

## **Pathogenesis of Virus-Induced Immune-Mediated Demyelination**

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### **Abstract**

Theiler's murine encephalomyelitis virus-induced demyelinating disease has been extensively studied as an attractive infectious model for human multiple sclerosis. Virus-specific inflammatory Th1 cell responses followed by autoimmune responses to myelin antigens play a crucial role in the pathogenic processes leading to demyelination. Antibody and cytotoxic T cells (CTL) responses to virus appears to be primarily protective from demyelinating disease. Although the role of Th1 and CTL responses in the induction of demyelinating disease is controversial, assessment of cytokines produced locally in the central nervous system (CNS) during the course of disease and the effects of altered inflammatory cytokine levels strongly support the importance of Th1 responses in this virus-induced demyelinating disease. Induction of various chemokines and cytokines in different glial and antigen presenting cells upon viral infection appears to be an important initiation mechanism for inflammatory Th1 responses in the CNS. Coupled with the initial inflammatory responses, viral persistence in the CNS may be a critical factor for sustaining inflammatory responses and consequent immune-mediated demyelinating disease.

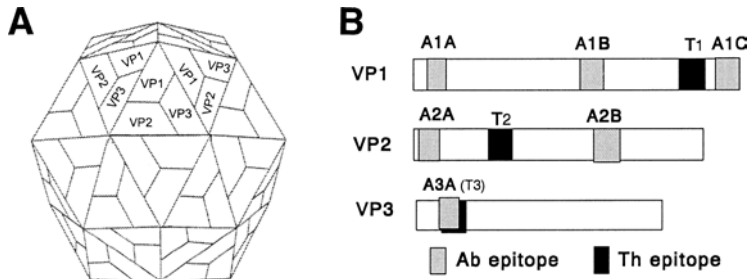
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### **Key Words**

Multiple Sclerosis  
Demyelination  
Theiler's virus  
Picornavirus  
Cytokines  
T cells

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**Fig. 1.** Viral capsid structure and the major epitopes for antibody as well as CD4<sup>+</sup> Th cells are schematically shown. VP1, VP2 and VP3 of Theiler's virus are external capsid proteins. Six major linear antibody epitopes common for resistant and susceptible mice and three predominant Th epitopes for susceptible SJL mice have been found by using overlapping fusion proteins and synthetic peptides. These three Th epitopes, one on each external capsid protein, represent the great majority (~80%) of Th responses to TMEV in SJL mice.

## Introduction

Multiple sclerosis (MS) is a neurological disease characterized by demyelination in the white matter of the brain and spinal cord mediated by inflammatory immune responses (1,2). Although the cause of MS is unknown, epidemiological evidence suggests that one or more infectious agents may be involved in the initial tissue damage leading to autoimmunity (1–3). Several virus-induced and autoimmune models have been used to study the underlying mechanisms of this disease (4–7). In particular, Theiler's murine encephalomyelitis virus (TMEV)-induced demyelination (6,8) provides an excellent infectious model for several reasons. First, intracerebral inoculation of TMEV into susceptible strains of mice results in a chronic immune-mediated demyelinating disease similar to human MS (9). Chronic inflammatory response is limited to the white matter of the CNS, and myelin breakdown is directly related to the clinical symptoms. Second, the virus infection is neurotropic and does not induce any other diseases except demyelination (6). Third, the susceptibility to disease is associated with gender (10), major histocompatibility complex genes (11,12) as well as T cell receptor

genes (13). Fourth, strong autoimmunity to myelin antigens is induced following the initial demyelination by virus-specific T cells as seen in MS (14). Finally, TMEV has only four capsid proteins as structural proteins and these proteins are primarily involved in induction of immune responses. The simple nature of this virus facilitates easy correlation between virus-specific immunity and disease progression (15).

## Antibody Response

To dissect the immune components involved in the protection and/or acceleration of demyelination, antibody responses to linear epitopes of viral capsid proteins were assessed by using fusion proteins and synthetic peptides (16–18). Six predominant linear antibody epitope areas, ranging from 13–26 amino acid residues were identified with antibodies from virus-infected mice (17; Fig. 1): A1A (VP1<sub>12–25</sub>), A1B (VP1<sub>146–160</sub>), A1C (VP1<sub>262–276</sub>), A2A (VP2<sub>2–16</sub>), A2B (VP2<sub>165–179</sub>), and A3A (VP3<sub>24–37</sub>). A time course study indicated that susceptible SJL mice intracerebrally infected with TMEV strongly and selectively recognize the A1C epitope of VP1 as compared to resistant BALB/c or C57BL/6 mice. The major

