Theiler’s Murine Encephalomyelitis Virus-Induced CNS Autoimmunity

Virus-induced molecular mimicry is part of a mouse model of multiple sclerosis that is providing insights about the disease in humans

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Multiple sclerosis (MS) is a progressive autoimmune disease affecting the central nervous system (CNS). The disease progresses because autoreactive T cells, especially CD4+ T cells, damage myelin sheaths surrounding neuronal axons in the brain and spinal cord, eventually paralyzing affected individuals.

MS is unmistakably immune mediated and myelin specific, and both genetic and environmental influences are involved in its development. Although its etiology is not fully defined and no particular virus can yet be said to cause this disease, epidemiologic studies indicate that viral infections may trigger and also sustain MS. Among the infectious agents purportedly associated with MS in humans are measles virus, simian virus 5, herpes simplex virus, parainfluenza virus 1, rubella virus, coronavirus, human T-lymphocyte retrovirus type I, Epstein-Barr virus, and human herpesvirus 6.

Investigators have proposed several different means by which viruses might induce and cause progressive damage in MS and other autoimmune diseases. According to one such proposal, called epitope spreading, an infection generates a virus-specific T cell-mediated host immune response, resulting in a localized inflammatory response, bystander damage to host tissues, and subsequent processing of and, ultimately, damaging autoreactive responses to self antigens. Although such antigens ordinarily are sequestered from the immune system, exposure to them in this case activates autoreactive T cells and leads to an autoimmune disease. The autoimmune response can also spread to encompass other self antigens as the disease progresses.

Additionally, virus-encoded epitopes that are shared or cross-reactive with the self epitopes may activate autoreactive T cells via a process termed molecular mimicry. Lastly, autoreactive T cells may be activated by bystander destruction of host tissues when self antigens secondary to necrotic and apoptotic tissue damage are released—either because infectious agents damage that tissue directly or through indirect effects when the innate immune response to an infection stimulates release of cytokines and chemokines.

TMEV Model for Virus-Induced Autoimmune Disease

Theiler’s murine encephalomyelitis virus (TMEV), which belongs to the cardiovirus group of the Picornaviridae, can infect susceptible mice, such as the SJL/J strain, leading to a chronic, progressive autoimmune demyelinating disease similar to MS in humans. For instance, when such mice are infected with the BeAn strain of TMEV, clinical signs first appear 30 days later when animals develop hind limb control difficulties, especially while walking (Fig. 1). These symptoms continue to increase in severity, leading progressively to paralysis of the hind limbs and front limbs and death. The axonal tracts in the spinal cords from infected mice are demyelinated and contain large numbers of infiltrating mononuclear cells.

The autoimmune component of TMEV-induced demyelinating disease is well defined. Beginning 45–60 days postinfection, CD4+ T cells specific for an immunodominant myelin epitope on myelin proteolipid protein, PLP139–151, can be identified in the spleens and spinal cords of infected mice, and responses to additional
myelin epitopes arise as disease progresses. When TMEV-infected mice are tolerized to a panel of myelin epitopes just before the onset of autoreactive responses (40 days postinfection), the autoimmune response does not appear and the disease symptoms fail to progress. Thus, autoreactive myelin epitope-specific CD4$^+$ T cells in large part mediate the chronic phases of TMEV-induced demyelinating disease, making this disease in mice a valuable model for MS in humans.

During the chronic phase of this demyelinating disease in mice, epitope spreading is the mechanism that initiates autoimmune responses. Shortly after TMEV infection, the mice develop both CD4$^+$ and CD8$^+$ T cell responses to viral antigens, without evidence of antemyelin autoimmune responses. However, these early responses do not clear the virus, meaning that infectious virions persist in microglial cells and macrophages within the CNS of these mice, leading to a chronic antivirus immune response. CD4$^+$ T cells specific for virus epitopes release inflammatory chemokines and cytokines that attract additional T cells and peripheral macrophages to the CNS. Inflammatory cytokines also activate CNS-resident microglia and infiltrating macrophages that produce additional proinflammatory substances, such as tumor necrosis factor $\alpha$ (TNF-$\alpha$), NO, and oxygen radicals, all of which are implicated in destroying myelin.

