MOLECULAR MIMICRY IN MULTIPLE SCLEROSIS

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One of the most common demyelinating central nervous system (CNS) diseases in humans is multiple sclerosis (MS). The disease can be very debilitating with vision loss, motor and sensory disturbances, and cognitive impairment. The clinical course may present as a relapsing-remitting disease course, a progressive disease course, or a combination thereof. The etiology of MS is unknown. Though many viruses have been shown to be associated with MS, no one virus has ever been demonstrated to be the cause of MS. In addition, MS is thought to have an autoimmune component. Molecular mimicry is one hypothesis put forth which could reconcile the diverse pathology and etiology of MS. Molecular mimicry occurs when peptides from pathogens share sequence or structural similarities with self-antigens. Infection with various pathogens, each with its individual molecular mimic to a CNS antigen, may explain the inability of investigators to link one specific virus to MS. Molecular mimicry may be mediated through human leukocyte antigen class I- and class II-restricted T cells and antibodies, which may explain the diversity in phenotype. Aspects of molecular mimicry will be discussed in relation to each of these immune system components. Examples of various molecular mimics will be discussed with a particular focus on the CNS and MS. Molecular mimicry alone may not be able to induce disease; priming of the immune system by infection with a pathogen that carries a molecular mimic to self may have to be followed by a later nonspecific immunologic challenge in order for disease to be initiated. Recent research into this priming and triggering of disease will be discussed in relation to an animal model for MS.

I. Introduction

Multiple sclerosis (MS) is the most common demyelinating disease in humans. MS has prevalence rates between 50 and 100 per 100,000 Caucasians; other ethnic groups have somewhat lower prevalence rates and women are more afflicted than men by a 2:1 ratio (Kurtzke, 1997). The inflammatory demyelinating lesions characteristic of MS are limited to the central nervous system (CNS) (Dejong, 1970). In most instances, MS patients have oligoclonal immunoglobulin (Ig)G bands in the cerebral spinal fluid (CSF), and a mild mononuclear pleocytosis may also be present. Clinical features of the disease include vision loss, motor and sensory disturbances, and cognitive impairment. The clinical course of MS can include relapses and remissions and may be progressive in nature. MS has been proposed to be mediated by autoreactive CNS-specific $CD4^+$ T cells (Markovic-Plese and McFarland, 2001; Noseworthy *et al.*, 2000). However, significant numbers of $CD8^+$ T cells are found in MS lesions (Hayashi *et al.*, 1988; Sobel, 1989) and are likely to be involved in pathogenesis.

There have been many studies which attribute the neuroinflammation and neurodegeneration in MS to $CD8^+$ T cells, $CD4^+$ T cells, or antibody recognition of self. In this chapter, we summarize some salient points on each of these components of the immune system as they relate to molecular mimicry and disease. However, first we will provide background information and define molecular mimicry in relation to other key concepts.

Molecular mimicry was first hypothesized to be a potential mechanism for the initiation of autoimmune disease in the early 1980s. It is said to occur when peptides from viral (Fujinami *et al.*, 1983) or bacterial (Zabriskie, 1986) proteins share sequence or structural similarities with self-peptides. This similarity could stimulate autoreactive immune responses causing the production of self-reactive antibodies. Fujinami, Oldstone, and colleagues (Fujinami and Oldstone, 1985; Fujinami *et al.*, 1983) initially showed that a cross-reactive epitope between the *Hepatitis B virus* polymerase and the encephalitogenic epitope of myelin basic protein (MBP) for the rabbit could induce an experimental autoimmune encephalomyelitis (EAE)-like disease when used as an immunogen. For EAE to occur, this cross-reaction had to occur at the level of induction/activation of autoreactive $CD4^+$ T cells. This was the first demonstration of autoimmune disease induced by a viral peptide (Fujinami and Oldstone, 1985).

In the past, peptide similarities had been identified by computer searches for similar amino acid sequences; however, based on more recent studies, molecular mimicry has also been shown to occur with incomplete sequence matching provided the major histocompatibility complex (MHC) and T-cell receptor (TCR) contact motifs are preserved (Lang *et al.*, 2002). Additionally, peptide elution studies have found that MHC molecules can potentially bind hundreds

of different peptides (Hunt *et al.*, 1992). The work by Lang *et al.* (2002) suggested that molecular mimicry occurs rather frequently having shown that different peptides bound to class II molecules can lead to cross-reactivity by the same TCR provided the complexes have similar charge distribution and overall shape. The flexibility of TCR recognition plays a major role in forming the T-cell repertoire through thymic selection, and is highly important in protecting the host against potential pathogen-derived antigens that are more wide ranging than the limited number of memory T cells (Casrouge *et al.*, 2000). TCR degeneracy raises the potential for cross-reactivity between pathogen-derived and self-antigens.

The various forms of molecular mimicry are illustrated in Fig. 1. There are many molecular mimics that have been identified in MS studies, both viral and bacterial, as shown in Table I, which supports the hypothesis that molecular mimicry frequently occurs.

