Immunological Reviews

Emily M. L. Chastain
Stephen D. Miller

Molecular mimicry as an inducing trigger for CNS autoimmune demyelinating disease

Authors' address
Emily M. L. Chastain[1], Stephen D. Miller[1]
[1]Department of Microbiology–Immunology and Interdepartmental Immunobiology Center, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA.

Correspondence to:
Stephen D. Miller
Department of Microbiology-Immunology
Northwestern University Feinberg School of Medicine
303 E. Chicago Avenue
Chicago, IL 60611, USA
Tel.: +1 312 503 764
Fax: +1 312 503 1154
e-mail: s-d-miller@northwestern.edu

Conflict of interest
The authors have no conflicts of interest to declare.

This article is part of a series of reviews covering Microbial Influences on Immune Function appearing in Volume 245 of *Immunological Reviews*.

Summary: Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) that affects about 0.1% of the worldwide population. This deleterious disease is marked by infiltration of myelin-specific T cells that attack the protective myelin sheath that surrounds CNS nerve axons. Upon demyelination, saltatory nerve conduction is disrupted, and patients experience neurologic deficiencies. The exact cause for MS remains unknown, although most evidence supports the hypothesis that both genetic and environmental factors contribute to disease development. Epidemiologic evidence supports a role for environmental pathogens, such as viruses, as potentially key contributors to MS induction. Pathogens can induce autoimmunity via several well-studied mechanisms with the most postulated being molecular mimicry. Molecular mimicry occurs when T cells specific for peptide epitopes derived from pathogens cross-react with self-epitopes, leading to autoimmune tissue destruction. In this review, we discuss an *in vivo* virus-induced mouse model of MS developed in our laboratory, which has contributed greatly to our understanding of the mechanisms underlying molecular mimicry-induced CNS autoimmunity.

Keywords: *cytotoxic T cells, Th1/Th2/Th17 cells, dendritic cells, autoimmunity, T-cell receptors, neuroimmunology*

Introduction

The breakdown of peripheral self-tolerance can result in the induction and progression of immune reactivity to self. This phenomenon of autoreactive pathology is the pathological basis for organ-specific autoimmune diseases. Although the downstream effects of this breakdown in tolerance are evidenced by disease pathology, little is understood regarding the initiation of the pathophysiological process in autoimmune disease.

One of the most prevalent neurologic autoimmune diseases is multiple sclerosis (MS) (1). This organ-specific autoimmune disease of the central nervous system (CNS) is characterized by the loss of the protective myelin sheath surrounding the axons of neurons, resulting in 'plaque' formation (2, 3). Plaques denote areas in which myelin has been lost. Loss of myelin and plaque formation is mainly attributed

Immunological Reviews 2012
Vol. 245: 227–238
Printed in Singapore. All rights reserved

© 2011 John Wiley & Sons A/S
Immunological Reviews
0105-2896

to myelin-specific $CD4^+$ T cells that cross the blood–brain barrier (BBB) and consequently destroy the myelin sheath (4–6). Upon destruction of myelin, saltatory axonal conduction is disrupted leading to loss of neurologic function. Symptoms include cognitive impairment, muscle weakness, spasms, ataxia, and paralysis (7).

Although the pathophysiology of MS has been extensively studied for many years, the etiology of the disease still remains a mystery. It is clear that genetics contributes significantly to disease susceptibility. Identical twins develop MS with a 25–30% concordance rate, and there is an increased chance of developing disease among siblings as compared to the incidence in the general population (8). Susceptibility is most closely linked to the human leukocyte antigen (HLA) DR [major histocompatibility complex (MHC) class II] locus, and more recently, disease has been more loosely linked to multiple immune-related genes, including *interleukin 2 receptor alpha* (*IL2RA*) *and IL7RA* alleles (9, 10). Thus, it is clear that genetics contributes importantly to MS susceptibility, but there is a large body of epidemiological evidence supporting an equally important role for infections as an inducing stimulus.

Numerous studies have shown a correlation between MS development and latitude, with an increased incidence of disease in temperate and colder climates compared to enhanced resistance in warmer climates closer to the equator (11). Outbreaks of MS have also occurred in isolated populations, for example, in native inhabitants of the Faroe and Shetland-Orkney Islands, after prolonged exposure to non-native individuals during World War II (11–13). This suggests that the possibility that exposure of genetically susceptible individuals to environmental pathogens may be involved in the induction of MS. More direct evidence for this hypothesis comes from reported isolation of viral proteins and RNA from MS plaques. Overall, over two dozen different viruses have been reportedly linked to MS over the past four decades (14, 15). Confirmation of the vast majority of these findings is lacking, and more recent reports have correlated MS with infections by human herpes virus type 6 and Epstein-Barr virus (EBV) (16).

There are multiple theories for how infections may induce autoimmunity. The three major hypotheses include (i) bystander activation, (ii) epitope spreading, and (iii) molecular mimicry (*Fig. 1*).

Bystander activation is a non-specific mechanism for virus-induced autoimmunity occurring within the inflammatory context generated by virus infection, especially chronic viral infection (16). Autoreactive T cells stimulated directly via pathogen-encoded superantigens or indirectly via tissue-resident antigen presenting cells (APCs) activated via Toll-like receptors (TLRs) and/or other pattern recognition receptors (PRRs) secrete inflammatory mediators, such as interferon-γ (IFN-γ), IL-1β, IL-12, IL-23, tumor necrosis factor-α (TNF-α), etc., which can non-specifically activate autoreactive T cells. These activated T cells can then initiate further tissue damage in the inflammatory milieu (14).

A functional consequence of bystander activation and local tissue damage is a phenomenon known as epitope spreading. This often occurs in the setting of persistent infection where a prolonged anti-pathogen specific immune response leads to tissue destruction resulting in release of endogenous or cryptic self-epitopes that are then engulfed and presented by APCs,

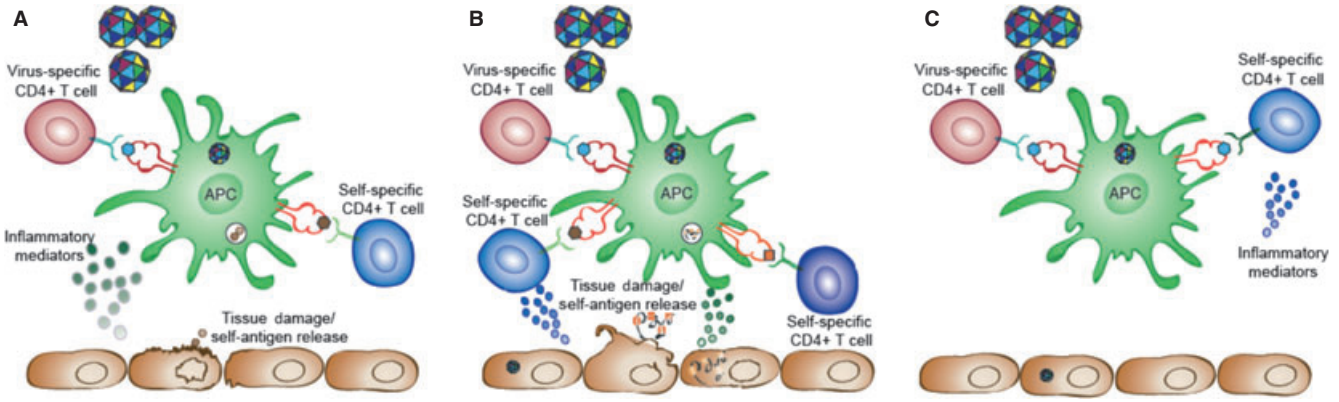

***Fig. 1.* Mechanisms of viral induced autoimmunity.** (A). Bystander activation. Chronic viral infection leads to activated lymphocytes that secrete inflammatory mediators mediating tissue damage. Once tissue is damaged, self-epitopes are released into the milieu, presented by APCs to autoreactive T cells which can mediate further tissue damage. (B). Epitope spreading. T-cell responses 'spread' from virus-specific to self-specific after continuous inflammation in a chronic virus setting. As tissue is destroyed, self-epitopes are released and present to T cells by APCs, which can mediate further damage to the tissue. Upon further tissue destruction, increasing self-epitopes are released and can be presented and activate naive autoreactive T cells. (C). Molecular mimicry. Tissue damage is mediated by autoreactive T cells that become activated after cross-reacting with viral epitopes presented by APCs.

