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Multiple sclerosis and virus induced immune responses: Autoimmunity can be primed by molecular mimicry and augmented by bystander activation

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Abstract
Polymicrobial infections have been associated with plausible immune mediated diseases, including multiple sclerosis (MS). Virus infection can prime autoimmune T cells specific for central nervous system (CNS) antigens, if virus has molecular mimicry with CNS proteins. On the other hand, infection of irrelevant viruses will induce two types of cytokine responses. Infection with a virus such as lymphocytic choriomeningitis virus (LCMV), can induce interferon (IFN)-α/β production and suppress autoimmunity, while infection with a virus, such as murine cytomegalovirus (MCMV), can activate natural killer (NK), NKT and dendritic cells, resulting in interleukin (IL)-12 and IFN-γ production. These cytokines can cause bystander activation of autoreactive T cells. We established an animal model, where mice infected with vaccinia virus encoding myelin protein can mount autoimmune responses. However, the mice develop clinical disease only after irrelevant immune activation either with complete Freund's adjuvant or MCMV infection. In this review, we propose that a combination of two mechanisms, molecular mimicry and bystander activation, induced by virus infection, can lead to CNS demyelinating diseases, including MS. Viral proteins having molecular mimicry with self-proteins in the CNS can prime genetically susceptible individuals. Once this priming has occurred, an immunologic challenge could result in disease through bystander activation by cytokines.

Keywords: *Autoimmune diseases, experimental allergic encephalomyelitis, immunological models, myelin proteolipid protein, virology, virus diseases*

Introduction

Virus infections and MS

Multiple sclerosis (MS) is the most common demyelinating disease in humans. The inflammatory demyelinating lesions characteristic of MS are limited to the central nervous system (CNS) [1]. In the cerebral spinal fluid (CSF), oligoclonal immunoglobulin (Ig)G bands and a mild mononuclear pleocytosis may be present. Clinical features include vision loss, motor and sensory disturbances and cognitive impairment. The clinical course can include relapses and remissions and/or progressive features of disease. MS has been suggested to be mediated by autoreactive CNS specific $CD4^+$ Th1 cells [2,3]. However, $CD8^+$T cells are found in MS lesions [4,5] and could also be involved in pathogenesis. MS has prevalence rates between 50 and 100 per 1,00,000 Caucasians; other ethnic groups have somewhat lower prevalence rates and women are more afflicted than men by a 2:1 ratio [6].

MS has been shown to have a genetic component, as monozygotic twins have about a 30% concordance rate [7–9]. Environmental factors also play a role in disease, with infections shown to contribute to the pathogenesis of MS. Interestingly, epidemiological data indicates that cases of MS are more prevalent in

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the northern latitudes in Europe and the United States (above the 37th parallel). Additionally, when individual countries were surveyed, a clustering of MS cases was found [6]. This clustering was also seen in the same areas a generation later when they were resurveyed. These data lead Kurtzke to suggest, "the occurrence of MS is intrinsically related to geography, and therefore, MS can be defined as an acquired, exogenous, environmental disease" [6]. Kurtzke also speculated that the spread of MS from Scandinavia to other regions was far too rapid to be due strictly to genetics, but that an environmental agent was likely the cause [6].

Infections have been associated with attacks of MS for a long time. Andersen et al. [10], Edwards et al. [11] and Panitch [12] found an association between upper respiratory tract viral infections and MS exacerbations. These studies confirmed work by Sibley et al. [13], who had previously studied 170 patients with MS over eight-years. These investigators concluded many viral-like infections were associated over time with exacerbations of MS; viral infections are closely linked with exacerbations [12,13]. However, although, over two-dozen viral agents have been isolated from MS patients [14], no single virus has been identified as the "MS virus".

Hypothesis: Two pathomechanisms induced by viral infections involved in MS?