The Welsh and Selin laboratories have investigated molecular mimicry, or the sharing of immunologic cross-reactive epitopes, between viruses, as opposed to between a pathogen and its host, using such viruses as *Lymphocytic choriomeningitis virus* (LCMV), *Pichinde virus* (PV), *Vaccinia virus* (VV), and murine cytomegalovirus (MCMV) (Brehm *et al.*, 2002; Chen *et al.*, 2001; Welsh *et al.*, 2000). They coined the term "heterologous immunity" to describe the protection provided by one
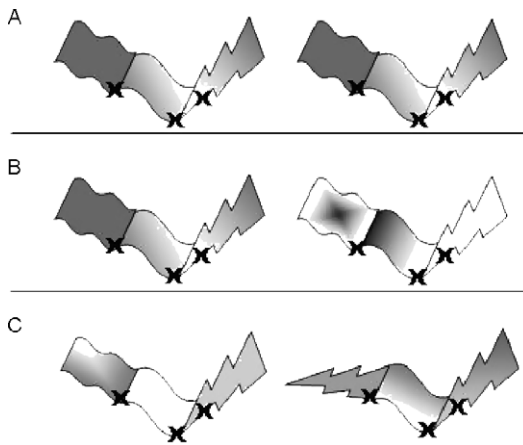
FIG. 1. Molecular mimicry with and without sequence homology. The two peptides shown in (A) share sequence homology as well as T-cell receptor (TCR) contact sites (marked as an "x"). The peptide sequences in (B) have the same overall shape and TCR contact residues, but do not share sequence homology. In (C), the sequences share TCR contact sites only; they do not share sequence homology and the overall shape is slightly different. Molecular mimicry may still occur due to the core region of TCR recognition; however, this recognition may be lower affinity.

TABLE I
MOLECULAR MIMICS IDENTIFIED IN MULTIPLE SCLEROSIS STUDIES

Molecular mimic	Infectious agent
Arginine-enriched domains of CNS proteins	Torque Teno Virus (Sospedra *et al.*, 2005)
Myelin basic protein	*Acinetobacter calcoaceticus* 4-carboxymuconolactone decarboxylase[a]
	Chlamydia pneumoniae (Lenz *et al.*, 2001)
	Epstein-Barr virus (Lang *et al.*, 2002)[b]
	Human coronavirus 229E[c]
	Human hepatitis B virus polymerase (Fujinami *et al.*, 1983)
	Human herpesvirus 6[d] (Tejada-Simon *et al.*, 2003)
	Maedi Visna virus[e]
	Pseudomonas aeruginosa γ-carboxymucono lactone decarboxylase[a]
Myelin oligodendrocyte glycoprotein	Butyrophilin[f]
	Rubella[g]
Myelin proteolipid protein	*Haemophilus influenzae* serine protease IV (Croxford *et al.*, 2005)

[a]Hughes *et al.* (2003).
[b]Ufret-Vincenty *et al.* (1998).
[c]Boucher *et al.* (2001).
[d]Cirone *et al.* (2002).
[e]Davies *et al.* (1996).
[f]Stefferl *et al.* (2000).
[g]Besson Duvanel *et al.* (2001).

infection against a different viral infection at a later time (Chen *et al.*, 2001). This protection to the second infection was mediated by $CD8^+$ T cells and the production of interferon (IFN)-γ (Brehm *et al.*, 2002). They also found that for $CD8^+$ T cells, cross-reactivity impacts T-cell kinetics and the hierarchy of T cells responding to epitopes encoded by the infecting virus, which changes T-cell responsiveness (Chen *et al.*, 2001; Selin *et al.*, 1998). Foreign proteins generally have several epitopes capable of being presented by MHC molecules; however, the cellular immune response is usually focused toward a narrow subset of these epitopes. This narrow response is known as immunodominance. When dominant epitopes have been deleted from a pathogenic organism, other epitopes emerge as dominant (Yewdell and Bennink, 1999). This phenomenon of immunodominance can be affected by prior infection with an organism encoding an epitope that generates T cells that cross-react with a second pathogen (Brehm *et al.*, 2002). The skewing of T-cell immunodominance may be beneficial or harmful to the host. Additional examples of cross-reactivity resulting in altered immune response

include: primary infection with influenza virus followed by Epstein-Barr virus (EBV), *Human papillomavirus* and *Human coronavirus*, and that which occurs between different strains of *Dengue virus* (Kim *et al.*, 2005). For example, dengue virus serotypes encode subdominant epitopes that share sequence homology between them. When a host with prior immunity to one of these serotypes is infected with another viral serotype, the weakly shared epitope dominates the immune response resulting in better recognition of the first serotype. This is due to memory T cells specific for the shared epitope proliferating and expanding, giving the subdominant epitope a distinct advantage (Mongkolsapaya *et al.*, 2003). Unfortunately, not all cases of heterologous immunity are as predictable. For instance, previous infection with LCMV has been shown to protect mice against VV infection resulting in reduced viral titers and a change in the T-cell-dependent pathology (Chen *et al.*, 2001; Selin *et al.*, 1998). In contrast, prior VV infection has little effect on LCMV immunity. It has been reported that VV infection of LCMV-immune mice results in expansion of MHC class I-restricted CD8$^+$ T cells with different epitopes dominating the response (Chen *et al.*, 2001; Kim *et al.*, 2002, 2005; Selin *et al.*, 1998). Following VV infection, LCMV-immune mice had a reduction in the frequency of LCMV-specific CD8$^+$ T memory cells to some epitopes while others remained constant or increased (Kim *et al.*, 2005).

