Published in final edited form as:
Pathophysiology. 2011 February ; 18(1): 31–41. doi:10.1016/j.pathophys.2010.04.011.

Theiler's Virus Infection: Pathophysiology of Demyelination and Neurodegeneration

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Abstract

Multiple sclerosis (MS) has been suggested to be an autoimmune demyelinating disease of the central nervous system (CNS), whose primary target is either myelin itself, or myelin-forming cells, the oligodendrocytes. Although axonal damage occurs in MS, it is regarded as a secondary event to the myelin damage. Here, the lesion develops from the myelin (outside) to the axons (inside) “Outside-In model”. The Outside-In model has been supported by an autoimmune model for MS, experimental autoimmune (allergic) encephalomyelitis (EAE). However, recently, 1) EAE-like disease has also been shown to be induced by immune responses against axons, and 2) immune responses against axons and neurons as well as neurodegeneration independent of inflammatory demyelination have been reported in MS, which can not be explained by the Outside-In model. Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease (TMEV-IDD) is a viral model for MS. In TMEV infection, axonal injury precedes demyelination, where the lesion develops from the axons (inside) to the myelin (outside) “Inside-Out model”. The initial axonal damage could result in the release of neuroantigens, inducing autoimmune responses against myelin antigens, which potentially attack the myelin from outside the nerve fiber. Thus, the Inside-Out and Outside-In models can make a “vicious” immunological cycle or initiate an immune cascade.

Keywords

Apoptosis; Autoimmunity; Microglia; Mouse Wld protein; Picornaviridae infections; Wallerian degeneration; CD4-Positive T-Lymphocytes; CD8-Positive T-Lymphocytes

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Introduction; anti-myelin autoimmunity in multiple sclerosis, the Outside-In model

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) [1–3]. In the United States, MS affects greater than 350,000 people with a prevalence rate of 85/100,000 persons and a ratio of women to men of 2.6:1 [4]. Although the precise etiology of MS is unknown, MS has been thought to be an immune-mediated disease, in which autoimmune responses against myelin antigens lead to production of inflammatory cytokines and chemokines, and upregulation of adhesion molecules, contributing to the pathogenesis of MS [5–9]. The autoimmune etiology of MS has been supported by an animal model for MS, experimental autoimmune (allergic) encephalomyelitis (EAE) [10].

In EAE, demyelination is induced by anti-myelin autoimmune responses, where both cellular ($CD4^+$ and $CD8^+$ T cells) and humoral immune responses play pathogenic roles (Table 1). $CD4^+$ T cells recognize antigens presented by major histocompatibility complex (MHC) class II on antigen presenting cells (APCs). In most EAE models, $CD4^+$ T helper (Th) 1 cells initiate CNS inflammation via delayed-type hypersensitivity (DTH) responses to myelin antigens in the presence or absence of epitope (determinant) spreading [11–14]. Myelin antigen-specific Th17 cells, a novel subset of $CD4^+$ T cells, also play an important role in the induction of EAE [15–17]. Interactions between these $CD4^+$ T cells and CNS APCs (i.e., microglia and macrophages) most likely damage myelin sheaths and myelin forming cells, oligodendrocytes, indirectly by production of cytotoxic factors, such as proinflammatory cytokines, since oligodendrocytes do not express MHC class II molecules [18]. Interferon (IFN)-γ and interleukin (IL)-17 are the major effector cytokines of Th1 and Th17 cells, respectively.

In some EAE models, MHC class I-restricted myelin-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) have been shown to induce an EAE-like disease [3,19,20]. In these models, myelin sheaths could be damaged by $CD8^+$ T cells either directly or indirectly. Oligodendrocytes can express MHC class I molecules during inflammation, whereas resting oligodendrocytes do not express MHC class I molecules [18,21]. Anti-myelin antibodies have also been shown to play a key role in some EAE models of primary progressive (PP-MS) and secondary progressive MS (SP-MS), where antibody deposition in the CNS and serum anti-myelin antibody responses were associated with disease progression [22]. Co-transfer of auto-antibodies with myelin-specific autoreactive T cells could also exacerbate EAE [23].