detectable antibodies in the CNS were also against A1C in virus-infected SJL mice. In addition, the level of antibodies to this epitope, capable of efficiently neutralizing virus *in vitro*, increased with the progression of disease. Further immunization with KLH conjugates of synthetic peptides containing individual antibody epitopes indicated that antibodies to only VP1 linear epitopes are significantly protective, and such protection may be restricted to the early stages of viral infection (18). Interestingly, mice immunized with A1C-KLH were significantly protected from the normal course of demyelination. This protection is conflicting with the appearance of anti-A1C antibodies which parallels disease progression. Thus, the exact role of antibodies specific for this VP1 epitope *in vivo* during the development of demyelinating disease is not yet clear.

### **CD8<sup>+</sup> T Cell Response**

The potential role of CD8<sup>+</sup> T cells in the pathogenesis of demyelination has been controversial. A research group has proposed that cytotoxic T cells (CTL) are necessary for clinical manifestation based on the lack of clinical symptoms in CTL-defective, perforin-deficient C57BL/6 mice (19,20). In contrast, we have shown that such mice are capable of developing clinical symptoms, especially following immunization with UV-inactivated virus (35% and 100%, respectively; 20a). We and others have also shown that viral infection can lead to clinical symptoms even in  $\beta_2$ -microglobulin-deficient mice lacking functional CD8<sup>+</sup> T cells (21,22). These results strongly suggest that virus-specific CTL are not necessary for the pathogenesis of demyelination in mice with C57BL/6 background genes. Moreover, treatment of susceptible SJL mice with anti-CD8 antibodies exacerbates the course of TMEV-induced demyelinating dis-

ease (23). Adoptive transfer of CD8<sup>+</sup> T cells also confers resistance to TMEV-induced demyelination in susceptible recipient mice of a BALB/c substrain (24), again consistent with a protective role of CD8<sup>+</sup> CTL. Therefore, the major role of virus-specific CTL in resistant as well as susceptible mice is most likely for protection via elimination of virus infected cells, which are a potential reservoir for viral replication.

Generally, it has been shown that one or two viral epitope(s) predominates the CTL response following viral infection (25). Similarly, it has been shown that TMEV infection also induces virus-specific CTL response toward a single dominant epitope in resistant C57BL/6 mice (26,27). In addition, two minor CTL epitopes were also identified in this mouse strain, one located near the predominant epitope on VP2, and the other on the VP3 capsid protein (Lyman et al., unpublished data). Although the role of CTL recognizing these minor epitopes is not yet clear, T cells recognizing these epitopes preferentially produce Th2-like cytokines, in contrast to Th1-like cytokines by T cells recognizing the predominant epitope. The level and specificity of CTL in susceptible SJL mice have not yet been well elucidated. A research group proposed differences in the level and kinetics of virus-specific CTL between resistant C57BL/6 mice and susceptible SJL mice: Rapid and high level of CTL efficient for removal of virus-infected cells in C57BL/6 mice and low and delayed response in SJL mice (28). Another group has suggested that virus-specific CTL are absent, whereas high levels of nonspecific cytotoxicity are observed in the CNS of SJL mice (29). Recently, we have identified the major and minor epitopes in the capsid proteins recognized by CNS infiltrating CD8<sup>+</sup> T cells from virus-infected SJL mice (Kang, Lyman and Kim, unpublished observation). A similar level of CD8<sup>+</sup> T cells recognizing viral epitopes in

conjunction with major histocompatibility complex (MHC) class I molecules was found in SJL mice as compared to that in resistant C57BL/6 mice, despite their failure to protect from viral persistence. Thus, it will be interesting to investigate the underlying mechanisms involved in the differential protection by CD8<sup>+</sup> T cells between these two different strains of mice. It was previously demonstrated that differences in H-2D rather than H-2K determine susceptibility of mice to the disease (11,12). This association is consistent with the fact that all the predominant and subdominant CTL from resistant C57BL/6 mice are restricted with H-2D, whereas H-2K restriction is preferred by CTL from susceptible SJL/J mice (Lyman et al., unpublished observation). The expression level of these class I molecules may be different and potentially influence CTL function in vivo. In addition, it is conceivable that potential differences are present in the CD8<sup>+</sup> T cell functions such as cytokine production, cytotoxicity, and/or regulatory function between resistant and susceptible mice.