© 2011 John Wiley & Sons A/S

which activate autoreactive T cells either directly in the target organ or in local lymph nodes draining the target organ (17, 18). Therefore, chronic viral infection and the accompanying inflammatory response poses a significant risk for inducing organ-specific autoimmune disease.

Molecular mimicry is the leading hypothesis for pathogen-induced autoimmunity. Molecular mimicry occurs when T cells bearing receptors (TCR) specific for epitopes derived from foreign pathogens (e.g. viruses) are activated during an infection and cross-react with self-antigens inducing autoimmune disease (19, 20). Mimicry has been postulated as the primary cause of many autoimmune diseases, including rheumatoid arthritis, diabetes, and MS (20–25).

Studies supporting molecular mimicry-induced autoimmune disease

Initial studies investigating the potential of foreign antigens to initiate autoimmune disease relied on immunizing animals with mimic peptides sharing varying degrees of sequence homology with self-epitopes and assaying T-cell responses and autoimmune disease symptoms. The initial report of T-cell molecular mimicry involved immunization of rabbits with a peptide from the hepatitis B virus epitope, which shared six amino acids with a sequence from myelin basic protein (MBP), in complete Freund's adjuvant. Immunization induced autoreactive T-cell responses to MBP; however, the animals failed to develop clinical disease (19). Subsequent studies employed transgenic mice expressing lymphocytic choriomeningitis virus (LCMV), viral antigens expressed in the pancreas under control of the rat insulin promoter. These animals failed to develop spontaneous autoimmune disease, but did develop clinical disease after subsequent LCMV infection (26, 27). Direct evidence for mimicry-induced autoimmunity was demonstrated upon infection with herpes simplex virus type 1 that leads to autoreactive $CD4^+$ T cells specific for corneal antigen and ultimate development of an autoimmune eye disease (28).

Our laboratory took a different approach by determining if infection with a normally avirulent CNS virus engineered to encode molecular mimics of myelin proteolipid protein (PLP) could induce CNS autoimmune disease. We employed a very well characterized virus that is studied as one of the primary animals models for MS, i.e. the use of the natural mouse pathogen Theiler's murine encephalomyelitis virus (TMEV) (29). TMEV is a single-stranded virus of the *picornoviridae* family, which is a natural enteric pathogen of mice and rats (30). TMEV is a neurotropic virus that can replicate and persist within the CNS. Intracerebral infection of susceptible inbred mouse strains (e.g. SJL/J) with wildtype TMEV can lead to the induction of a late-onset demyelinating disease, termed TMEV-induced demyelinating disease (TMEV-IDD) which is similar in pathology to MS, via the process of epitope spreading (17, 31). In contrast, disease-resistant mouse strains (e.g. C57BL/6) rapidly clear TMEV infection and develop no apparent CNS clinical manifestations.

Several strains of TMEV exist that differ in their neurovirulence. The two most commonly studied are the DA and BeAn strains. The DA strain is less virulent and results in a demyelinating disease approximately 140–180 postinfection (PI) (30). In our current model, we have employed the BeAn strain which results in chronic demyelinating disease with onset of paralytic symptoms approximately 30–40 days PI in SJL/J mice (30).

TMEV is classically characterized by two disease phases: early acute and late chronic demyelinating disease. Infection of both susceptible and resistant mouse strains induces an early acute disease marked by viral replication in the gray matter of the CNS that generally does not lead to clinical signs (30). Anti-virus immunity is marked by CNS infiltration of $CD4^+$ T cells specific to viral proteins, as well as a virus-specific $CD8^+$ cytotoxic T-lymphocyte (CTL) response in both the CNS and periphery. It has not been determined, however, which DCs are responsible for initiating these early adaptive T-cell responses.

In the TMEV-IDD susceptible SJL/J strain, early acute infection results in an ineffectual immune response that limits peripheral virus titers, but does not result in complete virus clearance. Inability to effectively clear the virus results in chronic CNS viral infection, with low viral titers detected for as long as a year or more PI. Typically, replication occurs in CNS-resident antigen-presenting cells and glial cells of the CNS (30, 32).

TMEV-IDD is thought to be initiated by the bystander CNS damage mediated by pathogenic proinflammatory virus-specific $CD4^+$ T cells, as evidenced by the fact that susceptibility is correlated with high class-II-restricted TMEV-specific delayed type hypersensitivity (DTH) (33). In addition, *in vivo* depletion of $CD4^+$ T cells in SJL mice resulted in decreased incidence of demyelinating disease and slower progression (34). In the SJL/J strain, immunodominant $CD4^+$ T cells are specific for the TMEV epitope $VP2_{70–86}$ (35). Sub-immunodominant epitopes include $VP1_{233–250}$ (36) and $VP3_{24–37}$ (37). Relevant to a potential role for molecular mimicry in inducing TMEV-IDD following infection with the wildtype BeAn virus strain in SJL/J mice, there appears to be no

cross-reactivity between the immunodominant TMEV CD4 epitopes and the immunodominant epitopes on PLP or other myelin proteins, including MBP and MOG (31), thus mimicry appears not to be involved in disease initiation in wildtype virus infection.

Disease progression is exacerbated by epitope spreading to self-myelin epitopes. When tissue damage reaches a critical mass, autoreactive $CD4^+$ T cells against the immunodominant $PLP_{139–151}$ epitope initially arise and mediate further myelin damage (38). $CD4^+$ T-cell responses against subdominant self-epitopes sequentially arise with further disease progression. We have shown that epitope spreading in susceptible SJL mice is initiated by activation of T cells in the CNS target organ by activation of naive T cells by peripherally derived myeloid dendritic cells (39, 40). Demyelination in the CNS results in chronic progressive hind limb paralysis, which can eventually lead to death.

TMEV-induced model of molecular mimicry-induced CNS autoimmunity

As detailed above, infection of SJL/J mice with wildtype TMEV leads to CNS autoimmunity via epitope spreading. To determine if natural virus infection can induce autoimmunity by molecular mimicry, we engineered a non-pathogenic strain of TMEV-BeAn to express self-peptide, peptide mimics, and eventually pathogen-derived self-mimics. This unique and direct model allowed us to examine the requirements for infection-induced cross-reactive activation of autoreactive T cells and *in vivo* autoimmune disease development.

