Chapter B2

TMEV AND NEUROANTIGENS: MYELIN GENES AND PROTEINS, MOLECULAR MIMICRY, EPITOPE SPREADING, AND AUTOANTIBODY-MEDIATED REMYELINATION

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Abstract: The Theiler's murine encephalomyelitis (TMEV) model has been used to study the interactions of virus, myelin and anti-neuroantigen autoimmunity. TMEV and myelin can interrelate during virus entry and persistence. On virus entry, TMEV might use peripheral myelin P₀ protein as a virus receptor. For persistence, TMEV seems to require myelin functional proteins or structural myelin itself. Here, myelin and oligodendrocyte loss and downregulation of myelin genes would lead to demyelination, but might limit virus spread in the central nervous system. Unlike experimental allergic encephalomyelitis (EAE), a pathogenic role of anti-myelin autoimmunity is unclear in TMEV infection. Anti-myelin autoantibodies have been detected in TMEV infection. Among them, only anti-galactocerebroside (GC) antibody is shown to be myelinotoxic, and has molecular mimicry with TMEV. Myelin-specific T cells play no role in initiation or progression of demyelination in the first two to three months after TMEV infection. However, cellular autoimmunity against several myelin antigens (epitope spreading) can be detected during the late chronic stage. Using the TMEV model, epitope spreading and autoantibody-mediated remyelination have been investigated by recombinant TMEV and anti-neuroantigen (natural) antibodies, respectively.

Key words: Animal models, Autoimmune diseases of the nervous system, Demyelinating diseases, Galactosylceramides, Myelin basic proteins, myelin proteolipid protein, Multiple sclerosis, Picornaviridae infections
1. INTRODUCTION

1.1 Overview

The Theiler's murine encephalomyelitis virus (TMEV) infection model of multiple sclerosis (MS) has provided excellent paradigms to study the interactions between the virus, myelin and immune system (1-5). In this chapter, we will first review the associations between TMEV and myelin genes and proteins. We will next discuss possible roles of anti-myelin immunity including molecular mimicry and epitope spreading in the pathogenesis of demyelination. Finally, we will introduce two unique model systems using TMEV infection (i) to study the pathogenic role(s) of molecular mimicry and (ii) to explore potential therapeutic agents, remyelination-promoting autoantibodies, in demyelinating diseases. An understanding of the diverse interactions between viruses, myelin and host immune responses to neuroantigens may shed light on the etiology and pathogenesis of MS.

1.2 Theiler's murine encephalomyelitis virus

In 1934, TMEV was isolated by Max Theiler, who was awarded a Nobel Prize for developing the first effective vaccine against yellow fever (6,7). TMEV belongs to the genus *Cardiovirus*, family *Picornaviridae* (2). Picornaviruses are small, nonenveloped negative stranded RNA viruses. Several picornaviruses, such as poliovirus and coxsackievirus, can cause neurological disease in humans (8). However, TMEV infects mice and rats in vivo and causes a demyelinating disease only in mice. Thus, TMEV has not been identified as a causative agent in any human disease; its association with dermatomyositis (9) and Vilyuisk encephalomyelitis (10,11) are thus far inconclusive.

The Theiler's original (TO) subgroup, which includes Daniels (DA), BeAn 8386, and WW strains, produces a biphasic disease in susceptible mice inoculated intracerebrally [reviewed in (2)]. During the first week (acute phase), the mice develop an acute polioencephalomyelitis where mice occasionally show clinical signs such as weight loss. Virus antigen is expressed primarily in neurons in the central nervous system (CNS) (12). Subsequently, about one month post infection (p.i.) (chronic phase), the mice show signs of spastic paralysis as a result of demyelination within the white matter of the spinal cord. Areas of demyelination are associated with perivascular infiltration of mononuclear cells (MNC). The disease progresses without evidence of remission, either clinically or pathologically, with the exception of CD-1 mice infected with the WW strain of TMEV.
During the chronic phase, viral antigens are expressed within oligodendrocytes, astrocytes and macrophage/microglia but not in neurons [reviewed in (4)].

TMEV-induced demyelinating disease is one of a few animal models for the progressive forms of MS (16). Although there are many animal models for MS, their clinical courses are either acute monophasic or relapsing-remitting (RR). These animal models are only useful as experimental models for RR-MS. Therefore, the TMEV model is a unique animal model to study the pathogenesis for the other three types of MS: primary progressive (PP)-, secondary progressive (SP)-, and progressive relapsing (PR)-MS (17).

The precise mechanisms of demyelination in TMEV infection are not clear. There are two major hypotheses: (i) direct virus infection of myelin forming cells, i.e. oligodendrocytes, and (ii) immune-mediated mechanisms. Infection with live TMEV is necessary to induce demyelination, since neither ultraviolet-inactivated TMEV nor cDNAs encoding individual TMEV capsid proteins alone cause demyelination (18). Virtually all immune cells, including T cells, B cells and macrophages, have been shown to play important roles [reviewed in (1-4)]. In contrast to experimental allergic encephalomyelitis (EAE) (3) and other virus-induced demyelinating diseases (19), adoptive transfer of uninfected immune cells from TMEV-infected mice does not induce demyelination in the recipient mice (20). In addition, acquired immunity is neither necessary, nor sufficient in some TMEV model systems; TMEV can persistently infect the CNS and induce demyelination in: major histocompatibility complex (MHC) class I-deficient mice (21,22), MHC class II-deficient mice (23), CD4-deficient mice (24), or CD8-deficient mice (24). TMEV also causes demyelination in the spinal cord of moth-eaten mice, lacking protein tyrosine phosphatase SHP-1, as early as five days p.i., when no acquired immune responses are normally generated (25).