### CD4<sup>+</sup> T Cell Response

The development and progression of demyelinating disease correlate well with the level of Th1 responses specific for viral epitopes (30–32). Administration of bacterial lipopolysaccharide (LPS) or IL-1 $\beta$  potentiating inflammatory Th1 responses into genetically resistant C57BL/6 mice infected with TMEV resulted in clinical symptoms (33). Similarly, LPS treatment of susceptible SJL/J mice, after infection with a nonpathogenic variant of TMEV, cause the mice to develop clinical symptoms (34). These results strongly suggest that an increase in the overall inflammatory Th1 response enhances the development of demyelinating disease in resistant as well as susceptible mice. The effects of addi-

tional representative inflammatory cytokines, such as IFN $\gamma$  and TNF $\alpha$ , have been further investigated. The presence of IFN $\gamma$  does not appear to be necessary for developing demyelination since the disease course was significantly accelerated in IFN $\gamma$ -receptor deficient mice (35) as well as after treatment with anti-IFN $\gamma$  antibody (36,37). Absence of IFN $\gamma$ -induced stimulation in these mice may result in reduced apoptosis controlling activated T cell levels (38,39). However, its presence can exacerbate the disease as shown by acceleration of the disease following intracerebral injection of recombinant IFN $\gamma$  (36). Similarly, treatment with antibodies to TNF $\alpha$  or IL-12 also delay the development and reduce the severity of TMEV-induced demyelination significantly (40–43). These results indicate that the reduction in the proinflammatory cytokine level generally provides a beneficial effect on virally induced demyelinating disease. However, IL-12 deficient mice on the resistant C57BL/6 background that display low levels of Th1 responses are not susceptible to demyelination (Lyman and Kim, unpublished observation). These results suggest that other gene effects also play an important role in the development of demyelinating disease.

The majority of CD4<sup>+</sup> helper T cell clones derived from demyelinating lesions of the spinal cords after viral infection react with VP1 or VP2 protein (30). In addition, the majority of T cells from virus-infected SJL mice recognize one of three predominant epitopes (VP1<sub>233–250</sub>, VP2<sub>74–86</sub> and VP3<sub>24–37</sub>) (44). Viral infection results in primarily Th1 responses to VP1 and VP2, in contrast to a Th2 response to the VP3 epitope peptide (30,32). Further immunization with VP1<sub>233–250</sub> or VP2<sub>74–86</sub> peptide exacerbates the disease whereas VP3<sub>24–37</sub> epitope does not, suggesting that T cells reactive to these VP1 and VP2 epitopes are likely to be involved in the patho-

genesis of demyelination. Production of Th1 cytokine messages in the CNS precedes that of Th2 cytokine messages in virus-infected SJL mice, suggesting that the initial establishment of a Th1 response is critical for the pathogenesis of TMEV-induced demyelination (34). Taken together, these experiments strongly suggest that CD4<sup>+</sup> helper T cells are primarily involved in the pathogenesis of TMEV-IDD, in particular, inflammatory Th1 responses to the VP1 and VP2 epitopes.

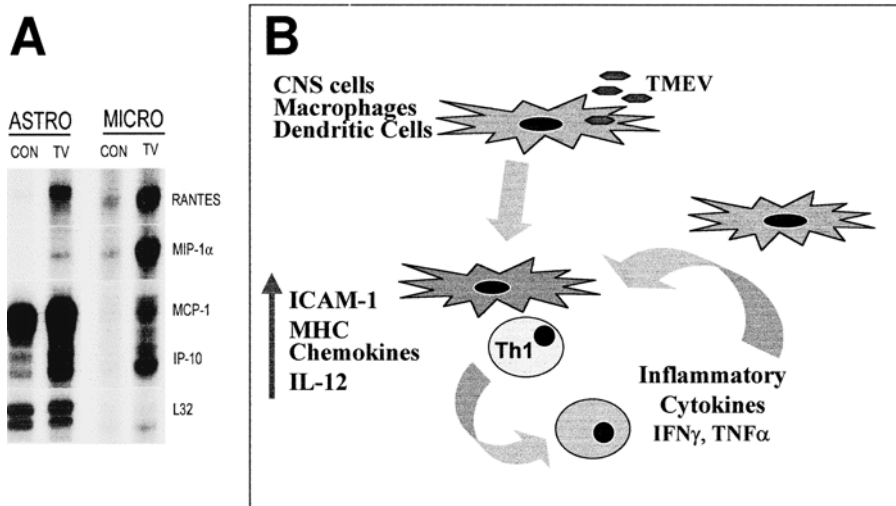
The CD4<sup>+</sup> T cell repertoire involved in the recognition of the representative pathogenic epitope, VP1<sub>233–250</sub>, appears to be diverse in virus-infected, susceptible SJL mice (45). Although close to 50% of the T cell hybridomas reactive to VP1<sub>233–250</sub> utilize the T cell receptor V $\beta$ 16, the CDR3 region is extremely heterogeneous among the T cell clones. The polymorphism in the TCR J $\beta$ 1 region differing between resistant C57L and susceptible SJL mice strongly affects susceptibility to virus-induced demyelinating disease (13). However, it is not yet clear how the J $\beta$ 1 polymorphism contributes in susceptibility to the disease. Analyses of TCR CDR3 regions utilized by CNS infiltrating T cells indicates that such virus-specific T cells are locally expanded in the CNS and some are maintained throughout the disease course (46). Nevertheless, the broad diversity and complexity of the T cell repertoire toward the predominant viral determinants on capsid proteins are apparent despite the local expansion of T cells and limited number of viral epitopes recognized by T cells.