We employed a mutant non-pathogenic BeAn strain of TMEV which had a *Cla*I restriction site inserted into the leader sequence of the virus resulting in a 23 amino acid deletion (Δ*Cla*I-TMEV) (29). SJL/J mice infected with the Δ*Cla*I-TMEV strain of TMEV did not develop demyelinating disease (*Fig.* 2B,

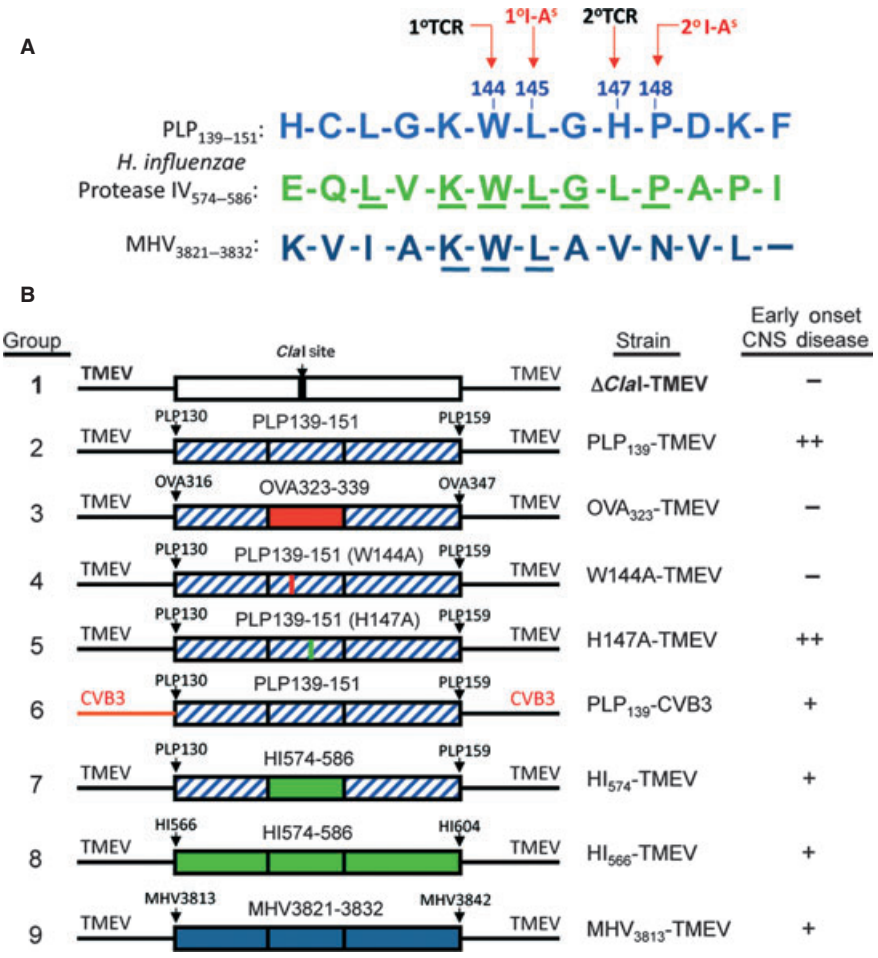

***Fig.* 2. Summary of induction of CNS autoimmunity in SJL/J mice by virus infection-induced molecular mimicry.** (A). Amino acid sequence of $PLP_{139–151}$ and molecular mimics. The amino acid sequence of $PLP_{139–151}$ and natural molecular mimics of this dominant encephalitogenic self-epitope encoded by *H. influenza* ($HI_{574–586}$) and mouse hepatitis virus ($MHV_{3821–3832}$). The primary and secondary TCR and MHC class II residues are indicated. (B). Pictorial representation of the ability of engineered TMEV encoding $PLP_{139–151}$ and varying molecular mimics to induce early onset demyelinating disease. Induction of early onset demyelinating disease in SJL/J mice following i.c. infection with the various viruses is indicated.

© 2011 John Wiley & Sons A/S

Group 1). The non-pathologic nature is due to the fact that the virus is not able to persist in the CNS of the infected animals. We then constructed additional viruses by inserting sequences encoding the native encephalitogenic self-myelin epitope [$PLP_{130–159}$ (PLP139-TMEV)], which encompasses the immunodominant encephalitogenic $PLP_{139–151}$ epitope, or altered peptide ligands of this epitope into the *Cla*I restriction site. The altered peptide ligands were generated by making point mutations at the primary and secondary TCR recognition sites, W144A and H147A, respectively (*Fig. 2A*). As a control, we also constructed encoding the non-self ovalbumin $OVA_{317–346}$ epitope (OVA323-TMEV). This model is unique because it allowed us to examine the processing of either native or APL sequences in the context of the larger VP2 protein (29).

Mice infected with the PLP139-TMEV (*Fig. 2B, Group 2*) developed clinical symptoms of disease (loss of tail tonicity and flaccid hind limb paralysis) rapidly (days 7–10 PI compared with normal delayed disease onset 30–40 days PI for wildtype TMEV) and with increased severity (clinical scores of three compared to two for wildtype). Inflammation and demyelination were observed in the lower lumbar spinal cord of these animals. This increased pathology was quite striking and was the first evidence that a self-epitope processed from whole virus could be presented to autoreactive T cells resulting in initiation of demyelinating disease. Significantly, it should be noted that innate signals provided by TMEV, a positive strand RNA virus, were sufficient to induce the early onset 'EAE-like' disease and did not require exogenous priming with other TLR agonists [e.g. complete Freund's adjuvant (CFA) containing *Mycobacteria*]. Mice infected with a TMEV variant encoding a 30-mer expressing the non-self $OVA_{323–339}$ epitope (OVA323-TMEV) failed to develop early onset disease due to molecular mimicry (*Fig. 2, Group 3*). However, these mice developed delayed onset spastic hind limb paralysis similar to SJL/J mice infected with wildtype TMEV. It was found that insertion of the OVA 30-mer into the leader deleted Δ*Cla*I virus restored CNS viral persistence consistent with this clinical finding (29). One potential explanation for restoration of virus persistence is a potential conformational change in Δ*Cla*I-TMEV upon insertion of the OVA 30-mer which promotes viral replication and CNS persistence. In fact, it was found that all strains which received insertions of 30-mer peptides were able to persist in the CNS and lead to late stage disease.

Similar disease kinetics were seen when mice were infected with the Δ*Cla*I-TMEV expressing the PLP peptide with H147A mutation (H147A-TMEV). These mice developed early onset EAE-like disease similar to mice infected with PLP139-TMEV (*Fig. 2B, Group 4*). However, mice infected with the W144A-TMEV virus strain (*Fig. 2B, Group 5*) failed to develop early onset disease (29). These results corresponded with previous studies in which SJL/J mice primed with the W144A peptide in CFA exhibited peptide (W144A)-specific $CD4^+$ T-cell response *in vitro*, but these mice did not develop EAE. However, mice primed with the H147A APL in CFA exhibited T-cell cross-reactivity to $PLP_{139–151}$ and develop clinical EAE (41–43). These results indicate that maintenance of the wildtype tryptophan residue at the primary TCR contact site at position 144 was critical for induction of disease via molecular mimicry, but that the residue at the secondary TCR contact site was not critical.

To determine if the initial virus infection had to occur in the eventual target organ of the autoimmune disease, the CNS in our model, we tested if the mice could be infected by different routes. Importantly, we determined that route of infection with these particular mutant viruses did not affect clinical disease outcome, in that infection by either the intravenous or intraperitoneal routes resulted in similar disease induction (29, 44). This is important because it suggests that infections of the periphery (and activation of peripheral T cells) can lead to autoimmunity within the CNS – a site distal from the initial infection. To determine whether infection of the CNS target organ was required for induction of infection-induced molecular mimicry disease, we inserted $PLP_{130–159}$ into Coxsackie virus B3 (PLP139-CVB3), which infects the heart and pancreas, but not the CNS. SJL/J mice infected with PLP139-CVB3 develop early onset mild demyelinating disease (*Fig. 2B, Group 6*) and concomitant activation of $PLP_{139–151}$-specific $CD4^+$ T-cell responses (unpublished), indicating that virus infection distal to the CNS target organ was sufficient to induce activation of myelin peptide-specific T cells via molecular mimicry, which can home to the CNS.