Heterologous immunity and molecular mimicry may be able to explain the low concordance rates for MS in monozygotic twins. With a 30% concordance rate for this group, it is clear that MS has a genetic component but that environmental factors also play a role in disease pathogenesis (Ebers *et al.*, 1986; Mumford *et al.*, 1994; Sadovnick *et al.*, 1993). Perhaps a series of infections that generate immune reactivity to otherwise subdominant epitopes mimicking self-proteins are required in a genetically predisposed individual in order for disease to occur. This response or T-cell reactivity would be different for identical twins since their TCRs are rearranged randomly.

An alternate hypothesis currently being explored states that environmental factors in fetal or neonatal stages of life cause genetically susceptible individuals to be primed for autoimmune disease. The déjà vu theory takes this step further stating that there is an initial (fetal or neonatal) viral infection that persists and primes the individual for autoimmune disease provided a subsequent infection shares T-cell epitopes with the persisting virus (Merkler *et al.*, 2006). In support of this, a study by Rothwell *et al.* (1996) of patients with type-1 diabetes found that disease prevalence was higher in patients born in the spring and early summer than during the winter months. However, this hypothesis is still controversial as studies from Slovenia (Ursic-Bratina *et al.*, 2001) and Israel (Laron *et al.*, 2005) had similar results with higher prevalence rates for type-1 diabetes in individuals born in the spring and early summer, but an analysis of data from Germany showed opposite results with fewer type-1 diabetes cases born between April and September (Neu *et al.*, 2000).

II. Class I Molecules and Mimicry

Thus far, MHC class II-restricted CD4^{+} T-cell responses to self have been more extensively investigated in MS patients and animal models than the MHC class I-restricted CD8^{+} T-cell response. However, clinical and experimental research indicates that CD8^{+} T-cell reactivity could be involved in the pathology of MS (Wekerle and Lassmann, 2005). Cytotoxic T lymphocytes (CTLs) could destroy oligodendrocytes leading to demyelination. *In vitro* experiments have shown that oligodendrocytes can be induced by IFN-γ to express MHC class I antigen but not class II antigen (Grenier *et al.*, 1989). In both MS and Theiler's murine encephalomyelitis virus (TMEV) infection, apoptosis of oligodendrocytes has been observed (Dowling *et al.*, 1997; Tsunoda and Fujinami, 1996, 1999; Tsunoda *et al.*, 1997). TMEV belongs to the family *Picornaviridae*. Infection with TMEV causes an extensive demyelinating disease in the CNS of persistently infected mice (Tsunoda and Fujinami, 1996, 1999). We monitored cytotoxic T-cell responses in SJL/J mice, which are susceptible to TMEV-induced demyelinating disease. We found that spleen cells and T-cell clones from TMEV-infected mice were highly cytotoxic to uninfected syngeneic cells, but not to allogeneic cells following stimulation with TMEV-infected antigen-presenting cells. These autoreactive cells were found to be CD3^{+}CD8^{+} by flow cytometry and antibody-blocking cytotoxicity assays (Tsunoda *et al.*, 2005). Double chamber ^{51}Cr release assays showed that direct cell-to-cell contact was required for lysis. It was also found that cell killing was mediated by the Fas-FasL pathway (Tsunoda *et al.*, 2002). Intracerebral adoptive transfer of activated TMEV-induced autoreactive cells and T-cell clones into naive mice resulted in degenerating lesions in the spinal cord, suggesting that these MHC class I-restricted CD8^{+} T cells could play an effector role in CNS pathology in this model (Tsunoda *et al.*, 2005). We went on to show that these CTLs could be efficiently induced by a recombinant VV encoding the TMEV viral capsid proteins (Tsunoda *et al.*, 2006). We hypothesized that the autoreactive CTLs recognize TMEV capsid proteins that share molecular mimicry with CNS antigen. We are in the process of narrowing down the TMEV capsid epitope and characterizing the mouse CNS antigen that is the target of these CTLs.

Previous studies on memory CD8^{+} T cells suggested that these cells were a resting, nondividing population lying in wait until reexposure to the antigen that resulted in their initial differentiation (Jamieson and Ahmed, 1989). Other studies suggest that memory CD8^{+} T cells continuously undergo a low level of homeostatic division for a long period of time following antigen clearance (Kos and Mullbacher, 1993; Tough and Sprent, 1994; Zimmerman *et al.*, 1996). This homeostatic division can be augmented by type I IFN, interleukin (IL)-15, and IFN inducers such as poly (I:C) as well as viral infection (Tough *et al.*, 1996;

