereas Th17 cells induced an EAE with a more severe clinical phenotype (Cua et al., 2003; Langrish et al., 2005; Jäger et al., 2009) often characterized by atypical manifestations (Stromnes et al., 2008; Domingues et al., 2010). There is also some recent evidence showing that Th1 cells can co-express IL-17 (Kurschus et al., 2010) and $IL\text{-}17^+$ $IFN\text{-}\gamma^+$ T cells have been identified in MS brains (Kebir et al., 2009). To understand the role of these cytokines, animal models, that are genetically deficient in IFN-γ or IL-17, have been developed and their susceptibility to EAE has been characterized. Some of these studies showed that IFN-γ is not required for EAE induction and it has been suggested that it may rather have suppressive activity (Ferber et al., 1996; Wensky et al., 2005). By contrast, in MS subjects it has been shown that the IFN-γ supplementation exacerbated disease manifestations, thus suggesting that IFN-γ has more disease-enhancing than disease-suppressing activity (Panitch et al., 1987). Regarding studies on Th17 cells, IL-17A- or IL-17 Receptor A-deficient mouse models showed a reduced incidence, severity and a delayed onset of EAE (Hofstetter et al., 2005; Komiyama et al., 2006; Hu et al., 2010). In addition, clinical trials in which RR–MS subjects were treated with a IL-17 neutralized monoclonal antibody, reported a reduction of lesion activity and a trend towards reduced relapse rates (Elain et al., 2014), supporting the central role of Th17 cells and IL-17 cytokine in the pathogenesis of MS.

The discovery of $CD4^+CD25^+$ T cells (Tregs), which express the master gene Foxp3 (forkhead box P3), as key players in the control of immune tolerance, allowed to establish an impaired number and functions of these cells in autoimmune disorders such as MS (Kleinewietfeld and Hafler, 2014). The majority of these studies was conducted in EAE mice where the adoptive transfer of this T cell subset reduced disease severity (Kohm et al., 2002) and, on the contrary, the administration of anti-CD25 antibody reduced Treg-mediated protection (Reddy et al., 2004; Zhang et al., 2004). Furthermore, the use of the Foxp3-GFP reporter mice facilitated detailed studies of Treg activity. Use of these mice showed that the population of Treg cells in the CNS is initially small but rapidly expands during EAE, and the majority of Tregs in the CNS of EAE mice were found to be antigen specific. Additional support for this approach came from studies that demonstrated impaired function of Tregs in patients with MS. Compared to healthy controls, Tregs isolated from peripheral blood and CSF of MS subjects have significantly reduced suppressive function (Frisullo et al., 2009; Viglietta et al., 2004; Haas et al., 2005). Tregs from MS subjects also exhibited a greater tendency for IFN-γ expression compared to healthy controls (Dominguez-Villar et al., 2011). Recently, CD25, CD127, and CD58, all of which contribute to Treg function, have been identified as risk alleles for susceptibility to MS, further suggesting an intrinsic Treg defect (Baranzini, 2009; Zenewicz et al., 2010; Broux et al., 2010; De Jager et al., 2009). Treating MS patients with IFN-β appears to restore suppressive function of Tregs (de Andrés et al., 2007; Korporal et al., 2008). Thus, the discovery that enhanced Tregs activity can ameliorate EAE, as well as studies of Tregs activity using reporter mice, have provided insight into current therapies and led to new therapeutic strategies for targeting pathways that enhance Tregs function. However, a note of caution has also emerged from studies using EAE models. Tregs isolated from the CNS during the peak of disease were able to suppress most peripheral effector T cells but failed to inhibit myelin-specific effector T cells isolated from the CNS. This observation suggests that effector T cells in the inflamed CNS may be resistant to suppression by Tregs (Korn et al., 2007).