Since antibodies against myelin-specific antigens as well as autoreactive T cells have also been identified in MS patients [5,24], CNS lesions in MS have been hypothesized to be induced by autoimmune responses against myelin sheaths as shown in EAE. In this theory, the primary target in MS is myelin itself (myelinopathy) or the oligodendrocytes (oligodendrogliopathy). Axonal degeneration, which is demonstrated in MS and EAE, is regarded as secondary damage following myelin destruction [25–27]. In this process, the lesion develops from the myelin (outside) to the axons (inside) “Outside-In model” [28,29]. In this Outside-In autoimmune model, immune responses against myelin and oligodendrocytes are initiators of CNS damage (Table 1).

Anti-axon autoimmunity in MS and EAE, the Inside-Out model

Recently, gray matter involvement and axonal damage in normal-appearing white matter (NAWM) have been demonstrated in MS [26,29–33]. Magnetic resonance spectroscopy (MRS) has been used to detect the decreased N-acetylasparate (NAA) signal, indicating

axonal and neuronal injury in the NAWM [34]. In addition, auto-antibodies against axonal and neuronal antigens, including contactin-2/transiently expressed axonal glycoprotein 1 (TAG-1), neurofilament light chain (NF-L), and neurofascin, have been identified in the serum and cerebrospinal fluid (CSF) of MS patients [5,35–40]. Derfuss et al. [41] showed contactin-2/TAG-1 as an auto-antigen for both Th1/Th17 cells and antibodies in MS patients. In MS, $CD8^+$ CTLs may also damage axons and neurons directly or indirectly; axons and neurons in demyelinating lesions have been shown to express MHC class I by some, but not all, research groups [18,42].

In animals, NF-L- and neurofascin-specific autoreactive T cells and autoantibodies have also been shown to induce axonal degeneration and gray matter inflammation with mild demyelination (Table 2) [40,41,43,44]. Interestingly, mice immunized with axonal and neuronal antigens demonstrated different lesion distribution, compared with those immunized with myelin proteins. For example, immunization with NF-L preferentially induced lesions in the dorsal funiculus of the spinal cord, whereas immunization with myelin oligodendrocyte glycoprotein (MOG) preferentially led to lesions in the lateral and ventral funiculi of the spinal cord [41,45]. Therefore, both in MS and EAE, autoimmune responses against axonal and neuronal antigens could lead to primary axonal and neuronal damage in the CNS, resulting in secondary demyelination. In this theory, the lesion develops from the axons (inside) to the myelin (outside) “Inside-Out model” [28,29]. The Inside-Out model can explain early neurodegeneration, including gray matter involvement and axonal degeneration in NAWM, in some patients with MS and as well as in animal models.

Immune-mediated demyelination in TMEV infection

Environmental factors, particularly viral infections, have been associated with induction or exacerbation of MS [46–49]. Viruses, including human endogenous retrovirus (HERV), Epstein-Barr virus, and human herpesvirus 6, have been linked with MS pathogenesis [50–54]. A mouse model of MS, Theiler's murine encephalomyelitis virus (TMEV) infection, has been widely used to elucidate this possible viral etiology [10,55]. TMEV is a non-enveloped, positive sense, single stranded RNA virus [56]. TMEV belongs to the genus Cardiovirus, family Picornaviridae, and is divided into two subgroups, GDVII and Theiler's original (TO), based on neurovirulence in mice [56]. Intracerebral infection with the GDVII subgroup leads to an acute fatal polioencephalomyelitis in all mouse strains. Infected mice show weight loss and encephalitic signs, including a hunched back and ruffled fur, and die within 10 days [57].

On the other hand, infection with the TO subgroup, such as Daniels (DA) and BeAn strains, causes a biphasic disease [57]. During the acute phase, 1 week postinfection, TMEV infects neurons, and infected mice develop acute polioencephalomyelitis pathologically. But most mice are clinically asymptomatic, and the pathological changes resolve by 2 weeks after infection [57]. Thereafter, virus clearance or persistence depends on the strain of mice. Resistant mouse strains, such as BALB/c and C57BL/6 mice, develop little or no chronic disease, since virus is eradicated from the CNS [58,59]. In contrast, susceptible mouse strains, such as SJL/J mice, develop a chronic inflammatory demyelinating disease in the white matter of the spinal cord with virus persistence in glial cells and macrophages, 1 month after infection (chronic phase) [60].