These results demonstrated the potential to induce CNS autoimmune disease as a consequence of a natural viral infection. Prior to the development of this model, studies had largely focused on immunization in adjuvant to examine cross-reactive T cells responses to potential mimic epitopes. The use of a non-pathogenic strain of TMEV as a vector for presenting the mimic epitopes supports the hypothesis that autoimmunity can be triggered by infection-induced molecular mimicry and enables the investigation of the underlying mechanisms by which a virus may present self-mimics and induce T-cell cross-reactivity and subsequent disease.

Infection with PLP-mimic viruses induces autoreactive Th1 responses

The induction of early onset EAE-like disease in SJL/J mice infected with PLP139-TMEV led us to investigate the immune mechanism(s) underlying disease induction. It was critical to determine if the disease was dependent on the activation of $PLP_{139–151}$-specific $CD4^+$ T cells subsequent to virus infection.

Responses to myelin epitopes in SJL/J mice infected with wildtype TMEV only appear 40–50 days PI following epitope spreading. In contrast, $PLP_{139–151}$-specific T-cell responses were triggered in PLP139-TMEV infected mice within 7–14 days PI, consistent with a major role for autoimmune pathology in disease induction (29, 44). These PLP peptide-specific T cells produced large amount of IFN-γ and IL-2. T-cell responses from mice infected with the $PLP_{139–151}$ mimics (W144A and H147A) had differential outcomes in cross-reactivity. T cells from H147A-TMEV activated cross-reactive $PLP_{139–151}$-specific $CD4^+$ T cells, whereas T cells from W144A-TMEV did not (29, 44). Thus, TCR contact residues of potential mimics are crucial for initiating self-reactive $CD4^+$ T-cell responses, an important consideration to consider when analyzing potential naturally occurring mimics.

To further determine the encephalitogenic capacity of the myelin peptide-specific T cells, we examined their ability to transfer disease, as well as the effects of $PLP_{139–151}$-specific tolerance in preventing disease onset in PLP139-TMEV infected mice. To examine their pathogenicity, we harvested spleens from animals infected with PLP139-TMEV. After reactivation *in vitro* with $PLP_{139–151}$ peptide, cells were transferred to naïve SJL/J mice. Mice receiving either bulk splenocytes or purified $CD4^+$ T cells developed clinical disease by day 10 post-transfer. This is clear evidence that highly pathogenic autoreactive T cells are induced early after infection with a virus expressing a self-epitope (44). The effector role of $PLP_{139–151}$-specific $CD4^+$ T cells was further shown using antigen-specific tolerance inducted by the i.v. injection of ethylenecarbodiimide (ECDI)-fixed, peptide-coupled splenocytes. This tolerance induction protocol is effective in inducing tolerance for prevention and treatment of various autoimmune disease models, e.g. the EAE of MS and the non-obese diabetic (NOD) mouse model of type 1 diabetes, as well as inducing tolerance to allogeneic transplants (45–47). In SJL/J mice with TMEV-IDD induced by infection with wildtype TMEV, tolerance induced with intact TMEV virions, which anergizes virus-specific $CD4^+$ T cells (48, 49), but not tolerance induced with splenocytes coupled with mouse spinal cord homogenate (containing a crude mixture of myelin epitopes) or $PLP_{139–151}$ (50), was able to inhibit disease induction indicating that virus-specific T-cell initiated disease. In contrast, in PLP139-TMEV-infected mice, previous tolerization with $PLP_{139–151}$-coupled splenocytes was able to significantly inhibit development of early onset demyelinating disease (44), clearly indicating that $PLP_{139–151}$-specific effector $CD4^+$ T cells were responsible for disease initiation. Taken together with the demonstration of induction of potent $PLP_{139–151}$-specific $CD4^+$ Th1 responses in PLP139-TMEV and H147A-TMEV-infected mice (44), these data clearly indicate that early onset clinical disease is due to the activation of myelin peptide-specific autoreactive T cells following infection with TMEV-expressing myelin peptides and myelin mimics.

Induction of CNS autoimmunity using viruses encoding natural $PLP_{139–151}$ mimic epitopes

Haemophilus influenza mimic

Our data show that TMEV encoding a 30-mer encompassing either the immunodominant encephalitogenic $PLP_{139–151}$ epitope or the altered peptide ligand (H147A) containing a conservative amino acid substitution in the primary TCR contact residue can trigger virus-induced CNS autoimmunity. However, to address the question of mimicry-induced autoimmunity in a more physiological manner, it was critical to determine if $PLP_{139–151}$ mimics encoded by natural pathogens could induce autoimmune disease. We thus used the same non-pathogenic strain of TMEV (Δ*Cla*I-TMEV) engineered to express a sequence from the *H. influenza* serine protease IV protein ($HI_{574–586}$). This protein is responsible for the degradation of proteins, peptides, and glycopeptides. Interestingly, this sequence is a naturally occurring mimic of $PLP_{139–151}$, as it shares 6 of 13 amino acids, including the primary TCR (position 144) and primary and secondary MHC (positions 145 and 148) sites (42) (*Fig. 2A*). However, it does not share the secondary TCR contact residue (position 147). Previous work had demonstrated that $HI_{574–586}$ was able to bind to $I\text{-}A^s$ and induce T-cell responses cross-reactive with $PLP_{139–151}$ in adjuvant primed SJL/J mice (42).

Mice infected i.c. with the PLP139-TMEV virus into which $HI_{574–586}$ was substituted for the core $PLP_{139–151}$ sequence (HI574-TMEV) were shown to develop early onset demyelinating disease, although with less severity compared to mice infected with PLP139-TMEV (*Fig. 2B, Group 7*). This disease was atypical compared to PLP139-TMEV disease. The clinical symptoms observed were ruffled fur, hunched back, and waddling gait, and these symptoms did not progress over time (51). Although the disease was milder, this was the first

© 2011 John Wiley & Sons A/S

demonstration that molecular mimics naturally expressed in an infectious pathogen could indeed induce clinical disease. Upon histologic examination of HI574-TMEV-infected mice, we observed infiltration of $CD4^+$ T cells into the thoracic region of the spinal cord; however, infiltration was mainly perivascular with limited infiltration into the parenchyma. This could potentially explain the limited disease seen in infected animals, i.e. T cells were unable to properly traffic to areas of the spinal cords to cause sufficient damage to induce progressive disease (51).

We also determined the nature of the T-cell responses induced by infection with HI574-TMEV. We found the temporal appearance of cross-reactive $PLP_{139–151}$-specific T cells which proliferated and produced IFN-γ in response to *in vitro* restimulation with $PLP_{139–151}$ peptide. *In vitro* responses to both TMEV peptide ($VP_{270–286}$) and $HI_{574–586}$ were also observed (51). This is the first model to directly demonstrate that a virus encoding a mimic of a self-myelin epitope can induce autoreactive $CD4^+$ T cells. To verify the pathologic contribution of myelin-specific (cross-reactive) T cells in disease induction in HI574-TMEV infected animals, we tolerized these animals to $PLP_{139–151}$ peptide before infection. Tolerance to the self-myelin peptide significantly reduced clinical disease in mice infected with the *H. influenza* mimic-encoding virus concomitant with a reduction in $PLP_{139–151}$-specific DTH and proliferative responses (52). Thus, CNS autoimmune disease in these animals was the result of cross-reactive induction of myelin peptide-specific T cells responses by molecular mimicry.