MYELIN GENES, VIRUS ENTRY, AND VIRUS PERSISTENCE

Peripheral nerve myelin protein for TMEV entry

During a natural infection, TMEV spreads from mouse to mouse by the oral-fecal route (26,27). In order to cause CNS infection by this route, the virus must get from the alimentary tract into the CNS, either hematogenously or intra-axonally. Since the strains of the TO subgroup of TMEV could be isolated from feces up to 154 days p.i. (28), invasion into
Table 1. Myelin gene, virus entry and virus persistence

| Virus Entry | Virus Persistence | Host |
|-------------|-------------------|------|
| CNS         | TMEV requires MBP and PLP functionally and/or requires myelin itself as a structure | dowaregulates MBP and PLP mRNA actively or passively and/or loses oligodendrocyte and myelin (demyelination) |
| Receptor unknown | | |
| PNS         | Evidence: No virus persistence in shiverer and rumpshaker mice | Evidence: in situ hybridization, TUNEL, myelin staining, EM |
| P0 protein as a virus receptor? | | |

**Evidence:**
- *in vitro* assays
- *in situ* hybridization, TUNEL, myelin staining, EM

**Abbreviations:** CNS, central nervous system; EM, electron microscopy; MBP, myelin basic protein; PLP, myelin proteolipid protein; PNS, peripheral nervous system; TMEV, Theiler’s murine encephalomyelitis virus; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling.

the CNS via the neural route from a persistent infection in the intestine may be one pathway, as originally suggested by Theiler and Gard (27). Myelin is a multiple lamellar membrane that sheathes axons and facilitates the saltatory conduction of nerve impulses. P0 protein is a member of the immunoglobulin (Ig) gene superfamily, and constitutes 50% of the total myelin proteins present in the peripheral nervous system (PNS). Using virus overlay and virus binding assays, Libbey et al (29) identified P0 protein as a potential receptor for TMEV (Table 1). They suggested that the use of the P0 protein in Schwann cells as a receptor may be one mechanism by which TMEV spreads from the gastrointestinal tract to the CNS in vivo.

In the CNS, TMEV receptors on neurons during the acute phase and on glial cells and macrophages during the chronic phase have not been identified.

### 2.2 Myelin protein requirement for TMEV persistence

Myelin basic protein (MBP) and myelin proteolipid protein (PLP) are the major component of CNS myelin; many other proteins and glycoproteins are present to a lesser extent. Bihl et al (30) demonstrated that myelin gene mutation could affect the persistence of TMEV. Susceptibility/resistance to chronic TMEV infection has been mapped to several loci including one tentatively located in the telomeric region of chromosome 18, close to the myelin basic protein locus (Mbp locus) (31,32). To determine if the Mbp gene influences viral persistence, Bihl et al (30) inoculated DA virus into C3H mice bearing the shiverer (shi) mutation, a 20-kb deletion in the gene. Whereas TMEV-infected wild-type C3H +/+ mice had intermediate levels of virus persistence on days 20 and 45 p.i., an increase in viral persistence was detected in heterozygous C3H +/shi mice, and no virus persistence was
detected in homozygous C3H shi/shi mice. The increased susceptibility of the heterozygous shiverer mice was also seen in (C57BL/6 x C3H +/shi) F1 mice (1), although C57BL/6 mice are resistant to TMEV persistence. Furthermore, no viral RNA was detected 20 days after DA virus infection of C3H/101H mice homozygous for a point mutation in the gene coding for PLP (PLP1, previously PLP), the rumpshaker (rsh/rsh) mutation (no data was available in control C3H/101H (+/+ ) mice, though) (30). In these studies, infection of oligodendrocytes, the presence of demyelination and anti-TMEV immune responses were not tested. The number of oligodendrocytes has been reported to be normal, or even slightly increased in uninfected shiverer and rumpshaker mutant mice, although the myelin is grossly deficient. Therefore, these results suggest that TMEV persistence is linked to myelin and functional alteration, for example, may change the virus's ability to replicate in, or traffic through, the myelin sheath. Although the MBP gene or some other MBP-linked locus has been suggested to influence susceptibility to MS, other studies have not demonstrated linkage between MS and the MBP gene (33,34).

