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Molecular Immunology 40 (2004) 1103–1108
Molecular Immunology
www.elsevier.com/locate/molimm

Innate and adaptive immune requirements for induction of autoimmune demyelinating disease by molecular mimicry

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Abstract

Molecular mimicry is the main postulated mechanism by which infectious agents induce autoimmune disease. A number of animal models have been utilized to establish a link between molecular mimicry and autoimmunity. However, a model of infectious disease whereby a natural pathogen expressing a known mimic epitope can induce autoimmunity to a known self-antigen leading to clinical autoimmune disease is still lacking. We have engineered a recombinant Theiler's murine encephalomyelitis virus (TMEV) to express an encephalitogenic myelin proteolipid protein $PLP_{139–151}$ epitope (PLP-TMEV) and a $PLP_{139–151}$ mimic peptide naturally expressed by *Haemophilus influenzae* (HI-TMEV). Infection of mice with either PLP-TMEV or HI-TMEV induces early-onset disease that is associated with the activation of cross-reactive $PLP_{139–151}$-specific immunopathologic $CD4^+$ Th1 cells. Based on results from this model, we hypothesize, due to the considerable degeneracy in the T cell repertoire, that induction of full-blown autoimmune disease via molecular mimicry is a tightly regulated process requiring multiple factors related to the pathogen expressing the potential mimic epitope. In this review, we will discuss how various factors related to the infectious environment control whether or not autoimmune disease is initiated. Contributing factors include the nature of the innate immune response to the pathogen which determines the immunopathologic potential of the induced cross-reactive T cells, the capacity of the mimic epitope to be processed and presented from its natural flanking sequences in the pathogen-encoded protein, the site(s) of the primary infection in the host and the ability of the pathogen to persist, and the potential requirement for multiple infections with the same or different pathogens.

Keywords: Molecular mimicry; T cell degeneracy; Multiple sclerosis; Theiler's virus; Innate immunity

1. T cell degeneracy and infection-induced autoimmune disease

Multiple sclerosis (MS), one of the most prevalent neurological diseases, is characterized by a loss of the myelin sheath surrounding axons in the central nervous system (CNS) (Steinman, 1996). MS is generally considered to be an autoimmune disease as demyelination is associated with elevated levels of $CD4^+$ T cells specific for major myelin proteins (Ota et al., 1990; Bernard and de Rosbo, 1991; Allegretta et al., 1990; Link et al., 1990; Sun et al., 1991). Although it is not known for certain what triggers the development of MS, epidemiologic evidence suggests that viral or bacterial infection may play an important role both in disease initiation and disease exacerbation (Kurtzke, 1993). Although most reports linking infection with MS development are circumstantial, epidemiological studies have consistently shown an increased risk for MS in certain climates, suggesting that pathogens endogenous to those regions may be responsible for triggering or propagating the disease (Fujinami, 2001). There are several potential ways in which an infectious agent can induce an autoimmune disease. T cells targeting the pathogen may induce bystander damage to the surrounding tissue and lead to autoimmunity via *epitope spreading* (Miller et al., 1997; Olson et al., 2001b), or normally sequestered tissue antigens may be released during infection and activate tissue-specific T cells (Horwitz et al., 1998). Pathogens can also trigger disease via *molecular mimicry*, wherein T cells generated against foreign epitopes respond in a cross-reactive manner with self epitopes.

Molecular mimicry remains the major postulated mechanism by which infections may trigger autoimmune tissue damage (Fujinami and Oldstone, 1985; Wucherpfennig and Strominger, 1995). Evidence for molecular mimicry stems mainly from experiments showing that mice expressing virus proteins as tissue-specific transgenes develop autoimmune disease after virus infection (Ohashi et al., 1991; Oldstone et al., 1991) and from reports showing the considerable degeneracy of the T cell recognition wherein viral peptides

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0161-5890/$ – see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.molimm.2003.11.010

sharing limited sequence homology with self peptides can stimulate autoreactive T cells in vitro (Wucherpfennig and Strominger, 1995; Hemmer et al., 1997; Gran et al., 1999). Although these findings demonstrate that molecular mimicry is a viable hypothesis explaining the link between infection and MS, the search for direct evidence of a role for this phenomenon in human autoimmune disease is ongoing.