Meanwhile, because the persistent infection continues to damage myelin, myelin-derived antigens are released into the immediate environment of the infected CNS—enabling infiltrating native CD4$^+$ T cells to encounter these self antigens on activated antigen-presenting cells within this milieu. The autoreactive CD4$^+$ T cells activated by the epitope spreading process continue to damage myelin, releasing additional self antigens and amplifying the autoimmune responses because of the involvement of additional myelin epitopes, further enhancing the CNS damage. As epitope spreading continues, the clinical symptoms grow more severe, eventually leading to complete paralysis and death.

**A Virus-Induced Model of Molecular Mimicry**

Other researchers studying molecular mimicry have focused on cross-reactive immune responses between mimic and self epitopes, but tended to bypass the infectious component of this mechanism for inducing autoimmune disease. Therefore, we developed a direct, TMEV infection-induced model for studying molecular mimicry, making use of a TMEV variant, $\Delta$Cla-BeAn, that contains a deletion in the virus leader and does not induce autoimmune demyelinating disease in mice. We determined that $\Delta$Cla-BeAn replicates initially within the host CNS, but does not persist and also does not induce disease symptoms.

This recombinant TMEV also contains an engineered restriction site that allows us to insert genetic material encoding foreign epitopes directly into the viral genome. For example, we
inserted an immunodominant myelin epitope, PLP139–151, into the ΔCla-BeAn viral genome and asked whether this virus (PLP139-BeAn) would induce an autoimmune response directed to the inserted self epitope.

Infecting SJL mice with the PLP139-BeAn virus results in an early-onset, severe demyelinating disease arising within 7–14 days, compared to the 30-day onset period in mice infected with wild-type BeAn virus (Fig. 2A). In contrast, mice infected with a control virus, encoding the nonself antigen ovalbumin 323–339, develop a late-onset disease similar to that induced by wild-type BeAn virus. The mice begin showing clinical signs of disease 7 days following infection with PLP139-BeAn virus, and their symptoms progress rapidly from a mild waddling gate to severe impairment in walking and righting abilities, followed progressively by paralysis of the hind limbs, paralysis of the front limbs, and death. During the course of this early-onset disease, CD4+ and CD8+ T cells, B cells, and macrophages infiltrate the spinal cord by 14 days following infection (Fig. 3). Clinical and histological signs of disease correlate with an early PLP139–151-specific CD4+ T cell response in the infected mice (Fig. 2B).

Further, in tolerance studies, this PLP139–151 CD4+ T cell response was proven to mediate the rapid-onset demyelinating disease. Tolerance is the process of rendering T cells unresponsive to a specific antigen. For instance, central tolerance occurs when cells develop in the thymus to educate the immune system to the difference between self and nonself, whereas peripheral tolerance is responsible for maintaining tolerance to self antigens that escaped thymic deletion.

In these mice, we induced peripheral tolerance to PLP139–151 by administering this peptide coupled to splenic antigen presenting cells to mice intravenously before we infected them with TMEV. This treatment causes such infected mice to develop significantly reduced demyelinating symptoms. Our ability to induce demyelinating disease in uninfected mice by transferring PLP139–151-specific CD4+ T cells isolated from PLP139-BeAn virus-infected mice provides further evidence that the early-onset disease is autoimmune.