Zhang *et al.*, 1998). One study used mice whose immune system had been reconstituted with labeled splenocytes from LCMV-immune mice and examined the fate of these cells following poly (I:C) treatment or infection with LCMV, PV, and VV (Kim *et al.*, 2002). Previous reports from this group indicated that LCMV-immune mice were partially resistant to PV and VV and that LCMV-immune splenocytes could provide resistance to both PV and VV following adoptive transfer into naive animals; however, this resistance was lost if CD8$^+$ T cells were depleted (Selin *et al.*, 1998). In the Kim *et al.* (2002) study, it was found that the heterologous viruses (PV and VV) had the capacity to induce several cycles of proliferative expansion of memory CD8$^+$ T cells which altered the antigen hierarchy of T cells specific to earlier pathogens. LCMV infection resulted in a greater than 300-fold increase in the number of CD8$^+$ T cells with a majority of cells being LCMV specific. In contrast, poly (I:C) treatment only resulted in limited cell division without a net increase in cell number or changes in the hierarchy. This phenomenon is likely due to poly (I:C) induction of apoptosis, which offsets cell division (Kim *et al.*, 2002). Therefore, it was concluded that memory cell division with a net increase in number is characteristic of antigen-specific stimulation while division without an increase in cell number is a characteristic of cytokine-induced bystander stimulation. Interestingly, it was also found that CTLs generated against either LCMV or PV were not capable of lysing cells infected with the heterologous virus, which indicated little cross-reactivity between the viruses (Brehm *et al.*, 2002). However, CTLs from PV-infected LCMV-immune mice were able to lyse cells infected with either virus (Selin *et al.*, 1994). It was found that PV encodes a subdominant epitope that shares six of eight amino acids with an LCMV epitope (Brehm *et al.*, 2002). The fate of memory cells was assessed following PV infection of mice reconstituted with LCMV-immune cells (Kim *et al.*, 2005). Results indicate heterologous infection caused an increase in the number of T cells specific for the subdominant PV epitope. In contrast, there were considerable differences between individual LCMV-immune mice in terms of what epitope-specific T cells were stimulated by VV infection. Even though these responses were unpredictable, patterns of epitope recognition did emerge, with three epitopes more likely to be dominant. These differences in epitope-specific response are thought to be due to private specificities of the TCR repertoire generated in each mouse following random rearrangement of the variable (V), diversity (D), and joining (J) TCR gene segments (Kim *et al.*, 2005). Studies examining the composition, maintenance, and alteration of the T-cell memory pool have important implications for MS, and may help to explain the low concordance rates seen in identical twins.

A novel transgenic mouse model was created by Oldstone and colleagues (Evans *et al.*, 1996) to examine whether molecular mimicry to a protein expressed in the CNS could lead to autoimmune disease. In this model, mice were generated that express nucleoprotein (NP) or glycoprotein from LCMV under the control of

the MBP promoter as self in oligodendrocytes (Evans *et al.*, 1996). These mice were infected with the Armstrong strain of LCMV and monitored for clinical signs of disease. It was found that LCMV infected tissues in the periphery but not the CNS, and that a vigorous $CD8^+$ CTL response cleared virus from all tissues by 7–14 days postinfection. At 3-weeks postinfection, the transgenic mice had 150–300 $CD8^+$ T cells per sagittal section of brain while nontransgenic control mice had less than 30 lymphocytes per section. Perivascular cuffing was seen in the transgenic mice and lymphocytes were seen in the parenchyma, brain stem, and spinal cord. Interestingly, brains of the transgenic mice infected with LCMV were found to contain these elevated levels of $CD8^+$ T-cell infiltrates 1 year after infection. At 3-months postinfection, a clustering of $CD4^+$ and $CD8^+$ T cells was found predominately in the white matter of the CNS including the corpus callosum, internal capsule, fimbria hippocampus, brain stem, and spinal cord. Clinical signs, including ruffled fur, weight loss, and balance difficulties, were also evident in 75% of the transgenic mice at 3-months postinfection. This study also investigated the effect of a second infection with LCMV or VV encoding the NP transgene on the transgenic mice 6 weeks after the initial LCMV infection. Stimulation of the memory response by these viruses resulted in enhanced pathology including an increase in the ratio of $CD8^+$ to $CD4^+$ T lymphocytes and a greater likelihood that these infiltrates would be localized to the white matter as shown by immunohistochemical staining (Evans *et al.*, 1996). This enhanced disease pathology, characterized by demyelination and motor dysfunction, following secondary viral infection is similar to human CNS autoimmune diseases, and these results suggest that infection by a virus that shares immune determinants with a protein expressed in oligodendrocytes can induce a chronic inflammatory disease of the CNS.