2.3 Myelin gene downregulation in TMEV infection

The genes encoding myelin proteins, such as MBP and PLP, are expressed by oligodendrocytes in the CNS, and their transcripts are markers for activity of maintenance or reproduction of myelin. Yamada et al (35) demonstrated the relationship between MBP and PLP mRNAs, viral RNA, and demyelination within the spinal cord during TMEV infection. At four weeks p.i., decreased levels of MBP and PLP mRNAs were associated with the presence of viral RNA and demyelination. By 8 or 12 weeks p.i., however, extensive demyelination was associated with a significant decrease of MBP and PLP mRNAs but not with the presence of viral RNA. These results indicate that a viral infection of white matter in the early phase of TMEV infection leads to demyelination, but that, later in the course of disease, some other mechanism, such as an immunopathologic process, drives extensive demyelination. Subsequent reports by other groups confirmed the decrease in PLP mRNA expression, not only in infected- but also in uninfected-oligodendrocytes in the spinal cord (36-38).

The decrease of MBP and PLP mRNAs in the lesions indicates either a loss of oligodendrocytes or suppression of the myelin metabolism of the oligodendrocyte without cell lysis. The former is supported by the finding of apoptosis of oligodendrocytes in demyelinating lesions in TMEV infection (39). The latter possibility is supported by the fact that some viruses are known to alter the "luxury" or differentiated function of a cell, without disturbance of its vital function, i.e., ability to survive (40). Using electron
microscopy, Rodriguez (15) demonstrated degenerative changes in the most distal processes of oligodendrocytes in TMEV infection. Although this 'dying-back oligodendrogliopathy' has not been directly linked to suppression of myelin metabolism, similar ultrastructural pathology has also been described in MS (41). Also, in the polyomavirus-induced human demyelinating disease, progressive multifocal leukoencephalopathy (PML), JC virus has been reported to cause demyelination not only by the lytic infection of oligodendrocytes, but also via the suppression of myelin gene expression [reviewed in (42)].

Although suppression of MBP and PLP mRNAs as well as demyelination results in neurological functional deficit, this might benefit the host by suppressing viral replication in the CNS. As we discussed in the previous section, persistent viral infection seems to require either functionally or structurally intact myelin. Therefore, suppression of myelin function and loss of myelin (demyelination) might serve a protective role by suppressing virus persistence in TMEV infection (Table 1). Demyelinating diseases might represent the oligodendrocytes' futile attempt to suppress viral replication, which ultimately results in potentially irreversible myelin damage. On the other hand, incomplete suppression of the oligodendrocyte functions might actually benefit the virus, since the heterozygous shiverer (+/shi) mutation resulted in an increase in viral persistence in the CNS.

3. HUMORAL AUTOIMMUNE RESPONSES TO MYELIN

3.1 Anti-myelin antibodies in TMEV infection

Damage to CNS tissue liberates CNS antigens into the cerebrospinal fluid (CSF) compartment. MBP can be detected in the CSF of patients with active myelin destruction caused by MS or other processes, such as CNS infarction. Rauch et al (43) found MBP in the CSF of 14 of 19 mice infected with DA virus 12 weeks p.i. They also detected serum anti-MBP antibody in 10 of 14 TMEV-infected mice. Welsh et al (44) also demonstrated that antibody responses to myelin developed in CBA mice by day 28 after BeAn virus infection and reached a peak between day 42 to 56 p.i. Anti-myelin antibody production was prevented by depletion of CD4+ T cells. However, there was no correlation between the titer of anti-myelin antibodies and the severity of clinical disease.

Using Western blot analysis, Cash et al (45) studied the specificity of Ig secreted by B cells isolated from the spinal cord of SJL/J mice infected with DA virus. They found antibodies directed against two unidentified nonviral
proteins with molecular weights of 26 and 28kDa; these were present only in the white matter of infected mice and were not found in purified myelin.

Rodriguez et al (46) showed that, like in MS, increased amounts of Ig in the CSF and oligoclonal IgG bands were detected in TMEV-infected mice on day 21 p.i. However, IgG in the serum and CSF was primarily directed at virus antigen rather than at normal myelin components, although rare examples of myelin sheaths positive for IgG were found by immunoelectron microscopy in TMEV-infected mice. On the other hand, Roos et al (47) demonstrated that the heterogeneity of anti-TMEV antibody in the CSF was less restricted than oligoclonal IgG bands seen in MS or subacute sclerosing panencephalitis (SSPE). Rodriguez et al (46) also showed that complement depletion on day 15 p.i. resulted in exacerbation of demyelination on day 21 p.i. Although the authors suggested no pathogenic role of humoral immunity from this study, the treatment can hamper antibody-mediated clearance of TMEV and day 21 might be too early to assess effector mechanisms in TMEV infection, where overt demyelination is generally seen later.

A direct pathogenic role for anti-MBP antibody in demyelination is unlikely, since anti-MBP antibody has been demonstrated not to be myelinotoxic either in vivo or in vitro (48). This is in contrast to antibodies against galactocerebroside (GC) or myelin oligodendrocyte glycoprotein (MOG), whose adoptive transfer to mice with EAE exacerbates demyelination (49). Functionally, Barbano and Dal Canto (20) demonstrated that sera from mice infected with TMEV for two to three months did not injure myelinating cultures in vitro. This study suggests that there is no myelinotoxic humoral factors in sera from TMEV-infected mice.