Moreover, infecting mice with PLP139-BeAn virus at various sites outside the CNS, such as by intraperitoneal, intravenous, or subcutaneous injection, leads to rapid-onset demyelinating disease. These findings suggest that infecting such animals with a self antigen-encoding virus...
Molecular Mimicry as a Model for Autoimmune Disease

To determine whether infection-induced molecular mimicry is a potential cause of autoimmune diseases, we introduced molecular mimics of the PLP139–151 sequence into the ΔCla-BeAn viral genome and tested for their ability to initiate demyelinating disease in mice infected with them. Initially, we introduced nonconservative amino acid substitutions into the PLP139–151 sequence at the primary and secondary sites for T cell receptor recognition—amino acids 144 and 147, respectively. Infecting mice with strains of TMEV that encode the peptide carrying a substitution at the primary T cell receptor recognition site (W144A) does not lead to early-onset demyelinating disease. Moreover, these infected mice do not activate T cell responses that cross-react with PLP139–151 (Fig. 2B). In contrast, when mice are infected with TMEV expressing a substitution at the secondary T cell receptor recognition site (H147A), the animals develop early-onset demyelinating disease as well as cross-reactive CD4+ T cell responses to PLP139–151. Therefore, infection-induced molecular mimicry requires conservation of amino acids at the primary T cell receptor recognition site.

Next, we constructed a BeAn virus containing a PLP139–151 mimic epitope from Haemophilus influenzae. In previous studies, researchers identified sequences from other pathogens that share sequence homology with the PLP139–151 epitope. Based on assays with PLP139–151-specific CD4+ T cells, we identified several mimic epitopes that stimulate cross-reactive responses, one of which is in the H. influenzae (HI) protease IV protein (specifically, amino acids...
Mice infected with the ΔCla-BeAn virus that expresses this epitope develop a mild, early-onset demyelinating disease and, importantly, cross-reactive CD4+ T cells specific for PLP139–151 (Fig. 2). Other experiments indicate that, when mice are tolerized to PLP139–151, they do not develop this HI574-BeAn virus-induced disease. These preliminary findings suggest that the pathology is due to infection-induced CD4+ T cell-mediated molecular mimicry.

According to this model, a virus infection activates specific CD4+ T cells, some of which express receptors capable of cross-reacting with self myelin epitopes and infection of CNS resident cells, including the microglia (1). Activated microglia can secrete chemokines, cytokines, and upregulate antigen presenting cell surface markers (2). Infected microglia secrete chemokines and cytokines that attract lymphocytes and macrophages from the periphery to the site of virus damage in the CNS (3). The activated microglia can upregulate costimulatory molecules, B7-1 and B7-2, and MHC class II enabling them to present antigen to T cells (4). These microglia also can secrete cytokines that promote inflammatory CD4+ T cell activation (4). Microglia and/or macrophages can process and present virus antigens to CD4+ T cells resulting in an inflammatory response in the CNS. The virus-specific CD4+ T cells proliferate and mount an immune response to the virus that can result in bystander damage of CNS tissue releasing self tissue antigens into an inflammatory environment (5). Self myelin antigens are processed and presented to virus-specific CD4+ T cells that are cross-reactive and recognize both the viral and myelin peptides (6). The autoreactive T cells continue to destroy the self myelin tissue perpetuating the autoimmune response even if the virus infection is eventually cleared.

Figure 4

Molecular mimicry model for virus-induced CNS demyelination. A virus infects the host resulting in activation of virus-specific CD4+ T cells, some of which express receptors capable of cross-reacting with self myelin epitopes, and infection of CNS resident cells, including the microglia (1). Activated microglia can secrete chemokines and cytokines that attract lymphocytes and macrophages from the periphery to the site of virus damage in the CNS (3). The activated microglia can upregulate costimulatory molecules, B7-1 and B7-2, and MHC class II enabling them to present antigen to T cells (4). These microglia also can secrete chemokines that promote inflammatory CD4+ T cell activation (4). Microglia and/or macrophages can process and present virus antigens to CD4+ T cells resulting in an inflammatory response in the CNS. The virus-specific CD4+ T cells proliferate and mount an immune response to the virus that can result in bystander damage of CNS tissue releasing self tissue antigens into an inflammatory environment (5). Self myelin antigens are processed and presented to virus-specific CD4+ T cells that are cross-reactive and recognize both the viral and myelin peptides (6). The autoreactive T cells continue to destroy the self myelin tissue perpetuating the autoimmune response even if the virus infection is eventually cleared.
specific Th1 cells that, in turn, stimulate the release of proinflammatory cytokines such as interferon gamma (IFN-γ), TNF-α, and lymphotoxin beta. Virus-infected, cytokine-activated microglia, along with macrophages, also secrete cytokines such as IL-12 and IL-18 that promote activation of a Th1 pro-inflammatory response.