Due to the high level of degeneracy in the T cell repertoire, cross-reactive activation of autoreactive T cells must be a tightly regulated process in order to protect the host from unbridled induction of autoimmune disease following common infections. Activation of autoreactive T cells secondary to infection-induced molecular mimicry is likely to require the following conditions in order to lead to full-blown autoimmune disease: (1) the infectious agent must encode a mimic epitope capable of activating the cross-reactive, self peptide-specific T cell repertoire; (2) the mimic epitope must be able to be processed from its native protein so that it can be presented by the host MHC class II molecules; (3) the infectious agent must be capable of providing the correct stimuli to the innate immune system to induce the expansion and differentiation of the pathologic cross-reactive, self-specific T cell repertoire; and (4) it is possible that an inflammatory response must occur in the target organ of the autoimmune disease to provide the requisite signals required to either initiate and/or sustain the pathologic autoreactive response. We have recently developed a novel model of a virus-induced demyelinating disease (Olson et al., 2001a) which will allow testing of the importance of the individual above conditions to the induction autoimmune disease following infection.

2. Virus-induced molecular mimicry model for MS

Initial studies addressing molecular mimicry as a possible way to induce autoimmunity were conducted using mice in which virus proteins expressed as transgenes were targeted as pseudo-self antigens causing tissue destruction following virus infection (Ohashi et al., 1991; Oldstone et al., 1991) and more recently with mimic peptides in complete Freund's adjuvant (Madsen et al., 1999). These studies provided evidence that a cross-reactive immune response could be initiated by mimic sequences, but fail to provide definitive evidence for infection-induced autoimmune disease. We decided to develop an infectious model of molecular mimicry wherein self-peptide mimic sequences are encoded by a virus pathogen (Olson et al., 2001a). Our studies were conducted with a picornavirus, Theiler's murine encephalomyelitis virus (TMEV). TMEV induces an autoimmune demyelinating disease that serves as a mouse model for multiple sclerosis. Infection of mice with the wildtype neurotropic BeAn strain of TMEV produces a chronic CNS infection that persists for the lifetime of the animal (Lipton, 1975). TMEV-induced demyelinating disease onsets with clinical signs around 30 days post-infection $CD4^+$ T cell responses to myelin epitopes arising via epitope spreading after initial CNS damage (45–60 days post-infection) (Miller et al., 1997).

To provide a model of virus-induced molecular mimicry, we constructed a recombinant strain of TMEV that had a 23 amino acid deletion and a ClaI restriction site inserted in the viral leader sequence (ΔCla-TMEV). ΔCla-TMEV failed to persist in the CNS of infected mice and did not induce autoimmune demyelinating disease nor activate $PLP_{139–151}$-specific $CD4^+$ T cell response in infected mice (Olson et al., 2001a). ΔCla-TMEV was then engineered to express $PLP_{130–159}$ in its leader sequence (PLP-TMEV). This 30-mer fragment contains the immunodominant encephalitogenic CNS myelin $PLP_{139–151}$ peptide as well as surrounding residues to allow for processing and presentation by antigen-presenting cells (APCs). Mice infected with PLP-TMEV developed severe paralytic disease with a significantly more rapid onset (7–14 days post-infection) than mice infected with wild-type TMEV infection (30–40 days post-infection), and developed $PLP_{139–151}$-specific $CD4^+$ Th1 responses within 7 days post-infection. Induction of tolerance to $PLP_{139–151}$ using $PLP_{139–151}$-coupled splenocytes prior to infection with the PLP-TMEV prevented development of demyelinating disease and reduced the $PLP_{139-151}$-specific $CD4^+$ T cell response (Olson et al., 2002). In addition, $PLP_{139–151}$-specific $CD4^+$ T cells isolated from the PLP-TMEV infected mice could transfer disease to naïve recipient mice. Mice infected with ΔCla-TMEV engineered to express a non-self epitope ($OVA3_{23–339}$) OVA-TMEV did not develop the early onset demyelinating disease, but developed late onset demyelinating disease similar to mice infected with wild type TMEV.