Over the last few years, a series of molecules known to play a function in metabolism has also been shown to play an important role in the regulation of the immune response. In this context, the adipocyte-derived hormone leptin has been shown to regulate the immune response in normal as well as in pathological conditions. Several studies in EAE mice were conducted to study the role of leptin in MS. It has been shown that leptin-deficient (*ob*/*ob*) mice were resistant to a series of experimentally induced autoimmune disorders, including EAE. Normal wild-type mice show increased secretion of leptin in serum upon EAE induction, and brain inflammatory infiltrates stain positive for leptin (Sanna et al., 2003). The same authors also showed an inverse relationship between leptin secretion and the frequency of Treg cells in patients with MS (Matarese et al., 2005) and leptin neutralization with leptin antagonists improves the EAE course by profoundly altering intracellular signaling of myelin-reactive T cells and increasing the number of Treg cells (De Rosa et al., 2006). These data suggest that leptin can be considered as a link among immune tolerance, metabolic state, and autoimmunity and that strategies aimed at interfering with the leptin axis could represent innovative, therapeutic tools for autoimmune disorders.

Recent observations revealed an increased intrathecal production of immunoglobulins (Ig) in the CSF of most patients with MS (Owens et al., 2001) thus suggesting a B cell involvement in MS and EAE pathogenesis. Both B cell deficient ($\mu MT^{-/-}$) mice and anti-CD20-mediated depletion have been used to investigate the role of B cells in EAE. B10.PL $\mu MT^{-/-}$ and wild-type mice exhibit similar susceptibility to MBP-peptide induced EAE (Wolf et al., 1996); however, EAE was induced by immunization with recombinant human MOG protein only in wild-type and not in $\mu MT^{-/-}$ C57BL/6 mice (Oliver et al., 2003; Lyons et al., 1999), thus suggesting a key role displayed by antibodies during immunization with human MOG protein to generate CNS-inflammation. Similar results were obtained in mice expressing a transgenic TCR specific for MOG which developed a very low incidence of spontaneous EAE (Bettelli et al., 2003). In a model of spontaneous RR-EAE in SJL/J mice, expressing a transgenic MOG-specific TCR, B cell depletion suppressed EAE in these mice, providing support for the pathogenic role of B cells. The efficacy of Rituximab, an anti-CD20 antibody, in reducing inflammatory lesions and clinical relapses in patients with RR–MS provides another support for a pathogenic role of B cells in MS (Hauser et al., 2008).

3.5. Limits of EAE

EAE model significantly contributed to our knowledge of autoimmunity and neuroinflammation, changing the course of MS understanding and thus allowing the development of novel therapeutic approaches for this disease. Nonetheless, there are also several limitations to the use of this animal model because of the differences in the pathogenesis of EAE compared to that of MS. More specifically:

1) EAE model provides very few information about MS progression.
2) The use of C57BL/6 mice does not allow the study of relapses rate.
3) Remyelination is difficult to be studied in EAE. Lesions occur stochastically with regard to timing and localization. Furthermore, mechanistic insights of myelin damage in EAE tissues have not been performed so far.
4) Studies aiming at evaluating the potential benefits of novel therapeutic treatments with neuronal growth and survival factors have been unsatisfactory, as most of the effects were off-target, making any results difficult to interpret (Oliver et al., 2003).
5) EAE is mainly a disease affecting the spinal cord white matter, whereas MS is mainly a brain disease with prominent demyelination of the cerebral and cerebellar cortex. Unfortunately, only very few studies analyzed the involvement of the cortex in EAE.
6) Most forms of EAE are generated by immunization with self-peptide that determines $CD4^+$ T cell activation. Very few studies have addressed the role of $CD8^+$ T cells, which on the contrary mainly act in MS lesions and show clonal expansion and activation (Lyons et al., 1999).
7) EAE studies did not extensively analyzed the role of B cells in the pathogenesis of the disease (Bettelli et al., 2003), despite recent clinical-trial studies have clearly shown their importance (Hauser et al., 2008).

