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Functional Activation of Myelin-Specific T Cells by Virus-Induced Molecular Mimicry

Julie K. Olson, Todd N. Eagar, and Stephen D. Miller

Molecular mimicry is the process by which T cells activated in response to determinants on an infecting microorganism cross-react with self epitopes, leading to an autoimmune disease. Normally, infection of SJL/J mice with the BeAn strain of Theiler’s murine encephalomyelitis virus (TMEV) results in a persistent CNS infection, leading to a chronic progressive, CD4+ T cell-mediated demyelinating disease. Myelin damage is initiated by T cell responses to virus persisting in CNS APCs, and progressive demyelinating disease (50 days postinfection) is perpetuated by myelin epitope-specific CD4+ T cells activated by epitope spreading. We developed an infectious model of molecular mimicry by inserting a sequence encompassing the immunodominant myelin epitope, proteolipid protein (PLP) 139–151, into the coding region of a nonpathogenic TMEV variant. PLP139-TMEV-infected mice developed a rapid onset paralytic inflammatory, demyelinating disease paralleled by the activation of PLP139–151-specific CD4+ Th1 responses within 10–14 days postinfection. The current studies demonstrate that the early onset demyelinating disease induced by PLP139-TMEV is the direct result of autoreactive PLP139–151-specific CD4+ T cell responses. PLP139–151-specific CD4+ T cells from PLP139-TMEV-infected mice transferred demyelinating disease to naive recipients and PLP139–151-specific tolerance before infection prevented clinical disease. Finally, infection with the mimic virus at sites peripheral to the CNS induced early demyelinating disease, suggesting that the PLP139–151-specific CD4+ T cells could be activated in the periphery and traffic to the CNS. Collectively, infection with PLP139–151 mimic encoding TMEV serves as an excellent model for molecular mimicry by inducing pathologic myelin-specific CD4+ T cells via a natural virus infection. The Journal of Immunology, 2002, 169: 2719–2726.

Autoimmune diseases have been suggested to be initiated and perpetuated as a result of virus infections. Three possible mechanisms have been suggested for initiating autoimmune disease following virus infection: bystander activation, molecular mimicry, and epitope spreading (reviewed in Ref. 1). Molecular mimicry is the process by which cross-reactive T cells are generated during a virus infection that recognizes both viral and self epitopes (2). Over the past decade, evidence for a role of molecular mimicry in the initiation of autoimmune diseases has resulted from a number of approaches. Initial molecular mimicry studies showed that transgenic mice expressing virus proteins in the context of self tissues developed autoimmune disease following infection with the corresponding virus (3, 4). Previous studies have also demonstrated that viral peptides sharing limited sequence homology with self myelin peptides could stimulate autoreactive T cell clones isolated from patients with multiple sclerosis (5, 6). Mimic epitopes have been identified in Borrelia burgdorferi that may be involved in Lyme arthritis and neuroborreliosis using patient T cell clones (7, 8), and heart disease was shown to be induced by immunizing mice with a Chlamydia pneumoniae peptide that shared sequence homology with heart-muscle protein (9). Although these studies provided support for a role of molecular mimicry in autoimmune disease, the evidence was indirect in that autoreactivity was not induced as a direct consequence of infection. Recently, several reports have provided more direct evidence of infection-induced molecular mimicry by showing that infection with HSV type I leads to the development of herpes stromal keratitis, an inflammatory eye disease (10), and that a Theiler’s virus variant engineered to encode a molecular mimic of an encephalitogenic proteolipid protein epitope (PLP139–151) could induce demyelinating disease upon infection (11).

Multiple sclerosis (MS) is a CD4+ T cell-mediated autoimmune demyelinating disease affecting humans that has a suspected virus etiology (12). Theiler’s murine encephalomyelitis virus (TMEV)-induced demyelinating disease serves as a relevant mouse model of MS due to numerous pathological and immunological similarities (13). Infection of susceptible SJL/J mice with the BeAn strain of TMEV results in a chronic progressive CD4+ T cell-mediated CNS demyelinating disease onseting 30–35 days postinfection and characterized by persistent infection of CNS microglia (14). Myelin destruction is initiated by virus-specific CD4+ T cells that eventually lead to the activation of myelin-specific, autoreactive CD4+ T cells via epitope spreading, with responses to PLP139–151 first detected at 50 days postinfection (15, 16). Therefore, TMEV-induced demyelinating disease is a highly relevant model for studying virus-initiated autoimmune disease.