To determine if the *H. influenza*-derived mimic epitope could be processed and presented from the native protease IV protein and induce CNS autoimmune disease, we tested the disease-inducing ability of a Δ*Cla*I-TMEV variant expressing a 39-mer sequence ($HI_{566–595}$, HI566-TMEV) derived entirely from the bacterial protein (52). This provides a more stringent test of molecular mimicry, in that the minimal mimic peptide epitope would have to be processed from its own flanking sequences in order, to be presented by SJL/J APCs to cross-activate self-specific pathogenic $PLP_{139–151}$-specific T cells. Interestingly, SJL/J mice infected with HI566-TMEV (*Fig. 2B, Group 8*) developed an early onset demyelinating disease (day 7–10 PI) similar to mice infected with HI574-TMEV. HI566-TMEV infected mice also displayed cross-reactive responses to $PLP_{139–151}$ as measure by DTH, proliferation, and cytokine production (52). Therefore, these results indicate that it is possible for mimic epitopes to be processed and presented in the context of the native protein sequence to lead to pathogen-induced autoimmunity.

Other explanations could explain the limited disease observed in SJL/J mice infected with either HI574-TMEV or HI566-TMEV. A discrepancy exists between the appearance of cross-reactive $PLP_{139–151}$ $CD4^+$ T cells and disease severity. Although cross-reactive T cells exist, the disease is mild and non-progressing. Potential explanations for this phenomenon include binding efficiency of the HI peptide, as well as differences in cross-reactive populations of T cells. First, it has been described that the $I\text{-}A^s$ molecule has lower affinity for the $HI_{574–586}$ peptide than for $PLP_{139–151}$. Therefore, *in vivo* processing, presentation of this peptide (from the larger virus protein) by endogenous APCs may induce suboptimal priming of cross-reactive $CD4^+$ T cells. A secondary hypothesis could be explained by differences in the $PLP_{139–151}$ and $HI_{574–586}$-specific $CD4^+$ T-cell populations. The $PLP_{139–151}$ T-cell population cross-activated in HI-TMEV infected mice may be less skewed toward a pathologic Th1/17 phenotype and/or include a regulatory phenotype. Therefore, the balance between harmful and protective T cells may be tipped such that disease progression is controlled. Further exploration of this secondary hypothesis would be interesting to determine if such a relationship exists.

Mouse hepatitis virus mimic

To complement our studies examining the potential of *H. influenza* as a naturally occurring $PLP_{139–151}$ mimic, we also examined the pathologic capacity of a mimic sequence derived from murine hepatitis virus (MHV). MHV is a single-strand RNA coronavirus consisting of multiple strains. Infection with the JHM strain of MHV can cause a chronic demyelinating disease similar in pathology to MS (53). In humans, there are reports showing coronavirus antigens within MS plaques (54, 55).

It has been previously described that MHV expressed a potential $PLP_{139–151}$ molecular mimic (42). $MHV_{3821–3832}$ is located in the *ORF1a* gene which encodes for the replicative polyprotein PL1-PRO, which is involved in transcription of RNA and cleaving the polyprotein into function products. Unlike the HI mimic that shares 46% (6/13 amino acid residues) homology, the MHV expressing mimic only shares 23% (3/13 amino acid residues) identity (*Fig. 2A*), but includes homology at the primary TCR (position 144) and primary MHC class II (position 145) contact residues.

We again engineered the Δ*Cla*I-TMEV strain to express a 30-mer peptide encompassing the potential myelin mimic (MHV3813-TMEV). Mice infected with MHV3813-TMEV developed an early onset (day 20 PI) demyelinating disease similar in severity to mice infected with PLP-TMEV (56)

(*Fig. 2, Group 9*). The clinical disease observed included a severe waddling gait and mild flaccid hind limb paralysis. *In vitro* upon restimulation with $PLP_{139–151}$ peptide, T cells from MHV3813-TMEV-infected animals produced large amounts of IFN-γ. Interestingly, T-cell responses *in vitro* were stronger toward $PLP_{139–151}$ than the mimic peptide ($MHV_{3821–3832}$) itself. This is likely due to the reactivation of endogenous $PLP_{139–151}$ $CD4^+$ T cells after myelin damage by myelin epitopes released into the inflammatory milieu. Using DTH as a measurement of *in vivo* T-cell activation, we determined that the MHV mimic is processed and presented by endogenous APCs (56). Thus, these data indicate that although this peptide mimic shares very little sequence homology with $PLP_{139–151}$, it is still able to induce demyelinating disease.

Comparison of mimicry induced by infection versus adjuvant priming

The most well-studied and characterized animal model of MS is experimental autoimmune encephalomyelitis (EAE). In this model, animals are primed with $PLP_{139–151}$ emulsified in CFA. Approximately 14 days after priming, mice exhibit a relapsing-remitting clinical disease (R-EAE) similar in pathology to MS. Clinical disease is attributed to both Th1 and Th17 $PLP_{139–151}$ $CD4^+$ T cells, as well as T cells specific for spread epitopes (57).

As mentioned above, many studies on molecular mimicry have been conducted by priming animals with mimics to examine the ability to induce clinical disease. We thus carried out studies comparing the ability to induce EAE using infection-induced molecular mimicry operating via virus delivered innate immune signals versus peptide/CFA. An important aspect of the studies was the comparison of infection with HI574-TMEV to priming with $HI_{574–586}$ in CFA. As described, mice infected with TMEV expressing the $HI_{574–586}$ sequence developed a reproducible early onset yet mild clinical disease; however, mice primed with HI/CFA did not develop clinical disease (51, 52, 56). This indicates that innate immune events associated with viral replication (cytokine production, activation of APCs, TLR/PKR signaling) were required for disease initiation, while priming with this mimic in CFA is insufficient in generating disease. Interestingly, although $HI_{574–586}$/CFA priming failed to invoke clinical disease, $PLP_{139–151}$-specific T-cell proliferation was observed, as well as a DTH response 14 days PI. However, the self-peptide-specific T cells produced significantly less IFN-γ than animals primed with $PLP_{139–151}$/CFA (51, 52). Perhaps 'lack of skewing' toward pathogenic Th1 or perhaps Th17 cells could explain the inability to develop disease. It will be interesting in the future to examine other T-helper phenotypes including Th17 and Treg cells.

We also investigated the possibility that the route of antigen exposure could explain the difference seen in priming versus infection with the HI mimic peptide, i.e. it is possible that innate activation of CNS cells is a prerequisite for disease development as CNS-resident cells (microglia) are activated following either i.c. or i.p. infection within the mimic peptide-expressing viruses, which is not true following s.c. peptide/CFA priming.

To address this question, SJL/J mice were infected with either the HI-TMEV or control OVA-TMEV strain of TMEV and subsequently primed with $HI_{574–586}$, $OVA_{323–339}$ or PBS in CFA. Interestingly, only mice preinfected with HI-TMEV and then primed with $HI_{574–586}$/CFA developed a *severe* rapid-onset disease (52). Mice preinfected with HI574-TMEV but then primed with $OVA_{323–339}$ developed clinical disease similar to HI574-TMEV infection alone (52). Therefore, this indicates that secondary challenge with the original mimic peptide is imperative to induce exacerbated clinical disease. We also determined that persistent infection is a prerequisite for disease development, as mice infected with the Δ*Cla*I-TMEV, which does not replicate/persist, did not result in severe disease in mice, subsequently primed with $HI_{574–586}$/CFA. Enhanced disease was accompanied by increased $PLP_{139–151}$-specific proliferation and IFN-γ production and increased CNS pathology (52).