In this review, we propose that a combination of two mechanisms, molecular mimicry and bystander activation, induced by virus infection, can lead to CNS demyelinating diseases, including MS. Nearly 20 years ago, we proposed the first mechanism, molecular mimicry [15]. Here, infection of viruses that have cross-reactive epitopes with self-determinants of myelin proteins can prime and activate autoreactive T cells, leading to autoimmune CNS disease [16]. More recently, the second mechanism, bystander activation, was proposed. Here, infection of irrelevant viruses, if they elicit strong cytokine responses locally, can lead to proliferation and activation of pre-existing autoreactive T cells. When they reach a critical mass/density, they migrate to the CNS and induce CNS damage that leads to disease [17]. It is not yet clear under this scenario whether local cytokine production in the CNS is required, or whether the activation events take place in the periphery. We hypothesize that viral proteins having molecular mimicry with self-proteins in the CNS can prime (fertile field) [18] genetically susceptible individuals for autoimmune CNS disease. Once this priming has occurred, an immunologic challenge could result in disease through bystander activation by cytokines and/or molecular mimicry.

Molecular mimicry

$CD4^+$ T cells

Myelin proteins, such as myelin basic protein (MBP), are potential auto-antigens in the CNS [19]. Myelin-specific T cells have been isolated from both MS patients and controls [20–26]. Studies where animals were injected with myelin protein administered in complete Freund's adjuvant (CFA) resulted in the induction of myelin specific $CD4^+$ Th1 cells [27], and the animals develop a CNS inflammatory demyelinating disease, experimental allergic (autoimmune) encephalomyelitis (EAE) (active EAE). Adoptive transfer of myelin specific T cells into naïve animals can also cause EAE in recipients (passive EAE). EAE can also be induced in animals using encephalitogenic peptides from myelin proteins administered in adjuvant. For example, injection of an encephalitogenic peptide derived from myelin proteolipid protein (PLP), $PLP_{139-151}$, administered in CFA into SJL/J mice leads to the induction of EAE with a relapsing-remitting (RR) clinical course [28].

We have shown that viruses have cross-reactive epitopes between themselves and self-proteins [15,16]. This cross-reaction occurs both in T cells [16] as well as in antibodies [15] and was dubbed "molecular mimicry". We initially showed that a cross-reactive epitope between the hepatitis B virus polymerase and the encephalitogenic epitope of MBP for the rabbit could induce an EAE-like disease. For EAE to occur the cross-reaction had to occur at the level of induction/activation of autoreactive $CD4^+$ T cells. This was the first demonstration that a viral peptide could induce a cell-mediated autoimmune disease in animals [16].

Based on recent studies, molecular mimicry can occur without complete sequence matching provided the major histocompatibility complex (MHC) and T cell receptor (TCR) contact motifs are preserved [29]. Lang et al. have shown that different peptides bound to different MHC class II molecules can lead to cross-reactivity by the same TCR as long as the complexes have similar charge distribution and overall shape, suggesting molecular mimicry occurs rather frequently [30].

$CD8^+$ T cells

$CD8^+$T cells has also been shown to recognize self proteins by molecular mimicry in diabetes studies [31–34]. Transgenic mice in which the rat insulin promoter (RIP) controls the expression of lymphocytic choriomeningitis virus (LCMV) nucleoprotein (NP) or glycoprotein (GP) in their pancreatic β cells develop insulin dependent diabetes mellitus following LCMV infection. The disease is mediated by virus-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) [35]. Interestingly, the adoptive transfer of these CTLs into

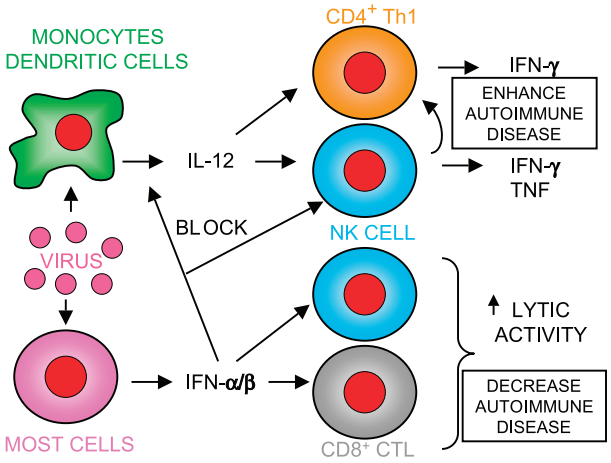

Figure 1. Two cytokine responses following virus infections. The first type of infection is where interleukin (IL)-12 is made. IL-12 would then facilitate interferon (IFN)-γ production from NK cells early after infection. IL-12 and IFN-γ would then activate Th1 autoreactive T cells. The second type is where high levels of IFN-α/β are produced, the activated NK cells have lytic function and later $CD8^+$ cytotoxic T cells are generated. IFN-α/β can also block IL-12 production.

uninfected RIP–LCMV recipients rarely resulted in hyperglycemia or insulitis. Other factors including the upregulation of MHC and interferon (IFN)-γ over-expression play a role in the destruction of β cells [35–37].