Anti-myelin antibody might indirectly affect myelin antigen presentation to class II-restricted T cell by receptor-mediated antigen uptake in TMEV infection (50). Myelin-specific surface Ig can serve to capture myelin antigens on the surface of B cells (51). The B cells could then internalize and present myelin antigen to specific T cells. In addition, professional antigen presenting cells (APCs) could exploit soluble anti-myelin antibody for antigen capture by Fc receptor mediated uptake of myelin-containing immune complexes. This would lead to augmentation of myelin-specific T cell responses, and also might contribute to determinant spreading (see a later section) in TMEV infection, as suggested for other autoimmune diseases (52). The potential beneficial role of anti-neuroantigen antibodies will be discussed in the last section of this chapter.
3.2 Molecular mimicry between TMEV and galactocerebroside

Molecular mimicry between host protein and microorganism has been known for many years. A cross-reacting immune response initially generated against a virus that also reacts with myelin components could lead to demyelination. However, Rauch et al (43) found that anti-MBP antibody from TMEV infected mice did not cross-react with TMEV antigens. Lack of cross reaction between MBP and capsid proteins VP1, VP2, and VP3 was also supported by a competition radioimmunoassay with sera from rabbit subcutaneously injected with TMEV capsid proteins (53).

On the other hand, Fujinami et al (54) raised a monoclonal antibody (mAb) H8 that reacts with TMEV and GC from spleen cells of BALB/c mice infected with DA virus intracerebrally. GC is a major lipid component of myelin. The mAb H8 reacts with viral capsid protein VP1 and efficiently neutralizes TMEV in vitro. Intraneural injection of mAb H8 into the sciatic nerve induced Schwann cell destruction and demyelination (55). When injected intravenously into mice with EAE, mAb H8 increased demyelinating lesions within the spinal cord by ten-fold (56). To determine whether antibodies similar to mAb H8 exist during viral infection, mice were infected with TMEV and sera were collected at various times after infection. Antibodies to GC were detectable ten days p.i. These results suggest that antibody(s) of the H8 type is generated and could contribute to demyelination in vivo in TMEV infection.

Interestingly, Fujinami et al (57) also demonstrated that another TMEV-neutralizing antibody (H7) could react with VP1 but not with GC. Passive administration of mAb H7 resulted in the clearance of virus and survival of athymic mice. Both H7 and H8 antibodies can neutralize TMEV, yet only H8 is myelinotoxic (Table 2). Further investigation is needed to clarify which immunologic factors, such as T helper cells, favors generation of each type of anti-VP1 antibody in vivo. The presence of “myelinotoxic activity” has been demonstrated in the CSF and sera in demyelinating diseases; only GC and MOG are identified as target molecules of demyelinating antibodies of which passive transfer exacerbates, but not initiates, demyelination in the CNS in vivo (58). Anti-GC antibody has also been detected in some MS patients (59).

Table 2. Destructive or beneficial autoimmunity in TMEV infection

| Effector Mechanism | P.I. Days | Destructive | Beneficial |
|--------------------|-----------|-------------|------------|
| Molecular mimicry  | 10 days   | Antibody to TMEV cross reacts with GC and exacerabtes demyelination | Virus neutralization |
| Epitope spreading  | 2-3 months| MBP-, PLP-, MOG-specific T cells induce DTH responses | CNS regeneration |
| Natural autoantibody| N.E.      | Autoantibody binds to CNS antigens | Remyelination |

Abbreviations: CNS, central nervous system; DTH, delayed-type hypersensitivity; GC, galactocerebroside; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; N.E., not examined in TMEV infection, but detected in SCH-immunized mice; P.I., TMEV post infection; PLP, myelin proteolipid protein; SCH, spinal cord homogenate; TMEV, Theiler's murine encephalomyelitis virus.
4. CELLULAR AUTOIMMUNE RESPONSES TO MYELIN

4.1 Myelin-specific T cell responses play no role in demyelination in the first two to three months after TMEV infection

Epitope spreading (determinant spreading) is defined as the diversification of epitope specificity from the initial dominant epitope-specific immune response to subdominant epitopes [reviewed in (60)]. Epitope spreading can play an active role in the relapse or progression of autoimmune diseases, as was first suggested in EAE by Lehmann et al (61). After initial immune-mediated tissue destruction, tissue debris is taken up by macrophages, dendritic cells or B cells, and presented to tissue-specific T cells. Spreading of the specificity of an immune response can be from one epitope to another on the same molecule (intramolecular epitope spreading, for example, from PLP_{139-151} to PLP_{178-191}) or from an epitope on one molecule to one on a different molecule (intermolecular epitope spreading, for example, from PLP_{139-151} to MOG_{92-106}) (60).

Although tissue destruction occurs less than one week after TMEV infection, epitope spreading is not observed until two to three months after TMEV infection [reviewed in (62)]. Barbano and Dal Canto (20) demonstrated that spleen cells from TMEV-infected mice did not injure myelinating cultures and did not proliferate in response to MBP or spinal cord homogenate (SCH) in vitro. In addition, these spleen cells did not produce disease in vivo by adoptive transfer into naïve mice, regardless of whether in vitro stimulation was with MBP or SCH. Similarly, Miller et al (63,64) demonstrated a lack of delayed-type hypersensitivity (DTH) responses and T cell proliferation against whole MBP and PLP, their encephalitogenic peptides MBP_{91-104} and PLP_{139-151}, and SCH in TMEV-infected mice 23, 60, 69, 90 and 127 days p.i.

