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Viral models of multiple sclerosis: Neurodegeneration and demyelination in mice infected with Theiler's virus

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ABSTRACT

Multiple sclerosis (MS) is a complex inflammatory disease of unknown etiology that affects the central nervous system (CNS) white matter, and for which no effective cure exists. Indeed, whether the primary event in MS pathology affects myelin or axons of the CNS remains unclear. Animal models are necessary to identify the immunopathological mechanisms involved in MS and to develop novel therapeutic and reparative approaches. Specifically, viral models of chronic demyelination and axonal damage have been used to study the contribution of viruses in human MS, and they have led to important breakthroughs in our understanding of MS pathology. The Theiler's murine encephalomyelitis virus (TMEV) model is one of the most commonly used MS models, although other viral models are also used, including neurotropic strains of mouse hepatitis virus (MHV) that induce chronic inflammatory demyelination with similar histological features to those observed in MS. This review will discuss the immunopathological mechanisms involved in TMEV-induced demyelinating disease (TMEV-IDD). The TMEV model reproduces a chronic progressive disease due to the persistence of the virus for the entire lifespan in susceptible mice. The evolution and significance of the axonal damage and neuroinflammation, the importance of epitope spread from viral to myelin epitopes, the presence of abortive remyelination and the existence of a brain pathology in addition to the classical spinal cord demyelination, are some of the findings that will be discussed in the context of this TMEV-IDD model. Despite their limitations, viral models remain an important tool to study the etiology of MS, and to understand the clinical and pathological variability associated with this disease.

Contents

Abbreviations: Ab, antibody; Ag, antigen; APC, antigen presenting cell; BBB, blood–brain barrier; CNS, central nervous system; COX-2, cyclooxygenase-2; CTL, cytotoxic T lymphocytes; dpi, days post-infection; DA, Daniels strain of Theiler's virus; EAE, experimental autoimmune encephalomyelitis; GALC, galactocerebroside; MBP, myelin basic protein; MNC, mononuclear cells; MHC, major histocompatibility complex; MHV, mouse hepatitis virus; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; NAA, N-acetylaspartate; NO, nitric oxide; PCR, polymerase chain reaction; PLP, myelin proteolipid protein; PPRs, pattern recognition receptors; SFV, Semliki Forest virus; SV, Sindbis virus; TMEV, Theiler's murine encephalomyelitis virus; TMEV-IDD, Theiler's murine encephalomyelitis virus-induced demyelinating disease; Tregs, regulatory T cells.

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1. Introduction

1.1. Multiple sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disorder of the human central nervous system (CNS), and it is considered to be the prototypical immune-mediated demyelinating disease. The involvement of the immune system in the pathogenesis of MS is irrefutable, and increased susceptibility to this disease has been associated with major histocompatibility complex (MHC) class II. MS is the main cause of non-traumatic neurological disability in young adults, affecting the most productive period of their lives, and it is characterized pathologically by focal plaques of demyelination in the white matter of the brain and spinal cord (Compston and Coles, 2008). Despite major advances in our understanding of the pathophysiology of MS, its etiology remains unknown. Most current therapies target the immune system or the inflammatory process. Indeed, clinical studies have demonstrated the therapeutic benefits of immunomodulatory or immunosuppressive treatments in patients in the early stages of the disease, which slow the progression of MS and the onset of permanent disability. However, no current therapies can cure or prevent the disease, highlighting the need for further studies to elucidate the etiology of MS.

1.1.1. Etiological aspects

MS is generally considered an autoimmune demyelinating disease, with inflammatory infiltrates representing a primary pathogenic element. While its etiology remains unknown, infectious agents have long been suspected as triggers of this disease (Ascherio and Munger, 2007a; Kurtzke, 1993). Moreover, evidence strongly suggests that myelin is targeted by an autoimmune reaction, although this still remains unproven. Oligodendrocyte apoptosis with microglial activation has been described in early MS lesions, in the absence of lymphocytes or myelin phagocytosis (Barnett and Prineas, 2004). Although inflammatory demyelinating lesions are a common pathological feature, the clinical course of MS is quite variable. Accordingly, the cause of MS appears to be multifactorial, suggesting that different etiological pathways converge to produce similar clinical and pathological outcomes. Epidemiological evidence suggests that MS is an environmentally triggered disease, and several viruses have been linked with MS pathogenesis, including human herpes virus type 6 (Tait and Straus, 2008), Epstein–Barr virus and endogenous retrovirus (Ascherio et al., 2001; Jilek et al., 2008; Martinez et al., 2007; Perron and Lang, 2010; Sargsyan et al., 2010). However, none of the viruses implicated to date (Table 1) can satisfactorily explain the etiology of all MS cases. Genetic and environmental factors are thought to interact to varying degrees, resulting in significant clinical and pathological variability (Ascherio and Munger, 2007a,b). It is generally assumed that in MS, the immune system erroneously identifies CNS myelin as foreign and thus, attempts to destroy it. This view is supported by studies in an animal model, experimental autoimmune encephalomyelitis (EAE) in which immunization with myelin, myelin proteins or peptides provokes immune-mediated destruction of CNS myelin. Although the mechanisms of T cell-mediated myelin destruction have been well described in these models, the mechanism by which the immune system identifies myelin as a foreign body in MS patients remains unknown. Imaging studies strongly suggest that MS begins as an inflammatory process but later develops a neurodegenerative component, which may progress independently of inflammation. An alternative view is that MS is a neurodegenerative process that is exacerbated by secondary inflammation that provokes demyelination. Resolving this question is critical to understand the disease and develop new treatments that target the underlying mechanism(s) in MS.

1.1.2. *Neurodegeneration* vs *inflammation*

The relationship between inflammation and neurodegeneration in the different stages of MS is somewhat controversial (Lassmann, 2010; Trapp and Nave, 2008). Axonal degeneration (Ferguson et al., 1997; Trapp et al., 1998) is now accepted as the major cause of irreversible neurological disability in MS patients. The lack of efficacy of current anti-inflammatory treatments in the progressive stage of MS and the low number of contrast-enhancing lesions are generally taken as evidence that neurodegeneration exists in the absence of inflammation. Although this view is partially supported by neuropathological studies, inflammation has been mainly quantified in the absence of immunocytochemistry with specific markers for T cells, B cells and macrophages. This approach is adequate to quantify perivascular inflammatory infiltrates but not to evaluate diffuse inflammation in the tissue parenchyma. More recently, the relationship between inflammation and neurodegeneration has been addressed in a large, detailed quantitative study of autopsy data from MS patients and controls (Frischer et al., 2009). One of the prominent findings of this study was that inflammation occurred in the brains of patients with progressive disease, both in lesioned areas and in the apparently healthy white matter and meninges. In general, a significant correlation was observed between inflammation and neurodegeneration in active demyelinating lesions, inactive lesions and apparently healthy white matter. While these observations clearly

Table 1
Virus associated to multiple sclerosis.

Family	Virus	Reference
Herpesviridae	Human herpes virus type 6	Knox et al. (2000)
	Herpes complex virus (VHS)	Perron et al. (1993)
	Varicella zoster virus (VVZ)	Brettschneider et al. (2009)
	Epstein–Barr Virus (EBV)	Lünemman et al. (2010)
	Marek's disease virus (MDV)	Bougiouklis (2006)
Retroviridae	Human T cell leukemia virus type 1 (HTLV-1)	Oger (2007)
	Human endogenous retrovirus	Perron et al. (2012)
Paramyxoviridae	Measles virus	Fujinami et al. (1983) and Ahlgren et al. (2012)
	Mumps virus	Tobler et al. (1982)
	Parainfluenza virus type 1	Rauch et al. (1975)
	Canine distemper virus	Haile et al. (1982)
	Simian virus type 5	Goswami et al. (1984)
Coronaviridae	Coronavirus	Boucher et al. (2007)

indicate a link between inflammation and neurodegeneration in MS, in all stages and among all lesion types, it remains unclear whether inflammation is the cause or a secondary consequence of neurodegeneration. Thus, it still remains to be proven that MS is primarily a neurodegenerative disease with secondary inflammatory demyelination.

1.1.3. Cortical demyelination

White matter demyelination can occur through distinct mechanisms that have been well characterized in the literature (Lucchinetti et al., 2000; Noseworthy et al., 2000). However, MS lesions can also affect the gray matter (Brownell and Hughes, 1962; Lumsden, 1970; Peterson et al., 2001). Although cortical demyelination was a surprise to MS community, it has been evident in the literature for decades (Brownell and Hughes, 1962; Lumsden, 1970). Three types of cortical lesions have been described on the basis of their location within the layers of the cortical gray matter: type I lesions are leukocortical areas of demyelination that contiguously occupy the subcortical white matter and cortex, and they consist of white matter plaques that extend into the lower layers of the cortex (Peterson et al., 2001); type II lesions are small perivascular areas; and type III lesions are strips of demyelination that extend from the pia, traversing several gyri to terminate in cortical layers 3 or 4. Type III lesions are the most abundant in the progressive stage of the disease, and while rare in acute and relapsing MS, they are prominent in patients with primary or secondary progressive MS (Kutzelnigg and Lassman, 2005). These lesions are predominantly located in the insular cortex, the frontobasal and temporobasal cortex and the gyrus cinguli (Kutzelnigg and Lassman, 2006), although cortical demyelination has also been described in the hippocampus and cerebellum (Geurts et al., 2007). It remains unclear whether cortical demyelination is mediated by immune effectors or if it compromises the blood–brain barrier (BBB). Demyelination in the cortex is associated with microglia activation, whereas T and B lymphocytes are sparse in the cortical parenchyma. However, systematic studies have revealed active cortical demyelination associated with profound meningeal inflammation (Lucchinetti et al., 2011), suggesting that inflammatory cells in the meninges produce a soluble factor that diffuses into cortical tissue and destroys myelin directly or indirectly *via* microglia activation. It is of great interest to determine whether similar subpial cortical lesions occur in MS models, and more importantly, how they can be induced.

1.2. Animal models of multiple sclerosis: an overview

Choosing an appropriate animal model to study a complex disease like MS presents several challenges, mainly associated with clinical and genetic heterogeneity. Two main animal model systems are used to study MS: experimental autoimmune encephalomyelitis (EAE), and experimental viral infection, commonly with Theiler's murine encephalomyelitis virus (TMEV). Both models have significantly advanced our understanding of MS and permitted preclinical testing of disease therapies. Moreover, these models have shed light on the immunological and inflammatory mechanisms involved in MS pathophysiology. Indeed, most of our current knowledge of MS has been derived from these animal models, particularly the EAE model. Nonetheless, one must remember that MS is highly heterogeneous in terms of its genetic basis, environmental triggers, clinical course, pathology and responsiveness to treatment (Friese et al., 2006). Ideally, an effective animal model should incorporate this heterogeneity. Although EAE must be induced by artificial immunization against myelin, most therapies tested in MS patients are based on concepts derived from the EAE model, which continues to be the model system of choice.