The Welsh and Selin laboratories [38–40] have also investigated cross-reactivity among various viruses such as LCMV, pichinde virus (PV), vaccinia virus (VV) and murine cytomegalovirus (MCMV). They found that prior infection by one virus could partially protect against another virus and have termed this "heterologous immunity" [40,41]. Protection was mediated by $CD8^+$ T cells and the production of IFN-γ. More recently they found, for $CD8^+$ T cells, that cross-reactivity impacts T cell immunodominance and can change the hierarchies of virus specific T cells during acute response and what is maintained within the memory population [40].

Antibodies

Molecular mimicry between virus and myelin antigen also causes humoral autoimmune responses [42]. Theiler's murine encephalomyelitis virus (TMEV) infection has been known to cause CNS demyelinating disease in mice [43]. We demonstrated that a TMEV neutralizing antibody, H8, could bind to the myelin component galactocerebroside, a lipid-like structure [44]. When H8 was injected intravenously into mice with acute EAE the size of demyelinated areas increased 10-fold within the spinal cords. This was the first report that an antibody could enhance demyelination during a CNS disease [45].

Bystander activation

Cytokine responses to viral infection

Over the past several years, Dr Biron's group has generated interesting data concerning innate immune responses to viral infections [46–48]. In contrast to other infections by bacteria and/or parasites, infection by viruses leads to the production of IFN-α/β, which appears to be a universal response by the infected host. Viruses can be divided into two groups based on whether high or low levels of IFN-α/β are induced (Figure 1). For example, LCMV infection causes high, sustained levels of IFN-α/β in mice with no detectable levels of serum interleukin (IL)-12 [46,47]. It has been shown that high levels of IFN-α/β can negatively regulate the active form of IL-12 [49] and IFN-α/β can promote a $CD8^+$T cell response at the expense of $CD4^+$ T cells [50]. This correlates with the observation that large numbers of $CD8^+$ CTLs are generated during LCMV infection [51–53]. In addition, IFN-α/β can facilitate the IFN-γ response produced by $CD8^+$ CTLs during the subsequent phase of LCMV infection. In contrast to LCMV, different host immune responses are seen with MCMV. Mice infected with MCMV produce low levels of IFN-α/β with a significant burst of IL-12. The IL-12 is released from monocytes/macrophages, dendritic cells and neutrophils during MCMV infection [54]. IL-12 promotes high levels of IFN-γ production from natural killer (NK) cells, early after infection with MCMV.

NK cells

Classical NK cells express asialo ganglio-*N*-tetraosylceramide (AGM)1 and are $CD56^+$ in humans and $NK1.1^+$ and DX5 $(CD49b, VLA\text{-}2)^+$ in mice. NK cells do not express TCR, or CD3. These cells are pivotal in focusing the direction of the early immune response during the different types of infection. They can undergo activation by IFN-α/β to perform perforin-dependent lysis of virus infected cells. NK cells are induced by IL-12 to secrete large amounts of IFN-γ, tumor necrosis factor (TNF), granulocyte macrophage-colony stimulating factor (GM-CSF), as well as chemokines, macrophage inflammatory protein (MIP)-1α and β and the factor regulated on activation normal T cell expressed and secreted (RANTES) [47]. NK cells have been shown to be central in the control of certain viral infections such as MCMV [55]. This is in part due to the NK cell's ability to kill virus-infected cells but also due to the immunoregulatory properties of the cytokines and chemokines produced by these cells [56].