We believe that studying molecular mimicry within the context of a replicating virus infection is critical for determining the capacity for a particular mimic to induce disease. Indeed, responses induced by mimic peptides in the 'artificial' context of CFA priming are not an accurate depiction of whether a particular peptide is able to act as a disease-inducing mimic. It is also likely that type of virus and its cell tropism are important factors for initiating disease. For instance, infection of APCs may be an important prerequisite for ability to induce clinical autoimmune disease.

Mimic peptides and cross-reactivity

Although our studies to this point mainly addressed our work with *H. influenza* peptides, we have also explored the potential of other $PLP_{139–151}$ peptide mimics to induce *in vitro* T-cell responses and examined the minimum requirement to induce cross-reactivity with $PLP_{139–151}$. We therefore used mimics derived from *Candida albicans* (CAN), *E. coli* (ECO), *Salmonella typhimurium* (SAL), and MHV that had been previously described by Carrizosa *et al.* (42).

© 2011 John Wiley & Sons A/S

Examination of the potential of these mimics to induce T-cell cross-reactivity revealed differential requirements for activating naive $PLP_{139–151}$-specific T-cell precursors versus reactivating $PLP_{139–151}$-primed T cells. $CD4^+$ T cells from mice primed with the MHV mimic peptide proliferated in response to $PLP_{139–151}$; however, the reverse was not true (58), i.e. T cells from $PLP_{139–151}$-primed mice did not proliferate in response to the MHV peptide. In contrast, mice primed with ECO did not proliferate when restimulated with $PLP_{139–151}$, whereas $PLP_{139–151}$-primed T cells cross-reacted and proliferated in response to the ECO peptide (58). We found that peptides best recognized by $PLP_{139–151}$-primed T cells, all contained a proline residue at the 10th position which is the secondary $I\text{-}A^s$ binding site.

One of the observations that we made was the degree to which mimic-primed T cells cross-reacted to other mimics *in vitro*. In other words, disease appearing to be attributed to one mimic (based on *in vitro* response) may in fact have been induced by a different mimic. Thus, this system is somewhat 'leaky', and disease causation cannot strictly be attributed to a T-cell response to a given pathogen. In addition, autoreactive cells may also cross-react with pathogens (*in vitro*), although they were induced by another means (such as autoimmune disease).

We found somewhat different results when we infected the animals with TMEV expressing the peptide mimics, instead of priming with CFA. Again, we generated ΔClaI-TMEV strains that expressed three of the mimics (ECO-TMEV, CAN-TMEV, and SAL-TMEV). Increased cross-reactivity (determined by proliferation) was seen to $PLP_{139–151}$ when mice were infected with the mimic strains (58), and all three strains were able to induce significantly more severe clinical signs of disease than in animals infected with OVA323-TMEV-infected animals. However, we did observe the cross-reactivity to other pathogen mimics. For example, mice infected with MHV-TMEV (which induces an early onset demyelinating disease) proliferated poorly to $MHV_{3821–3832}$ yet responded more vigorously to both $HI_{574–586}$ and $PLP_{139\text{-}151}$ (58).

Again addressing the idea of cross-reactivity and cross-specificity, we made an interesting observation with animals' tolerized with $HI_{574–586}$ peptide. Using ECDI splenocytes coupled to $HI_{574–586}$ peptide, we tolerized animals and then primed them with $PLP_{139–151}$ in CFA. Interestingly, tolerization with the mimic epitope was able to significantly suppress EAE compared to OVA coupled splenocytes (52). $HI_{574–586}$ tolerization was as efficient as $PLP_{139–151}$ tolerization at suppressing disease, indicating bi-directional cross-reactivity between these two epitopes.

Molecular mimicry in a humanized mouse model

Many studies have been conducted to identify the specificity of autoreactive T-cell clones isolated from in MS patients. Whereas $PLP_{139–151}$ is the immunodominant myelin protein epitope in SJL/J mice, $MBP_{85–99}$ is the $CD4^+$ immunodominant epitope in humans. Double transgenic mice expressing a humanized $MBP_{85–99}$-specific TCR and human HLA-DR2 (MHC class II) on the C57BL/6 mouse background can be induced to develop a demyelinating disease similar to MS when primed with $MPB_{85–99}$/CFA (59).

We employed two different strains of humanized mice to examine the ability of bacterial MBP mimics to induce EAE. The two strains we used were on genetically different backgrounds, which interestingly resulted in differential outcomes in disease. The first strain was generated on the C57BL/6 background with a null mutation for mouse MHC class II (H2-A) ($dTgH2A^{neg}$) (60). When primed with $MPB_{85–99}$/CFA [plus pertussis toxin (PT)], these mice developed a severe monophasic disease. In contrast, humanized mice on the mixed DBA/2xC57BL/6 ($dTgH2A^{pos}$) background with intact mouse MHC class II developed a relapsing disease course when primed with $MPB_{85–99}$/CFA (61).

To determine the ability of bacterial mimics to induce CNS autoimmune demyelinating disease in the humanized mice, we primed both strains with five bacterial peptide-mimics – *Staphylococcus aureus* (SAU), *Mycobacterium avium* (MBA), *Mycobacterium tuberculosis* (MBT), *Bacillus subtilis* (BACS), and *Haemophilus influenza* (HI) (*Table 1*). Homology to MBP ranged from 20 to 33% (3–5/15 AA residues), and all mimics shared at least two of three primary TCR contact residues. This appears to be important for disease induction, whereas fidelity between the primary MHC contact residues was variable. Disease was induced by all mimics in $dTgH2A^{neg}$ mice, with the exception of HI and MBT. Similar to $MBP_{85–99}$-induced EAE, the mice exhibited a monophasic disease, although the clinical severity was milder. In $dTgH2A^{pos}$ mice, all mimics, including HI, induced R-EAE; however, HI disease was extremely mild (62).

We examined the infiltrate in the brains and spinal cords of infected mice. In both transgenic strains, $CD4^+$ T cells were seen in the cerebellum, albeit around the periphery, in mice primed with the mimics (62). The degree of infiltration correlated with disease; mice primed with the HI mimic peptide (in the $dTgH2A^{neg)}$ did not have any infiltration in either the cerebellum or lower lumbar spinal cord. There was a slight difference between strains, in that more $F4/80^+$ infiltrates

***Table 1.* Amino acid sequences of $MBP_{85–99}$ and its bacterial mimics**

Peptide source (abbreviation)	Amino acid residue/sequence														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Myelin Basic Protein (MBP)	**E***	**N**	**P**	**V**	**V**	**H**	**F**	**F**	**K**	**N**	**I**	**V**	**T**	**P**	**R**
Staphylococcus aureus (SAU)	V	L	A	R	L	**H**	**F**	Y	R	N	D	V	H	K	E
Mycobacterium avium (MBA)	Q	R	C	R	V	H	F	L	R	N	V	L	A	Q	V
Mycobacterium tuberculosis (MBT)	Q	R	C	R	V	**H**	**F**	M	R	N	L	Y	T	A	V
Bacillus subtilis (BACS)	A	L	A	V	L	H	F	Y	P	D	K	G	A	K	N
Haemophilus influenzae (HI)	D	F	A	R	**V**	**H**	**F**	I	S	A	L	H	G	S	G

Boldface indicates residues shared with the self-human myelin basic protein epitope. Residues 5 and 8 are primary MHC contact residues; residues 6, 7, and 9 are primary TCR contact residues (63).

were found in the cerebellum of $dTgH2A^{pos}$ compared to $dTgH2A^{neg}$ (62). Perhaps this could partially explain this difference in disease phenotype seen between the two strains. However, in both strains, the immune infiltration was observed mainly in the lower lumbar spinal cord. We also observed demyelination in clinically affected mice of both strains.