4. Theiler's murine encephalomyelitis virus (TMEV)

Epidemiological studies have suggested that a viral infection early in life, in the presence of a specific genetic background, may induce an immune-mediated attack against CNS (Poser, 1986; Dal Canto and Lipton, 1977; McFarlin and McFarland, 1982; Kurtzke, 1980), however, there is no specific virus that has been identified as a potential cause or contributor to MS, to date. More recently Epstein–Barr virus (EBV) infection has been linked to MS as a critical environmental susceptibility factor (De Jager et al., 2008; Ascherio and Munger, 2007; Ascherio et al., 2001). Viral infections of the CNS can induce demyelination in mice and the best studied are the picornavirus, such as Theiler's murine encephalomyelitis virus (TMEV) and certain strains of the coronavirus, such mouse hepatitis virus (MHV). TMEV is a non-enveloped, positive sense, single stranded RNA virus (Tsunoda and Fujinami, 2010) (Table 1) and represents one of the neurotropic viral infection models for MS (Libbey and Fujinami, 2003). TMEV is divided into two subgroups, GDVII and TO, based on the ability to cause disease in the CNS. The GDVII subgroup (strains GDVII and FA) is highly neurovirulent for mice, as it induces death within 1 to 2 weeks. The DA and BeAn8386 (BeAn) strains of the TO subgroup induce acute polioencephalomyelitis. Unlike EAE, the disease is always chronic-progressive in susceptible mice and TMEV can induce inflammatory demyelinating disease only in mice (Owens, 2006) and not in other different species, such as rodents and primates. GDVII virus predominantly infects neurons (Tsunoda et al., 1996) and dying neurons display chromatin condensation and apoptotic (fragmented) nuclei (karyorrhexis) in the absence of inflammatory mononuclear cell (MNC) recruitment. In contrast to GDVII infection, parenchymal as well as perivascular and subarachnoidal MNC infiltrates, including $CD3^+$ T cells, are present in the gray matter of the brain (Tsunoda et al., 2007a), during the acute phase of DA infection, while during the chronic phase of DA infection (a month or more after infection), the inflammation in the gray matter of the CNS subsides (Ure and Rodriguez, 2005).

Although axonal damage is observed in MS and its animal model (EAE), it is believed that axonal damage occurs secondarily to severe inflammatory demyelination, where lesions develop from the outside (myelin) to the inside (axon; outside-in model; Tsunoda et al., 2007b). On the contrary, in TMEV infection, axonal damage precedes demyelination (Tsunoda et al., 2003) (inside-out model) and the distribution of damaged axons observed during the early phase corresponds to regions, where subsequent inflammatory demyelination occurs during the chronic phase (Table 1). This evidence suggests that axonal degeneration triggers recruitment of T cells and macrophages into the CNS, leading to subsequent loss of myelin.

4.1. Immune system activation

TMEV persistently infects macrophage/microglia lineage cells, oligodendrocytes and astrocytes during the chronic phase (Lipton et al., 1995). Macrophages have been suggested to play an effector role in demyelination, since their depletion ameliorates TMEV-induced demyelination and intracerebral inoculation with a TMEV-infected macrophage cell line induces acute focal demyelination (Rossi et al., 1997; Rodriguez and Quddus, 1986). Confirming the role of humoral immunity in the pathogenesis of TMEV-induced neurodegeration, serum anti-TMEV neutralizing antibody responses have been detected within 1 week after infection and high neutralizing antibody titers are seen in mice with persistent TMEV infection (Tsunoda et al., 1996). Moreover, adoptive transfer of neutralizing antibody into TMEV-infected nude mice resulted in viral clearance (Fujinami et al., 1989), suggesting that virus-specific antibody can play a role in viral protection and clearance *in vivo*.