NK cells can recognize and kill not only virus infected cells but also activated T cells [57,58]. This data coincides nicely with the Kastrukoff [59] data showing a strong inverse relationship between NK cell

lytic activity and the number of active lesions identified by magnetic resonance imaging (MRI) in MS (discussed below). In addition IFN-β, which has become a standard therapy for RR-MS, is known to increase cytotoxicity of NK cells. It has also been reported that the blockade of TNF-related apoptosis-inducing ligand (TRAIL), through which NK cells can induce target cell lysis, exacerbates EAE [60]. Furthermore, remission, but not relapse, of MS is associated with a strong bias of NK cells toward producing IL-5 (NK type 2) and decreased expression of IL-12 receptor (IL-12R) β2 [61]. This data suggests NK cells play a vital role in T cell mediated autoimmune diseases and MS.

Based on the above findings, we propose possible association of NK cell responses in virus infection with Th1 autoreactive responses (Figure 1). There appear to be two pathways for NK cell involvement in virus infection depending on the virus. The first type of viral infection is where high levels of IFN-α/β are produced which activate NK cells that have lytic function and later generate CTLs [47,56,62]. These $CD8^+$ CTLs also have lytic function on virus infected cells, contributing to virus clearance. IFN-α/β also block IL-12 production. In contrast, in the second type of infection, high levels of IL-12 are made [56,63]. IL-12 then facilitates IFN-γ production by NK cells, early after infection. IL-12 and IFN-γ could then activate pre-existing Th1 autoreactive T cells (bystander activation). IL-12 and IFN-γ can also activate newly primed autoreactive T cells if virus has molecular mimicry with host proteins.

NKT cells

NKT cells have characteristics of classical NK cells and T cells, and are $CD3^+CD56^+$ in humans and $CD3^+NK1.1^+DX5^+$ in mice. The NKT cells express an invariant TCR α chain paired with particular V_β segments: $V_\alpha24\text{-}J_\alpha Q$ paired with $V_\beta11$ in humans, $V_\alpha14\text{-}J_\alpha281$ paired with $V_\beta8.2$ and 7 in rodents. NKT cells recognize a glycolipid antigen associated with CD1, a non-classical MHC class Ib molecule [64,65]. Upon antigen recognition, NKT cells produce large amounts of IL-4 and/or IFN-γ [66]. A second NKT cell population bears an invariant AV19-AJ33 TCR ($V_\alpha7.2\text{-}J_\alpha33$ in humans, $V_\alpha19\text{-}J_\alpha26$ in mice) and would involve a non-classical class I-related molecule MR1.

Illés et al. [67] showed that $V_\alpha7.2\text{-}J_\alpha33$ NKT cells are accumulated in some of the CNS lesions of MS and in the majority of the sural nerve samples from chronic inflammatory demyelinating polyneuropathy (CIDP), a demyelinating disease in the peripheral nervous system (PNS). $V_\alpha24\text{-}J_\alpha Q$ NKT cells were often found to be infiltrated in sural nerve lesions in CIDP, but MS plaque lesion in the CNS rarely expressed the $V_\alpha24\text{-}J_\alpha Q$ TCR [68]. $V_\alpha24\text{-}J_\alpha Q$ NKT cells were reduced in the peripheral blood from MS, but $V_\alpha7.2\text{-}J_\alpha33$ NKT cells were not reduced in the peripheral blood from MS. In addition, rodent NKT cells can rapidly migrate to and accumulate in inflammatory lesions [69]. In type 1 diabetes and MS, CD1d-restricted NKT cells have been shown to be affected in number [68,70–73] or function [71,73]. It has recently been reported that stimulation of NKT cells with glycolipid ligands could lead to suppression of the Th1 mediated autoimmune disease in an EAE model by inducing IL-4 [74]. The CD1d-restricted NKT cells expanded from MS patients in remission produce a larger amount of IL-4 than those from patients with active MS and healthy controls [66]. These expanded NKT cells displayed a Th2 bias based on the IL-4/IFN-γ balance, suggesting that like NK cells NKT cells play a regulatory role in MS.