Recently, we developed a molecular mimicry model for initiation of autoimmune demyelination following virus infection with TMEV containing a self myelin epitope (11). Native or mimic sequences of the immunodominant PLP139–151 epitope were inserted into a nonpathogenic TMEV variant. Infection of SJL mice...
with TMEV encoding native mouse PLP139–151, an altered peptide ligand of PLP139–151, or a PLP139–151 mimic present in the protease IV protein of *Hemophilus influenzae* induced a rapid onset demyelinating disease associated with the induction of PLP139–151-specific CD4+ T cell responses. The current studies were conducted to determine whether the early onset demyelinating disease occurred directly through early activation of PLP139–151-specific CD4+ T cells. The results show that the PLP139–151-specific CD4+ T cells induced in PLP139-TMEV-infected mice were encephalitogenic, as demonstrated by serial transfer of these T cells to naïve mice, and that infection of mice with PLP139-TMEV at sites peripheral to the CNS resulted in activation of PLP139–151-specific CD4+ T cells leading to CNS demyelination. Most compellingly, PLP139–151-specific tolerance induction before PLP139-TMEV infection resulted in reduced clinical demyelinating disease corresponding to a reduced PLP139–151-specific CD4+ T cell response. Collectively, these results demonstrate that infection with a virus expressing a self epitope mimic can directly induce autoreactive T cells with pathologic potential in the absence of CFA.

**Materials and Methods**

**Construction of mimic BeAn viruses**

The cDNA encoding the BeAn strain of TMEV was modified to contain a ClaI restriction site at bp 1137, resulting in the deletion of 23 aa from the virus leader (designated ΔCla-BeAn), as previously described (11). Briefly, PCR methods were used to insert ClaI sites into PLP DNA at both ends of a 30-aa piece, PLP139–159, which encompassed the encephalitogenic PLP139–151 epitope. This piece was then inserted into the ClaI site of the ΔCla BeAn cDNA. Viral RNA was produced from the cDNA for each recombinant using a T7 promoter transcription kit (Roche Molecular Biochemicals, Indianapolis, IN) and then transfected using Lipofectin (Life Technologies, Rockville, MD) into BHK-21 cells to produce infectious virus. BHK-21 cells were infected with virus in serum-free DMEM medium (Sigma-Aldrich, St. Louis, MO) and then transfected using Lipofectin (Life Technologies, Rockville, MD) into BHK-21 cells to produce infectious virus. BHK-21 cells were repeatedly infected with virus in serum-free DMEM medium (Sigma-Aldrich, St. Louis, MO) until a viral titer of 1 × 10⁶ PFU/ml was obtained for the viral construct.

**Infection and clinical evaluation of mice**

Five- to 6-week-old female SJL mice were purchased from Harlan Labs (Bethesda, MD) and housed in the Northwestern University animal facility. Mice were infected by intracerebral (i.c.) injection with 3 × 10⁶ PFU virus. Mice were scored at daily intervals on a clinical scale of 0–5; score 0, mice show no signs of disease; score 1, mice show mild gait abnormalities; score 2, mice show more severe gait abnormalities; score 3, mice had paralysis in one limb; score 4, mice had more than one paralyzed limb; score 5, mice were moribund.

**Peptides**

PLP139–151 (HSLOGKWLGHPDVK), PLP178–191 (NTWTTCCQSAFPSS), VP2 70–86 (WTTSQEAFSHIRPLPH), and OVA323–339 (ISQAVHAAHAEINEAGR) were purchased from Peptides International (Louisville, KY). The amino acid composition was verified by mass spectrometry, and purity was assessed by HPLC.

**Delayed-type hypersensitivity response**

Delayed-type hypersensitivity (DTH) assays were performed by injecting mice s.c. with 5 µg challenge peptide, PLP139–151 or VP2 70–86, into the ears. The ear thickness was determined before injection using a Mutoyto model 7326 engineers micrometer (Schlesinger’s Tools, Brooklyn, NY). After 24 h, the ears were again measured, and differences in ear swelling over prechallenge thickness were expressed in units of ± SEM.