In this context also TMEV-specific $CD4^+$ T cells play an important role in demyelination, as testified by their infiltration into demyelinating lesions and by the evidence that *in vivo* depletion of these cells diminished the severity of demyelination. However Gerety et al. (1994) have shown that TMEV specific $CD4^+$ Th1 cells alone cannot induce demyelinating, but that the homing of virus specific T cells into the CNS requires previous virus infection in the CNS. Moreover it has been recently shown that treatment of TMEV infected mice with *ex vivo* generated induced Tregs (iTregs) worsened clinical signs of MS when the treatment was performed in the early phase of the disease, but was protective when the treatment was performed in the chronic phase, as it increased IL-10 production from B cells, $CD4^+$ T cells and dendritic cells, which may contribute to the decreased CNS inflammation (Martinez et al., 2014).

With regard to $CD8^+$ T cells, it has been shown that these cells infiltrate the demyelinating lesions, that *in vivo* administration of CD8 antibody diminishes demyelination, that CD8-deficient SJL mice showed minimal deficits with no effect on the extent of demyelination (Murray et al., 1998) and that MHC class I molecules are up-regulated in the CNS of TMEV-infected mice, thus suggesting that $CD8^+$ T cells play an effector role in TMEV-induced demyelination. However, recent reports have also demonstrated that TMEV infection can result in induction of autoreactive CTLs that recognize both virus and host antigens, potentially leading to CNS pathology (Tsunoda et al., 2002). In TMEV infection, intercellular adhesion molecule (ICAM)-1, leukocyte function associated antigen (LFA)-1 and vascular cell adhesion molecule (VCAM)-1 are upregulated in the CNS (Olson et al., 2001); their inhibition resulted in the suppression of demyelinating disease in SJL mice (Mestre et al., 2009). In addition, a decreased number of $CD4^+$ and $CD8^+$ T cells in the brains of the adhesion-molecule deleted mice as compared to control mice has been detected (Njenga et al., 2004).

4.2. Limits and positive aspects of TMEV models

The TMEV model and EAE display several important differences, such as a requirement for viral persistence, immune system activation, neuropathogenetic mechanism, and clinical courses.

The main positive aspect of TMEV model are as follows:

1) Its virus-induced pathology has clear similarities to MS, as the clinical manifestation is very similar to those observed in human chronic progressive MS.
2) Pathological features of virus-induced demyelinating disease are in general mediated by the activation of immune system and not by a direct toxic effect mediated by the virus on the target cells.
3) The TMEV model can be useful for the testing of new therapeutic approaches, particularly for therapies targeting adhesion molecules, axonal degeneration, and immunosuppression.

The main negative aspects (which limit its use) are as follows:

1) Unlike EAE, which is inducible in several different species, such as rodents and primates, TMEV can induce inflammatory demyelinating disease only in mice. Specifically it does not cause pathology in humans. This aspect raised the question whether it is rational or not to use a non-human pathogen to characterize a human disease, such as MS.
2) The pathogenesis of TMEV-induced demyelination in part differs from that in human MS, where persistent viral infection of the CNS has not been demonstrated.