Comparative studies using the EAE model induced by neuroantigens also demonstrated no pathogenic role of myelin specific T cells during the early chronic phase of TMEV infection. EAE is induced by subcutaneous injection of myelin antigens emulsified with complete Freund’s adjuvant (CFA), while emulsification with incomplete Freund’s adjuvant (IFA) can induce anergy to myelin antigens, rendering resistance to subsequent EAE induction. Following MBP or SCH emulsified in IFA injection, Lang et al (65) injected mice with DA virus or SCH/CFA for EAE induction. They found that pretreatment with myelin antigens/IFA suppressed EAE induction, but not TMEV-induced demyelination at 45 days p.i. Another protocol for neuroantigens-specific tolerance induction that can suppress
EAE also failed to alter the development of TMEV-induced demyelinating disease on day 65 p.i. (64). Similarly, using a treatment regimen reported to suppress EAE, Drescher et al (66) demonstrated that oral tolerization with myelin did not alter TMEV-induced demyelination at day 45 p.i., supporting a non-autoimmune mechanism of TMEV-induced demyelination. Although TMEV-infected mice develop demyelinating disease one month after infection and disease progresses continuously from then on, myelin-specific cellular immunity contributes to neither initiation nor progression of demyelinating disease at least for the first two to three months after TMEV infection.

4.2 Epitope spreading during the late chronic phase of TMEV infection

Although TMEV-infected mice develop extensive inflammatory demyelination with progressive disability by two to three months after infection, neurological disease seems to progress for the rest of the life of infected mice. While most studies on TMEV-induced demyelination have used mice infected with TMEV for one to two months p.i., the Miller group has examined the role of epitope spreading in initiating anti-myelin autoimmune responses in SJL/J mice during the late chronic phase of disease, more than two to three months after BeAn strain of TMEV infection (60). As reviewed in the previous section, before three months p.i., TMEV-infected mice develop CD4+ T cells specific for TMEV, but not for neuroantigens. However, the Miller group demonstrated PLP139-151-specific T cell proliferation in TMEV-infected mice 87 days, but not 33 days, after infection, and DTH response to PLP139-151 was first demonstrable at day 52 (67). Peripheral T-cell responses to myelin epitopes develop during progressive disease in a hierarchical order, beginning with the dominant PLP139-151 peptide, but only after the onset of demyelination. As disease progresses, responses to various other less-dominant encephalitogenic myelin epitopes, such as PLP178-191, PLP56-70 and MOG92-106 develop. Although there are some inconsistencies as to the onset date of the anti-neuroantigen specific immune response between the reports by Miller's group, overt anti-myelin immune responses seem to be detectable around three months after TMEV infection, which argues against these responses contributing to myelin damage before this time.

Autoreactivity could be initiated, in part, by the processing of myelin debris and subsequent presentation of myelin epitopes by infiltrating macrophages and resident perivascular cells/microglia in the CNS. Macrophages and microglia harvested from the CNS during the early chronic phase, 40-42 days after TMEV infection, presented viral peptides,
but not myelin peptide (68). However, by 90 days post-infection, CNS APCs presented both viral and myelin epitopes to Th1 and Th2 cell lines and hybridomas specific for TMEV or PLP peptides in the absence of exogenous antigen in vitro, indicating these APCs could endogenously process and present both viral and myelin antigens (68,69). Importantly, anti-CD86 (B7-2) antibody predominantly inhibited PLP_{139-151}-specific T cell proliferation mediated by endogenous antigen presentation by CNS APCs from TMEV-infected mice (72% inhibition), compared with anti-CD80 (B7-1) (44% inhibition). This is in contrast to CD80 predominance in SJL/J mice with RR-EAE. Therefore, the costimulatory dependence of epitope spreading differs between TMEV infection and RR-EAE. In vivo costimulatory blockade of CD28 (ligand for CD80 and 86) on days 0, 2, 4, 6 and 9 after TMEV infection inhibited anti-TMEV immune responses and increased virus persistence, accelerating epitope spreading (70). This CD28 blocking would have been more intriguing, if it had been administered in TMEV-infected mice during the early chronic phase, possibly during the priming period of anti-PLP responses, where the treatment could inhibit epitope spreading without suppression of anti-TMEV immunity.

Most recently, Miller's group demonstrated that the treatment of TMEV-infected mice with an MP4 fusion protein, consisting of PLP and MBP, mildly attenuated disease progression (40-110 days p.i.) and resulted in decreased inflammatory cell infiltration in the CNS (71). Paradoxically, PLP_{139-151}-specific splenic T cell proliferative and interferon (IFN)-γ responses were enhanced in the MP4-treated mice, while PLP_{139-151}-specific DTH responses were downregulated. In addition, after 120-140 days p.i., MP4-treated mice showed disease severity comparable to control animals. These results indicated that MP4-treatment in TMEV infection did neither induce tolerance via deletion or anergy, nor immune deviation from Th1 to Th2 responses. In contrast, the MP4 treatment completely inhibited both the acute attack and relapse of PLP- and MBP-induced EAE with significant suppression of PLP- and MBP-specific lymphoproliferative responses (72). Administration of MP4 also markedly ameliorates the course of established EAE. These results suggest that the immunological parameters of epitope spreading differ between TMEV infection and EAE.