To further validate the role viruses may play in the induction of autoimmune disease, a molecular mimicry model utilizing an H-2D^b-restricted immunodominant epitope of LCMV, $NP_{396–404}$ was used to identify structurally homologous murine self-proteins (Hudrisier *et al.*, 2001). The MHC class I-restricted $CD8^+$ T-cell response against the LCMV NP protein has been shown to be directed against this immunodominant $NP_{396–404}$ epitope. Typically in an LCMV-infected mouse that has cleared the virus, there is activation and proliferation of $CD8^+$ T cells which stay at a high level throughout the animal's life despite the fact that there is no detectable virus or viral antigens. These CTLs represent a potential source of autoreactivity, especially if their cytolytic activity remains functional and intact. Six nonameric sequences from endogenous proteins which shared structural and functional homology with LCMV $NP_{396–404}$ were identified through a database search. Five of these peptides were shown to have high H-2D^b-binding affinity and shared the main TCR contact site with LCMV $NP_{396–404}$. Three of these five peptides also shared an auxiliary TCR contact residue and one of these, tumor necrosis factor (TNF) receptor $I_{302–310}$, also shared a third residue with the

LCMV peptide resulting in a marked functional similarity between the self and viral epitopes. Despite the presence of TCR contact residues, none of the epitopes identified were able to stimulate antiviral CTLs in cytotoxicity assays. However, these peptides did behave as antagonists of lysis by LCMV-specific CTLs which indicated that they were capable of interacting with the TCR, though the affinity was relatively low. Low affinity recognition of self-MHC promoted thymocyte survival, and cells cultured with peptides were maintained for more than 2 months, suggesting these epitopes were in fact low-affinity molecular mimics. Importantly, these self-peptides were shown to allow LCMV-specific and potentially autoreactive $CD8^+$ T cells to maintain their cytolytic function in the absence of viral antigens over a period of months (Hudrisier *et al.*, 2001).

III. Class II Molecules and Mimicry

In the 1930s Rivers and colleagues (Rivers and Schwentker, 1935; Rivers *et al.*, 1933) published reports on induction of encephalomyelitis using CNS homogenates; and throughout the 1950s and 1960s research continued on myelin proteins in EAE and MS. In the 1940s it was shown that injection of myelin proteins with adjuvant into naive animals could cause relapsing-remitting, acute, or chronic encephalomyelitis (Freund *et al.*, 1947; Kabat *et al.*, 1946, 1947; Kopeloff and Kopeloff, 1947; Morgan, 1946, 1947; Morrison, 1947). Early studies also determined that EAE could be transferred by adoptive transfer of $CD4^+$ T cells (Pettinelli and McFarlin, 1981; Zamvil and Steinman, 1990).

MHC class II was found to have a genetic link with a variety of autoimmune diseases including MS (Jersild *et al.*, 1973a,b). MHC class II molecules present self-peptides to $CD4^+$ T cells; therefore, $CD4^+$ T cells are thought to play a major role in the initiation and/or progression of many autoimmune diseases. Several animal models of MS and human studies using blood and CSF from MS patients implicate $CD4^+$ T cells, mimicry, and various microbes such as *Chlamydia pneumoniae*, influenza virus, Torque Teno virus (TTV), EBV, and *Human herpesvirus 6* (HHV-6) (Croxford *et al.*, 2005; Lang *et al.*, 2002; Markovic-Plese *et al.*, 2005; Sospedra *et al.*, 2005; Sriram *et al.*, 1999; Tejada-Simon *et al.*, 2003).

C. pneumoniae has been associated with MS (Sriram *et al.*, 1999). In one study, CSF from 17 patients with relapsing-remitting MS, 20 patients with progressive MS, and 27 patients with other neurological diseases was tested for *C. pneumoniae* (Sriram *et al.*, 1999). Bacterial DNA was detected in 97% of these MS patients compared to only 18% of patients with other neurological diseases. They were also able to isolate *C. pneumoniae* from the CSF of MS patients in addition to identifying anti-*C. pneumoniae* antibodies. This work inspired Lenz *et al.* (2001) to identify a protein from *C. pneumoniae*, Cnp0483, with similarity to MBP. Cnp0483,

an MBP$_{68-86}$ homologue peptide, was used to sensitize Lewis rats. Animals began showing signs of disease approximately 12-days postsensitization. Pathologically, perivascular cuffing and mononuclear cell infiltrates were found in the spinal cords of Cpn0483-immunized rats. Additionally, splenocytes from Cpn0483-injected mice that had been cultured with peptide caused disease when they were transferred into naive animals. Interestingly, the Cpn0483 peptide shares only seven amino acids with MBP$_{68-86}$. This small degree of similarity happens to constitute a structural motif that permits interaction of the peptide with MHC class II gene products (Lenz *et al.*, 2001).

Influenza virus is an example of a virus infection where there is flexibility in TCR recognition and the degree of sequence and structural similarity necessary for cross-reaction. One group derived a CD4$^+$ T-cell clone from an MS patient's peripheral blood mononuclear cells (PBMCs) during an acute respiratory infection with influenza-A (Markovic-Plese *et al.*, 2005). They determined that this T-cell clone, GP5F11, reacted with the immunodominant influenza hemagglutinin (Flu-HA) epitope 306–318. GP5F11 was found to be a high avidity pathogen-specific T-cell clone that had demonstrated cross-reactivity against 14 Flu-HA variants, 11 viral, 15 human, and 3 myelin-derived peptides. Of the 11 viral mimics, it was found that equine influenza-A HA peptide had greater than 70 times higher stimulatory potential than the human influenza peptide, suggesting that there is a high potential for cross-recognition even for a T-cell clone with a stringent (high avidity) TCR response. Of 12 potentially stimulatory myelin-derived peptides, 2 myelin oligodendrocyte glycoprotein (MOG) peptides and one 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase)-derived peptide were found to be extremely potent stimulators of the T-cell clone. Interestingly, the CNPase-derived peptide had no sequence similarity to the Flu-HA peptide (Markovic-Plese *et al.*, 2005). This broad range of cross-reactivity of a single TCR would assure protection against much more than the original infecting influenza virus with the potential side effect of autoimmune responses against self-antigens having structural or sequence similarity to pathogenic peptides.