**T cell proliferation and cytokine assays**

Spleens were removed from infected mice at various times following infection. Splenocytes were cultured at 1 × 10⁶ cells/well (in 96-well microtiter plates) in HL-1 medium (BioWhittaker, Walkersville, MD) supplemented with 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin. Peptides were added to the wells at increasing concentrations from 1 to 100 µM. Plates were incubated at 37°C for 72 h and then pulsed with 1 µCi [3H]Tdr for 24 h before harvesting and counting. Proliferation was determined with triplicate wells for each peptide concentration and then expressed as cpm ± SEM. The stimulation index (SI) was calculated by dividing cpm in experimental wells by the cpm in PBS-containing wells.

For cytokine analysis, a duplicate set of proliferation wells was used to collect supernatants at 48 and 72 h. Supernatants were analyzed for IL-2, IFN-γ, or IL-5 secretion by ELISA (Endogen, Cambridge, MA).

**Coupled cell tolerance**

Spleens were removed from naive mice and mechanically disrupted, and the RBCs were lysed. The splenocytes were incubated with ethylcarbodi- midel (150 µg/ 3.2 × 10⁶ cells) and peptide (1 mg/ml PLP139–151 or OVA 323–339) on ice, shaking periodically for 2 h. The coupled cells were washed and centrifuged to remove cell clumps. The single cell suspension was resuspended in BSS, and 5 × 10⁵ coupled cells were injected i.v. into each recipient mouse. Recipient mice were given peptide-coupled spleno- cytes at day 7 before infection and again at day 3 following infection with PLP139-TMEV virus.

**Serial disease transfer**

Female SJL mice 5–6 wk old were infected with PLP139-TMEV virus, as described above. At 21 days postinfection, spleens were removed from the mice and mechanically disrupted, and the RBCs were lysed. The resulting splenocytes were cultured with 20 µg/ml peptide (PLP139–151 or VP2 70–86) for 4 days in DMEM supplemented with 10% FCS, 0.1 mM non-essential amino acids, 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 5.5 µM 2-ME. After the incubation, some of the cells were incubated with anti-CD4-coated MACS beads (Miltenyi Biotec, Auburn, CA), and then sorted on the AutoMacs (Miltenyi Biotec) column to separate out the CD4+ T cells. Both the whole splenocytes and the sorted T cells were washed and resuspended in BSS. Then 3 × 10⁶ blast cells were transferred i.v. to each mouse.

**Viral plaque assay**

The brains and spinal cords were removed from the infected mice at the indicated days postinfection. The organs were homogenized and then di- luted in serum-free DMEM for the assay. BHK-21 cells were seeded in tissue culture dishes for 2 days and grown to confluency. The cells were washed with serum-free DMEM before the addition of the homogenized tissue dilutions. The cells were then incubated at room temperature for 1 h with occasional swirling. A 2% agar solution was added in equal volume to 2× DMEM supplemented with 2% serum, plus 5 mM L-glutamine, 200 µg/ml penicillin, and 200 µg/ml streptomycin. Following a 1-h incubation, the DMEM/agar mixture was added to the cells, and the cells were incu- bated at 34°C for 6 days in a humidified environment, at which time the agar layer was removed from the cells. The cells were fixed with methanol, and then stained with crystal violet solution (0.12% crystal violet in 20% ethanol). The plaques were counted on each plate and multiplied by the dilution and the amount of homogenate added to the plate to determine the PFU/ml. The weight of the tissue (mg/ml homogenate) was then used to calculate PFU/mg.

**Results**

**Infection with PLP139-TMEV results in a rapid onset severe demyelinating disease**

Intracerebral injection of mice with the wild-type (WT) BeAn strain of TMEV leads to the development of a chronic progressive demyelinating disease with clinical signs appearing ~30–40 days postinfection (Fig. 1). A nonpathogenic variant of the BeAn virus, ΔCla BeAn, was constructed by removing a 23-aa coding region from the leader sequence and inserting a ClaI restriction digest site into which the immunodominant myelin epitope PLP139–151 was then inserted to produce PLP139-TMEV (11). Mice infected with PLP139-TMEV developed an earlier onset severe demyelinating disease compared with WT TMEV-infected mice (Fig. 1). As a control, a virus was constructed with an irrelevant epitope OVA233–339 inserted into the ΔCla BeAn virus, and mice infected with this virus developed a late onset demyelinating disease similar to the WT BeAn virus.
PLP139-TMEV virus infection results in encephalitogenic PLP139–151-specific CD4+ T cells