To determine if the disease could be induced by mimics of the $PLP_{139–151}$ epitope, additional studies utilized TMEV which expressed mimic $PLP_{139–151}$ sequences containing non-conservative amino acid substitutions in either the primary or secondary TCR recognition sites (Olson et al., 2001a). Infection of mice with TMEV containing $PLP_{139–151}$ with a non-conservative substitution in the primary TCR site (residue 144) did not result in an early onset demyelinating disease or cross-reactive $PLP_{139–151}$-specific $CD4^+$ T cell response, however these mice developed the late onset demyelinating disease similar to the wild type TMEV infected mice. Meanwhile, mice infected with TMEV containing $PLP_{139–151}$ with a substitution at the secondary TCR recognition site (residue 147) developed early onset demyelinating disease and cross-reactive $PLP_{139–151}$-specific $CD4^+$ T cell response similar to the mice infected with the native PLP-TMEV, proof-of-principle for molecular mimicry. As more direct evidence for infection-induced molecular mimicry, PLP-TMEV was then engineered to express a $PLP_{139–151}$ mimic peptide derived from the protease IV protein (*sppA*) of *Haemophilus influenzae*, $HI_{574–586}$ (HI-TMEV) (Carrizosa et al., 1998). This peptide shares 6/13 amino acids with $PLP_{139–151}$ including the primary TCR contact residue at position 144 and the primary and secondary MHC class II binding residues

(positions 145 and 148). Although disease onset was not as rapid nor clinical symptoms as severe as that seen in PLP-TMEV-infected mice, HI-TMEV infected mice developed early-onset paralytic disease (Olson et al., 2001a). Disease induction was characterized by the activation of $CD4^+$ IFN-γ-producing Th1 cells responsive to both $HI_{574–586}$ and $PLP_{139–151}$ and by CNS infiltration of activated $CD4^+$ T cells and $F4/80^+$ macrophages/microglia. Further, peripheral tolerance to either $PLP_{139–151}$ or $HI_{574–586}$ decreased clinical disease (unpublished observations). These studies definitively indicate that infection of the CNS with a pathogen containing a mimic epitope for a self myelin antigen, can induce a cross-reactive $CD4^+$ T cell response resulting in autoimmune demyelinating disease.

3. Innate immune response to pathogens in molecular mimicry-induced autoimmune disease

The virus-induced model of molecular mimicry described above provides a model for addressing the requirement of the innate immune response to the infection on the development of the mimic T cell response. Most interestingly, our preliminary results (unpublished observations) indicate that SJL mice immunized with the core $HI_{574–586}$ mimic peptide in complete Freund's adjuvant did not develop EAE-like clinical disease. $HI_{574–586}$ mimic peptide immunization induced a T cell proliferative response that was cross-reactive with $PLP_{139–151}$, but, unlike the mice infected with HI-TMEV, these cross-reactive T cells failed to produce significant amounts of IFN-γ upon re-simulation with the self $PLP_{139–151}$ epitope. These results suggest that the virus infection creates an environment that promotes the activation of cross-reactive Th1 cells leading to autoimmune demyelinating disease.

The innate immune response to the pathogen is critical in determining the immune environment in which the mimic T cell response is activated. Toll-like receptors (TLRs) are the innate immune receptors that recognize pathogen-associated molecular patterns (PAMPs) which are molecular patterns unique to pathogens (Medzhitov and Janeway, 1997). TLR engagement has been associated with an intracellular signaling pathway that results in NF_kB activation leading to a cascade of cytokine and chemokine expression as well as increased expression of additional immune system receptors. We have shown that TMEV can persistently infect microglia, the CNS resident antigen-presenting cell population (Olson et al., 2001c). Upon TMEV infection, microglia become activated to express innate immune cytokines, such as IFN-α and IFN-β, IL-12 and TNF-α, and chemokines that are important for attracting T cells, B cells, and macrophages to the CNS. Virus-infected microglia also upregulated expression of B7-1 and B7-2 costimulatory molecules, as well as MHC class I and MHC class II on their cell surface enabling the virus-infected microglia to process and present both viral and myelin antigens to $CD4^+$ Th1 T cells. Thus, TMEV infection of microglia initiates an innate immune response that activates them to induce T cell responses against both virus and self myelin antigens. Further, we have also shown that microglia express numerous Toll-like receptors (unpublished data). In addition to TMEV infection, microglia stimulated with TLR ligands such as lipopolysaccharide, peptidoglycan, CpG DNA, and polyI:C, are activated to express innate immune cytokines (unpublished data).

These results suggest that the innate immune response to an infecting pathogen is critical for directing the development of a cross-reactive immunopathologic Th1-mediated autoimmune response. Infection with an identical mimic carried by a pathogen which cannot provide the critical innate immune signals may lead either to an autoimmune response which is non-pathogenic (e.g. Th2) or even tolerize the autoreactive T cells (Barnett et al., 1996). Thus, it is likely that molecular mimicry can either promote or protect against the development of autoimmune disease depending on the context in which a mimic peptide is presented to naïve T cells during an infection.