Another series of experiments found that one CD4$^+$ T-cell clone (MN19) isolated from the CSF of an MS patient during an exacerbation was stimulated by arginine-enriched protein domains from common viruses as well as the non-pathogenic TTV (Sospedra *et al.*, 2005). The T-cell clones used in this experiment were found to be expanded during exacerbations of MS and reduced during periods of remission. Biometric analysis was used to predict stimulatory peptides for the T-cell clones and then peptides were selected from human infectious agents. Many peptides were identified using this method; however, following normalization only two related DNA viruses were selected. These viruses were TTV and TTV-like mini virus, both of which are recognized as ubiquitous, nonpathogenic viruses in the human population. There were several stimulatory peptides identified from TTV, and surprisingly, the majority were mapped to a 74-amino acid

sequence in the N-terminal region of open reading frame 1. This N-terminal region is enriched in positively charged amino acids, mainly arginine. Other stimulatory peptides were also identified for the T-cell clone from other human viruses, including adenovirus and papillomavirus. The adenovirus peptide was from the pVII protein which is enriched in arginine and performs a histone-like function. The papillomavirus peptides were found in the minor capsid protein L2, which is enriched in basic amino acids. Human stimulatory peptides from the human CNS proteins adrenergic receptor α-1B, α-1A, and α-2C, arginine-rich protein, and dopamine D2 receptor were also identified for the MN19 T-cell clone. Interestingly, arginine-rich domains are frequent in viruses as well as eukaryotes and prokaryotes (Sospedra et al., 2005). This recognition of an arginine-enriched conserved domain may be involved in inducing and perpetuating autoimmune responses against arginine-rich self-antigens.

EBV has long been associated with MS and viral reactivation is linked to disease activity (Bray et al., 1983; Larsen et al., 1985). EBV is a large DNA virus that causes mononucleosis in humans and has been shown to persist in B cells. Infection with EBV late in life has been correlated with an increased risk of developing MS. Lang et al. (2002) used a $CD4^{+}$ T-cell clone isolated from an MS patient with a relapsing-remitting disease course to examine the TCR contact surfaces of a cross-reactive TCR that recognizes both the MBP_{85-99} peptide and an EBV DNA polymerase peptide ($EBV_{627-641}$). They found that MBP_{85-99} was recognized in the context of DRB1*1501 and $EBV_{627-641}$ was recognized in the context of DRB5*0101 based on relative binding affinities (Lang et al., 2002). DRB1*1501, DRB5*0101, as well as DQB1*0602 are MHC class II alleles of the DR2 haplotype that have been shown to be risk factors for MS. Transgenic mouse studies determined that spleen cells from TCR-DRB1*1501 double-transgenic mice only responded in the form of proliferation and cytokine production to the MBP_{85-99} peptide, while spleen cells from TCR-DRB5*0101 double-transgenic mice only responded to the $EBV_{627-641}$ peptide. Spleen cells from triple-transgenic mice with TCR-DRB1*1501-DRB5*0101 responded to both the MBP_{85-99} and $EBV_{627-641}$ peptides. These transgenic mice recognized the MBP and EBV peptides in the context of two different MHC class II molecules. Next, the crystal structures of both of these MHC-peptide complexes were determined and compared. It was found that the TCR contact surfaces of the DRB5*0101-$EBV_{627-641}$ and DRB1*1501-MBP_{85-99} complexes were structurally very similar. The nature of MHC class II binding to peptide, with the peptide chain held in a highly conserved extended conformation, is thought to allow for structural mimicry in TCR recognition (Lang et al., 2002).

Clinical studies extending the in vitro work of Lang et al. (2002) on EBV have found that $CD4^{+}$ T cells present in the CSF of an MS patient cross-react with $EBV_{627-641}$ and the immunodominant peptide MBP_{85-99} (Holmøy et al., 2004). The presence of $EBV_{627-641}$-specific $CD4^{+}$ T cells in the CSF proves that

EBV-specific T cells can gain access to the intrathecal compartment and suggests that they could target MBP in the CNS (Holmøy *et al.*, 2004).