Serial transfer experiments were conducted to determine whether infection with PLP139-TMEV virus in the absence of CFA induces the differentiation of encephalitogenic PLP139–151-specific CD4+ T cells. Previous studies have demonstrated that PLP139–151-specific CD4+ T cells isolated from spleens of mice immunized with PLP139–151/CFA can transfer experimental autoimmune encephalomyelitis (EAE) to naive recipient mice (17). Splenocytes from SJL mice 21 days postinfection with PLP139-TMEV were reactivated in vitro with either PLP139–151 peptide or viral peptide, VP2 70–86, as a control. One group of PLP139–151-reactivated splenocytes was magnetically sorted to isolate CD4+ T cells. Peptide-activated splenocytes or sorted CD4+ T cells were transferred i.v. to naive mice. Mice that received the PLP139–151-reactivated splenocytes developed clinical signs of demyelinating disease onsetting ~10 days posttransfer (Fig. 2, A and B). However, mice that received VP2 70–86-activated splenocytes did not develop demyelinating disease, despite the fact VP2 70–86-reactive cells are found in the spleens of PLP139-TMEV-infected mice (11). Furthermore, purified PLP139–151-reactivated splenic CD4+ T cells also were able to transfer disease to naive mice similar to the PLP139–151-reactivated splenocytes. Meanwhile, the reactivated splenocytes that were not CD4+ (those remaining after the CD4+ purification) were unable to transfer disease to naive recipients (data not shown). Next, the CD4+ T cell responses were examined in the transfer recipients (Fig. 3). Mice that received the PLP139–151-reactivated splenocytes or CD4+ T cells displayed specific CD4+ T cell responses to PLP139–151, but not to virus peptide. Therefore, PLP139–151-specific CD4+ T cells that arise during infection with PLP139-TMEV virus can directly mediate demyelinating disease. These experiments demonstrated that infection with a virus that encodes a self epitope directly activates self-reactive CD4+ T cells with the potential to initiate an autoimmune disease.

PLP139-TMEV virus infection at peripheral routes results in the development of CNS demyelinating disease

PLP139-TMEV virus administered directly to the CNS, via i.c. injection, results in demyelinating disease in the CNS. In an attempt to determine whether local CNS inflammation at disease onset could initiate local T cell responses, SJL mice were infected with PLP139-TMEV virus and observed for clinical signs of disease. SJL mice infected with PLP139-TMEV virus showed signs of disease onset ~10 days postinfection (Fig. 1). Moreover, mice that received PLP139–151-reactivated splenocytes or CD4+ T cells displayed specific CD4+ T cell responses to PLP139–151, but not to virus peptide. Therefore, PLP139–151-specific CD4+ T cells that arise during infection with PLP139-TMEV virus can directly mediate demyelinating disease. These experiments demonstrated that infection with a virus that encodes a self epitope directly activates self-reactive CD4+ T cells with the potential to initiate an autoimmune disease.
initiation was a prerequisite for the development of virus-induced molecular mimicry, we asked whether PLP139–151-specific CD4⁺ T cells and clinical disease could be activated by PLP139-TMEV infection at sites peripheral to the CNS. SJL mice were infected with PLP139-TMEV virus via i.v., i.p., and s.c. routes and followed for development of clinical disease signs. As shown in Fig. 4, SJL mice infected at peripheral sites developed early onset demyelinating disease with similar incidence of disease and kinetics as by the i.c. route, but with slightly reduced severity. Therefore, infection with epitope mimic PLP139-TMEV virus at sites peripheral to the CNS target organ leads to development of organ-specific autoimmune disease.