1.2.1. Experimental autoimmune encephalomyelitis

EAE is a well established model used to investigate the possible autoimmune etiology of MS (Baxter, 2007). This model originated with Louis Pasteur's vaccinations with spinal cord from rabies-infected rabbits from 1885. This acute demyelinating disorder was later found to occur due to contamination of the inoculums by spinal cord components (Rivers et al., 1933). Model organisms, ranging from mice to monkeys, were immunized against myelin, myelin components or related synthetic peptides, including myelin oligodendrocyte glycoprotein (MOG), myelin basic protein (MBP) and myelin proteolipid protein (PLP). The course of the resulting autoimmune inflammatory demyelinating disease ranged from acute monophasic to chronic progressive or relapsing-remitting, depending on a combination of several factors including the immunogen peptide, age, sex and the commercial source of the animal (De Luca et al., 2010). The immune response elicited in EAE is primarily due to the activation of myelin-reactive $CD4^+$ Th1 cells, leading to inflammatory demyelination (Zamvil and Steinmann, 1990). In all EAE models, $CD4^+$ cells predominate in the lesions and they are crucial for the adoptive transfer of the disease to naïve healthy animals. Nonetheless, myelin-specific $CD8^+$ T cells also play a role as suppressor/regulatory T cells or effector T cells in EAE pathogenesis. Studies employing either antibody (Ab) depletion of $CD8^+$ T cells or genetic knockout of the CD8 gene suggest a regulatory role of $CD8^+$ T cells in EAE (Goverman et al., 2005). An effector role for $CD8^+$ T cells has also been proposed, as adoptively transferred MBP specific $CD8^+$ cytotoxic T lymphocytes can mediate autoimmune disease (Huseby et al., 2001). Moreover, MOG specific $CD8^+$ T cells can act as effector cells in EAE and mediate demyelination (Sun et al., 2001). While macrophages, mast cells, eosinophils and $CD8^+$ cells have been implicated in EAE, the disease is considered a mainly class II $CD4^+$ cell-mediated process. Pathophysiological variations between specific EAE models correlate with distinct predominating aspects of the immune response. Various EAE models have been used to dissect out the molecular mechanisms of the autoimmune inflammatory response and to develop new therapies for MS. The main limitation of this model is the artificial induction of a myelin-specific immune response, which does not mimic the spontaneous disease and may bypass key pathogenic mechanisms operating in human MS. However, since the key target autoantigens of MS are unknown, this is still difficult to verify. Currently, it remains unclear why very few therapies that show promise in pre-clinical EAE trials have not shown similar efficacy in MS patients. Nevertheless, EAE remains a useful model to aid the development of novel MS treatments.

1.2.2. Viral models

Both genetic and environmental factors have been implicated in MS, with greater importance attributed to the latter (Ascherio and Munger, 2007a,b). Epidemiologic research has identified external risk factors in this multi-factorial setting, and has implicated viral and other microbial agents. Although most reports linking infection with MS are circumstantial, efforts are ongoing to identify specific pathogens that may be important in disease development (Stüve et al., 2004). A possible viral association with MS is suggested by epidemiological studies (Allen and Brankin, 1993; Kurtzke, 1993; Soldan et al., 1997) and by the detection of viral antigens (Ag) and virus-specific Abs in the majority of MS patients. For example, Ags derived from human herpes virus type 6 have been observed in MS plaques but not in tissues from patients with other neurological disorders (Cermelli et al., 2003; Challoner et al., 1995; Soldan et al., 1997; Virtanen et al., 2011). Similar findings have been reported for Epstein–Barr virus, which is in turn implicated in MS (Jilek et al., 2008; Lucas et al., 2011; Munger et al., 2011; Sargsyan et al., 2010; Tzartos et al., 2012). In addition, many studies have modeled the interactions between viruses and the

host immune response *in vivo* by inoculating mice with different viral strains, as outlined in Table 1. The three major hypotheses for how infections may induce immunity include: bystander activation, epitope spreading and molecular mimicry (Fig. 1). Bystander activation is a non specific mechanism for virus induced autoimmunity occurring within the inflammatory context generated by virus infection, especially chronic viral infection that leads to activated lymphocytes to secrete inflammatory mediators that mediate tissue damage. Stimulation of autoreactive T cells bearing particular Vβ receptors by virus encoded superantigens or indirectly *via* tissue resident APCs activated *via* Toll-like receptors (TLRs) or other patterns recognition receptors (PRRs) may drive tissue injury due to cytotoxic inflammatory molecules. A functional consequence of bystander activation and local tissue damage is the epitope spreading. In the setting of persistent infection where a prolonged anti-viral-immune response leads to tissue destruction resulting in the release of cryptic self-epitopes that are then engulfed and presented by APCs to T lymphocytes leading to activated autoreactive T cells. In summary, endogenous *de novo* activation of autoreactive T cells by sequestered Ags released secondary to tissue destruction directly by virus or by virus specific T cells is the basis for epitope spreading. Finally, the molecular mimicry, the mechanisms most often proposed for virus trigger autoimmunity, involves immunological cross reactivity between self-epitopes and epitopes from a foreign pathogen (Chastain and Miller, 2012; Fujinami et al., 1983).

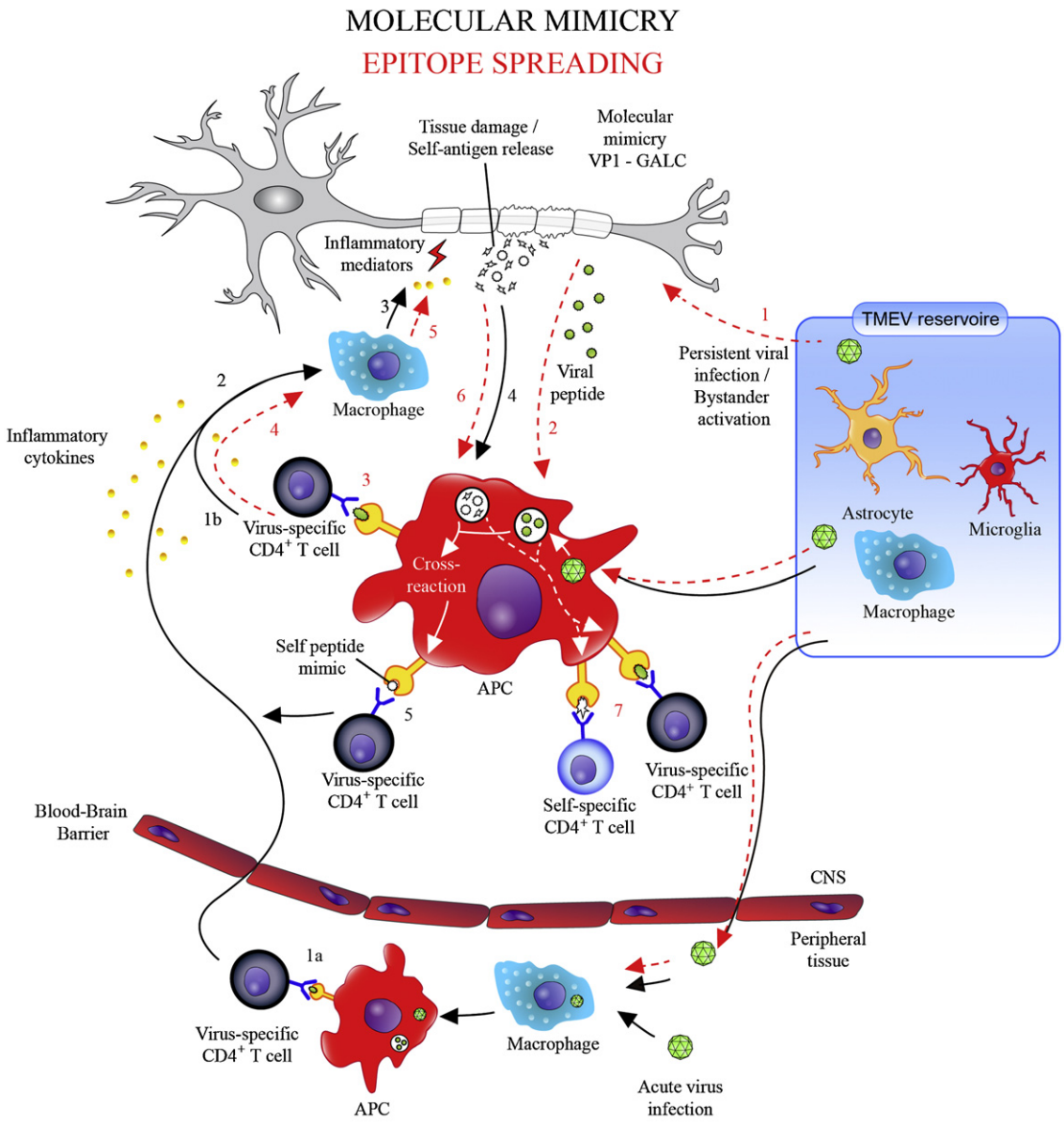

Fig. 1. Scheme of molecular mimicry and epitope spreading models of viral induced autoimmunity. In black (continuous line): after an acute viral infection, macrophages or APC process viral peptides and present antigen to virus epitope-specific CD4 T cells in the periphery (1a) or in the CNS (1b). Activated peripheral cells cross the blood–brain barrier, releasing proinflammatory cytokines and chemokines (2) that activate and recruit monocytes and macrophages, which cause tissue damage also by bystander activation allow self-antigens release (3). The CNS persistence of virus and the presence of self-antigens in the inflammatory context cause the self-peptides processing (4). The similarity between some self-peptides (galactocerebroside, GALC) and viral peptides (VP1) causes a cross-reaction (molecular mimicry) (5). In red (dashed line): in the epitope spreading model, after a persistent viral infection (1), APCs process the antigen (2) and present it to virus epitope-specific CD4 T cells (either in the nervous tissue or in peripheral tissue, in which case the T cell would have to cross the blood–brain barrier) (3). This activation causes a release of proinflammatory cytokines and chemokines, recruiting and activating monocytes and macrophages (4), which initiate the self-tissue destruction (5). A prolonged inflammation and processing self-antigens (6) induced activation of self-epitope-specific CD4 T cells and virus-specific CD4 T cells (7), causing an immune-mediated disease prolonged in time.

1.2.2.1. Theiler's murine encephalomyelitis virus (TMEV). TMEV is a single-stranded virus of the *Picornaviridae* family, which is a natural enteric mouse pathogen that behaves as a neurotropic virus that can replicate and persists within the CNS. Intracerebral infection of susceptible inbred mouse strains (SLJ/J) with TMEV leads to the induction of a late-onset demyelinating disease, termed TMEV-induced demyelinating disease (TMEV-IDD) which is similar in pathology to MS. TMEV is a well characterized model used to study the potential role of pathogenic agents in the development of MS. Several strains of TMEV exist that differ in their neurovirulence. TMEV is subdivided into two subgroups, GDVII and TO. The GDVII subgroup is neurovirulent and its representative strain is the GDVII virus. The TO subgroup is less virulent and the two representative and most commonly used strains are the Daniels (DA) and BeAn strains that promote a biphasic disease in which the chronic progressive phase is associated with demyelination in the white matter of the spinal cord (Dal Canto and Lipton, 1975). This chronic phase is considered a model of primary progressive MS. The persistence of TMEV in the CNS of susceptible mice is demonstrated as viral RNA and viral Ags can be detected throughout the animal's lifespan (Miller et al., 1997; Oleszak et al., 2004; Rodriguez et al., 1983). In TMEV-IDD, CNS damage is mediated by antiviral responses mediated by pathogenic proinflammatory virus-specific $CD4^+$T cells and is characterized by immune cell infiltration into the CNS, causing demyelination and disease signs. Pro-inflammatory cytokines recruit monocytes and macrophages into the CNS, which cause myelin damage. Reactivity to myelin does not appear until after the onset of disease signs (Katz-Levy et al., 1999). Thus, the chronic phase of TMEV-IDD is characterized by autoimmune processes due to epitope spreading from viral determinants to self-myelin determinants, demonstrating that viruses can trigger autoimmune reactions. Tolerance to myelin proteins after TMEV infection significantly inhibits TMEV-IDD, indicating the importance of the myelin-specific immune response in disease progression (Karpus et al., 1995; Neville et al., 2002).