CNS tissue—damaged either directly by the virus infection or indirectly because of these virus-specific immune responses—releases myelin that is then processed and presented by the local antigen-presenting cells. If the infecting virus contains an epitope that shares critical residues with self myelin peptides, a portion of the virus-specific CD4⁺ T cells activated to the mimic epitope can recognize those processed myelin peptides, thereby enhancing the local tissue damage and perpetuating disease even when the virus infection is cleared. Importantly, molecular mimicry and epitope spreading can occur sequentially—releasing additional endogenous myelin antigens and further enhancing disease symptoms.

Other Questions Regarding Molecular Mimicry Are Being Addressed

Outstanding questions regarding the molecular mimicry model include whether T cell receptors indeed recognize self epitopes, the extent to which viruses harbor sequences that mimic those found in hosts, and whether such sequences can induce a sufficient autoimmune response that leads to disease.

Recent studies address the ability of T cells to recognize self epitopes and survive deletion during thymic development. For example, it is relatively easy to isolate PLP139–151-specific T cells from naive SJL mice—that is, animals that had not been injected with this antigen—suggesting that such T cells are induced by self antigen and then escape thymic deletion. T cell receptors also exhibit significant degeneracy, meaning they can recognize peptides with many sequence variations as long as the primary T cell receptor recognition sites and MHC class II binding sites remain relatively constant.

Second, investigators identified several viral and bacterial sequences by computer analysis that share critical residues with self-myelin peptides, responses to which are found in MS patients. The TMEV model of virus-induced molecular mimicry provides a basis for studying responses to other mimic sequences that can be found in viral pathogens infecting mice, humans, or other species.

Third, for a mimic sequence to induce an immune response during an infection, the proteolytic machinery of the host antigen-presenting cells needs to process that sequence from the pathogen-encoded protein that expresses the specific cross-reactive epitope. Furthermore, depending on the context in which a mimic sequence is recognized by the host immune system, antigen-specific T cells may be activated without causing disease symptoms. For instance, in our mimicry model, although mice infected with TMEV expressing the HI574–586 peptide develop early-onset autoimmune disease, mice immunized with HI574–586 peptide in complete Freund’s adjuvant do not develop demyelinating disease, even though they produce HI-specific CD4⁺ T cell responses. Therefore, antigen recognition within a particular inflammatory microenvironment accompanied by delivery of necessary pathogen-delivered innate immune signals may be required to push the autoimmune response sufficiently to lead to the development of clinically apparent symptoms.

Epitope spreading and molecular mimicry describe two plausible mechanisms whereby pathogens may induce autoimmune responses. Indeed, the molecular mimicry mechanism that is at work in mice provides definitive evidence that infecting such animals with a virus encoding a mimic sequence for a myelin epitope induces an autoimmune, demyelinating disease by directly activating self-reactive CD4⁺ T cells.

Although infections appear linked to the development of autoimmune diseases in other mammalian species, the process by which such infection triggers autoimmune responses in humans is not fully understood. We are trying to identify infectious agents that trigger or exacerbate human MS and are currently analyzing myelin sequences expressed by human pathogens to determine whether these potential molecular mimics can induce CNS disease when they are introduced into transgenic mice that encode and produce human-type MHC class II molecules and human myelin epitope-specific T cell receptors. We hope that these studies lead to the development of new ways for preventing and treating MS and other autoimmune diseases.
SUGGESTED READING