4. Antigen processing and presentation in infection-induced molecular mimicry

Molecular mimicry-induced autoimmune disease is also dependent on antigen processing and presentation. Two important factors are required for proper antigen processing and presentation of mimic peptide to autoreactive T cells during an infection. The first factor is the type of antigen presenting cell that initiates the response to the mimic peptide during the infection. APCs in a tissue-specific autoimmune disease may be infiltrating professional APCs, such as macrophages, dendritic cells, and B cells, or they may be local APCs, such as microglia, that reside in the tissue and become activated upon infection and/or exposure to cytokines. The professional APCs are more efficient than the local APCs at presenting antigen in a context leading to activation of naïve T cells. However, infection in the target organ may activate the local APCs to be more efficient APCs enabling them to present mimic epitopes to naïve T cells and possibly present self-epitopes to the cross-reactive T cells. As described above, microglia become functional APCs following virus infection or exposure to pro-inflammatory cytokines (Olson et al., 2001c). In addition, some pathogens directly infect APCs, both professional and tissue-resident, which may directly affect the ability of these cells to process and present the mimic peptide to T cells, either by activating or inhibiting their APC function. Thus, the presence of a mimic peptide in an inflammatory environment, may not necessarily result in mimic-specific T cell activation.

The second factor required for the induction of autoimmunity via molecular mimicry is the necessity that the mimic peptide be capable of being processed from the native pathogen protein. HI-TMEV contains the $HI_{574–586}$ epitope between the flanking regions of PLP130-159, and therefore

is capable of being processed by APCs from SJL mice. To test whether the $HI_{574–586}$ mimic can function as a natural mimic epitope, we constructed a virus (HI39-TMEV) encoding a 39-mer derived entirely from the *H. influenzae* protease IV protein which encompasses the $HI_{574–586}$ core epitope. Preliminary studies have shown that infection of mice with HI39-TMEV induces early cross-reactive immune responses as measured by T cell proliferation, DTH, and IFN-γ secretion, to both $PLP_{139–151}$ and $HI_{574–586}$ (unpublished observations). Therefore the $HI_{574–586}$ sequence appears to be immunogenic epitope in the SJL mouse and the data further suggests that molecular mimicry is a viable mechanism for the induction of autoimmunity.

5. Is infection of the target organ required for initiation of autoimmune disease via molecular mimicry?

Autoimmune diseases such as MS are organ-specific with the autoimmune T cell response developing to tissue-specific self antigens in the specific organ. For molecular mimicry-induced autoimmune disease, it is not known whether the infection must occur primarily in the target organ or whether it can occur in a distal site. Direct infection of the target organ may be important for providing an inflammatory environment for local activation of cross-reactive T cells and may be important for tissue damage to release self-antigens into the inflammatory environment. Potential requirement for infection of the autoimmune disease target organ may relate to the previously discussed role of local APCs in processing and presenting the mimic peptide to infiltrating T cells. Further, the innate immune environment created following infection may be required for local activation of autoreactive T cells. A further requirement may relate to local tissue destruction as a direct or indirect result of the infection. Self-antigens, including the self mimic epitope, released following tissue damage may be required to sustain the autoimmune response.

An additional infectious requirement for molecular mimicry is whether the infection needs to be persistent either in the target organ or in a different organ. A persistent infection with a mimic containing pathogen would provide continual antigen stimulation required for sustained activation of the autoreactive T cells. Continued tissue damage by the mimic-specific T cells may then gradually lead to induction of a clinically apparent autoimmune disease such as MS, where disease development occurs over many years. Additionally, persistent infection of the target organ would lead to continued release of self-antigens which could be processed and presented to the cross-reactive T cells.

TMEV is a natural mouse pathogen that is administered by intracerebral injection and results in a persistent infection in the CNS throughout the lifetime of the mouse. Thus, in the molecular mimicry-induced TMEV model described above, the virus is injected into the CNS target organ and remains persistent in the CNS (Olson et al., 2001a). To attempt to address the necessity for infection of the target organ, we infected mice with PLP-TMEV by intraperitoneal, intravenous, and subcutaneous routes (Olson et al., 2002). These peripherally infected mice developed mild demyelinating disease associated with $PLP_{139–151}$-specific $CD4^+$ T cell responses suggesting that infection at sites distal from the eventual CNS target organ could induce disease. However, detailed analysis of these peripherally infected mice showed that PLP-TMEV was detectable at low titers in the CNS as early as 7 days post-infection regardless of the route of infection. To more directly test this postulate, we are currently inserting $PLP_{139–151}$ and appropriate mimics into related picornaviruses which are not tropic for the CNS and testing whether cross-reactive T cells activated in peripheral sites can traffic to the CNS and mediate autoimmune demyelinating disease.