1.2.2.2. Murine hepatitis virus (MHV). Like TMEV, MHV is a natural mouse pathogen that infects all cells types within the CNS (Bergmann et al., 2006). Particular strains of MHV, such as John Howard Mueller (JHM), display a distinct CNS tropism leading to severe acute encephalitis (Bender and Weiss, 2010). Strains with a less pronounced neurotropism, such as the gliatropic MHV-A59 strain generally establish a persistent CNS infection, leading to chronic neuroinflammation and demyelination (Hosking and Lane, 2010; Knobler et al., 1981). Mice inoculated intracerebrally or intranasally with the JHM virus or MHV-A59 strains mount a robust immune response resulting in an influx of immune cells that largely clear the virus, although low level viral infection persists in animals surviving the acute infection (Adami et al., 1995). In contrast to TMEV, susceptible mice infected with MHV develop a single major symptomatic episode (ataxia, hindlimb paresis, paralysis), from which the majority recover. Demyelination begins about one week post-infection, peaking at week 3–4, after which lesion repair and remyelination may occur (Lavi et al., 1984; Jordan et al., 1989).

Although the mechanism mediating demyelination remains unclear, extensive evidence suggests that immune responses are central to this process. MHV infects and replicates within oligodendrocytes, suggesting that oligodendrocyte damage is the main cause of demyelination (Hosking and Lane, 2010; Liu and Zhang, 2007). However, mice exposed to immunosuppressive doses of irradiation following JHM infection exhibit little demyelination despite the presence of the virus in oligodendrocytes, and demyelination is restored when irradiated mice are reconstituted with splenocytes from non-irradiated infected mice (Fleming et al., 1993). Similarly, T and B cell-deficient RAG-1 deficient mice are resistant to demyelination but exhibit demyelination after adoptive transfer of splenocytes from MHV-infected mice, a process that involves the recruitment of activated macrophages/microglia to demyelinated regions of the spinal cord (Wu and Perlman, 1999). Most studies suggest that macrophages are primarily responsible for the destruction of myelin, while others have proposed that the presence of either $CD8^+$ or $CD4^+$ cells is required for demyelination, but not both subsets simultaneously (Wu et al., 2000) Both β2-microglobulin and MHC class II-deficient mice exhibit demyelination after MHV infection. However, demyelination is less severe in CD4-deficient *vs* CD8-deficient mice, in agreement with the observed recruitment of macrophages to the CNS in these animals (Pewe et al., 2002). There is no evidence to date of self-specific immunity in the CNS of MHV-infected mice (Marten et al., 2001). Taken together, findings in the MHV model suggest that demyelination in human MS may be induced in an Ag non-specific manner if the virus is tropic for cells of the CNS.

1.2.2.3. Semliki Forest virus (SFV). SFV is a neurotropic alphavirus of the family *Togaviridae* that infects CNS neurons and oligodendrocytes (Fazakerley et al., 1993; Pathak and Webb, 1983). In adult C57BL/6 and BALB/c mice the virus is largely cleared from the CNS by 6 days post-infection (dpi). Demyelination peaks around day 14 and subsequently wanes, with sporadic and mild clinical symptoms (Fazakerley and Webb, 1987). The demyelination observed in SFV-infected mice appears to involve T cells, as demyelination does not occur in nude or SCID mice (Amor et al., 1996). Indeed, in BALB/c mice, depletion of $CD8^+$ but not $CD4^+$ T cells abolishes demyelinating lesions (Subak-Sharpe et al., 1993). Demyelination may also occur following cytolytic damage of virus-infected oligodendrocytes (Mokhtarian et al., 2003; Pathak and Webb, 1983). In this model in C57BL/6 mice, molecular mimicry may also play a role in demyelination, as infected mice exhibit proliferative T cell responses to MBP, and Abs reactive to MBP and MOG (Smith-Norowitz et al., 2000). Indeed, it has been proposed that the demyelinating lesions are generated mainly *via* Ab responses, which are cross-reactive to MOG and the SFV E2 protein.

1.2.2.4. Sindbis Virus (SV). Infection of SJL mice with SV is another model of demyelination that has not been extensively studied, but that provides further proof-of-principle that pathogen infection can lead to autoimmune disease. Infected SJL mice develop EAE-like paralysis beginning at 6 dpi and lasting up until 8 weeks post-infection (Mokhtarian et al., 1989). Cyclophosphamide treatment ameliorates disease signs despite increasing the CNS viral load, indicating that paralysis in these mice is mediated by the immune response. CNS lymphocytes collected at 7 dpi are specific for SV but not for MBP (Rowell and Griffin, 2002). Interestingly, MBP-specific T cell and Ab responses are detected in the periphery at 8 weeks post-infection, suggesting that anti-myelin responses arise due to bystander damage *via* epitope spreading (Metcalf and Griffin, 2011). The rapid onset of symptoms following SV infection indicates that demyelination is not the primary cause of paralysis but may contribute to chronic disease.

To summarize this first part of the review, viruses can induce demyelination and autoimmunity *via* multiple pathways. Although one of the main mechanisms of virus-induced autoimmunity in many of the animal models discussed here are epitope spreading and molecular mimicry, in other cases such as the MHV infection the virus does not induced autoimmunity. The underlying mechanisms mediating myelin destruction may differ from one model to another and still not fully clarified. Moreover, the different clinical manifestations of each model reflect the broad spectrum of symptoms experienced by patients. Of the MS models

discussed, the TMEV-IDD model has clear advantages over the other models of virus-induced demyelination as it permits the analysis of initial viral exposure and clearance, and the progression from viral recognition by the immune system to autoimmunity. This model has proved effective in the development of clinical therapies for MS. Below we will review the characteristics of the TMEV model and discuss its novel aspects with respect to pathogenic mechanisms and the evolution of TMEV-induced brain and spinal cord damage.

2. TMEV-IDD: models and strains

TMEV infection of mice is one of the better characterized neurotropic viral infection models of MS. Since Max Theiler first isolated TMEV from the CNS of mice with spontaneous paralysis of the hind legs (Theiler, 1937), our understanding of TMEV-IDD has advanced greatly. In 1952, demyelination of the spinal cord of mice infected with the Daniels (DA) strain of TMEV was described (Daniels et al., 1952). Several years later Lipton demonstrated the utility of TMEV infection as an experimental model of MS (Lipton, 1975).

TMEV belongs to the genus *Cardiovirus* of the family *Piconaviridae* and it is a positive strand RNA virus, like poliovirus, rhinovirus or coxsackievirus. TMEV strains are divided into two subgroups, GDVII and TO, based on the severity of the CNS disease they induce. The GDVII subgroup (strains GDVII and FA) is highly neurovirulent in mice and causes fatal encephalitis, resulting in death within one to 2 weeks (Yamada et al., 1991). The TO subgroup (DA and BeAn strains) is much less virulent and induces mono or biphasic disease. The monophasic disease consists of transient meningoencephalomyelitis, peaking one week after infection. In resistant mouse strains the virus is cleared after approximately 3 weeks by an intense inflammatory response that protects against viral persistence and gives way to a transient stage, after which mice generally recover without persistent neurological disease (Lindsley and Rodriguez, 1989; Njenga et al., 1997). However, in susceptible mouse strains (SJL/J), TMEV induces a biphasic disease characterized by acute encephalomyelitis followed by a chronic inflammatory demyelinating disease that begins 35–45 dpi (BeAn strain). Most of the demyelinating lesions are found in the spinal cord, as seen in the EAE models although in EAE also occur brain lesions (Centonze et al., 2009; MacKenzie-Graham et al., 2009; Mandolesi et al., 2012). This phase is considered a valid model of chronic progressive MS. TMEV persists in the CNS for the entire lifespan of the animal and disease severity depends on the strain, sex and age of the mouse (Kappel et al., 1990; Steiner et al., 1984), as well as the dose and strain of the virus. TMEV persists indefinitely to varying degrees in different cell types including monocyte/macrophages, microglia, oligodendrocytes and astrocytes (Clach et al., 1990; Jelachich et al., 1995, 1999). Resistance to persistent CNS infection by TMEV is controlled by multiple genetic loci, with the strongest linkage observed for the MHC class I H-2D region (Brahic et al., 2005; Rodriguez et al., 1986).

Although similarities are observed, the disease induced by the DA virus differs in several aspects to that induced by the BeAn strain, including the cell types infected and the associated pathology (Kang et al., 2002; Zoecklein et al., 2003). During the acute phase of DA infection viral antigen-positive neurons are found in the gray matter, including the cerebral cortex and hippocampus. Some apoptotic neurons and parenchymal, perivascular and subarachnoidal MNC infiltrates, including $CD3^+$ T cells, are observed (Schlitt et al., 2003). During the chronic phase of DA infection, inflammation persists in the white matter of the CNS. Demyelination with perivascular and subarachnoidal MNC inflammation is observed in the ventral and lateral funiculi, particularly at the ventral root exit zone of the spinal cord. During the chronic phase, viral Ag and viral genome have been demonstrated by immunohistochemistry and *in situ* hybridization in oligodendrocytes, microglia/macrophages and astrocytes, but not in neurons (Oleszak et al., 2004). Infectious virus and viral genome have also been quantified in plaque assays and by reverse transcription polymerase chain reaction (PCR), respectively. CNS cells infected with DA virus contain 100–500 copies of viral RNA, and quantification of positive and negative strand RNAs in infected CNS cells confirms that RNA replication is blocked at the level of negative-strand RNA synthesis, as postulated for other picornaviruses in which the main mechanism of persistence *in vivo* is restriction of viral RNA replication (Cash et al., 1988). Differences between DA and BeAn viral infection in mice may explain the divergent disease courses previously described for these viruses (Kang et al., 2002; Oleszak et al., 2004). In particular, the level and avidity of virus-specific $CD8^+$ T cells infiltrating the CNS could be different after the infection with these two strains of TMEV and may differentially influence the pathogenic and/or protective outcome (Kang et al., 2002). Infection with DA virus results in a greater demyelination, more viral RNA, and more antigen-positive cells in the spinal cord as compared with the BeAn virus (Zoecklein et al., 2003). There was a higher incidence of demyelination in the spinal cord of mice infected with DA, but the extent of demyelination was similar for both viral strains when compared with mice those developed demyelination. While no differences in brain infiltrating immune cells are observed between DA and BeAn-infected mice, the latter exhibit higher titers of TMEV-specific Ab. Functional deficits measured by rotarod performance are more severe in DA-infected mice (Zoecklein et al., 2003). Although the capsid proteins of the BeAn and DA strains Exhibit 93% amino acid homology, the disease induced by the BeAn strain in SJL/J mice differs from that induced by the DA strain (Tsunoda and Fujinami, 2010); the early acute disease is attenuated in BeAn-infected mice as opposed to the gray matter disease induced by the DA strain of TMEV. Furthermore, although both strains induce late chronic demyelinating disease, the kinetic of the disease induced by each strain differs considerably. BeAn-infected SJL mice exhibit clear disease signs at 30–45 dpi, including waddling gait and hind leg paralysis, in function of the viral dose and the age of the animals. By contrast, DA-infected SJL/J mice develop these signs much later, at approximately 90–120 dpi (Njenga et al., 1999).

2.1. Pathogenesis: TMEV-IDD

2.1.1. Pathophysiology of demyelination

Intracerebral infection of susceptible mouse strains with TMEV results in a biphasic disease of the CNS, consisting of early acute disease and subsequent chronic demyelination. During the acute phase, the white matter is unaffected, but multifocal inflammation involving cerebral and spinal cord gray matter is observed (Lipton and Dal Canto, 1976; Rodriguez et al., 1987b). Cellular infiltrates (predominantly T cells and monocyte/macrophages) are detected in the leptomeninges and the cerebral cortex, although inflammation is mainly concentrated in the subcortical gray matter, in particular the thalamus, hippocampus and basal ganglia. In the spinal cord, inflammation is restricted to the anterior horns of the gray matter, although infiltration of the leptomeninges also occurs. During the chronic phase (30–35 dpi), mononuclear cell infiltrates of the spinal cord white matter consisting predominantly of T cells and monocyte/macrophages coincide with the initiation of chronic demyelination, and microglial activation in the white matter is observed (Arévalo-Martín et al., 2003; Oleszak et al., 2004). The demyelination process is multifocal and although it is evident throughout the rostrocaudal length of the spinal cord, it preferentially affects the thoracic segments (Rodriguez et al., 1996).