Another virus that encodes proteins with peptide mimics to human brain proteins is HHV-6, the agent responsible for infantile exanthem. The virus has a tropism for $CD4^+$ T cells and can reactivate from its latent state following immunosuppression (Braun *et al.*, 1997; Salahuddin *et al.*, 1986). Challoner *et al.* (1995) found that oligodendrocytes from MS patients expressed HHV-6 virion proteins. A protein found in both the A and B variants of HHV-6, known as U24, has amino acid sequence similarity with MBP. Two 13-mer peptides corresponding to residues 1–13 of the HHV-6 U24 protein and MBP_{93-105} were synthesized and used to determine whether T cells would cross-react with the two peptides or were specific for only one of the sequences (Tejada-Simon *et al.*, 2003). T-cell cultures from PBMCs of MS patients were incubated with MBP_{93-105} or HHV-6 $U24_{1-13}$ for 1 week, tested for specificity, and characterized as MBP or HHV-6 reactive or cross-reactive. The frequency of T cells reactive to both HHV-6 and MBP peptides was determined to be significantly higher in MS patients than in the control group (Tejada-Simon *et al.*, 2003). The cytokine profiles of the peptide-specific and cross-reactive T-cell lines generated from MS patients were found to display a Th1 phenotype, predominately producing IFN-γ and TNF-α, but not IL-4 and IL-10, regardless of peptide specificity. These cells were shown to be $CD4^+$ by flow cytometry.

IV. Antibody and Mimicry

The presence of oligoclonal antibodies in the CSF of patients with MS is a consistent immunologic marker of the disease (Link, 1978; Link and Kostulas, 1983). Two or more oligoclonal bands consisting of IgG are routinely seen by isoelectric focusing followed by immunoblotting of CSF in up to 95% of MS patients (Link and Huang, 2006). Each band represents antibody, though identification of the antigen that these antibodies bind has been difficult in part because of the low amount of antibodies contained in the CSF. Some of the antigens to which the oligoclonal IgG antibodies have been found to be specific include MBP, HHV-6, measles, rubella, Varicella-zoster virus, and Herpes simplex virus 1 (HSV-1) (Cruz *et al.*, 1987; Derfuss *et al.*, 2005; Reiber *et al.*, 1998).

One method that has been used as an alternative source of antigens to study the specificity of antibodies is phage-displayed random peptide libraries (RPL) (Cortese *et al.*, 1996; Dunn, 1996; Smith, 1991). The peptides in these libraries are referred to as mimotopes because it is not required for them to have sequence similarity, but rather they mimic binding properties or conformation of natural epitopes. There have been reports of mimotopes from RPL selection that were

recognized by antibodies from the CSF of MS patients (Cortese *et al.*, 1996, 1998; Dybwad *et al.*, 1997). Interestingly, some of these antibodies were detected in sera from normal individuals as well as MS patients, suggesting that the mimotopes mimic common antigens (Cortese *et al.*, 2001). One mimotope family, MS17, was chosen because of its reactivity with CSF antibodies and high frequency of reactivity with the sera of MS patients. Two CSF samples from MS patients that recognize MS17 were tested for reactivity in an enzyme-linked immunosorbent assay (ELISA) to neurotropic viruses, including EBV, measles, mumps, rubella, HSV-1, and cytomegalovirus (Cortese *et al.*, 2001). Both CSF samples tested positive against measles and HSV-1; however, only the reactivity to HSV-1 was competitively inhibited by MS17 phage. In addition, whole cell extract from an HSV-1-infected cell line preincubated with CSF from the MS patients completely abolished recognition of the phage (Cortese *et al.*, 2001). A consensus sequence profile was then derived from multiple alignments of all HSV-1 isolates, and this was used to search for homology between eight members of the MS17 family and HSV-1 protein sequences. The highest score from this profile came from the N-terminal region of the HSV-1 envelope glycoprotein B (gB). The MS17 mimotope corresponds to the sequence of HSV-1 gB between amino acid positions 474–482. Antibodies against MS17 were raised in rabbits, and extensive characterization of these antibodies demonstrated that the MS17 phage is both an antigenic and immunogenic mimic of the HSV-1 gB epitope. Using the anti-MS17 antibodies in place of the CSF samples, the antibodies were found to specifically interact with an as yet uncharacterized protein present in protein extract from the brain of an MS patient (Cortese *et al.*, 2001). These studies represent an important step in identification of the natural antigens that antibodies from MS patients recognize as well as providing an example of molecular mimicry between a viral and CNS epitope that is mediated through antibody. The RPL technique has the potential to aid in explaining the pathogenesis of autoimmune diseases.

Monoclonal antibodies generated against various virus-specific proteins have been shown to cross-react with host cell components, thus serving as additional examples of molecular mimicry at the level of antibodies. Studies have shown that monoclonal antibodies generated against viral proteins from measles, HSV-1, and VV react with host cell components (Dales *et al.*, 1983; Fujinami *et al.*, 1983); common determinants existed between viruses and cellular- or tissue-specific elements (Srinivasappa *et al.*, 1986). In another report, a monoclonal antibody, H8, to the TMEV Daniels (DA) strain reacted with both TMEV viral protein-1 and lipid-like moieties including galactocerebroside, a major component of myelin (Fujinami *et al.*, 1988). In mouse brain cultures, cells that bound the TMEV monoclonal antibody, H8, were also labeled with antibody to MBP (Yamada *et al.*, 1990). This double staining indicated that the H8 antibody recognized epitopes on oligodendrocytes. When the antibody was injected into mice with EAE, H8 caused an increase in the area of demyelination within spinal cords. Furthermore,

a competition ELISA for galactocerebroside and TMEV found that sera from mice contained antibody with the same specificity as H8. These results indicate that the immune response which generates antibodies specific for TMEV that cross-react with myelin and oligodendrocytes could contribute to demyelination through an antibody-mediated process (Yamada *et al.*, 1990).