5. Toxic models of MS

In addition to the well-characterized experimental approach to induce demyelination in mice, such as autoimmune inflammatory-induced demyelination in EAE, also viral-induced demyelination and toxic demyelination can be performed (Pachner, 2011) (Table 1). While EAE is the most commonly used model to reflect the autoimmune origin of MS, toxic demyelination is more suitable to study the de- and re-myelination processes (Blakemore and Franklin, 2008). Two are the most common agents utilized to induce demyelination: cuprizone and lysolecithin. Cuprizone (bis-cyclohexanone-oxaldihydrazone) is a copper chelating reagent which, supplemented to normal rodent chow, causes oligodendroglial cell death with subsequent demyelination, together with a profound activation of astrocytes and microglia (Matsushima and Morell, 2001) (Table 1). Specific targets of cuprizone are mature oligodendrocytes, which fail to fulfill the extensive metabolic demand and consequently undergo apoptosis, while the other cell types are not affected. The main reason for the metabolic failure is copper deficiency due to the copper chelation properties of cuprizone, but the reason why oligodendrocytes are the only cellular subset susceptible to this effect still remains unclear (Liu et al., 2010; Lucchinetti et al., 2000). Once demyelination is complete, new oligodendrocytes, generated from the pool of oligodendrocyte progenitors (OPC), begin to form new myelin sheaths soon after the removal of the cuprizone from diet (Matsushima and Morell, 2001). Despite the experimental use of cuprizone, the specific mechanism of action and the reason underlying oligodendrocyte damage is not completely known. Indeed, copper administration together with cuprizone administration, does not reduce the toxic effects and, for this reason, the copper-chelating property of cuprizone does not seem to be the only mechanicistical explanation. Many evidence suggest that oligodendroglial apoptosis mainly relies on mitochondrial disturbancies, since enlarged "giant" mitochondria are observed in both liver and brain of cuprizone-treated mice (Suzuki, 1969; Hemm et al., 1971; Komoly et al.,1987). Recently, a strong reduction of the mitochondrial potential was also reported in cuprizone-treated oligodendrocytes *in vitro* (Benardais et al., 2013). Taken together all these evidences suggest that a regular mithocondrial function is essential for oligodendroglial bio-energetic demand requiring a large amount of oxygen and adenosin triphosphat (ATP) to support an extensive membrane synthesis. Although not appropriate to study autoimmune mediated demyelination, the cuprizone model is a suitable tool to study basic mechanisms during de- and remyelination in absence of primarily immune-mediated phenomena (Matsushima and Morell, 2001). When cuprizone is fed continuously remyelination is abortive and demyelination persists until the end of the diet (chronic demyelination). In this case, the remyelination capacity retains after withdrawal from cuprizone diet but is strongly decreased (Matsushima and Morell, 2001).

Lysolecithin is an activator of phospholipase A2 which induces focal areas of demyelination upon injection into the spinal cord in several animals, including cat, rabbit, rat and mouse (Jeffery and Blakemore, 1995) (Table 1). Demyelination occurs due to primary toxic effects of detergent on myelin sheaths, rather than to secondary effects on oligodendrocytes (Hall, 1972). Lysolecithin triggers a rapid and highly reproducible form of demyelination in the CNS, without producing much damage to adjacent cells and axons; it is not immune-mediated since it occurs even in immune-deficient mice. However, chronic inflammation in lesions is minimal if young animals are used, and complete remyelination occurs in 5–6 weeks; on the contrary, repair in older animals is much slower (Shields et al., 1999). In the acute phase immediately following the lysolecithin injection, lesion sites are infiltrated with T cells, B cells, macrophages and neutrophils which seem to be involved in CNS repair (Bieber et al., 2003). Infiltration and activation of macrophages and microglia begin within hours after injection and last many days. The role that these cell types plays in establishing an environment in which remyelination can occur is not completely known. It is well accepted that the T-cell response promotes the expression of different neurotrophins by macrophages and astrocytes, that sustain neuronal protection and survival. During the remyelination process, several growth factors are produced and T cells might play a similar role in supporting oligodendrocyte remyelination, both directly and indirectly, by stimulating the activity of CNS glia. Moreover, depletion of macrophages impairs *per se* oligodendrocyte remyelination, thus suggesting a key role for this population in the myelin repair process. Taken together all this evidence suggests that toxin induced demyelination models, compared to EAE and virus induced demyelinating syndrome, do not reflect MS disease, but are mainly established systems to study the process of de- and remyelination (Blakemore and Franklin, 2008).