The immunological mechanisms of TMEV-IDD imply the involvement of different cellular components of the immune system. At first, the innate immune response affects the development of the autoimmune response (Olson and Miller, 2009). The immune response is initiated by the presentation of persistent viral Ags to $CD4^+$ T cells by resident CNS Ag presenting cells (Olson et al., 2001; Olson and Miller, 2004). Pro-inflammatory cytokines recruit monocytes and macrophages into the CNS, which cause myelin damage. The subsequent release of myelin Ags and their uptake by APCs leads to the emergence of myelin-specific $CD4^+$ T cells. Reactivity to myelin does not appear until after the onset of clinical symptoms (Katz-Levy et al., 1999). Both, $CD4^+$ and $CD8^+$ T cells appear to play important roles in the pathogenesis of late chronic demyelinating disease. Although the infiltrating $CD4^+$ T cells during the early chronic phase are specific for the virus, in the late chronic phase $CD4^+$ T cells can recognize host or viral Ags, as elegantly revealed Miller et al. (1997) by demonstrating epitope spreading to self-myelin epitopes in TMEV-IDD. $CD4^+$ T cell responses to the immunodominant myelin proteolipid protein ($PLP_{139–151}$) are present during late chronic phases and as the disease progresses further, $CD4^+$ T cell responses to multiple myelin epitopes occur in an ordered progression (Katz-Levy et al., 1999; McMahon et al., 2005). Therefore, autoimmune responses against myelin are only evident during the late chronic phases of TMEV-IDD. The possibility that a natural TMEV infection can induce CNS autoimmunity by molecular mimicry was first demonstrated in elegant experiments conducted by Fujinami's group that showed the existence of molecular mimicry between VP1 and lipid like structure of myelin such as galactocerebroside (GALC) (Fujinami et al., 1988). Results from the same group also showed that antibodies generated by immune response to TMEV could react with myelin and oligodendrocytes contributing to demyelination (Yamada et al., 1990). Additionally, Miller's group developed a model where the immunodominant myelin epitope $PLP_{139–151}$ was inserted into a nonpathogenic variant of TMEV to produce demyelination (Olson et al., 2001). In that case, they used engineered recombinant TMEV strains and not natural TMEV strains and the use of different molecular constructs can be unrealistic from the point of view of real TMEV infection and the model of TMEV-IDD. However, they showed that a naturally infectious virus when encoding a myelin epitope mimic can initiate autoimmunity *via* molecular mimicry associated with the appearance of cross-reactive Th1 cell responses to the endogenous myelin protein. It is important to note that molecular mimicry requires the infectious agent to encode a mimic epitope with structural and sequential similarity to the self-peptide and must be capable of being processed from its native protein by APCs in the infected hosts providing the necessary accessory molecules required for autoreactive T cell activation.

To summarize both, epitope spreading and molecular mimicry are the main mechanisms underlying virus-induced CNS autoimmunity (Chastain and Miller, 2012; Croxford et al., 2002; Fujinami et al., 1983) and both mechanisms have been demonstrated in TMEV-IDD.

The role of $CD4^+$ T cells in the development of demyelinating disease is supported by several lines of evidence: (i) treatment of TMEV-infected susceptible mice with anti-IA Abs results in decreased demyelination (Friedmann et al., 1987); (ii) $CD4^+$-mediated delayed hypersensitivity to TMEV Ags is associated with myelin damage (Miller and Rodriguez, 1995); (iii) *in vivo* depletion of $CD4^+$ T cells during established disease diminishes the severity of demyelination (Rodriguez and Lindsley, 1992); and finally, (iv) certain $CD4^+$ T cells generated during TMEV infection with either the BeAn or the DA strain (Jin et al., 2009) specifically recognize predominant viral peptides ($VP1_{233–250}$, $VP_{274–286}$, $VP3_{34–37}$). However, it remains unclear how deficiencies in class II gene products contribute to viral persistence and the degree of demyelination. TMEV-IDD has been reported in class II knockout mice (Njenga et al., 1996), CD4 deficient mice (Murray et al., 1998) and nude mice (Fujinami et al., 1989; Rosenthal et al., 1986)

MHC class I-restricted $CD8^+$ T cells have been proposed to play effector roles in the development of demyelinating disease since (i) $CD8^+$ T cells infiltrate demyelinating lesions, (ii) *in vivo* administration of CD8 antibody diminishes demyelination, and (iii) MHC class I molecules are upregulated in the CNS in TMEV-infected mice (rev by Drescher et al., 1997). Depletion of $CD8^+$ T cells during chronic phases greatly reduces myelin damage in the CNS of TMEV-infected mice (DA strain: Rodriguez and Sriram, 1988), while CD8-deficient mice infected with the BeAn strain of TMEV display greater susceptibility to infection and greater pathological alterations during demyelination (Begolka et al., 1998). Interestingly, autoreactive TMEV-specific $CD8^+$ T cells have been described by Tsunoda and Fujinami's group (Tsunoda et al., 2002, 2005, 2006). They tested whether an autoreactive cell induced by TMEV infection mediated cytotoxicity using a ^{51}Cr release assay in SJL/J mice and demonstrate that intracerebral inoculation of $CD8^+$ T cell clones effector cells into naïve mice caused meningitis and perivascular cuffing in the brain and spinal cord with no evidence of viral antigen positive cells (Tsunoda et al., 2002, 2005).

As disease resistance partly maps to the H-2D MHC class I locus, the $CD8^+$ T cell response has been extensively studied. The requirement of $CD8^+$ cytotoxic T lymphocytes (CTL) for clearing the virus may explain the above findings. Several years ago, the Rodriguez group analyzed antiviral immune responses and their modulation of TMEV-IDD (Drescher et al., 1997), and they found that VP1 and VP2 capsid Ags of TMEV are critical in determining resistance/susceptibility to immune-mediated demyelination. Mice with H-2Db haplotype are resistant and resolve TMEV-induced encephalitis following a strong $CD8^+$ T cell response, which clears the virus and prevents viral persistence (Lin et al., 2002). The tentative association between the presence of functional $CD8^+$ T cells and resistance to TMEV-IDD has prompted direct comparison of $CD8^+$ T cell responses to TMEV in SJL/J and C57BL/6 mice. Although these responses exhibit some qualitative differences, they are quantitatively quite similar (Lyman et al., 2004), and thus the basis for the differential virus clearance observed in the two mouse strains remains unclear. Recent studies using transgenic mice that express VP1, VP2 or $VP2_{121–130}$ Ags have analyzed immune responses to specific capsid Ags in the TMEV-IDD model (DA strain: Denic et al., 2011; Pavelko et al., 2007). Expression of specific capsid Ags is able to induce susceptibility to demyelination in the normally resistant strain (Pavelko et al., 2007). Moreover, susceptible transgenic mice exhibit demyelination, inflammation and axonal loss as compared with wild type mice, despite suffering no apparent increase in viral load, supporting the view that expression of viral capsid proteins influences the extent of axonal pathology following TMEV infection (Denic et al., 2011). On the other hand, a recent study using the BeAn strain demonstrated that adoptive transfer of activated $CD8^+$ $VP3_{159–166}$-specific T cell blasts shortly after TMEV infection to boost the early antiviral response leads to clearance of CNS virus and protection from subsequent TMEV-IDD (Getts et al., 2010).

It was recently proposed that differential induction of regulatory T cells (Tregs) mediates susceptibility to TMEV-IDD. Indeed, infection of disease-susceptible SJL/J, but not resistant B6 mice leads to rapid activation and expansion of Tregs, resulting in an unfavorable CNS ratio of Treg:T effector cells (Richards et al., 2011). Moreover, anti-CD25-induced inactivation of Tregs in susceptible SJL/J mice, but not in resistant B6 mice, induces a significant decrease in clinical disease severity concomitant with enhanced antiviral $CD4^+$, $CD8^+$ and antibody-mediated antiviral

immune responses, resulting in decreased CNS viral titers. The delay in onset and progression of disease following inactivation of Tregs in susceptible mice prior to TMEV infection would be related to better virus clearance as a significant decrease in viral load in the brain was observed at day 12 post-infection. As disease progresses, the virus spreads to the spinal cord, however, by day 28 post-infection the viral load in anti-CD25 treated and control treated mice is equivalent. It is important to note that inactivation of Tregs with anti-CD25 mAb is temporary and functional Tregs return to near frequencies within 7–10 days after the final mAb treatment. The fact that Treg cells proliferate preferentially in TMEV-susceptible mice resulting in a CNS $CD8^+$ T effector:Treg (1:4 at day 7 post-infection) less favorable for virus clearance than the ratio (1:1.2 at day 7 post-infection) in resistant mice during the acute TMEV infection may control the efficiency of the antiviral-immune response resulting in virus persistence. This is the first report demonstrating that TMEV-induced Treg activation differentially regulates susceptibility to immune-mediated disease in different mouse strains and provides new mechanistic insights into infection-induced autoimmunity.

In addition to their role in TMEV persistence, the action of macrophages/microglia as effectors of demyelination is well documented (Oleszak et al., 2004). Several lines of evidence suggest that macrophages (Pena-Rossi et al., 1997), and to a lesser extent glial cells, serve as the site of TMEV persistence (Clach et al., 1990; Dal Canto and Lipton, 1982; O'Shea et al., 1997; Zheng et al., 2001). Active viral replication in macrophages is indicated by the immunohistochemical detection of virus accompanied by cytoskeletal changes in macrophages (Dal Canto and Lipton, 1982), the presence of a large TMEV Ag burden and the detection of the viral genome within macrophages (Lipton et al., 1995). Resident CNS cells such as astrocytes and microglia are important reservoirs of TMEV (Zheng et al., 2001). Within the CNS, APCs like microglial cells (Mack et al., 2003; Olson and Miller, 2004) act as a first-line of defense against TMEV infection and inflammation, but in the chronic phase they may help sustain the self-destructive environment by secreting inflammatory factors and/or presenting Ag to T cells. Furthermore, dendritic cells, though not CNS-resident cells, have emerged as key APCs, even within CNS (Chastain et al., 2011). Astrocytes are the most abundant cell type in the brain and they are not commonly associated with immunological responses but rather, they play a regulatory role in TMEV-IDD (Carpentier et al., 2008). Inflammation, involving the infiltration of blood-derived cells, particularly T and B lymphocytes and monocytes, together with the activation of resident microglia and astrocytes, is the earliest pathologic event in the spinal cord of infected mice, slightly preceding demyelination. Monocyte-derived macrophages and activated microglia account for over half of the inflammatory cells in spinal cords, as evaluated by FACS of isolated inflammatory cells (Olson et al., 2001). The onset of demyelination in the spinal cord coincides with the inflammatory response, which co-localize in areas of viral expression, as evident by *in situ* hybridization and immunohistochemistry for TMEV. Demyelinated lesions are concentrated in the thoracic and cervical spinal cord areas, and while the ventral and lateral funiculi are consistently lesioned, the dorsal funiculus is more variably affected (Ure and Rodriguez, 2002).