### **Initiation of Inflammatory Response Following Viral Infection**

One of the most important questions in virus-induced inflammatory diseases is how viral infection initiates local or systemic inflammatory responses. To address this ques-

tion, we have begun to analyze the consequences of viral infection in host cells by using various primary cell cultures and cell lines (46a). Viral infection of astrocyte cultures selectively upregulated RANTES and IP-10 genes resulting in consequent chemokine production (Fig. 2A). Additional chemokine genes including MIP-1 $\alpha$  and MIP-1 $\beta$  were activated in oligodendrocyte and microglia cultures (Fig. 2A). An identical pattern of chemokine expression was found in astrocytes and total brain cultures after TMEV infection. Similarly, only RANTES and IP-10 expression was significant in the brain of virus-infected SJL/J mice as early as 1 and 3 d postinfection, suggesting the possibility that RANTES and IP-10 are the predominant chemokines induced at the site of initial viral infection. Therefore, these glial cells are likely to play an important role in the initial stages of virus-induced inflammatory responses via chemokine production for CNS recruitment of inflammatory cells, including Th1 cells (Fig. 2B).

Overlapping chemokines are also induced in astrocyte cultures after treatment with proinflammatory cytokines: Both RANTES and IP-10 by IFN $\gamma$  and RANTES only by TNF $\alpha$  (46a). Thus, Th1 cells infiltrating the CNS in response to chemokines produced initially by activated glial cells upon viral infection will most likely be further stimulated to secrete various proinflammatory cytokines (47). Moreover, similar to proinflammatory cytokines, viral infection also induces MHC class I and II molecules, ICAM-1, as well as IL-12 in astrocytes which are directly involved in the activation of inflammatory T cell responses. Subsequently these cytokines including IFN $\gamma$  and TNF $\alpha$  may activate additional glial cells (48–50) to produce similar chemokines (Fig. 2B), resulting in further recruitment and retention of virus specific, inflammatory cells in the CNS, leading to



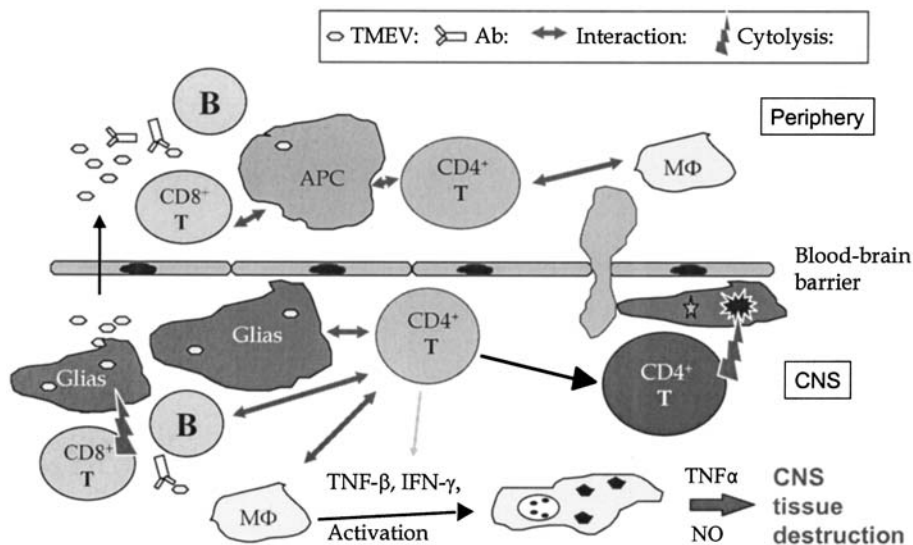
**Fig. 2.** Activation of glial cells following viral infection and potential mechanisms involved in the initiation of infiltrating inflammatory responses. **(A)** Induction of various chemokines by TMEV-infection in isolated CNS resident glial cells. Results of astrocytes and microglial cells are shown here as examples. The chemokine specific mRNA levels 5 hr after viral infection were assessed by RNase protection assays using a chemokine multiprobe set (PharMingen). **(B)** A schematic presentation of potential mechanisms for initiation and amplification of inflammatory responses in the CNS.