In TMEV infection, immunopathology (immune-mediated tissue injury) has been shown to play a pathogenic role in the CNS (Table 3). Cellular immune responses seem to play both protective and pathogenic roles in TMEV infection. $CD4^+$ T cells have a vital protective role in the early stage postinfection; mice depleted of $CD4^+$ T cells prior to infection with TMEV die within 3–5 weeks [61]. However, during the early chronic phase, $CD4^+$ T cell-mediated

DTH responses against virus in the CNS have been proposed to damage myelin sheaths in a "bystander" fashion [62]. During the late chronic phase, $CD4^+$ T cell responses against myelin antigens, such as myelin proteolipid protein (PLP), can be induced by epitope spreading, and this autoimmunity has been suggested to exacerbate demyelination [63,64].

$CD8^+$ T cell responses have also been associated with both pathogenesis and viral clearance in TMEV infection [65,66]. $CD8^+$ MHC class I-restricted virus-specific CTLs have been shown to contribute to viral clearance. However, TMEV infection can result in induction of autoreactive CTLs that recognize both virus and host antigens, which can potentially lead to CNS pathology [66–68]. Similar to cellular immune responses, humoral immune responses against TMEV can play dual roles. Although anti-TMEV antibody contributes to viral clearance [69], anti-TMEV antibody has been demonstrated to cross-react with a major myelin lipid component, galactocerebroside, and passive transfer of anti-TMEV antibody results in augmentation of demyelination in mice with EAE [62,70].

TMEV persistently infects macrophage/microglia lineage cells, oligodendrocytes and astrocytes during the chronic phase [56,71]. Macrophages have been suggested to play an effector role in demyelination, since 1) depletion of macrophages ameliorates TMEV-induced demyelination and 2) intracerebral inoculation with a TMEV-infected macrophage cell line induces acute focal demyelination [57,72,73].

Oligodendrocyte death in TMEV infection

On the other hand, virus-induced pathology (direct infection of neuronal cells) can also play a role in demyelination during TMEV infection. Since TMEV infects oligodendrocytes, the myelin-forming cells, both in vivo (during the chronic phase) and in vitro, direct lytic infection of oligodendrocytes could result in demyelination in the absence of immune cells. Roos et al. demonstrated that nude mice, which have a limited T cell response, developed demyelinating lesions following TMEV infection [74]. While the precise mechanism of oligodendrocyte death in vivo is not clear, TMEV leader (L) protein, but not capsid proteins, has been shown to trigger apoptosis in mammalian cells in vitro [75]. Further studies on the role of the L protein as well as other regions of the TMEV genome, such as IRES, will clarify the mechanism of oligodendrocyte apoptosis in vivo (see Box. 1).

Although death of oligodendrocytes induced by direct viral infection can lead to demyelination, autoimmune responses and other patho-mechanisms may also lead to death of oligodendrocytes. Oligodendroglial apoptosis is observed in several demyelinating diseases, including MS and EAE, by terminal deoxynucleotidyl-transferase-mediated dUTP-biotin nick-end labeling (TUNEL) [10,76–80]. During the chronic phase of TMEV infection, TUNEL-positive nuclei were double-stained with both oligodendrocyte and macrophage/microglia markers, but not with viral antigen or the astrocyte marker, glial fibrillary acidic protein (GFAP) [80,81]. Interestingly, oligodendrocyte apoptosis was detected in the white matter of the spinal cord, as early as 1 week after infection with either GDVII or DA virus, without infection seen in oligodendrocytes [82]. Since 1) the apoptotic oligodendrocytes were detected adjacent to degenerated axons during the acute phase of TMEV infection, and 2) the distribution of axonal damage present during the early phase of TMEV infection corresponds to regions where subsequent demyelination occurs during the chronic phase [82], these results suggest that there is an association between oligodendrocyte death and axonal degeneration (discussed below).