As mentioned above, myelin degeneration in TMEV-IDD is probably mediated by a range of processes, including direct phagocytosis of myelin. Cytotoxic factors are also likely to be involved, including proteases, cytokines, complement, nitric oxide (NO) metabolites and reactive oxygen species, generated in part by macrophages/microglia of the myelin sheath, sparing oligodendrocyte cell bodies (Langrish et al., 2005; Liuzzi et al., 1995; Oleszak et al., 2004). Alternatively, demyelination may be occurring secondary to a primary insult to the oligodendrocyte cell body. Oligodendrocytes exhibit a degenerative pathology in lesions, including swelling and vacuolation, and decreased myelin protein immunoreactivity provides an index of myelin disruption (Rodriguez, 1985). In addition, some demyelination is associated with Wallerian degeneration, as axonal loss is observed in chronically diseased mice, although this issue remains controversial. Finally, excitotoxicity has emerged as another possible mechanism underlying demyelination and axonal damage in the TMEV model. We demonstrated the beneficial effect of administration of NBQX, a classical AMPA/kainate receptor antagonist, in TMEV diseased mice, probably due to the blockade of excessive glutamate release (Docagne et al., 2007). These results are consistent with previous findings in the acute EAE model (Pitt et al., 2000), the chronic relapsing EAE model (Smith et al., 2000) and in MS patients (Srinivasan et al., 2005). In this scenario, oligodendrocyte death and myelin disruption may also occur due to TMEV-induced cytolysis or apoptosis. Cyclooxygenase-2 (COX-2) is expressed in dying oligodendrocytes at the onset of demyelination in TMEV-IDD (Carlson et al., 2006), increasing their susceptibility to excitotoxic death (Carlson et al., 2010). TMEV infects oligodendrocytes both *in vivo* (during the chronic phase) and *in vitro*, as the DA strain of TMEV kills oligodendrocytes in culture (direct lytic infection). Several studies have also demonstrated infection of oligodendrocytes by the DA strain *in vivo*, which is localized in degenerating cells (Ghadge et al., 2011). Nude mice with a limited T cell response develop demyelinating lesions following TMEV infection, although the overall extent of demyelination is limited (Roos and Wollmann, 1984). Oligodendroglial apoptosis has also been described during the chronic phase of TMEV infection, as revealed by double staining of TUNEL-positive nuclei with oligodendrocyte and macrophage/microglia markers (Rose et al., 1998; Tsunoda et al., 1997). While the pathogenic mechanisms associated with TMEV-IDD appear to be more complex than previously thought, most studies suggest that the immune system plays a prominent role in demyelination, although a wide variety of cells and molecules regulate demyelination and axonal damage in the TMEV model (Fig. 2).

2.1.2. Pathophysiology of neurodegeneration: axonal damage

Neurodegeneration, axonal and/or neuronal damage has been recognized as a component of MS since the pioneering studies of Charcot (1868). However, MS, EAE and most TMEV studies have typically focused on demyelination, and axonal injury has only recently attracted the attention of researchers. Pathological studies have highlighted the existence of axonal injury in MS patients (Ferguson et al., 1997; Trapp et al., 1998; Trapp and Nave, 2008), although, the temporal profile of demyelination and axonal loss in MS patients, and their respective contribution to the clinical and electrophysiological abnormalities, are not completely understood. In the TMEV model of progressive MS, demyelination in the spinal cord is followed by the loss of medium-to-large myelinated axons, it is associated with electrophysiological abnormalities and is strongly correlated with spinal cord atrophy and reduced motor coordination (McGavern et al., 2000).

Axonal injury in TMEV-IDD was first described during the chronic phase of the disease (DA strain) by Dal Canto and Lipton (1975). It was believed that axonal damage occurred secondary to severe inflammatory demyelination, whereby the myelin and/or oligodendrocytes are injured first, and the lesions subsequently extend from the myelin (outside) to the axon (inside). In the last 20 years, interest in axonal damage as the potential cause of the irreversible neurological deficits in MS has led to further analyses of TMEV-induced axonal degeneration. This research is focused along two main lines: that led by the group of Tsunoda who proposes that early axonal damage occurs prior to demyelination (Tsunoda and Fujinami, 2002), while the Rodriguez group

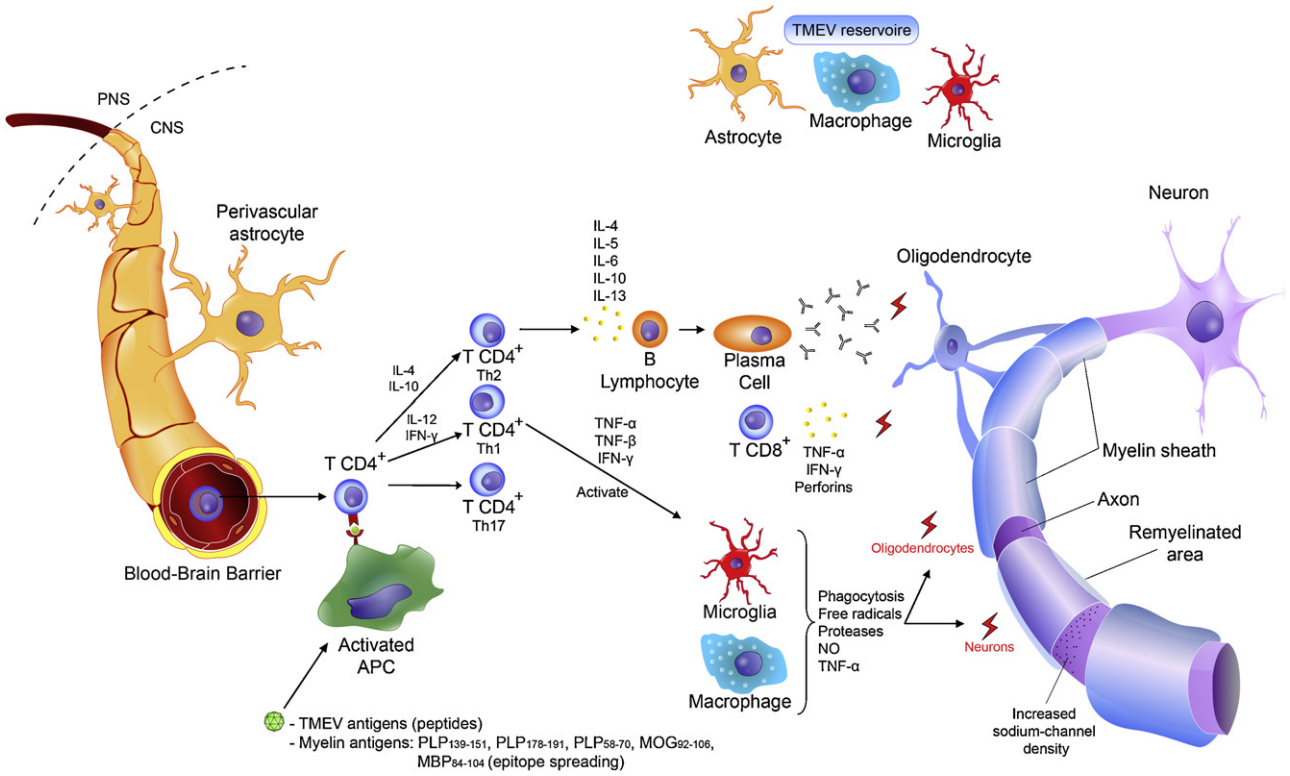

Fig. 2. Pathogenic mechanisms involved in demyelination in the TMEV-IDD model. Cells of the immune system potentially involved in demyelination. APCs can take up antigens from TMEV or from self-tissue (myelin or oligodendrocyte proteins). Antigens are processed and presented to T cells *via* the TCR in the context of MHC class I or class II. Activated cytolytic T cells (MHC class I) induce damage by direct lysis of the target. Th cells (activated by MHC class II) release inflammatory cytokines that also activate monocytes/macrophages and microglial cells. B cells recognize surface antigen and when facilitated by T cells (Th2), secrete antibodies specific for foreign and self-epitopes.

postulates that axonal loss follows primary immune-mediated demyelination in the CNS and that the severity of axonal loss is closely correlated with the degree of spinal cord atrophy and neurological deficits (McGavern et al., 2000). Tsunoda and Fujinami labeled non-phosphorylated filaments to reveal damaged axons in the white matter of the spinal cord one week after TMEV infection (DA strain: Tsunoda and Fujinami, 2010). This axonal damage was independent of T cell infiltration and the presence of virus-infected cells. At this time point (7 dpi), T cells and virus-infected cells are present in the gray matter of the brain. During the presymptomatic phase (2–3 weeks post-infection) axonal damage in the spinal cord white matter is associated with activation of microglia and macrophages, while there is little inflammation and no viral Ags are detected. Because the magnitude of demyelination increases at 4–5 weeks post-infection, axonal degeneration was proposed to precede demyelination in the TMEV model (Tsunoda et al., 2003). Based on the above findings and other more recent observations (Tsunoda et al., 2007a), an inside-out lesion model of TMEV infection was proposed as a self-defense mechanism against viral spreading (Sato et al., 2011). In this scenario, the lesion develops in the axon (inside) and extends to the myelin (outside), and thus, axonal damage represents the primary event that is followed by secondary demyelination (Fig. 3).

During the chronic phase of TMEV infection with the DA strain, Rodriguez and co-workers described, in a detailed and elaborated study, spinal cord atrophy in the ventral and lateral funiculi but not in the dorsal area, with a significant decrease in the number of medium-to-large myelinated axons at 195–220 dpi (McGavern et al., 1999, 2000). As spinal cord atrophy was not observed in resistant C57BL/10 mice, the authors suggested that spinal cord atrophy occurs as a consequence of demyelination during the chronic phase, and that it is unrelated to neuronal infection during the acute phase, which occurs in both resistant and susceptible mice. However, the significant decrease observed in medium-to-large myelinated axons in normally myelinated areas during the chronic phase of TMEV infection (Sathornsumetee et al., 2000) suggests that axonal degeneration may be independent of demyelination in at least some CNS areas during the late chronic phase. There is no consensus about the timing and the precise mechanism of axonal damage in the TMEV-IDD model of MS. Nevertheless, the observed axonal and functional preservation in chronically demyelinated mice genetically deficient in perforin (Howe et al., 2006), and the preservation of motor function (Rivera-Quinones et al., 1998) and axon integrity (Ure and Rodriguez, 2002) seen following genetic disruption of MHC class I function during chronic demyelination, strongly support the immune-mediated injury model. In MS lesions, $CD8^+$ T cells are the most abundant lymphocytes (Babbe et al., 2000) and their presence is correlated with axonal injury (Bitsch et al., 2000). $CD8^+$ T cells directed against a TMEV viral peptide, $VP2_{121-130}$, contribute to the loss of motor function by disrupting axonal transport in IFN-$\gamma R^{-/-}$ mice, a viral model of rapid demyelination (Howe et al., 2007). However, despite the significant advances in our understanding of TMEV pathogenesis in recent years, the precise mechanisms underlying axonal neurodegeneration in TMEV infection remain unclear in terms of timing of occurrence, or if it is the cause or the consequence of demyelination. Demyelination may be necessary but not sufficient condition for motor deficits associated to TMEV infection. The idea most consolidated is that demyelination creates a permissive environment wherein the naked axon becomes susceptible to immune-mediated injury.