T cell activation and interferons in MS

In RR-MS, clinical attacks are unpredictable and remission periods are of undetermined lengths. Conducting serial cranial MRIs on patients with RR-MS showed that disease was almost always active with old lesions disappearing and new lesions appearing despite patients presenting as clinically well. The majority of new lesions represent areas of local inflammation and edema. Okuda et al. [75] showed that the percentage of $CD4^+$ memory T cells was greater in active vs inactive patients with MS. They also found that these $CD4^+$ memory T cells were more prevalent during relapses than during remissions in the same patients. On the other hand, $CD8^+$ memory T cells were more common in MS patients than in healthy controls. Interestingly, there were lower percentages of $CD8^+$ memory T cells in active patients compared with inactive MS patients [75]. The authors conclude that the activation of memory $CD4^+$ T cells is associated with the exacerbation of MS and the activation of $CD8^+$ memory T cells reflects the dysregulation of the immune system in MS patients. Why these cells are more or less prevalent during MS exacerbations needs to be explored as the role of $CD4^+$ and $CD8^+$ T cells in MS pathogenesis is still incompletely understood.

A multi-center clinical trial of RR-MS patients found that IFN-β was effective in reducing relapses [76]. The MRI results showed there were significant decreases in lesion activity determined by the number of new lesions and frequency of new lesion development. Recently, the four-year data was released from the prevention of relapses and disability by interferon β-1a subcutaneously in multiple sclerosis (PRISMS) study group. Patients with RR-MS were given 22 or 44 μg IFN-β-1a or placebo for 2 years. After this initial phase patients receiving IFN-β-1a treatment continued at the same dose and the placebo group was re-randomized to receive either 22 or 44 μg

of IFN-β-1a [77]. Treatment with IFN-β-1a three times a week over the course of 2 years was shown to decrease the rate of relapse and fewer lesions were present based on MRI [77]. This is in contrast to an IFN-γ treatment study, where MS attacks occurred at higher rates in MS patients receiving IFN-γ vs placebo control patients [78–80]. This IFN-γ trial suggests that systemic IFN-γ had a rapid effect on immune responses in the CNS, although, there are many controversies in evaluation of this study, such as small number of patients examined, dose response and recovery and relapse rate during the follow-up [81].

Kastrukoff et al. [59] followed RR-MS and control patients every 6 weeks for 2 years. They found that, in MS patients, NK cell cytolytic activity, as measured by the ability to kill K562 target cells, was much lower vs matched control patients. Interestingly, this decrease in cytolysis by NK cells correlated significantly with clinical disease. In addition, there was a correlation with new or enlarging lesions as determined by MRI. In control subjects, treatment with IFN-β enhanced NK cytolytic activity [82]. Treatment of peripheral blood mononuclear cells (PBMCs) from RR-MS patients with IFN-α enhanced NK lytic activity [83]. Treatment of RR-MS or progressive MS patients with either IFN-α or β resulted in enhanced NK cytolytic activity within 48 h of treatment and was maintained for approximately 7 days [59,83,84].

In *in vivo* IFN localization studies within the CNS, IFN-γ was detected in macrophages, lymphocytes and astrocytes at the margins of active plaques but not in unaffected white matter [85,86]. Brod et al. [87] found that IFN-β treatment decreased TNF and to a lesser degree IFN-γ production. In studies with MS patients on IFN-β therapy, investigators found that there was marked reduction in IL-1β, IFN-γ and TNF secreting cells vs cells from a placebo group [88,89]. Also, two studies [90,91] found that IFN-β treatment of MS patients reduced the number of IFN-γ producing cells. In other studies, NK cells producing IFN-γ were required for the generation of myelin oligodendrocyte glycoprotein (MOG) specific Th1 T cells in promoting EAE [92] and IFN-β reduced IFN-γ and could modulate EAE in rodents [93]. Taken together these data are consistent with the observation that IFN-α/β enhances NK cell cytotoxic activity and down-regulates NK cell IFN-γ production. The high levels of IFN-β would also decrease IL-12 production leading to fewer Th1 cells.