Another group also demonstrated myelin specific-DTH responses during the late chronic phase (36-48 weeks p.i.), but not during the early chronic phase (4 to 12 weeks p.i.), indicating autoimmune responses do not play a major role in the initiation of demyelination in TMEV infection (73). There was no correlation between clinical score and the strength of the DTH response to myelin autoantigens in mice tested at 36-48 weeks. Therefore, the authors concluded that anti-myelin autoimmunity might be an additional factor that contributes to lesion progression during the late chronic stage,
although autoimmunity to myelin might represent only an epiphenomena, being induced only after damage to myelin produced by other mechanisms.

It is unknown why epitope spreading can be detected only during the late chronic stage of TMEV infection. In addition to virally induced tissue injury and immunomodulation, mouse strain and aging might contribute to epitope spreading in TMEV-infected SJL/J mice. Naïve SJL/J mice have a high frequency of PLP\textsubscript{139-151} specific T cells, and it increases with age (74,75). Moreover, aged SJL/J mice tend to have immunological abnormalities: a high incidence of reticulum cell sarcoma and paraproteinemia with enlargement of lymph nodes and splenomegaly (76).

A pathological role for epitope spreading is controversial even in EAE (74) and difficult to verify. Epitope spreading might simply reflect release of autoantigens secondary to myelin destruction, and may not have pathogenic significance. However, Dal Canto \textit{et al} (77) showed that lymph node cells harvested from mice 70 days after TMEV infection produced demyelination in organotypic cultures after stimulation with PLP\textsubscript{139-152} but not after stimulation with whole MBP. The authors suggested that antigen spreading to PLP, but not to MBP, might play an important role in demyelination. It would be intriguing to test whether adoptive transfer of the PLP peptide-stimulated lymphocytes from TMEV-infected mice could cause demyelination in naïve mice \textit{in vivo}, while possible contamination with live transferred virus in cultures and the effect of whole PLP or MBP peptide stimulation of lymph node cells should also be addressed in future experiments. On the other hand, autoimmunity in the CNS, including MBP-specific T cells, has been demonstrated to have a protective or beneficial nature under certain circumstances (78). Therefore, one cannot rule out the possibility that anti-myelin autoimmunity might play a neuroprotective role during the late chronic phase of TMEV infection, a time when severe axonal injury and loss has been demonstrated [reviewed in (5)].

5. TMEV INFECTION AS MODELS TO STUDY MOLECULAR MIMICRY AND REMYELINATION

5.1 Recombinant TMEV as a molecular mimicry model

Using TMEV, Olson \textit{et al} (79) established a virus-induced molecular mimicry model of MS. They engineered a nonpathogenic TMEV (ΔC\textsubscript{IAI}-BeAn) encoding PLP\textsubscript{139-151}. Infection with the PLP\textsubscript{139-151}-encoding TMEV (PLP\textsubscript{139-BeAn}) led to rapid-onset of paralytic disease within ten days. As early as 14 days p.i., inflammatory demyelinating lesions were detected in
the spinal cord, and PLP$_{139-151}$-specific T cell proliferation and DTH response were detected in the spleen. Similar early-onset disease with anti-PLP responses was also observed in mice infected with a TMEV encoding altered peptide ligand (APL) of PLP$_{139-151}$ with an amino acid substitution at the T cell receptor contact residue (H147A PLP139-BeAn). PLP$_{139-151}$-Specific T cells, but not TMEV-specific T cells, from PLP139-BeAn-infected mice transferred demyelinating disease to naïve recipients, and induction of PLP$_{139-151}$-specific tolerance before infection prevented clinical disease (80). The authors suggest that both PLP139-BeAn and H147A PLP139-BeAn viruses induced PLP specific T cells, leading to early onset of demyelinating disease. No information is available about pathology and viral replication one week after PLP139-BeAn virus infection. It is also unknown whether this model is similar to TMEV infection or PLP$_{139-151}$-induced EAE clinically and histologically.

In this study, TMEV expressing an encephalitogenic PLP epitope as well as an APL of PLP could induce early-onset disease in the absence of a stimulus associated with CFA. This is in contrast to the infection of mice with a vaccinia virus construct expressing the entire coding region of PLP, which failed to induce CNS disease without a second challenge by CFA (81). Thus, TMEV might provide sufficient signals to induce autoreactive T cells in some circumstances. Importantly, the APL of PLP that is encoded in H147A PLP139-BeAn is not encephalitogenic even when administered with CFA in mice (82). This may mimic the situation of a recent clinical trial in MS, in which administration of an APL of MBP into MS patients resulted in induction of MBP-specific T cell proliferation and exacerbation of clinical signs (83,84).