There are several other examples of molecular mimics at the level of antibodies in autoimmune diseases other than MS. They include Guillain-Barre syndrome in which antibody cross-reactivity between peripheral nerve and ganglioside has been shown (Ogino *et al.*, 1995; Yu *et al.*, 2006), and rheumatic heart disease in which antistreptococcal antibodies cross-react with *N*-acetyl-*β*-D-glucosamine, myosin, and other self-epitopes (Guilherme *et al.*, 2006; Mertens *et al.*, 2000). In addition, autoantibody development is seen in systemic lupus erythematosus (Poole *et al.*, 2006). EBV has been associated with lupus through serological and DNA studies and antibodies specific for Epstein-Barr nuclear antigen-1 (EBNA-1) cross-react with lupus-associated autoantigens (Poole *et al.*, 2006). Alternately, infection with human T-lymphotropic virus type-1 (HTLV-1) can cause HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), an immune-mediated disease of the CNS (Lee *et al.*, 2006). Molecular mimicry has been found in antibodies that cross-react with heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1), a protein found in CNS neurons, and the HTLV-1 tax protein. Two core epitopes within the C-terminal region of hnRNP A1, both functionally important regions, were found to react with antibodies purified from HAM/TSP patients. Additionally, monoclonal antibodies raised against HTLV-1 tax protein also reacted with the two core epitopes from hnRNP A1 (Lee *et al.*, 2006).

V. Discussion

Potentially, infection with viruses that have molecular mimicry with CNS antigens can prime autoreactive immune cells specific for the CNS in hosts. Our laboratory has explored whether a virus having molecular mimicry with self-CNS antigens could induce an autoimmune disease in mice which had previously been inducible only by injection of CNS antigen in complete Freund's adjuvant (CFA) (Theil *et al.*, 2001). Three recombinant VVs were constructed: encoding myelin proteolipid protein (VV$_{PLP}$), encoding myelin-associated glycoprotein (VV$_{MAG}$), and encoding glial fibrillary acidic protein (VV$_{GFAP}$). Mice infected with VV$_{PLP}$, VV$_{MAG}$, or VV$_{GFAP}$ showed no clinical or histological signs of CNS disease. This suggests that molecular mimicry alone cannot result in high enough numbers or activation of CNS-specific autoimmune cells for induction of CNS disease. Thus, 5 weeks after the first infection, when VV was cleared, we challenged mice nonspecifically with CFA to activate CNS-specific autoimmune cells (bystander activation).

Clinically, some mice showed paralysis in the tail, similar to EAE, following the CFA challenge. At 1-month post-CFA challenge, mice were sacrificed and CNS tissues were examined for pathological changes. All mice (15/15) were found to have inflammatory lesions with $CD3^+$ T cells in the CNS (Theil *et al.*, 2001). As a negative control, mice were infected with VV_{SC11}, a recombinant VV that encodes β-galactosidase, which has no known molecular mimicry with CNS antigens; following CFA challenge, no inflammatory changes were seen in the CNS (Theil *et al.*, 2001). These data indicate that infections with viruses encoding molecular mimics can substantially prime animals for autoimmune disease and at a later time a nonspecific immunologic challenge could initiate the disease.

As reviewed by Trinchieri (1995), MCMV infection in mice causes a significant burst of IL-12, which promotes the development of Th1 cells. Therefore, we have tested whether VV_{PLP}-sensitized mice developed clinical disease following a second, unrelated viral challenge with MCMV. Preliminary findings suggest this to be the case. Five out of nine mice primed with VV_{PLP} and challenged with MCMV were found to have meningitis and perivascular cuffing in the CNS. In contrast, mice primed with VV_{PLP} and challenged with the wild-type strain of VV were found to have no obvious lesions. In another experiment, mice primed with VV_{PLP} and challenged with MCMV showed marked weight loss and had righting reflex disturbances, compared with mice injected with phosphate-buffered saline (PBS) or VV_{SC11} followed by MCMV challenge (Ikuo Tsunoda, Jane E. Libbey, and Robert S.Fujinami, unpublished data). Thus far, the definitive experiments to determine whether disease in this model is caused by MHC class I or class II cells have not been completed. However, these experiments are important in that they provide a working model mirroring what may be occurring in human patients with MS.

Molecular mimicry has been found in many autoimmune diseases on a variety of levels. There is significant evidence that autoreactive $CD4^+$ and $CD8^+$ T cells as well as autoantibodies can contribute to disease progression in various animal models of MS and other autoimmune diseases. However, it has yet to be shown that a single molecular mimic is responsible for initiation of disease. None of the autoimmune diseases described here are simple cause-and-effect diseases and as such the mechanisms responsible for their manifestation are likely to be a combination of molecular mimicry, bystander activation, epitope spreading, and heterologous immunity.

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