Panitch [12] found that upper respiratory tract infection preceded attacks of MS. Serum antibody titers suggested that herpes viruses, influenza viruses, parainfluenza viruses and adenoviruses were positive among those individuals having attacks [12]. These viruses can induce large amounts of IL-12 during infection [46,62,94–96]. Interestingly, Beck et al. [97] found in a longitudinal study that increases of IFN-γ and TNF were seen 2 weeks prior to clinical symptoms. In benign MS cases, the increases disappeared rapidly before the appearance of symptoms. These data support the hypothesis that certain kinds of viral infections that can promote IL-12 production, possibly through NK cell IFN-γ and TNF production, are responsible for exacerbating MS through bystander mechanisms.

IL-12 family in EAE

IL-12 production has been shown to contribute to the preferential development of Th1 responses over Th2. Seder et al. [98] showed that IL-12 could facilitate naïve $CD4^+$ T cells to produce IFN-γ, a proinflammatory cytokine. Similarly, T cells specific for *Dermatophagoides pteronyssinus*, when derived in the presence of IL-12, produced more IFN-γ and very little IL-4 [99]. In contrast, when T cells specific for purified protein derivative (PPD) from *Mycobacterium* were derived in the presence of IL-12 antibody, T-cells produced IL-4 with very little IFN-γ. The development of the IFN-γ secreting cells was markedly reduced when NK cells were removed. The authors concluded that IL-12 and NK cells have inhibitory effects on the development of IL-4 producing cells and promote Th1-like responses [99]. Other studies by Macatonia et al. [100] found that IL-12 could replace the requirement for macrophages in Th1 cell development. These $CD4^+$ T cells produced high levels of IFN-γ and their induction was dependent on IL-12 and IFN-γ. In studies on the developmental commitment to a Th phenotype, Szabo et al. [101] showed that early elimination of IL-12 or IL-4 treatment resulted in the loss of IL-12Rβ2 subunit. IFN-γ treatment of early developing Th2 cells maintained IL-12Rβ2 subunit and resulted in these cells becoming responsive to IL-12. The authors [101] speculated that IFN-γ might prevent early Th cells from premature commitment to the Th2 pathway. Mice deficient in IL-12 have impaired IFN-γ production and defective Th1 T cell responses [102].

Leonard et al. [103] demonstrated involvement of IL-12 in the development of EAE. They found that adoptive transfer experiments of PLP primed T cells resulted in enhanced EAE when cells were activated in the presence of IL-12. Therefore, cellular recruitment and generation of the inflammatory lesion is facilitated by IL-12. When mice were treated with IL-12 antibody, adoptive transfer of EAE using $CD4^+$ T cells was markedly reduced. It has been reported that staphylococcal enterotoxins can reactivate EAE [104] and that administration of IL-12 antibody could inhibit enterotoxin-induced relapses [105]. Bright et al. [106] have demonstrated that lisofylline, an inhibitor of IL-12 function, reduced the clinical and pathological features of EAE. The decrease in disease correlated with a decrease in IFN-γ secreting Th1 cells. Pagenstecher et al. [107] showed that transgenic

mice expressing IL-12 in astrocytes reduced the spontaneous development of activated Th1 cells and NK cells in the CNS. Furthermore, there was marked cerebral expression of IFN-γ, TNF and IL-1 genes. When these IL-12 expressing mice were sensitized with an encephalitogenic MOG peptide, mice developed EAE sooner and lesions were more severe than in control mice [107].

Non-vertebrate DNA, which contains CpG motifs more frequently than vertebrate DNA, has been shown to induced IL-12 and IFN-γ and NK cell activation. We demonstrated that plasmid DNA, pCMV, which contains CpG motifs, can induce IL-12, IFN-γ and IL-6 production as well as activate NK cells [108]. Injection of the pCMV exacerbated RR-EAE with increased IFN-γ and IL-6 responses. Importantly, pCMV injection also exacerbated demyelinating disease induced with TMEV, a viral model for MS. This suggests that immunostimulation by viral and bacterial CpG DNA could be one of the contributing factors for exacerbation of MS.