We also compared T-cell responses between naive and primed animals to determine the degree of cross-reactivity among the mimic peptides. A second goal was to determine whether the degree of response of naive transgenic T cells would be indicative of later disease outcome. *In vitro* assays were employed to examine proliferation and cytokine production. In both strains, the greatest response was seen when naive T cells were cultured with $MBP_{85–99,}$ however, both naive and primed T cells proliferated (cross-reacted) with all mimics. Cytokine production, however, was not consistent with proliferation. While low levels of IFN-γ, IL-17, and IL-2 were seen with most mimics, SAU induced large amounts of IFN-γ (even from naive T cells) and high levels of IL-17 (in primed $dTgH2A^{neg}$ T cells). MBT also skewed toward a Th1 response with production of IFN-γ in naive $dTgH2A^{neg}$ T cells (62). Overall, this model is extremely relevant to human disease, as the mice express humanized receptors and the pathogen-derived mimics employed are routinely encountered by humans.

Conclusions

Our studies indicate that molecular mimicry is a viable explanation for the induction of a wide variety of autoimmune diseases that have been shown to have both a genetic predisposition and are associated with environmental influences. Given the wide genetic diversity of individuals displaying a particular autoimmune disease, it is highly unlikely that any one infectious agent will be found to be exclusively associated with a disease. Depending on the genetics of the individual, it is possible that an autoimmune disease could be triggered by a variety of infectious agents contingent on many variables. These variables could include the number of potential cross-reactive epitopes encoded by the pathogen, the ability of the infectious agent to activate the innate immune system via stimulation of pattern recognition receptors, such as TLRs, the cellular/tissue tropism of the agent, the route of infection, and the age and immune repertoire of the affected individual at the time of infection. Significantly, progression of mimicry-induced autoimmune disease can be mediated by additional mechanisms, including epitope spreading, thus these two potential mechanisms for initiation of infection-induced autoimmune disease are not mutually exclusive. In conclusion, much still remains to be learned about the interaction of genetics, environment, and immune effector function/regulation in the induction and progression of autoimmune disease.

References

1. Steinman L. Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. Cell 1996;**85**:299–302.
2. McFarland HF, Martin R. Multiple sclerosis: a complicated picture of autoimmunity. Nat Immunol 2007;**8**:913–919.
3. Wekerle H. Immunopathogenesis of multiple sclerosis. Acta Neurol 1991;**13**:197–204.
4. Sun JB, et al. Autoreactive T and B cells responding to myelin proteolipid protein in multiple sclerosis and controls. Eur J Immunol 1991;**21**:1461–1468.
5. Ota K, Matsui M, Milford EL, Mackin GA, Weiner HL, Hafler DA. T-cell recognition of an immunodominant myelin basic protein epitope in multiple sclerosis. Nature 1990;**346**:183–187.
6. Bernard CC, de Rosbo NK. Immunopathological recognition of autoantigens in multiple sclerosis. Acta Neurol 1991;**13**:171–178.
7. Whitaker JN, Mitchell GW, Raine CS, McFarland HF, Tourtellotte WW. Clinical features of multiple sclerosis. Multiple sclerosis: clinical and pathogenetic basis. London: Chapman and Hall, 1997: 3–19.
8. Sospedra M, Martin R. Immunology of multiple sclerosis. Annu Rev Immunol 2005;**23**:683–747.
9. Hafler DA, et al. Risk alleles for multiple sclerosis identified by a genomewide study. N Engl J Med 2007;**357**:851–862.