To determine the relative efficiency of activation of PLP139–151-specific CD4⁺ T cell responses in mice infected by the different routes, splenic proliferative responses of PLP139-TMEV-infected mice were analyzed at varying times postinfection (Fig. 5). Mice infected by all the different routes of infection developed significant CD4⁺ T cell responses to the major viral epitope, VP2 70–86, and to PLP139–151 by 14 days postinfection. Interestingly, proliferative responses in the mice infected by the peripheral routes, particularly i.v., were somewhat higher than those in mice infected by the i.c. route (Fig. 5A). This same pattern was observed when CD4⁺ T cells were analyzed for secretion of the Th1 cytokines, IFN-γ and IL-2, in response to viral and myelin epitopes at 14 days postinfection (Fig. 6). Splenic proliferative and cytokine responses to both VP2 70–86 and PLP139–151 persisted in all groups at 28 and 56 days postinfection. Responses in mice infected by the i.v. route remained greater than the other routes at later times postinfection. Mice infected with PLP139-TMEV virus by the various routes did not result in induction of CD4⁺ T cells that secreted IL-4 or IL-5 in response to either viral or myelin peptides (data not shown). These results suggest that infection with the mimic virus at sites peripheral to the CNS can activate CD4⁺ Th1-type T cells in the periphery, resulting in the development of autoimmune demyelinating disease in the CNS.

TMEV is a CNS-tropic virus that is naturally transmitted to the CNS secondary to infection via the gut mucosa. The infection eventually spreads to the CNS, where the virus can persist, leading to the development of autoimmune demyelinating disease. Therefore, mice infected at the sites peripheral to the CNS were analyzed by plaque assays on brains and spinal cord tissue to determine the relative ability of the virus to spread to the CNS. At 21 days postinfection, mice infected by i.v., i.p., or s.c. routes have similar virus loads in both the brain and spinal cord (Table I). However, the virus loads in the mice infected by the peripheral routes were significantly lower than those in the brains and spinal cords from mice infected i.c. with PLP139-TMEV, which had similar virus load as WT BeAn-infected mice. Therefore, PLP139-TMEV mimic virus can spread to the CNS in mice infected at sites peripheral to the CNS, but at significantly reduced levels compared with mice infected in the CNS, even though the severity of the disease course in mice infected by the peripheral and CNS routes is similar. This suggests that the rapid onset CNS disease is primarily due to the
The various treatment groups were next examined for CD4+ T cell responses. Mice infected with PLP139-TMEV virus develop CD4+ T cell responses, as measured by both DTH and T cell proliferation to the dominant virus epitope, VP2 70–86, and to the myelin mimic epitope, PLP139–151, at early times postinfection, and the responses continue through later times postinfection (Fig. 8). PLP139–151-tolerized mice exhibited normal CD4+ T cell-
specific responses to VP2 70–86, but significantly reduced responses to PLP139–151 at early as well as at later times post-
PLP139-TMEV infection. In contrast, mice tolerized to control
OVA133–339 peptide developed CD4+ T cell-specific responses to
both VP2 70–86 and PLP139–151 similar to untreated mice. Simi-
larly, PLP139–151, but not VP2 70–86-induced Th1 (IL-2 and
IFN-γ) cytokine responses were inhibited in PLP139–151-SP-
tolerized mice (Fig. 9). These results directly demonstrate that the
early demyelinating disease in mice infected with PLP139-TMEV
virus is dependent on the PLP139–151-specific CD4+ Th1 re-
sponses, as demonstrated by specifically reduced PLP139–151-
specific CD4+ T cell responses accompanied by reduced demy-
elinating disease in PLP139–151-tolerized mice.

Discussion
These studies demonstrate that a nonpathogenic strain of TMEV
encoding a self myelin epitope (PLP139-TMEV) can induce an
early onset autoimmune demyelinating disease via activation of
autoreactive PLP139–151-specific CD4+ T cells upon virus infec-
tion of SJL mice in the absence of CFA. PLP139–151-specific
CD4+ T cells activated upon infection with PLP139-TMEV were
encephalitogenic, as demonstrated by their ability to transfer au-
toimmune disease to naive mice. Second, infection with the
PLP139-TMEV by routes peripheral to the CNS resulted in acti-
vation of PLP139–151-specific CD4+ T cells in the periphery,
which could initiate autoimmune demyelinating disease in the
CNS. Finally, PLP139–151 epitope-specific tolerance before infec-
tion with PLP139-TMEV resulted in the specific reduction of
PLP139–151-specific CD4+ Th1 responses that directly correlated
with a significant reduction in the incidence and severity of the
early onset demyelinating disease.