IL-23 was identified as a new member of the IL-12 family of cytokines. IL-23 and IL-12 are heterodimeric cytokines which share the p40 subunit, but which have unique second subunits, IL-23p19 and IL-12p35. The p40–p19 complex is secreted by activated dendritic cells and macrophages. Recently, IL-23 has been shown to be more critically involved in EAE susceptibility than IL-12. In contrast to IL-12, IL-23 preferentially stimulates memory as opposed to naïve T cell populations in both humans and mice [109]. As with IL-12, IL-23 induces the production of IFN-γ by both NK cells and $CD4^+$ T cells. Mice deficient for the IL-23 specific p19 subunit are resistant to EAE, while mice deficient in IL-12 production or responsiveness develop severe EAE. Interesting new studies have shown that IL-12p35 deficient mice, lacking IL-12 but not IL-23, have similar or more severe EAE disease compared with wild type controls [110–112]. These IL-12p35$^{-/-}$ mice were shown to mount a Th1 response to MOG_{35-55} in the spleen and draining lymph nodes. These mice had normal levels of TNF and IL-2 but lower than normal IFN-γ production. Additionally, IL-12 receptor knockout mice (IL-12Rβ2$^{-/-}$), which are unable to respond to IL-12, had severe and often fatal EAE [113]. IL-12Rβ2$^{-/-}$ mice also exhibited elevated proliferative response to autoantigen, increased production of proinflammatory molecules TNF-α, GM-CSF and nitric oxide, as well as increased expression of IL-23p19 mRNA.

Recently, Cua et al. [110] utilized IL-23p19$^{-/-}$ mice, which lack only IL-23, in experiments that showed IL-23p19$^{-/-}$ mice were resistant to EAE. They also found that intracerebral injection of IL-23 gene transfer vectors reconstituted disease susceptibility in both p19$^{-/-}$ mice and p40$^{-/-}$ mice, although, the p40$^{-/-}$ mice had delayed disease onset and reduced disease severity. Treatment of p40$^{-/-}$ mice with recombinant IL-12 intraperitoneally from day 0 to 18 or with IL-12 gene transfer vectors intracerebrally at day 8 did not facilitate disease. When p40$^{-/-}$ mice were given IL-12 intraperitoneally from day 0 to 7, followed by intracerebral IL-23 gene transfer at day 8, intense EAE, comparable to that seen in wild-type controls was induced. The authors suggested that IL-12 promotes development of Th1 cells, whereas IL-23 is necessary for subsequent CNS inflammatory events, for example, recruitment and/or reactivation of T cells in the CNS or activation of inflammatory and CNS-resident macrophages [110].

IL-27 is the newest member of the IL-12 family and is a heterodimer of Epstein–Barr virus induced gene 3 (EBI3) with protein p28 [114]. EBI3 is homologous to IL-12p40 and p28 is related to IL-12p35 [114]. IL-27 is produced by antigen presenting cells (APCs) and induces proliferation of naïve T cells. Li et al. [115] have shown that at the peak of clinical disease in mice with EAE, IL-27 mRNA is up-regulated in APCs of the CNS and lymph nodes. In addition, the IL-27 receptor (WSX-1) expression was up-regulated during the early stages of EAE as well as during the peak of disease in APCs found in the CNS and lymph nodes [115].

Association of molecular mimicry and bystander activation

Autoreactive T cells primed via molecular mimicry and bystander-activated

Theoretically, infection with viruses that have molecular mimicry with CNS antigens can prime autoreactive cells specific for the CNS in hosts. We have explored whether encephalitogenic peptides could be replaced with a virus having molecular mimicry with self-CNS antigens [116]. Three recombinant vaccinia viruses were constructed: VVplp, encoding PLP; VV_{MAG}, encoding myelin-associated glycoprotein (MAG) and VV_{GFAP}, encoding glial fibrillary acidic protein (GFAP). We infected mice with VVplp, VV_{MAG}, or VV_{GFAP}. Clinically and histologically, we did not see CNS disease. This suggests that molecular mimicry alone cannot result in high enough numbers of CNS specific autoimmune cells for induction of CNS disease. Then, 5 weeks after the first infection when VV was cleared, mice were non-specifically challenged with CFA. Clinically, some of mice showed paralysis in the tail, similar to EAE, after the CFA challenge. At one-month post CFA challenge, mice were sacrificed and CNS tissues were examined for pathologic changes. All mice (15/15) were found to have inflammatory lesions with $CD3^+$T cells in the CNS [116]. As a negative control, mice were infected with VVsc11, a recombinant VV that encodes β-galactosidase, which has no known