6. Development, success and failure of novel therapies for MS tested in animal models

EAE models have historically been used pre-clinically to assess and define the utility of novel MS therapies. The spectrum of agents showing promising results in EAE is extensive and ranges from natural compounds to modern genetic manipulation of the immune system with cytokines and antigen. The most important examples in this context are represented by glatiramer acetate, mitoxantrone and natalizumab (Kieseier and Hartung, 2003; Steinman and Zamvil, 2006). The glatiramer acetate preparation is a random polymer consisting of repeated sequences of four amino acids, which has been shown to suppress EAE progression, probably through the stimulation of Th2-mediated anti-MBP immune response (Aharoni et al., 1997, 2008), or by inducing killing of antigen-presenting cells (APCs) and generation of Treg cells (Racke et al., 2010). Mitoxantrone has first been proven to be a powerful immunosuppressive drug in EAE (Ridge et al., 1985), and it is now a second-line component of MS therapy (Hartung et al., 2002). Cytotoxic effects on lymphocytes and induction of apoptosis of APC have been proposed as the major mechanism of action of this drug (Neuhaus et al., 2005; Vollmer et al., 2010). Also Natalizumab, which is a monoclonal antibody (mAb) that inhibits the transmigration of immune cells into the inflamed parenchyma of lymphatic organs and the CNS, has been shown to be effective in preventing EAE (Rice et al., 2005; Yednock et al., 1992). It was of the first mAb approved for therapeutic trials in MS (Polman et al., 2006) and indeed now it represents a second-line drug for MS therapeutic approach.

A recent criticism of EAE has been raised by the fact that several therapeutical approaches that showed promising results in this mouse model, have been shown to be either inefficient or in some cases harmful in human MS (Steinman and Zamvil, 2006). For example, the blockade of three different cytokines, whose activity has been reported to be important in inflammation in EAE, such as TNF-a, BAFF, and IL-23, was found either to worsen MS (Meinl et al., 2011) or to have no effect in humans (Longbrake and Racke, 2009). The same holds true for the neuroprotective polypeptide hormone ciliary neurotrophic factor (CNTF), which elicited an acute-phase response in rat liver (Dittrich et al., 1994), or for the anti-adhesion molecule mAb anti-CD54 (Morrissey et al., 1996), and the phosphodiesterase-4 inhibitor rolipram, which despite its effective role in suppressing EAE, failed to suppress inflammatory activity in a pilot trial in patients with relapsing–remitting MS (Bielekova et al., 2009). Moreover, in clinical studies aimed at inducing oral tolerance or antigen-specific tolerance to a potential encephalitogenic autoantigen such as MBP, either worsening of disease or no change in the clinical course (Bielekova et al., 2000; Kappos et al., 2000) have been reported. Likewise, there was no beneficial effect of anti-CD4 antibody therapy on the progression of MS, despite the profound decrease of $CD4^{+}$ T cells in peripheral blood (van Oosten et al., 1997; Lindsey et al., 1994). The reasons for the discrepant results obtained in the animal and human systems could be due to their different genetic natures, different pathogenetic mechanisms or kinetic (temporal differences of immune reactivity and response to therapy). Additionally, in MS the blood–brain barrier (BBB) may be insufficiently disrupted as compared to EAE thereby preventing therapeutic molecules to reach their target within the CNS. For all these reasons, EAE has been considered sometimes a misleading model of MS (Sriram and Steiner, 2005), and some scientists truly think that EAE is more suitable for the studies of immunogenetics and histopathology rather than for screening of new treatments (Mix et al., 2010).

7. Concluding remarks

Animal model of EAE will continue to play a key role as a first-line model system in the development of novel therapeutic approaches for MS, especially for shedding light on specific mechanistic questions. Nonetheless, a great number of animal models, developed for MS, have garnered consistent criticism, often resulting in disappointing failures. It is important to remember that there is no a single animal model that can reflect the entire spectrum of heterogeneity of MS and this research field lacks a focused disease model for progressive MS. To approach the complexity of MS, current progress in humanizing the entire immune system in rodents will surely provide substantial advantages for exploring novel immune-modulatory approaches in more appropriate models. In conclusion, despite the clearly existing limitations, basic science on MS will continue to rely on these models for new drug development and for a better comprehension of the different pathogenic mechanisms of MS.

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