© 2011 John Wiley & Sons A/S

10. Haines JL, et al. A complete genomic screen for multiple sclerosis underscores a role for the major histocompatability complex. The Multiple Sclerosis Genetics Group. Nat Genet 1996;**13**:469–471.
11. Kurtzke JF. Epidemiologic evidence for multiple sclerosis as an infection. Clin Microbiol Rev 1993;**6**:382–427.
12. Kurtzke JF, Hyllested K. Multiple sclerosis: an epidemic disease in the Faroes. Am Neurol Assoc 1975;**100**:213–215.
13. Kurtzke JF, Hyllested K. Multiple sclerosis in the Faroe Islands. I. Clinical and epidemiological features. Ann Neurol 1979; **5**:6–21.
14. Olson JK, Croxford JL, Miller SD. Virus-induced autoimmunity: Potential role of viruses in initiation, perpetuation, and progression of T cell-mediated autoimmune diseases. Viral Immunol 2001;**14**:227–250.
15. Fujinami RS. Can virus infections trigger autoimmune disease? J Autoimmun 2001;**16**:229–234.
16. Munz C, Lunemann JD, Getts MT, Miller SD. Antiviral immune responses: triggers of or triggered by autoimmunity? Nat Rev Immunol 2009;**9**:246–258.
17. Vanderlugt CL, Miller SD. Epitope spreading in immune-mediated diseases: implications for immunotherapy. Nat Rev Immunol 2002;**2**:85–95.
18. Miller SD, et al. Epitope spreading leads to myelin-specific autoimmune responses in SJL mice chronically infected with Theiler's virus. J Neurovirol 1997;**3**(Suppl. 1):S62–S65.
19. Fujinami RS, Oldstone MB. Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. Science 1985; **230**:1043–1045.
20. Wucherpfennig KW, Strominger JL. Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. Cell 1995; **80**:695–705.
21. Basu D, Horvath S, Matsumoto I, Fremont DH, Allen PM. Molecular basis for recognition of an arthritic peptide and a foreign epitope on distinct MHC molecules by a single TCR. J Immunol 2000;**164**:5788–5796.
22. Basu D, Horvath S, O'Mara L, Donermeyer D, Allen PM. Two MHC surface amino acid differences distinguish foreign peptide recognition from autoantigen specificity. J Immunol 2001;**166**:4005–4011.
23. Kouskoff V, Korganow AS, Duchatelle V, Degott C, Benoist C, Mathis D. Organ-specific disease provoked by systemic autoimmunity. Cell 1996;**87**:811–822.
24. Kaufman DL, Erlander MG, Clare-Salzler M, Atkinson MA, Maclaren NK, Tobin AJ. Autoimmunity to two forms of glutamate decarboxylase in insulin- dependent diabetes mellitus. J Clin Invest 1992;**89**:283–292.
25. Lang HL, et al. A functional and structural basis for TCR cross-reactivity in multiple sclerosis. Nat Immunol 2002;**3**:940–943.
26. Ohashi PS, et al. Ablation of ''tolerance'' and induction of diabetes by virus infection in viral antigen transgenic mice. Cell 1991; **65**:305–317.
27. Oldstone MB, Nerenberg M, Southern P, Price J, Lewicki H. Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. Cell 1991;**65**:319–331.
28. Zhao ZS, Granucci F, Yeh L, Schaffer PA, Cantor H. Molecular mimicry by herpes simplex virus-type 1: Autoimmune disease after viral infection. Science 1998;**279**:1344–1347.
29. Olson JK, Croxford JL, Calenoff M, Dal Canto MC, Miller SD. A virus-induced molecular mimicry model of multiple sclerosis. J Clin Invest 2001;**108**:311–318.
30. Oleszak EL, Chang JR, Friedman H, Katsetos CD, Platsoucas CD. Theiler's virus infection: a model for multiple sclerosis. Clin Microbiol Rev 2004;**17**:174–207.
31. Miller SD, et al. Persistent infection with Theiler's virus leads to CNS autoimmunity via epitope spreading. Nat Med 1997;**3**:1133–1136.
32. Dal Canto MC, Kim BS, Miller SD, Melvold RW. Theiler's murine encephalomyelitis virus (TMEV)-induced demyelination: a model for human multiple sclerosis. Methods 1996;**10**:453–461.
33. Clatch RJ, Lipton HL, Miller SD. Characterization of Theiler's murine encephalomyelitis virus (TMEV)-specific delayed-type hypersensitivity responses in TMEV-induced demyelinating disease: correlation with clinical signs. J Immunol 1986;**136**:920–927.
34. Welsh CJ, Tonks P, Nash AA, Blakemore WF. The effect of L3T4 T cell depletion on the pathogenesis of Theiler's murine encephalomyelitis virus infection in CBA mice. J Gen Virol 1987;**68**:1659–1667.
35. Gerety SJ, et al. Class II-restricted T cell responses in Theiler's murine encephalomyelitis virus-induced demyelinating disease. V. Mapping of a dominant immunopathologic VP2 T cell epitope in susceptible SJL/J mice. J Immunol 1994;**152**:908–918.
36. Yauch RL, Kim BS. A predominant viral epitope recognized by T cells from the periphery and demyelinating lesions of SJL/J mice infected with Theiler's virus is located within VP1(233-244). J Immunol 1994;**153**:4508–4519.
37. Yauch RL, Kerekes K, Saujani K, Kim BS. Identification of a major T-cell epitope within VP3 amino acid residues 24 to 37 of Theiler's virus in demyelination-susceptible SJL/J mice. J Virol 1995;**69**:7315–7318.
38. Tompkins SM, Fuller KG, Miller SD. Theiler's virus-mediated autoimmunity: local presentation of CNS antigens and epitope spreading. Ann NY Acad Sci 2002;**958**:26–38.
39. McMahon EJ, Bailey SL, Castenada CV, Waldner H, Miller SD. Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis. Nat Med 2005;**11**:335–339.
40. Bailey SL, Schreiner B, McMahon EJ, Miller SD. CNS myeloid DCs presenting endogenous myelin peptides 'preferentially' polarize CD4(+) T(H)-17 cells in relapsing EAE. Nat Immunol 2007;**8**:172–180.
41. McRae BL, Miller SD. Fine specificity of CD4 + T cell responses to the dominant encephalitogenic PLP 139-151 peptide in SJL/J mice. Neurochem Res 1994;**19**:997–1004.
42. Carrizosa AM, et al. Expansion by self antigen is necessary for the induction of experimental autoimmune encephalomyelitis by T cells primed with a cross-reactive environmental antigen. J Immunol 1998;**161**:3307–3314.
43. McRae BL, Karandikar NJ, Miller SD. Degenerate antigen recognition by CD4 + effector T cells in experimental autoimmune encephalomyelitis. J Neuroimmunol 1997;**75**:156–162.
44. Olson JK, Eagar TN, Miller SD. Functional activation of myelin-specific T cells by virus-induced molecular mimicry. J Immunol 2002;**169**:2719–2726.
45. Smith CE, Miller SD. Multi-peptide coupled-cell tolerance ameliorates ongoing relapsing EAE associated with multiple pathogenic autoreactivities. J Autoimmun 2006; **27**:218–231.
46. Turley DM, Miller SD. Peripheral tolerance Induction using ethylenecarbodiimide-fixed APCs uses both direct and indirect mechanisms of antigen presentation for prevention of experimental autoimmune encephalomyelitis. J Immunol 2007; **178**:2212–2220.
47. Luo X, et al. ECDI-fixed allogeneic splenocytes induce donor-specific tolerance for long-term survival of islet transplants via two distinct mechanisms. Proc Natl Acad Sci USA 2008;**105**:14527–14532.
48. Karpus WJ, Pope JG, Peterson JD, Dal Canto MC, Miller SD. Inhibition of Theiler's virus-mediated demyelination by peripheral immune tolerance induction. J Immunol 1995;**155**:947–957.
49. Karpus WJ, Peterson JD, Miller SD. Anergy *in vivo*: down-regulation of antigen-specific CD4 + Th1 but not Th2 cytokine responses. Int Immunol 1994;**6**:721–730.
50. Miller SD, et al. Class II-restricted T cell responses in Theiler's murine encephalomyelitis virus (TMEV)-induced demyelinating disease. III. Failure of neuroantigen-specific

immune tolerance to affect the clinical course of demyelination. J Neuroimmunol 1990;**26**:9–23.
51. Croxford JL, Anger HA, Miller SD. Viral delivery of an epitope from *Haemophilus influenzae* induces central nervous system autoimmune disease by molecular mimicry. J Immunol 2005;**174**:907–917.
52. Croxford JL, Olson JK, Anger HA, Miller SD. Initiation and exacerbation of autoimmune demyelination of the central nervous system via virus-induced molecular mimicry: implications for the pathogenesis of multiple sclerosis. J Virol 2005;**79**:8581–8590.
53. Haring J, Perlman S. Mouse hepatitis virus. Curr Opin Microbiol 2001;**4**:462–466.
54. Murray RS, Brown B, Brian D, Cabirac GF. Detection of coronavirus RNA and antigen in multiple sclerosis brain. Ann Neurol 1992;**31**:525–533.
55. Stewart JN, Mounir S, Talbot PJ. Human coronavirus gene expression in the brains of multiple sclerosis patients. Virology 1992;**191**:502–505.
56. Croxford JL, Ercolini AM, Degutes M, Miller SD. Structural requirements for initiation of cross-reactivity and CNS autoimmunity with a PLP139-151 mimic peptide derived from murine hepatitis virus. Eur J Immunol 2006;**36**:2671–2680.
57. Schreiner B, Bailey SL, Miller SD. T-cell response dynamics in animal models of multiple sclerosis: implications for immunotherapies. Exp Rev Clin Immunol 2007;**3**:57–72.
58. Ercolini AM, Ludovic Croxford J, Degutes M, Miller SD. Cross-reactivity between peptide mimics of the immunodominant myelin proteolipid protein epitope PLP139-151: Comparison of peptide priming in CFA vs. viral delivery. J Neuroimmunol 2007;**186**:5–18.
59. Gregersen JW, Holmes S, Fugger L. Humanized animal models for autoimmune diseases. Tissue Antigens 2004;**63**:383–394.
60. Ellmerich S, et al. Disease-related epitope spread in a humanized T cell receptor transgenic model of multiple sclerosis. Eur J Immunol 2004;**34**:1839–1848.
61. Madsen LS, et al. A humanized model for multiple sclerosis using HLA-DR2 and a human T- cell receptor. Nat Genet 1999;**23**:343–347.
62. Greene MT, Ercolini AM, Degutes M, Miller SD. Differential induction of experimental autoimmune encephalomyelitis by myelin basic protein molecular mimics in mice humanized for HLA-DR2 and an MBP(85-99)-specific T cell receptor. J Autoimmun 2008;**31**:399–407.
63. Hausmann S, Martin M, Gauthier L, Wucherpfennig KW. Structural features of autoreactive TCR that determine the degree of degeneracy in peptide recognition. J Immunol 1999;**162**:338–344.

© 2011 John Wiley & Sons A/S