tissue damage and demyelination. However, viral persistence may be necessary to sustain continuous inflammatory responses for the pathogenesis of disease as these are dependent on the continuous viral infection and/or viral antigenic stimulation.

### Mechanisms of Demyelination

Accumulation of virus-specific Th1 cells in the CNS appears to be critical for the pathogenesis of demyelination (30). In addition, initial inflammatory responses to viral antigens are followed by autoimmune responses to the major myelin components (14). The ability of this virus to directly lyse host cells may contribute to the release of autoantigens for induction of these autoimmune responses. Furthermore, viral infection of professional antigen presenting cells such as dendritic cells and macrophages, preferentially induces produc-

tion of IL-12, promoting inflammatory Th1 responses (Kim and Palma, unpublished observation). The local presentation of viral epitopes to infiltrating Th1 lymphocytes in the CNS by activated resident glial cells (47) may also be a critical step for both the initiation and progression of the immune-mediated tissue damage following viral infection (Fig. 3). Proinflammatory cytokine-activated astrocytes may present viral antigens and further activate virus-specific CD4<sup>+</sup> T cells, leading to Fas-mediated apoptosis by the activated T cells (47). Such apoptosis of astrocytes may contribute to the pathogenesis of TMEV-induced demyelination by compromising the integrity of the blood-brain barrier. In addition, activation of astrocytes induces a variety of immunoregulatory cytokines (51), chemokines attracting various inflammatory cells (52), as well as adhesion and costimulatory molecules (53,54). Similarly, virus infection itself appears



**Fig. 3.** Potential pathogenic mechanisms involved in immune-mediated demyelination induced following TMEV infection. Viral infection may lead to viremia and stimulate various immune responses including B cell/antibody, Th cell and cytotoxic T cell responses to viral antigens. Such activated, virus-reactive T cells then infiltrate in the CNS. Resident glial cells may also be activated by inflammatory cytokines and become competent antigen presenting cells that further amplify the inflammatory Th1 cell response in the CNS. Activated Th1 cells may consequently lead to further breaching of the blood brain barrier by direct apoptosis of CNS cells and/or cytokine-dependent mechanisms. In addition, such inflammatory cytokines may also activate monocytes and macrophages which participate in local tissue damage. The tissue destruction may lead to further release of autoantigens for autoimmunity. Virus-specific antibody and CTL may be involved in the neutralization of virus infection and elimination of virus-infected resident CNS cells and macrophages, respectively.

to activate a variety of immune response genes in glial cells, including chemokines, cytokines such as IL-12 and MHC molecules (Fig. 2). Overall, these may promote the influx of inflammatory cells into the CNS and further amplify immune-mediated demyelination. This chain of immune stimulation appears to be dependent on viral persistence and/or autoimmunity, which are likely required to sustain the levels of inflammatory responses (Fig. 3). In addition, antibody and CTL responses may be involved in restricting viral persistence. Although preexisting antibodies may be able to prevent virus persistence and consequent inflammatory responses, antibodies are not very effective in eliminating established viral

persistence. CTL function rather than the level of CD8<sup>+</sup> T cells may be most important in controlling viral persistence, although this issue has to be clarified by further studies. We believe that these studies will continuously provide important information regarding the pathogenic mechanisms involved in virally induced inflammatory diseases and, consequently, strategies for prevention or therapy of such virus-induced diseases.

### Acknowledgments

This work was supported by United States Public Health Service Grants, NS28752, NS33008 and NS23349, and by a National Multiple Sclerosis Society grant, RG3126-A4.

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