Organ-specific autoimmune diseases have been strongly sug-
gested to be initiated and/or exacerbated by virus infection (1). The
possible mechanisms by which virus infection can trigger an au-
toimmune response include molecular mimicry, bystander activa-
tion, and epitope spreading. Molecular mimicry involves the de-
ovo activation of autoreactive T cells due to the cross-reactivity
between self epitopes and viral epitopes during a virus infection
(2). Bystander activation is the nonspecific activation of autoreac-
tive T cells resulting from the direct inflammatory and/or necrotic
effects of virus infection on tissue in the target organ (20). Epitope
spreading is the activation of autoreactive T cells due to the tissue
damage following virus infection releasing self epitopes as a result
of the immune response to an organ-localized persistent virus infec-
tion (15, 21).

Molecular mimicry theoretically results from infection with a
virus expressing a peptide determinant(s) that shares homology
with a self peptide, resulting in activation of T cells that can cross-
react with the self epitope. TCR degeneracy has been ascribed as
the reason for recognition of multiple peptides by the same TCR.
Recent studies have shown degeneracy in the TCR specific for the
human myelin basic protein MBP85–99 peptide, with the TCR
requiring only a few critical residues for recognition (22). T cell
clones specific for MBP85–99 established from MS patients were
shown to cross-react with viral peptides expressed by a number of
viruses, including HSV, adenovirus, reovirus, and human papillo-
mavirus (5). Likewise, a few critical residues were shown to be
necessary for recognition of PLP139–151 by its TCR (23, 24).
PLP139–151-specific T cell hybridomas derived from SJL mice
were also shown to cross-react with peptides expressed by various
mouse pathogens, demonstrating degeneracy in the PLP139–151
TCR (25). Therefore, myelin-specific T cells have been shown by

**FIGURE 8.** PLP139–151-specific CD4+ T cell responses are specifically inhibited in mice tolerized with PLP139–151-SP before infection with
PLP139-TMEV virus. DTH and splenic T cell proliferation were used to assess T cell responses in mice tolerized with PLP139–151-SP or control treated
with OVA133–339-SP 7 days before and 3 days after i.c. infection with PLP139-TMEV virus on day 0. DTH was determined upon ear challenge at days
14 (A) and 28 (B) postinfection with 5 μg PLP139–151 or with the immunodominant TMEV peptide VP2 70–86. *, Indicates significant DTH response
over naive mice, p < 0.05. Splenic CD4+ T cell proliferative responses were performed in cultures stimulated with the myelin peptides, PLP139–151 and
PLP178–191, or viral peptide VP2 70–86, and at days 14 (C) and 35 (D) postinfection. *, Indicates significant increase over PBS controls for each group
(SI ≥ 3). Results shown are from a representative experiment of four replicates.
in vitro studies to have the potential to cross-react with viral epitopes, supporting the molecular mimicry model described in these studies.

EAE is a well-established experimental model for studying self myelin peptide-induced autoimmune demyelinating disease. PLP139–151/CFA immunization of SJL mice results in a relapsing-remitting demyelinating disease mediated in the initial phase by PLP139–151-specific CD4+ Th1-type T cells with the following relapses mediated by myelin-specific Th1 cells specific for endogenous myelin epitopes such as PLP178–191 and MBP84–104 activated via epitope spreading (18, 21, 26). The pathologic significance of epitope spreading is illustrated by the finding that tolerance to PLP178–191, but not to PLP139–151, is required to prevent disease relapses in mice treated following recovery from acute disease (18). Additionally, PLP139–151-specific CD4+ T cells from the immunized mice are encephalitogenic and can transfer disease to naive recipient mice (18, 27). However, induction of EAE initiated by either the native mouse myelin peptides or mimic peptides requires the presence of CFA to induce this hierarchical cascade of peptide-specific autoimmune responses. CFA creates an artificial inflammatory milieu that does not reflect the natural environment in which self or mimic peptides would be normally encountered; thus, an infectious model as described in the current studies is more appropriate for the study of molecular mimicry.