Axonal degeneration induced by TMEV infection

Historically, as in EAE and MS studies, most TMEV studies had focused on how oligodendrocytes and myelin sheaths are damaged; axonal degeneration had not drawn much

attention until recently. Dal Canto and Lipton first demonstrated axonal damage in susceptible SJL/J mice during the chronic phase of DA virus infection [83]. Axonal degeneration caused by TMEV infection has been investigated by two groups; Tsunoda et al. conducted a time course study of damaged axons from 1 week to the early chronic phase, while Rodriguez et al. investigated axonal loss during the late chronic phase (Table 4) [82,84–86].

Tsunoda et al. have demonstrated that axonal damage heralds demyelination in TMEV infection [55,82,87]. Damaged axons were detected in the white matter of the spinal cord, using immunohistochemistry against non-phosphorylated neurofilaments, as early as 1 week after DA virus infection. At 1 week postinfection, spinal axonal damage in the white matter was accompanied by neither T cell infiltration nor virus-infected cells; T cells and infected cells were mainly present in the gray matter of the brain. During the subclinical phase (2 to 3 weeks after infection), the extent and distribution of axonal damage in the white matter of the spinal cord were associated with microglia and macrophage activation, but minimal or no inflammation and no viral antigens were detected [82]. Thus, neither T cell nor direct viral attack of axons seems to be required for the induction of this early axonal degeneration. Since obvious demyelination is not observed until 4–5 weeks post-infection, these results demonstrate that axonal degeneration precedes demyelination in TMEV infection (Inside-Out lesion development) (Table 4). In addition, during the early chronic phase, the level of axonal loss is greater than the level of demyelination, which further supports the Inside-Out model in TMEV infection (Fig. 2).

During the late chronic phase, Rodriguez et al., using plastic-embedded 1-μm sections, observed spinal cord atrophy in the ventral and lateral funiculi, but not in the dorsal funiculus [84–86]. A significant reduction in spinal cord areas was observed at 195–220 days after TMEV infection, including a 25% reduction in the ventral and lateral funiculi, compared with a 12% area reduction seen at 45 and 92–100 days postinfection. Since spinal cord atrophy was not observed in resistant C57BL/10 mice infected with TMEV, the authors speculated that spinal cord atrophy occurred following demyelination during the chronic phase, but not neuronal infection during the acute phase, since acute neuronal infection occurs in both resistant and susceptible mice. Interestingly, however, the same research group demonstrated that a significant decrease in medium to large myelinated axons in normally myelinated areas occurred on days 195–220 post TMEV infection. They also found increased intra-axonal mitochondria, an indicator of axonal injury, in normally myelinated, remyelinated, and demyelinated axons [86]. Therefore, axonal degeneration may also be independent of demyelination in some areas during the late chronic phase.

The precise mechanism of axonal damage, particularly in NAWM, in TMEV infection in susceptible SJL/J mice, remains unclear. Deb et al. demonstrated that perforin-producing $CD8^+$ T cells play a central role in the induction of axonal degeneration during the late chronic phase, 6 months after virus infection, in mice with a resistant C57BL/6 genetic background (Table 4) [88]. In another viral model for MS, mouse hepatitis virus (MHV) infection, axonal loss seems to occur due to direct attack, rather than occurring secondary to demyelination [89].

Axonal degeneration itself can contribute to secondary neuropathology

In general, axonal degeneration is considered to be an end product in most neuropathological insults. However, experimental and clinical findings demonstrate that axonal degeneration itself can trigger secondary patho-mechanisms, 1) recruitment of inflammatory cells and 2) induction of oligodendrocyte apoptosis. In TMEV infection, axonal damage in the CNS has been shown to contribute to the recruitment of inflammatory

cells into the site of axonal degeneration, exacerbating lesion development [90]. In TMEV infection, the distribution of damaged axons observed during the early phase corresponds to regions, where subsequent inflammatory demyelination occurs during the chronic phase [82]. This suggests that axonal degeneration triggers recruitment of T cells and macrophages into the CNS, leading to subsequent loss of myelin. If this is the case, axonal injury recruits inflammatory cells into sites of wallerian degeneration, leading to inflammatory demyelination.