Although axonal degeneration is considered the end result in most neuropathologies, experimental and clinical findings demonstrate that axonal degeneration can itself trigger secondary

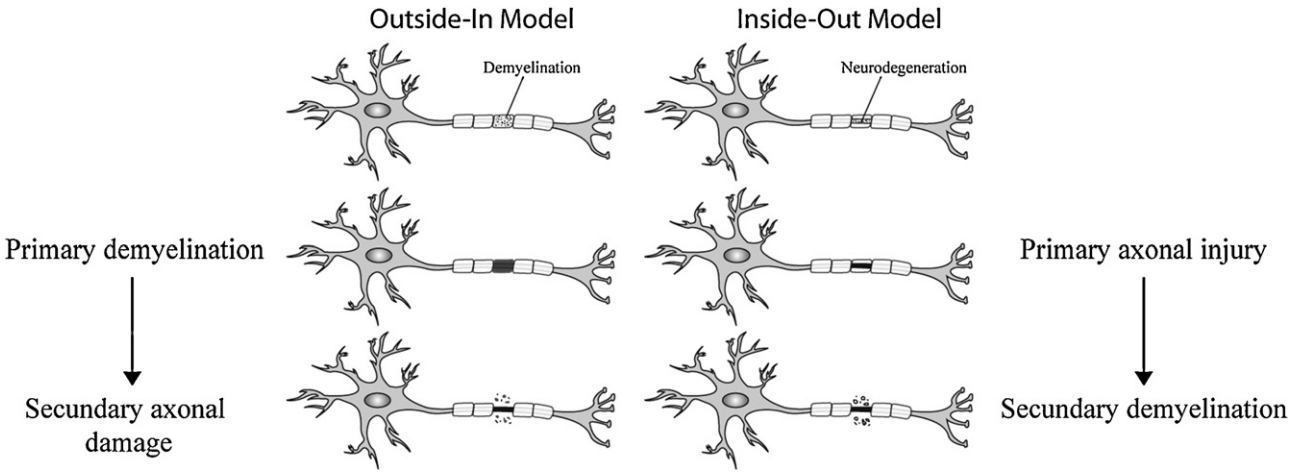

Fig. 3. Models proposed to explain the axonal degeneration and demyelination in the TMEV model. In the Outside-In model, the lesion develops from the outside (myelin) and extends inwards (to the axon). Myelin and/or oligodendrocytes represent the primary targets for injury and after the primary destruction of myelin axonal damage is a secondary consequence. In the Inside-Out model, lesions extend from the axon to myelin. The primary target in this scenario is the axon or its cell body, the neuron, and the primary axon injury drives secondary demyelination.
Adapted from Tsunoda and Fujinami (2002).

pathological events, such as oligodendrocyte apoptosis. In cases of axonal degeneration induced by spinal cord injury at sections distal to the transection site (Wallerian degeneration), oligodendrocyte apoptosis is observed along the degenerating fiber tracts and it even extends into regions at a distance from the lesion (Tsunoda et al., 2007a). Multiple mechanisms have been implicated in oligodendrocyte apoptosis, including microglial activation with the release of cytotoxic mediators, glutamate mediated excitotoxicity and the disruption of cross-talk between axons and oligodendrocytes (Jack et al., 2007; Li et al., 2005; Matute et al., 2001).

According to the inside-out model, TMEV first infects neurons in the gray matter of the CNS, damaging their axons, which in turn leads to the degeneration of the distal stumps of the axons of the spinal cord as early as one week after DA infection. In the spinal cord, axon degeneration and/or apoptotic oligodendrocytes may activate local microglia and macrophages, which can induce demyelination through the secretion of inflammatory cytokines, chemokines and proinflammatory mediators. Activated microglia and macrophages phagocytose degenerated oligodendrocytes, myelin and axons, resulting in persistent viral infection and tissue damage. Infected degenerated oligodendrocytes phagocytosed by macrophages/microglia may contribute to persistence of immune viral responses by presenting viral antigens or myelin epitopes to T cells. However, several important questions remain unanswered in this model, including: what are the neuronal populations and specific brain structures that contribute to such rapid axonal degeneration in the spinal cord. Like the murine neurotropic virus, TMEV uses axonal transport to spread through the CNS. As such, the axonal degeneration observed in the host may represent a protective response to prevent TMEV from spreading, further blocking disease expansion (Tsunoda et al., 2007b; Tsunoda, 2008). However, no definitive data have demonstrated that this specific sequence of events occurs. Chronic spinal cord pathology and axonal injury may occur as a result of virus-induced autoimmunity (Miller et al., 1997), or due to bystander damage by microglia/macrophages or T cells present in the immune repertoire (Rodriguez et al., 1996). It should be noted that demyelination predisposes bare axons to subsequent injury by cytokines, inflammatory cells and nitric oxide, thus creating a microenvironment conducive to possible irreversible deficits. Not all axonal degeneration in MS can be explained by the two models proposed above. It is likely that both scenarios exist at different stages and contribute to the development of an immunopathological cycle leading to continuous disease progression (Fig. 3).

An important aspect not considered in the aforementioned studies is the temporal pattern of brain pathology following TMEV infection in susceptible SJL/J mice. In the classical TMEV model, the disease induced by the less virulent viral strains (DA and BeAn) is characterized by transient meningoencephalomyelitis, which peaks about 7 dpi and clears from the brain after approximately 3 weeks. This gives way to a chronic demyelinating stage with progressive neurological deficits in which most of demyelinating lesions affect the spinal cord (Dal Canto et al., 1996). Accordingly, we will focus our discussion on the expression of specific markers for microglia (Iba-1; Wako Pure Chemical Industries, Ltd., Osaka, Japan), axonal density (pan neurofilament; Enzo Life Sciences UK Ltd., UK) and the myelin protein CNPase (myelin 2′,3′-cyclic nucleotide 3′-phosphodiesterase; Sternberger Monoclonals Incorporated, MD, USA) at various time points following TMEV infection with the DA strain in two brain structures, the motor cortex and brainstem.

The expression profile of microglia staining in the motor cortex of TMEV-infected mice reveals that as expected, the highest levels of activation occur in the acute phase (Oleszak et al., 2004) due to the injection of virus. However, in the chronic stages (180 dpi), microglia activation is greater than in sham animals (Fig. 4), accompanied by a decrease in axonal density (staining with pan neurofilament) and reduced expression of CNPase (Fig. 5). Therefore, in the chronic phases of TMEV infection might be occurring cortical demyelination and activation of microglia, as described in MS patients, but it is necessary to perform additional histological analysis with Luxol fast blue and electronic microscopy to reinforce the possibility of cortical demyelination presence in the TMEV model. In the brainstem, two waves of microglial activation occur in the acute and chronic phases (180 dpi), indicating that inflammation persists throughout the disease course (Fig. 6). Moreover, we observed decreased axonal density in the brainstem and loss of CNPase immunoreactivity in the early preclinical phase (35 dpi) and in the late phase (180 dpi), indicating that brain pathology persists after the acute phase of the disease (Fig. 7). These findings indicate that in the classic SJL/J mouse model of demyelination, brain pathology occurs in specific brain areas, including the cortex and brainstem, demonstrating

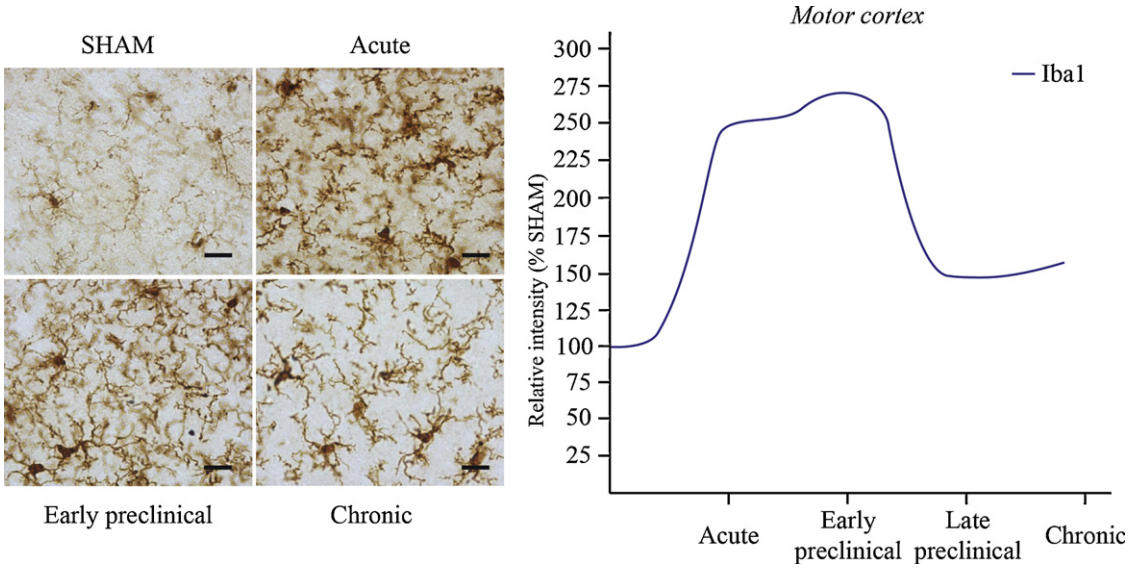

Fig. 4. Microglial activation in the motor cortex of TMEV-infected susceptible SJL/J mice. (A) Representative sections (frozen sections obtained by criostate) of the motor cortex showing microglia staining by Iba-1 in sham mice and in the acute (19 dpi), early preclinical (35 dpi) and chronic phases (180 dpi) of TMEV-IDD. Scale bars 100 μm. (B) Scheme showing the time course of Iba-1 expression throughout the disease course in susceptible SJL/J infected mice.

that inflammation associated with viral inoculation is not resolved within 3 weeks, as previously proposed (Oleszak et al., 2004). Our findings also suggest that disease signs observed during the chronic phase of the disease may be not exclusively due to the spinal cord pathology.

As expected, we observed that TMEV-infected mice exhibit spinal cord inflammation, demyelination, axonal loss and motor deficits in the chronic stages (Arévalo-Martín et al., 2003; Loría et al., 2010; Ortega-Gutiérrez et al., 2005). Pathological alterations in the brain, including those affecting the gray matter structures, have recently been described in the EAE model (immunization with MOG_{35-55}), mainly in the cortex, striatum and cerebellum (Centonze et al., 2009; MacKenzie-Graham et al., 2009; Mandolesi et al., 2012). However, few studies of the expression of markers of inflammation and neurodegeneration in the brain of TMEV-infected mice have demonstrated a dynamically regulated temporal expression pattern after viral infection. In an analysis of the expression of the protease Kallikrein 6 in the TMEV-IDD model, brain pathology was described at early (40 dpi) but not late chronic stages (180 dpi: Scarisbrick et al., 2012), in contrast to our findings. Using a grading scale of 0–4 to quantify the pathological parameters (Denic et al., 2011; Pavelko et al., 2007), the higher magnitude of brain pathology observed in our study is likely to be due to the higher viral dose used: 2×10^6 plaque-forming unit (pfu) (brain pathology at 180 dpi) *vs* 2×10^5 pfu (no brain pathology at 180 dpi). Interestingly, another study in the TMEV model reported $CD45^+$ cell activation, a marker of inflammation, in the forebrain, which was mainly observed in the subventricular zone, but also in the anterior cortex, hippocampus, brainstem and other brain areas during the preclinical, early onset and chronic phases of the disease (Goings et al., 2008). However, the highest levels of activation were observed in the cervical spinal cord during the chronic phase. These observations raise novel questions about the existence of pathological changes exclusively in the spinal cord during chronic phases of the disease, and suggest that alterations in brain structures may also contribute to the neurological deficits that have been classically associated with spinal cord demyelination/neurodegeneration. In fact, recent neuroimaging studies

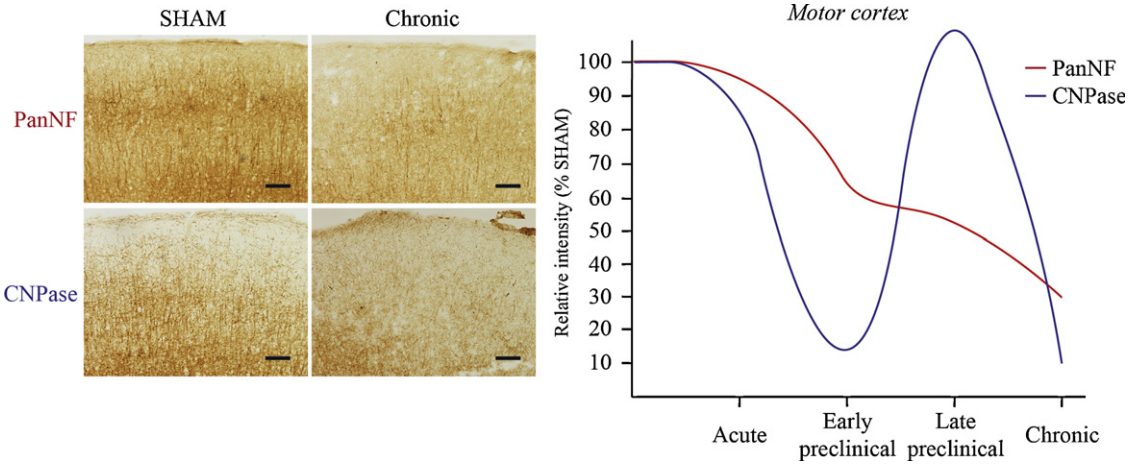

Fig. 5. Axonal density and myelin expression in the motor cortex of TMEV-infected susceptible SJL/J mice. (A) Representative sections (frozen sections obtained by criostate) of the motor cortex showing axonal density (pan neurofilament: pan NF staining) and CNPase expression (Scale bars: 200 μm) in sham animals and TMEV-infected mice during the chronic phase (180 dpi). (B) Scheme showing the time course of pan NF and CNPase expression throughout the disease course in susceptible SJL/J infected mice.