5.2 Anti-neuroantigen antibodies as a therapeutic agent in TMEV infection

In contrast to the classical view of the anti-myelin immune responses playing a pathogenic role in CNS demyelinating disease, autoimmunity against myelin might play a beneficial role by promoting CNS regeneration: axonal regeneration and myelin regeneration (remyelination). In the EAE model, injection of a myelin component with IFA ameliorates clinical and pathological demyelinating disease. This therapeutic effect could be due to: 1) suppression of Th1 cell-mediated autoimmunity and 2) production of anti-myelin antibody that plays a direct role in remyelination. This latter role is in agreement with in vitro observations of antibody-mediated oligodendrocyte stimulation. Based on the initial observations by Traugott et al (85) of remyelination in EAE, Lang et al (86) conducted similar experiments with mice infected with the DA strain of TMEV. At three months p.i., mice
treated with subcutaneous injections of MBP and GC emulsified in IFA showed substantial CNS remyelination compared with control treated animals. In the following paper, however, they demonstrated that pretreatment of mice with SCH/IFA or MBP/IFA did not suppress clinical and histological disease in TMEV infection (65).

The discovery that certain autoantibodies can be beneficial in an animal model of CNS demyelination suggests that the presumed pathogenic role of antibodies in MS may need to be reevaluated. Using the TMEV model, Rodriguez and colleagues have shown a beneficial role of anti-neuroantigen antibodies due to their promotion of remyelination [reviewed in (87)]. Spontaneous remyelination is impaired in SJL/J mice infected with the DA strain of TMEV. Rodriguez and Lennon (88) observed that passive transfer of either antiserum or purified IgG from uninfected syngeneic animals immunized with SCH promoted remyelination by oligodendrocytes (CNS-type), but not by Schwann cells (PNS-type), in TMEV-infected mice. While astrocytes were common within areas of remyelination, inflammatory cells were less numerous in remyelinating areas than in demyelinating lesions. In vivo autoradiography with \[^3\text{H}\] thymidine demonstrated that the majority of radiolabeled cells were MNCs, while remyelinated areas contained GC--proliferating cells that might be immature oligodendrocytes, morphologically (89). However, subsequent experiments demonstrated that a similar extent of oligodendrocyte proliferation was also detected in mice treated with control sera, suggesting proliferation of oligodendrocytes is not sufficient for new myelin synthesis (90). Anti-SCH serum also stimulated proliferation of primary glial cell cultures in vitro (91). The effect of the treatment on anti-TMEV antibody production and virus persistence seemed to be inconclusive. A mild increase of anti-TMEV antibody titer was detected by an enzyme-linked immunosorbent assay (ELISA), but not by a plaque reduction assay; an increase of viral persistence was reported by immunohistochemistry against viral antigen, but not by virus plaque assays of CNS homogenates (92). Rodriguez et al (93) also demonstrated that treatment with antiserum or purified IgG directed against MBP promoted remyelination after TMEV infection without altering virus persistence or serum TMEV-specific antibody responses.

The Rodriguez group also generated a remyelination-promoting IgM mAb, SCH94.03, from the spleen cells of mice injected with SCH. SCH94.03 showed reactivity toward a red blood cell antigen, spectrin and several other protein antigens, astrocytes and oligodendrocytes, but not with MBP (94, 95). SCH94.03 injection suppressed T cell infiltration and increased TMEV persistence in the CNS, while levels of TMEV-specific IgG were similar to controls (96). SCH94.03 neither reacted with any TMEV proteins as determined by Western blot, nor neutralized TMEV
in vitro (97). Treatment with SCH94.03 also reduced relapse rate, demyelination and meningitis in SJL/J mice with EAE induced by adoptive transfer of MBP91.103-specific T cells (98). These results are consistent with the hypothesized immunomodulatory function of autoantibodies, although neither immune responses to MBP nor extent of remyelination was explored in this EAE model. Interestingly, however, SCH94.03 increased the rate of oligodendrocyte remyelination in a nonimmune, toxin-induced model of lysolecithin-induced demyelination (99).

Other IgM mAbs, SCH79.08, O1, O4, A2B5, and HNK-1, have also been shown to promote remyelination in TMEV models. SCH79.08 was established from mice immunized with SCH, reacts with MBP and shows intracellular, but not cell surface, staining of oligodendrocytes (100). O1, O4, A2B5, and HNK-1 antibodies recognize differentiation stage-specific surface antigens on oligodendrocytes. We do not know, however, whether these antibodies against oligodendrocyte precursors actually recognized the cells in vivo. Immunoperoxidase staining for markers expressed on presumed glial progenitor cells (O4, A2B5, and Gd3) was not successful in the adult mouse spinal cord in TMEV infection, even though the reagents worked in the neonatal rat optic nerve system in vitro (90).

Several antibodies join a list of immunoglobulins that enhance remyelination in the Theiler's virus model (101). The list includes pooled human intravenous IgG (IVIg), polyclonal human IgM, and two human IgM mAbs, sHlgM22 and sHlgM46, both of which were isolated from Waldenstrom's macroglobulinemia. Neither IVIg nor human IgM bound to human oligodendrocytes in vitro, while the two IgM mAbs bound to oligodendrocytes. Polyclonal human IgM and sHlgM22, but neither polyclonal IgG, anti-SCH IgG, nor IVIg, accelerated the rate of remyelination following lysolecithin-induced demyelination in SJL/J mice (99,102). Therefore, the authors suggested that IgG and IgM antibodies might function to enhance remyelination through different mechanisms.