6. Multiple infections in autoimmune disease following induction by molecular mimicry

Epidemiological evidence suggests MS has a viral etiology based on migration studies, twin studies, and epidemics of MS in Iceland and the Faroe Islands (Kurtzke and Hyllested, 1986; Kurtzke et al., 1993; Kurtzke, 1997; Sadovnick et al., 1993). MS presents in most patients with symptoms between 20 and 40 years of age, and some MS patients have underlying clinical signs such as optic neuritis or fine limb tremor for years before the disease develops into more severe symptoms. Thus, one hypothesis is that an early childhood infection with a mimic containing virus may result in an expanded repertoire of autoreactive T cells that may become autoreactive memory T cells. The activation of these autoreactive T cells from the initial infection may result in mild neurological defects over a period of time, but without further stimulation, disease may not progress. This initial infection may allow for the ability of subsequent infections with pathogens encoding the same or another cross-reactive mimic epitope to reactivate the autoreactive memory T cells resulting in disease exacerbation/progression. MS patients often report a number of particular circumstances, including stress, trauma following an accident, or respiratory infections, preceding the onset of MS symptoms. Therefore, these secondary infections could actually serve to restimulate autoreactive memory T cells already present from a childhood infection.

We have preliminarily investigated the role of secondary infections on the clinical disease severity of mice infected with HI-TMEV, $PLP_{139–151}$ mimic-expressing virus. Mice re-infected with the HI-TMEV virus 14 days after the initial infection develop severe clinical disease resulting in spastic paralysis and severe inflammation of the CNS (unpublished observations). Interestingly, mice re-infected with OVA-TMEV also developed a more severe disease, although less severe than HI-TMEV re-infected mice. Therefore we

suggest that following the initial activation of cross-reactive T cells, re-infection of the target organ with either the primary or a secondary virus can initiate a cascade of events, which leads to an exacerbation of clinical disease. From these results, there are numerous possibilities with which to test the hypothesis that molecular mimicry can induce severe autoimmune disease.

Other stimuli which cause the release of myelin antigens in an inflammatory context such as mechanical damage following head injury or stroke, or other related or unrelated infections, may also reactivate myelin-specific memory T cells. Significantly, autoreactive T cells from MS patients may be less dependent upon costimulation than naïve T cells (Scholz et al., 1998). Therefore childhood infection may result in the activation and expansion of autoreactive T cells by molecular mimicry. This may result in mild disease for a number of years, however, following a secondary stimuli, these autoreactive memory T cells may be more easily reactivated by subsequent infections leading to a more severe disease.

7. Conclusions

Although many studies have provided evidence for the role of molecular mimicry-induced autoimmunity, further elucidation of the mechanisms involved are required. We have developed a virus infection model for molecular mimicry in which a virus encodes a mimic sequence. With the infection model, we have addressed and are continuing to address multiple questions related to molecular mimicry-induced autoimmune diseases. TCR recognition of the mimic epitope is the first identification point for molecular mimicry, however due to the significant degeneracy of the T cell repertoire, multiple factors related to the infectious agent encoding the mimic sequence are required for induction of a full-blown autoimmune disease. First, the Th1/Th2 nature of the immune response to the pathogen is critical. The innate immune response induced by the infectious agent creates an environment that dictates the type of adaptive immune response to the pathogen and the mimic-specific autoreactive T cell response. Second, the ability of the mimic sequence to be correctly processed and presented leading to activation of the cross-reactive, self-specific T cells is dependent on the APCs presenting the peptide, as well as on the sequences in the pathogen-encoded protein that flank the core mimic epitope. Third, the site of the primary infection may also be critical in organ-specific autoimmune responses. The site of infection may be required to be in the same organ to which the mimic T cell response is directed. Further, a persistent infection may be required for tissue damage and to provide a continuous source of mimic and/or self peptide. Lastly, multiple infections may be required to activate the autoreactive T cell response to a level sufficient enough to cause autoimmune pathology.

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