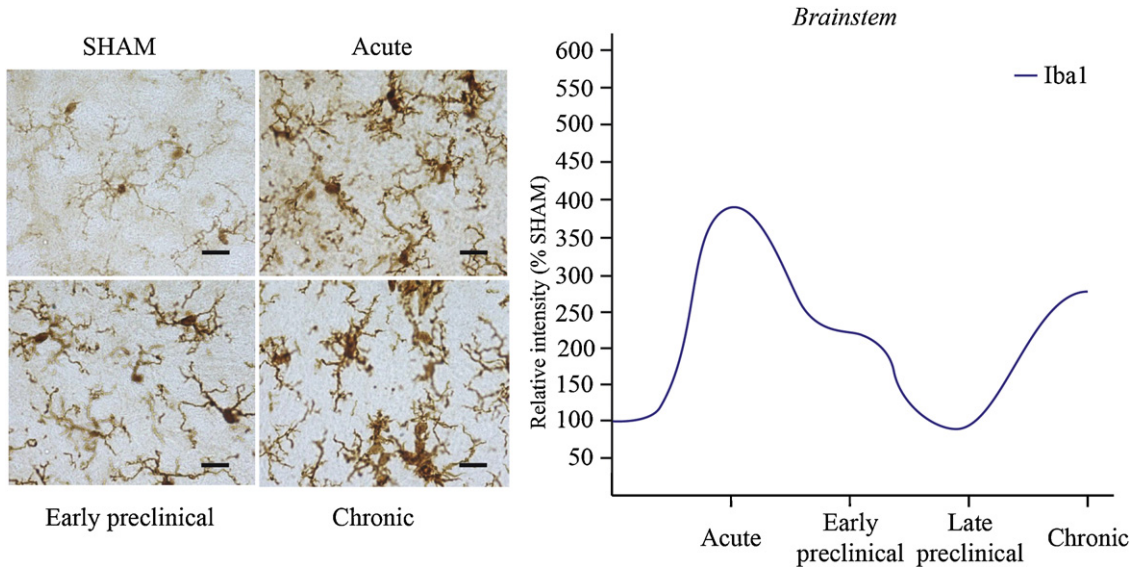

Fig. 6. Microglial activation in the brainstem of TMEV-infected susceptible SJL/J mice. (A) Representative brainstem sections (frozen sections obtained by criostate) showing microglia stained with Iba-1 in sham mice and in the acute (19 dpi), early preclinical (35 dpi) and chronic phases (180 dpi) in TMEV-IDD mice. Scale bars: 100 μm. (B) Scheme showing the time course of Iba-1 expression throughout the disease course in susceptible SJL/J infected mice.

showed the presence of T2 hypointensity in deep gray nuclei, most notably the thalamus, showing a strong correlation with disability (Pirko et al., 2009). Additional volumetric MRI studies demonstrate that significant brain atrophy accompanies the development of the progressive TMEV-induced disease. In that case also brain atrophy correlates with disability assessed by rotarod assay (Pirko et al., 2011). Other studies related to TMEV infection in other strain of mice such as the C57/BL6 strain showed severe hippocampal degeneration and spontaneous seizures at 2 months post-infection indicating that viral infection of the CNS can lead to long-term neurologic defects, including increased risk for the development of epilepsy (Stewart et al., 2010).

2.2. Remyelination in TMEV-IDD

Various theories have been advanced to explain the oligodendrocyte damage observed in MS lesions. The prevailing, albeit circumstantial, view is that this damage is incurred *via* a variety of immunological mechanisms, involving anti-MOG Abs, the production of proinflammatory cytokines by monocytes/macrophages and lymphocytes, T-cell-mediated injury (*via* $CD8^+$ MHC class I-restricted cytotoxicity), immunoglobulins and activated complement components, apoptosis and other oligodendrogliotoxic factors (Dhib-Jalbut, 2007).

An important question is whether myelin repair can restore neurological function following a progressive CNS demyelinating disease that induces axonal damage. In human MS, remyelination can occur after demyelination (Hirano, 1989) and may contribute to the recovery observed after exacerbation (McDonald, 1974). In early MS lesions, myelin repair mediated by oligodendrocytes (Brück et al., 1994) and Schwann cells can occur. Indeed, in patients with recent onset of MS, oligodendrocytes are found at high densities in lesions (Brück et al., 1994; Raine et al., 1981), while the number of oligodendrocytes is decreased and limited remyelination occurs in the lesions of patients who have had MS for many years (Ozawa et al., 1994). Remyelination frequently occurs following recruitment and repopulation of the plaque by oligodendrocytes, and migration of oligodendrocyte precursor

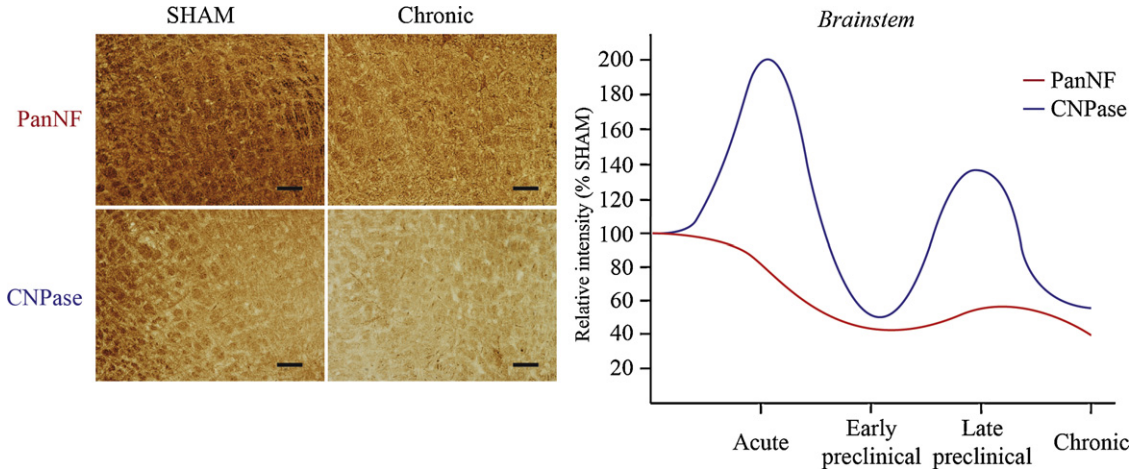

Fig. 7. Axon density and myelin expression in the brainstem of TMEV-infected susceptible mice. (A) Representative brain stem sections (frozen sections obtained by criostate) showing axon density (pan neurofilament: pan NF staining) and CNPase expression (Scale bar: 200 μm) in sham animals and in TMEV-infected mice during the chronic phase (180 dpi). (B) Scheme showing the time course of Pan NF and CNPase expression throughout the disease course in susceptible SJL/J infected mice.

cells toward demyelinated lesions has been reported recently (Blakemore, 2008; Nait-Oumesmar et al., 2007). As a rule, new lesions undergo remyelination to varying degrees, which is either interrupted or confounded by recurrent activity. Depletion of oligodendrocytes or their progenitors may be one explanation for the lack of myelin repair observed following chronic CNS demyelination (Franklin, 2002). Thus, enhancing remyelination is an important therapeutic strategy for MS that requires further study.

Although remyelination can occur in the TMEV-IDD model, it is generally incomplete. Lesions from chronically infected susceptible mice reveal minimal spontaneous myelin repair (Bieber et al., 2005; Dal Canto and Lipton, 1975; Rodriguez and Lennon, 1990), despite an increase in the number of progenitor cells (Prayoonwiwat and Rodriguez, 1993). Dysregulation of oligodendroglial progenitor cell differentiation may account for the lack of regeneration in this MS model (Ulrich et al., 2008). As observed in MS, there is clear ultrastructural evidence of attempted but generally abortive remyelination in TMEV-IDD, and different approaches have indicated that immune responses may underlie the lack of remyelination. Pioneering work described that remyelination in mice infected with the DA strain appears late and is incomplete (Dal Canto and Lipton, 1975), however, inoculation of the cell adapted WW strain of TMEV to outbred CD1 mice develop a remitting relapsing course characterized by extensive remyelination during the periods of remission (Dal Canto and Barbano, 1984; Dal Canto and Lipton, 1980). In that case authors show that Schwann cells, in association with a lack of gliosis, were the predominant myelinating cells in the outer white matter, but oligodendrocytes were numerous and very active in the inner portions of the spinal cord column being able to complete remyelination. It seems that the host inflammatory response to DA virus strain is responsible for myelin injury subsides, impeding remyelination, in this TMEV model (Dal Canto and Lipton, 1980). Classical studies using immunosuppressive drugs such as cyclophosphamide demonstrated that myelin repair is enhanced (Rodriguez and Lindsley, 1992), suggesting that myelin repair may be a natural phenomenon that is suppressed by a chronic inflammatory response to Ags present in the CNS.