To prove the hypothesis, Tsunoda et al. used an approach for induction of wallerian degeneration in the CNS, which involves injecting Ricinus communis agglutinin (RCA) I (a toxic lectin) into the peripheral nervous system (PNS) [90]. In this experimental system, RCA I, which is injected into the sciatic nerve, is transported axonally (retrogradely), causing cell death of dorsal root ganglion cells and wallerian degeneration of the dorsal funiculus in the spinal cord. Three weeks after TMEV infection, RCA I was injected into the sciatic nerve of SJL/J mice. Neuropathologically, control mice that received TMEV alone, but no RCA I, had inflammatory demyelinating lesions only in the ventral and lateral funiculi, while the other control mice receiving RCA I alone had wallerian degeneration without inflammatory demyelination only in the dorsal funiculus. In contrast, RCA I injection in TMEV-infected mice induced inflammatory demyelinating lesions not only in the ventral and lateral funiculi but also in the dorsal funiculus. This suggests that axonal degeneration itself contributes to recruitment of inflammatory cells into the CNS, targeting lesion development. In this case, lesions are triggered from the axons (inside) to the myelin (outside) “Inside-Out model” (Table 4) [28,90,91].

Similarly, in a passive EAE model, Konno et al demonstrated that adoptive transfer of encepahlitogenic immune cells resulted in inflammatory demyelinating lesion development in accord with the distribution of artificially induced axonal degeneration with activated MHC class II^+ microglia; i.e., in the ipsilateral thalamus after cortical cryoinjury, and in the ipsilateral optic nerve, the contralateral optic tract and superior colliculus after unilateral eye ball enucleation [92,93]. Here, the EAE locus may be targeted by axonal degeneration. In MS, the NAWM damage has been correlated with abnormalities in connected gray matter atrophy [94]. These results suggested that, in these regions, there may be a link between the pathological processes occurring in the two compartments.

Axonal degeneration has also been shown to induce oligodendrocyte apoptosis. For example, spinal cord injury (SCI) induces axonal (wallerian) degeneration in the part of the spinal cord distal to the transection site; wallerian degeneration is defined as the changes, such as fragmentation, occurring distal to the site of transaction of a nerve fiber. Then, apoptosis of a substantial number of oligodendrocytes occurs along the fiber tracts undergoing wallerian degeneration, even extending into regions remote from the lesion [28,95,96]. The exact mechanisms of oligodendrocyte apoptosis following SCI remain unclear and are still controversial. Oligodendrocytes have been shown to express apoptosis-related molecules p53, p21, Bcl-2, and Bax, as early as 30 min after experimental SCI [97]. Since axonal degeneration itself can activate resident microglia, oligodendrocyte apoptosis may be caused by soluble toxic factors, such as inflammatory cytokines, produced from activated microglia. Alternatively, oligodendrocyte apoptosis may be induced by glutamate-mediated excitotoxicity [98]. Glutamate receptors are expressed not only on neurons but also on oligodendrocytes and these cells are vulnerable to glutamate mediate excitotoxicity [99]. Glutamate spillover from injured axons has been suggested to damage oligodendrocytes [100].

An alternative mechanism for oligodendrocyte apoptosis occurring as a result of axonal degeneration is the disruption of cross-talk between axons and oligodendrocytes; a failure of

cell communication between axons and oligodendrocytes has been proposed in both axonal and oligodendroglial pathology (Table 4) [101]. The survival of the oligodendrocytes depends on the presence of axons, at least during development [102]. Oligodendrocyte apoptosis occurs selectively in transected neonatal optic nerves in which the axons degenerate. The cell death does not occur in optic nerves, if the same experiment is performed in C57BL/Wld (wallerian degeneration slow mutant) (Wld) mice, which are a substrain of C57BL/6 mice and have prolonged survival of the distal stumps of transected axons (see Box 2). Purified neurons, but not neuron-conditioned culture medium, promote the survival of purified oligodendrocytes in vitro [103]. Thus, the cell-to-cell contact between axons and oligodendrocytes is required for the survival of the oligodendrocytes. This supports the hypothesis that oligodendrocytes compete for axon-derived survival signals, helping to adjust the number of oligodendrocytes to the number of axons that require myelination.