TMEV is a natural mouse pathogen that upon infection can lead to the development of an autoimmune demyelinating disease in which the chronic phase of disease is mediated by myelin-specific CD4+ Th1 cells (21, 28). Previous studies have shown that the development of autoimmune demyelinating disease is dependent on persistent virus infection of the CNS, predominantly in microglial cells (29). Initially following infection with the WT BeAn strain of TMEV, mice display CD4+ T cell responses only to virus Ags; however, beginning ~50 days postinfection, CD4+ T cell responses to immunodominant PLP139–151 myelin epitope develop. Subsequently, as the disease progresses, epitope spreading leads to development of CD4+ T cell responses to a variety of additional myelin epitopes (15). Furthermore, the demyelinating disease develops independent of MHC class I presentation to CD8+ T cells (30). Tolerance to intact TMEV virions coupled to syngeneic splenocytes before TMEV infection anergizes virus-specific CD4+ T cell responses, resulting in a reduction of incidence and severity of demyelinating disease (31, 32). In contrast, induction of peripheral tolerance to mouse spinal cord homogenate before TMEV infection did not affect the initiation of TMEV-induced clinical disease and had no effect on the development of virus-specific CD4+ T cell responses (33). Together, these studies indicate that the initial virus-specific T cell response and continued response to persistent virus infection are critical for the activation of the myelin-specific CD4+ T cell response and the development of the chronic progressive autoimmune demyelinating disease.

Recently, our laboratory developed a virus-induced model of molecular mimicry for the induction of autoimmune demyelinating disease (11). A nonpathogenic TMEV variant (∆CiaI-BeAn) was used to construct a virus that contained a self myelin sequence, PLP130–159, within the virus coding region. Mice infected with this virus developed an early onset autoimmune demyelinating disease as well as displaying early CD4+ T cell responses to both TMEV epitopes and PLP139–151, unlike infection with WT TMEV, in which early T cell responses are only seen in response to virus epitopes. In addition, mimic PLP139–151 sequences were constructed in which amino acid substitutions were made at the primary (aa 144) or secondary (aa 147) TCR contact residues. Infection with the virus carrying a substitution in the secondary TCR contact residue induced early onset demyelinating disease and activated cross-reactive PLP139–151-specific CD4+ T cells. In contrast, infection with the virus substituted at the primary TCR contact residue (position 144) failed to induce early demyelinating disease or activation of cross-reactive PLP139–151-specific CD4+ T cells. An additional mimic virus was constructed by inserting a sequence from H. influenzae that shared only 6 of 13 aa with the core PLP139–151 epitope (11). More significant to a role for molecular mimicry in induction of autoimmune disease, infection with this mimic virus resulted in early onset demyelinating disease and activation of Th1 cells cross-reactive with the native PLP139–151 determinant. This model is the first to directly demonstrate that a virus encoding a mimic of an encephalitogenic self myelin epitope could induce an autoreactive CD4+ T cell response leading to a CNS demyelinating disease.

As illustrated by the ability of PLP139–151-specific CD4+ T cells recovered from PLP139-TMEV-infected mice to transfer disease to naive recipients and by the ability of peptide-specific tolerance to inhibit induction of PLP139-TMEV-induced disease, the current results clearly demonstrate that the early onset demyelinating disease is due to the activity of the autoreactive PLP139–151-specific Th1 cells and not due to any direct or indirect effects of the virus infection. The failure of splenic T cells reactivated in vitro with VP2 70–86 to transfer disease (Fig. 2) also indicates there is no cross-reactivity between the immunodominant TMEV epitope and any myelin epitopes. Our previous report (11) showed that insertion of even a nonsense epitope (OVA317–346) into the nonpathogenic TMEV parental virus led to a late onset (50 days postinfection) disease, indicating that reintroduction of nonspecific 30 mer into the leader of the ∆CiaI-BeAn parental strain restored the ability of the virus to persist in vivo. Mice infected with...
Reference

2726 MYELIN-SPECIFIC AUTOIMMUNITY INDUCED BY VIRUS INFECTION

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In summary, these studies provide direct evidence that TMEV encoding PLP139–151 causes early onset CNS demyelinating disease due to the direct activation of autoreactive T cells. Using appropriate HLA-DR and human TCR transgenic mice (34), this model system should allow the functional identification of pathogen-encoded mimic epitopes for a variety of human myelin proteins that may be involved in the pathogenesis of MS. A final advantage of the TMEV molecular mimicry model that inserts 30 mers into the virus coding region is that induction of the autoimmune disease will require that the mimic epitope be processed from its native flanking regions in addition to the requirement that the core epitope be presented in an appropriate fashion to activate the self-reactive Th1 response.

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