The precise mechanisms of remyelination by the autoantibodies are unknown. It is unclear whether there is one single common mechanism which explains promotion of remyelination in all remyelination-promoting sera, IgG, and IgM antibodies. Because many of the remyelination-promoting antibodies bind to oligodendrocytes and/or myelin, a direct effect on the recognized cells has been hypothesized. However, since the remyelination-promoting autoantibodies have varying specificities, it is unlikely that each of the antibodies functions directly through a common antigen or receptor. The authors also suggested opsonization of myelin debris due to IgM antibody by phagocytes might enhance clearance of cellular debris from the damaged area, allowing the normal process of CNS repair to progress. Since Fc receptors on phagocytes do not generally bind
to the Fc portion of IgM, IgM likely interacts with C3b via its Cμ1 domain, thereby allowing antibody-antigen complexes containing IgM to indirectly mediate phagocytosis. Although by this mechanism, C3b, once fixed, can promote uptake via complement receptors on macrophages, the roles of complement and Fc receptor have not been tested in this model. Immunomodulation by autoantibody is also hypothesized. However, no data are available as to how autoantibody treatment might influence cellular immune responses to virus or myelin antigen.

Several other studies need to be performed to clarify the mechanism of remyelination, such as immunological and histological time course studies over a four-week antibody treatment period and in vivo trafficking of injected antibody. In addition, we do not know why autoantibodies stimulate CNS-type remyelination by oligodendrocytes, but not PNS-type remyelination by Schwann cells, despite the fact that the PNS also expresses antigens, including MBP, recognized by the autoantibodies (95). It should be noted that most remyelination-promoting studies used mice four to six months after TMEV infection, in contrast to the other TMEV research groups who use mice one to two months after TMEV infection. As discussed earlier, the mechanisms of demyelination might be different at these two time points.

Interestingly, among the autoantibodies, several (SCH94.03, SCH79.08, O1, O4, HNK-1), but not all (A2B5, shlgM22), monoclonal antibodies have been shown to have characteristics of natural autoantibodies (100,101,103). Natural autoantibodies, usually IgMs whose physiological significance is unknown, are present in the serum of normal unimmunized animal, and represent a substantial fraction of the total Ig repertoire (104). Natural autoantibodies show polyreactivity toward multiple self and nonself antigens, and are typically encoded by unmutated germ line Ig genes (103). It has been shown that some viruses may act as polyclonal B cell activators, may release antigens not ordinarily recognized by the host immune system, or may augment the response to many antigens (105,106). Any of these mechanisms may explain the induction of autoantibodies or an increase of the levels of natural autoantibodies.

It is attractive to hypothesize that the presence or absence of remyelination-promoting antibody might alter the disease course in demyelinating diseases. Here, a lack of the autoantibodies would prevent remyelination in DA virus-induced progressive disease in SJL/J mice. It may be intriguing to test whether the autoantibody is involved in the pathogenesis of CD-1 mice infected with the WW strain of TMEV, who develop a relapsing remitting disease course, characterized by extensive PNS- and CNS-type remyelination (13).

Clinically, comparison of the levels of autoantibodies in patients with RR-MS versus PP- and SP-MS might be important. From a patient with MS,
a clone of a possible natural antibody was established (107). This oligodendrocyte-reactive IgM monoclonal antibody, DS1F8, is polyreactive and its antigen-binding domains are encoded by germ line genes. In addition, Matsiota et al (108) found that MS patients often had elevated CSF antibody levels against many autoantigens, but not against MBP, suggesting a local expansion of B cells producing natural autoantibodies. Whether these antibodies have any effect on demyelination is unknown.

6. CONCLUSION

In this chapter, we have reviewed the interactions of TMEV, myelin and anti-myelin immune responses. On virus entry, TMEV might use peripheral myelin protein P₀ as a virus receptor, and require myelin genes functionally or myelin itself structurally for virus persistence. In turn, the host down-regulates myelin gene expression and/or loses oligodendrocytes and myelin. While this leads to demyelination, it might also be beneficial to the host since it prevents virus spread in the CNS. Anti-TMEV humoral immune responses can help virus clearance, while some anti-viral antibodies might

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**Figure 1.** Myelin infection, anti-myelin responses, and other effector mechanisms during TMEV disease course.
cross-react with host myelin molecules, including GC, leading to exacerbation of demyelination. During the early chronic phase, cellular immune responses to TMEV, but not to myelin, were detected. However, epitope spreading seems to develop during the late chronic phase, and T cell responses to encephalitogenic epitopes of PLP, MBP and MOG, can be detected. Impaired remyelination could also contribute to disease progression. Here, the presence or absence of remyelination-promoting antibody might be important in disease remission. Of all the above interactions between virus, myelin, and autoimmune responses, no single mechanism may be solely responsible for demyelination. However, all of the interactions potentially contribute to progression of demyelinating disease in which a variety of different pathogenic mechanisms, such as oligodendrocyte infection and apoptosis, axonal injury and anti-TMEV immune responses, seem to act in parallel or sequence to cause myelin destruction (Figure 1).

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B2. TMEV and Neuroantigens

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