There are currently no therapeutic strategies available to promote remyelination and enhance repair mechanisms in MS. However, experimental data in the TMEV-IDD model gave rise to the hypothesis that immunoglobulins may promote remyelination. Thus, immunoglobulins directed against spinal cord homogenates and intravenously administered polyclonal immunoglobulins (IVIg) facilitate remyelination in the TMEV model (Rodriguez et al., 1987a; Rodriguez and Lennon, 1990). There is no doubt about the immunomodulatory and anti-inflammatory properties of IVIgs that might be the basis for their capacity to promote remyelination in TMEV-IDD. Although IVIgs do not influence the function of oligodendroglial cells *in vitro* (Warrington et al., 2000) could protect oligodendrocytes against complement-mediated injury and thus provide more cells that could engage in remyelination. In addition, IVIgs could modulate microglial function, may be creating a microenvironment permissive for remyelination. In additional studies by Dr. Rodriguez's group, monoclonal antibodies (mAbs) generated against spinal cord antigens were generated and tested in TMEV-infected mice. The first remyelinating-promoting mAb identified was SCH 94.03 (Miller et al., 1994). In the process of characterizing this IgM, they found that it bound to glycolipids on the surface of live oligodendrocytes. A second mouse IgMkappa monoclonal antibody (mAb) (SCH79.08) raised against normal mouse spinal cord homogenate, reacts with myelin basic protein and also promotes remyelination. Because these two mAbs recognize different oligodendrocyte antigens, several previously identified oligodendrocyte-reactive IgMkappa mAbs (O1, O4, A2B5, and HNK-1), each with distinct antigen specificities, were evaluated and found to promote remyelination (Asakura et al., 1995, 1998). Interestingly, these mAbs had relatively conserved germline sequences suggesting that the mechanism of action of IgMs to facilitate remyelination requires recognition of oligodendrocyte plasma membrane lipids. The shared characteristics of these mAbs are an IgM isotype and the capacity to bind oligodendrocytes. The following step was the identification of human antibodies that promote remyelination on the basis that natural antibodies present in human population bound oligodendrocytes (Rodriguez et al., 2009; Warrington et al., 2000). Two humans IgMs (sHIgM22 and sHIgM46) promoted significant remyelination in TMEV-infected mice. These human monoclonal Abs that bind to the surface of rat and human oligodendrocytes were shown to exhibit remyelinating properties not only in the TMEV model but also in lysolecithine-induced demyelination model (Bieber et al., 2002; Warrington et al., 2004, 2007). When testing IVIG and polyclonal human IgM for the ability to promote remyelination in the TMEV model, it was clear that polyclonal IgMs and the two human monoclonal IgMs were superior. IVIG may be effective in the TMEV model by modulating the immune response, whereas the IgMs may act directly on the cells of the nervous system (Warrington et al., 2000). It is proposed that the mechanism of action of IgMs is through lipid microdomain signaling by transient calcium influx in glial cells (Soldán et al., 2003), however although it is clear that IgM-mediated repair required binding to specific antigens on the oligodendrocyte membrane, initiating a signal that may result in oligodendrocyte proliferation and/or protection, the exact mechanism of action is not fully understood. Importantly, these studies suggest that oligodendrocyte-reactive natural autoantibodies may provide a powerful therapeutic way to induce remyelination in multiple sclerosis patients.

Clinical trials with IVIg have so far failed to demonstrate clinical improvement in MS patients, although these studies only employed IgGs preparations. However, recent experimental data from *in vivo* and *in vitro* studies underline the importance of IgM for remyelination (Trebst and Stangel, 2006; Wright et al., 2009). Further clinical trials will therefore be required to evaluate the remyelination potential of IgM in human disease. The design of monoclonal Abs capable of promoting remyelination provides a basis for the development of new specific therapies derived from biological products such as polyclonal immunoglobulins (Warrington and Rodriguez, 2010).

3. The TMEV model: a preclinical model of MS

No effective cure has yet been designed for MS, although several genetic and environmental risk factors have been identified and treatments are now available that effectively modify the disease course. However, to elucidate the mechanism of disease onset and progression, and to evaluate therapeutic and reparative approaches, new animal models of MS are needed. As MS is a complex disease of unknown etiology a single animal model is unlikely to represent all pathological and clinical features. In addition to EAE, TMEV-IDD represents a valid autoimmune model of primary progressive MS with viral etiology that has been used for preclinical drug-screening (Denic et al., 2010). Indeed, it is an ideal model to screen drugs designed to promote remyelination and protect axons.

3.1. Therapies developed in the TMEV-IDD

The ultimate aim of research using animal models of human disease is to understand the pathogenesis of the disease and translate these findings into rational therapeutic approaches. The two main animal models of MS, EAE and TMEV-IDD, have improved our understanding of this disease and facilitated the development of clinical therapies. While both models exhibit

many of the clinical and histological features of MS, it should be stressed that MS is a uniquely human disease. The analysis of therapeutic success or failure may aid the development of more directed, effective treatments for MS, with fewer adverse effects. Despite extensive screening for new targets in EAE and TMEV-IDD models, only a few of the established MS therapies have been developed in animal models. Successful therapies developed in the EAE model include glatiramer acetate (first line therapy), mitoxantrone and natalizumab (second line therapies: Kieseier and Hartung, 2003; Steinman and Zamvil, 2005). In the TMEV model anti-adhesion molecule therapy was efficacious too (Inoue et al., 1997). One of the most successful therapies for MS is interferon (IFN) β, a treatment classically linked to viral infection and resistance (Paty and Li, 1993). Analysis of IFN-β treatment in the TMEV model demonstrated its modulation of MHC class I expression in the brain (critical for $CD8^+$ T cells) and identified its short and long-term effects (Njenga et al., 2000). Intravenous administration of polyclonal human immunoglobulins exerts reparative effects in the TMEV model (Warrington et al., 2007). Natural auto-Abs were recently developed that bind to oligodendrocytes and stimulate these cells to produce new myelin (Warrington and Rodriguez, 2010). However, it takes many years to translate an effective therapeutic treatment from the bench to the bedside, and despite the differences between humans and animals, it is unlikely that any progress in the treatment of MS will be possible without the knowledge obtained from animal research. We compare (Table 2) the outcome of MS related clinical trials to outcome in the TMEV model showing that TMEV-IDD may be an accurate predictor of response to therapies.

3.2. Unsuccessful MS therapies

Unfortunately, many agents that effectively modify disease activity in the animal models have no therapeutic effects or produce adverse effects in MS patients. These discrepancies led to the publication of several papers suggesting that the animal models used, particularly the EAE model, are not accurate models of MS (Sriram and Steiner, 2005; Steinman and Zamvil, 2005). There may be several explanations for these divergent findings, including genetic (species differences, characteristics of inbred animals), pathogenetic (individual variations between MS patients), environmental and even kinetic differences (different ontogeny and biorhythms, temporal differences in immune reactivity and response to therapies) between animal models and humans. Nonetheless, important aspects of the etiopathogenesis of MS, including susceptibility genes, mechanisms of immune cell activation, immunoregulatory circuits, and mechanisms of axonal damage and repair have been elucidated by studying animal models. Moreover, several emerging MS therapies are currently in the preclinical testing phase in EAE or TMEV-IDD models (Weber et al., 2007). However, further improvements in the animal models used to study MS are required so that they may better reflect the pathogenesis and heterogeneity of this disease (Baker and Amor, 2011; Moreno et al., 2012).

4. Revising concepts and perspectives

In the last decade, the concept that TMEV-IDD constitutes a chronic progressive inflammatory disease of the CNS, with spinal cord demyelinating lesions and secondary neurodegeneration, has been challenged by evidence suggesting that axonal damage may occur independently of demyelination. In addition to the more commonly described white matter demyelination and axonal atrophy in the spinal cord, lesions can also affect the gray matter, both in the acute polioencephalomyelitic phase (acute phase) and the late chronic phase, in which brain pathology is detected in areas including the motor cortex and brainstem.

Several findings have enhanced the interest in the role of $CD8^+$ T cells in MS. In TMEV-IDD, $CD8^+$ T cells play dual roles. Resistant strains of mice mount a strong $CD8^+$ T cell response to eliminate the virus from the brain and prevent spinal cord pathologies (Mendez-Fernandez et al., 2003). In susceptible strains, $CD8^+$ T cells help to clear the virus from the CNS during the acute phase of TMEV-induced disease, thereby fulfilling a partially protective role. However, during the chronic phase, both $CD4^+$ and $CD8^+$ T cells exert harmful effects (Deb et al., 2009, 2010). Recent studies support the view that $CD8^+$ T cells injure denuded axons after recognizing axonal MHC class I. Accordingly, deletion of β-2 microglobulin ameliorates spinal cord lesion load in one of two susceptible mouse strains, preserving axons and motor function, and promoting recovery of brainstem N-acetylaspartate (NAA) levels. These findings point to $CD8^+$ T cells as the primary mediator of axonal injury in the TMEV model (Denic et al., 2012) and suggest that these cells may induce long term injury in MS.

Natural regulatory T cells (nTregs – $CD4^+CD25^+Foxp3^+$) generated in the thymus can limit the activation, trafficking, and effector functions of $CD4^+$ and $CD8^+$ T cells. Recently, several TMEV-IDD studies have investigated whether these cells can mediate genetic susceptibility to TMEV-induced autoimmune disease, resulting in increased activation of Tregs in susceptible mice, which may dampen the virus-specific CTL response. Inactivation of Tregs prior to infection of susceptible mice results

Table 2
Comparison of treatment efficacies in Theiler's murine encephalomyelitis virus and multiple sclerosis.

Treatment	Treatment effect in TMEV	Reference	Treatment effect in MS	Reference
IFN-β	Effective	Njenga et al. (2000)	Effective	IFNβ MS Study Group, Neurology 1993 Paty and Li (1993)
IFN-γ	Effective	Rodriguez et al. (2003)	Exacerbated	Panitch et al. (1987)
Glatiramer acetate	Effective	Ure and Rodriguez (2002)	Effective	Johnson et al. (1995)
Linomide	Ineffective	Drescher et al. (1998)	Toxic	Noseworthy et al. (2000)
Anti-TNF α	Exacerbated	Paya et al. (1990)	Exacerbated	Lenercept MS Study Group, Neurology 1999
Intravenous immunoglobulin	Effective	Rodriguez and Lennon (1990)	Effective	Fazekas et al. (1997)
Anti-CD4	Effective	Welsh et al. (1987)	Ineffective	Van Oasten et al. (1997)
Anti-CD8	Effective	Rodriguez and Sriram (1988)	Untested	–
Cyclophosphamide	Effective	Lipton and Dal Canto (1976) and Rodriguez and Lindsley (1992)	Moderately effective	Weiner et al. (1993)
Cyclosporine	Moderately effective	Rodriguez and Quddus (1986)	Moderately effective	Cyclosporine MS Study Group, Annals of Neurology 1990
Anti-VLA4	Effective	Inoue et al. (1997)	Effective	Fernandez et al. (2012) and Melin et al. (2012)
Cannabinoid derivatives	Effective	Croxford and Miller (2003) and Arévalo-Martín et al. (2003)	Moderately effective	Zajicek et al. (2003)

in delayed disease onset and progression, increased viral clearance, and increased adaptive antiviral CD4, CD8 and Ab immune responses (Richards et al., 2011). These findings indicate that Tregs may control genetic susceptibility to infection-induced autoimmune disease and provide a novel potential mechanism to explain the induction of a variety of human autoimmune diseases secondary to viral or bacterial infection. The preferential expansion of Tregs in TMEV-infected SJL/J mice, as compared with disease-resistant C57BL/6 mice, suggests that in some instances, genetic susceptibility to certain autoimmune diseases may occur due to 'regulatory mimicry'. In such a scenario a clonotype of nTregs expressing a self-antigen-specific T cell receptor on a particular MHC background could be activated and expand in response to a cross-reactive epitope expressed by an infectious agent. This expanded population of Tregs could then delay the clearance of the infectious agent in the target organ of the immune-mediated disease, promoting the development of autoimmunity *via* mechanisms such as molecular mimicry or epitope spreading.

In summary, although the TMEV-IDD model presents both advantages and disadvantages, it is undoubtedly a valid model for preclinical studies of MS. This model allows the initial viral exposure and clearance to be analyzed, as well as the progression from immune system viral recognition to autoimmunity. The attack and destruction of axons by $CD8^+$ T cells has been demonstrated using the TMEV model and there is strong circumstantial evidence suggesting that a similar process occurs in MS. Moreover, axonal injury may precede demyelination following TMEV infection, resulting in the release of neuroantigens and the induction of autoimmune responses. As such, the TMEV-IDD model provides a highly useful tool to determine whether axonal degeneration itself can contribute to secondary neuropathologies, as suggested for MS. Both the EAE and TMEV models have proven useful in the development of clinical therapies for MS, and further integration of these models will help advance our understanding of the etiology and treatment of MS.

Conflict of interest

Authors declare no conflict of interest.

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