Axonal degeneration leads to demyelination: the Inside-Out model

Based on the above findings, we propose a possible patho-mechanism of demyelination and neurodegeneration (axonal degeneration) in TMEV infection, i.e. the Inside-Out model, in which the lesion develops from the axons (inside) to the myelin (outside) (Fig. 3). First, TMEV infects neurons in the gray matter of the CNS and damage axons. This leads to degeneration of the distal stumps of axons in the white matter of the spinal cord. Oligodendrocyte apoptosis is induced either by axonal degeneration itself or by direct virus infection [74]; TMEV is able to traffic from the axon into the surrounding myelin [104]. Axonal degeneration and/or (infected) oligodendrocyte apoptosis activate local microglia and macrophages. Activated microglia and macrophages can induce demyelination immunopathologically by secreting inflammatory cytokines, such as tumor necrosis factor (TNF)-α. Activated microglia and macrophages phagocytose degenerated oligodendrocytes (infected or uninfected), myelin and axons, resulting in persistent infection as well as viral antigen presentation by microglia and macrophages [28]. Activated microglia can also produce chemokines and up-regulate adhesion molecules in the CNS, leading to transvascular migration of T cells to areas of axonal degeneration.

These activated microglia and macrophages act as APCs, which present neuroantigens and viral antigens to $CD4^+$ T cells. $CD4^+$ T cells mediated DTH response against virus and/or myelin, leading to further demyelination [28]. $CD4^+$ T cells produce cytokines, helping anti-myelin antibody production by B cells as well as killing of oligodendrocytes by $CD8^+$ CTLs. These autoimmune T cells and auto-antibodies may attack myelin according to the Outside-In mechanism. At this time point, the lesion develops from the outside (myelin) to the inside (axons) (Fig. 3), although the initial step of demyelination developed from the inside to the outside. Here, the Inside-Out and Outside-In models are not mutually exclusive but act in synergy, resulting in a reinforcing immune cascade reaction and further disease progression.

Conclusion

Axonal pathology may precede or be independent of demyelination in some patients with MS and EAE models. We reviewed findings that support axonal degeneration preceding demyelination in TMEV infection, “Inside-Out model”. We then discussed the process whereby the Inside-Out and Outside-In models can collaborate to create a “vicious” immunopathological cycle, initiating a reinforcing cascade of events, leading to disease progression. Theoretically, therapeutic strategies targeting each step (axonal degeneration, inflammation, and demyelination), in these models may interfere with or arrest this cascade reaction. We believe that the concept of the Inside-Out and Outside-In models will be

helpful in designing therapeutic strategies to prevent disease progression, particularly in primary and secondary progressive MS.

Box 1 A possible role of IRES in TMEV infection

Most of the cellular protein synthesis is initiated by cap-dependent translation which is mediated by the binding of the mRNA 5'-end cap structure and eukaryotic translation initiation factors (eIFs) [105] (Fig. 1 left). During infection with some eukaryotic viruses, host cell mRNA translation is selectively inhibited. A unique viral RNA structure, internal ribosomal entry site (IRES), which allows efficient translation when the cap-dependent translation is inhibited, is identified in 5' UTR of TMEV genome. The IRES structure recruits ribosomes without the binding of cap structure and eIFs, thereby bypassing the cap-dependent translation [106] (Fig. 1 right).

IRES was first discovered in several picornaviruses, whose RNA are naturally uncapped [107]. Later, IRES has also been identified in other viral families and is known as a critical mechanism to maintain viral activity while protein synthesis was shut off in viral-infected host cells [108]. Recently, IRES-dependent translation has also been found even in some cellular mRNAs, such as XIAP, Apaf-1, and Bcl-2, which are involved in inhibition of apoptosis [109–111]. These IRES-dependent gene expressions can help host cell survival when global cap-dependent protein synthesis is inhibited. If the viral infection itself switches the translation mechanism from cap-dependent to IRES-dependent, the virus might obtain advantages by producing simultaneously both viral protein and cellular proteins that are involved in cell survival.

Efficient IRES-dependent translation requires auxiliary cellular proteins, IRES trans-acting factors (ITAFs). These proteins bind to the IRES structure and stimulate the recruitment of ribosomes. TMEV induces apoptosis in neurons during the acute phase and in oligodendrocytes during the chronic phase in vivo. Fan et al. demonstrated that TMEV leader (L) protein triggers apoptosis in mammalian cells in vitro [75]. On the other hand, TMEV mutant virus, H101, which has mutations in the 5'UTR sequence with no mutations in L protein, has been shown to induce apoptosis in vivo only in the meninges, and not in neurons or oligodendrocytes [112]. These findings suggest that the structural difference of 5'UTR might cause alteration of the IRES-dependent translation of L protein, which is mediated by the binding of ITAFs, and that these changes influence induction of apoptosis in different cell types. Research in this field may elucidate the mechanism whereby some neurons seem to survive even after extensive neuronal infection with TMEV.

Box 2 Axonal degeneration as a self destructive defense mechanism against virus infection

A novel role of axonal degeneration has been proposed from findings of TMEV infection in Wld mice, which are a substrain of C57BL/6 (B6) mice and have prolonged survival of the distal stumps of transected axons [113–115]. Transected axons from Wld mice survive for up to 4 weeks, support action potentials for at least 2 weeks, and continue anterograde and retrograde transport of proteins for similar amounts of time [116–118]. TMEV-infected B6 and Wld mice had similar neuropathology and virus replication in the CNS at 1 week postinfection [119]. However, at 3 weeks after infection, only Wld mice showed clinical signs, substantial inflammation, and viral persistence. Since TMEV is known to infect neurons and spread using axonal flow, delayed axonal degeneration in Wld mice could favor virus transport and persistence in the CNS. In contrast, rapid induction of axonal degeneration in B6 mice prevents viral dissemination in the CNS

[119]. Since axonal degeneration has been shown to be a self-destructive physiological process during the development, axonal degeneration in TMEV infection may be a self-destructive defense mechanism that protects from the transport of toxic substances, including virus, in the CNS [87,114].

Acknowledgments

The authors thank J. Steven Alexander, PhD, Tomoko Tanaka, MD, and Stephen L. Jaffe, MD for many helpful discussions, and Daniel Doty and Krystal D. Porter BS for excellent technical assistance. This work was supported by the National Institutes of Health (R21NS059724).

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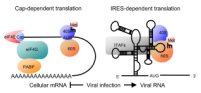

Fig. 1.

Cap-dependent translation and IRES dependent translation. (Left) eIF4E binds to the cap structure at the 5' end of cellular mRNA. This binding initiates recruitment of ribosomes and methionin-loaded initiator tRNAs on mRNA. (Right) Viral RNAs do not have cap structure. IRES structures recruit the ribosomes and methionin-loaded initiator tRNAs without eIFs. Viral infection can switch the translation mechanism from cap-dependent to IRES-dependent. eIF: eukaryotic translation initiation factor, PABP: poly (A) binding protein, 40S: 40S ribosomal subunit, 60S: 60S ribosomal subunit, Met: methionin-loaded initiator tRNA.

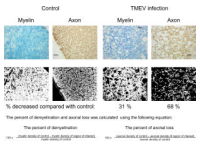

Fig. 2.

The percent of demyelination (myelin loss) and axonal loss during the chronic phase of TMEV infection. Normal axons were immunostained using an antibody cocktail against phosphorylated neurofilament, SMI 312, with diaminobenzidine (DAB) as the chromogen. Myelin was stained using Luxol fast blue. Densities of myelin sheaths and axons were compared in the white matter of the spinal cord between controls and TMEV-infected mice, using Image PloPlus. Sections of TMEV-infected spinal cord showed 31% myelin loss and 68% axonal loss, compared with control sections. This result supports the Inside-Out model of lesion development, whereby axonal damage heralds demyelination in TMEV infection. Scale bars = 20 μm.

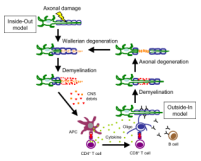

Fig. 3.

In TMEV infection, axonal degeneration precedes demyelination “Inside-Out model”; lesions develop from the axons (inside) to the myelin (outside). Virus first infects neurons and damages axons, which lead to wallerian degeneration of the distal stumps of axons, and activation of microglia and macrophages. Then, the axonal degeneration itself as well as virus-infected glial cells and activated macrophages recruit inflammatory cells to the site of wallerian degeneration, leading to demyelination. Damaged CNS debris is captured by microglia and macrophages, which function as APCs, activating $CD4^+$ T cells. This leads to production of inflammatory cytokines, antibody production by B cells, and $CD8^+$ T cells that attack oligodendrocytes (Oligo). At this time point, the autoimmune cells can attack myelin sheaths from the outside, leading to secondary axonal degeneration, “Outside-In model”. Here, the Inside-Out and Outside-In models can collaborate in a “vicious” cycle, initiating a reinforcing cascade.

Table 1

Immune-mediated primary demyelination in EAE: possible patho-mechanisms in the Outside-In model

Effector	Patho-mechanism	References
$CD4^+$ T cell	Th1 and Th17 cells against myelin antigens damage myelin sheaths	[11,12,17]
	Anti-myelin specific Th1 cells induced by epitope spreading	[13,14]
$CD8^+$ T cell	CTLs against myelin antigens attack MHC class I^+ oligodendrocytes	[3,19,20]
B cell	Anti-myelin antibodies exacerbate demyelination	[22,23]

CTLs, cytotoxic T lymphocytes; EAE, experimental autoimmune (allergic) encephalomyelitis; MHC, major histocompatibility complex; Th, T helper

Table 2

Immune-mediated secondary demyelination in EAE: possible patho-mechanisms in the Inside-Out model

Effector	Patho-mechanism	References
$CD4^+$ T cell	NF-L- or contactin-2/TAG-1-specific Th1 and Th17 cells attack axons and neurons	[41,43,44]
$CD8^+$ T cell	CTLs attack axons and neurons directly or indirectly	[42]
Bcell	Anti-axon and neuron antibodies attack axons and neurons	[40,43]

NF-L, neurofilament light chain; TAG, transiently expressed axonal glycoprotein

Table 3

Virus- and/or immune-mediated demyelination in TMEV infection: possible patho-mechanisms in the Outside-In model

Effector	Patho-mechanism	References
$CD4^+$ T cell	DTH responses against virus antigens damage myelin sheaths in a "bystander" fashion	[61,62]
	Anti-myelin specific Th1 cells induced by epitope spreading	[63,64]
$CD8^+$ T cell	Anti-virus CTLs kill virus-infected and uninfected oligodendrocytes	[3,65–68]
B cell	Anti-virus antibodies cross-reacts with myelin lipid, galactocerebroside	[62,70]
Virus	Direct virus infection of oligodendrocytes	[74]

DTH, delayed type hypersensitivity; TMEV, Theiler's murine encephalomyelitis virus

Table 4

Virus- and/or immune-mediated neurodegeneration in TMEV infection: possible patho-mechanisms in the Inside-Out model

Effector	Patho-mechanism	References
Virus	Direct virus infection in neurons leads to wallerian degeneration	[55,82,87]
Virus/microglia	Axonal degeneration leads to oligodendrocyte apoptosis by the disruption of cross-talk between axons and oligodendrocytes or by locally activated microglia	[10,95,101]
Inflammatory cells, microglia/macrophages	Axonal injury recruits inflammatory cells at the site of wallerian degeneration, leading to inflammatory demyelination	[90,93]
$CD8^+$ T cell	$Perforin^+$ CTLs damage axons and neurons	[3,88]