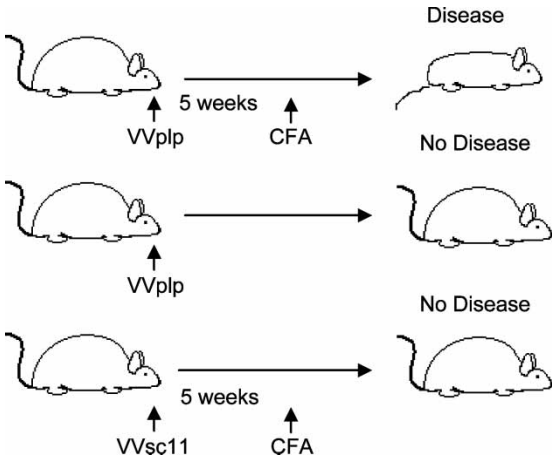

Figure 2. Mice were sensitized with vaccinia virus encoding myelin proteolipid protein, VVplp, or control vaccinia virus, VVsc11. Five weeks after infection mice were challenged with complete Freund's adjuvant (CFA) or observed without the challenge. Only the mice sensitized with VVplp and subsequently challenged with CFA developed clinical disease (top). Mice infected with VVplp alone (middle) or mice challenged with CFA following VVsc11 (bottom) do not develop disease.

molecular mimicry with CNS antigens. Following CFA challenge, no inflammatory changes were seen in the CNS (Figure 2) [116]. These data indicate that infections having molecular mimicry can substantially prime animals for autoimmune disease and at a later time a non-specific immunologic challenge could initiate an attack.

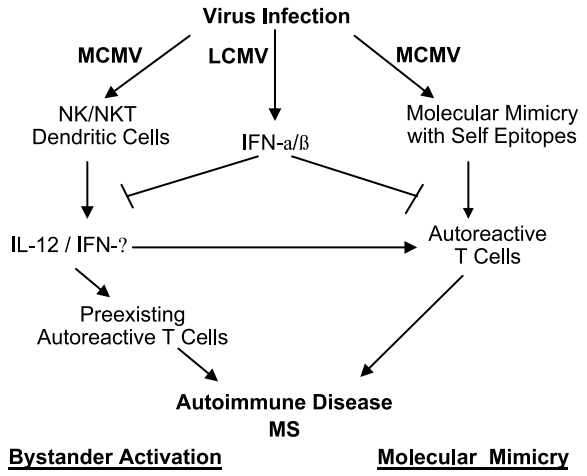

Figure 3. Two mechanisms induced by virus infection can trigger autoimmune diseases, including MS. Infection with virus that has molecular mimicry with myelin antigen can prime autoimmune T cells (right). Infection with a virus, including murine cytomegalovirus (MCMV), can cause the activation of NK, NKT and dendritic cells, resulting in interleukin (IL)-12 and interferon (IFN)-γ production (left). These cytokines can cause the bystander activation of autoreactive T cells, leading to autoimmune diseases, such as MS. On the other hand, infection with a virus such as LCMV can suppress these pathways by triggering the production of IFN-α/β (center). MCMV infection may also result in molecular mimicry with self antigens [118,119] leading to the activation of autoreactive T cells and autoimmune disease through TCR engagement with the induction of IL-12 driving T cell development.

Table I. Mice primed with VVplp followed by virus challenge.

Prime	Challenge	Lesion/total mice
VVplp	pVV-20	0/8
VVplp	LCMV	0/8
VVplp	MCMV	5/9

Three groups of mice were infected with VVplp intraperitoneally. Three weeks later, mice were infected with wild-type vaccinia virus, pVV-20, LCMV or MCMV intraperitoneally. All mice were killed 12 days after second virus challenge.

As described above, MCMV infection in mice causes a significant burst of IL-12. Therefore, we have tested whether VVplp sensitized mice developed clinical disease following a second, unrelated viral challenge with MCMV. Preliminary findings suggest this to be the case. As shown in Table I, five out of nine mice primed with VVplp and challenged with MCMV were found to have meningitis and perivascular cuffing in the CNS. In contrast, mice primed with VVplp and challenged with either LCMV or the wild-type strain of vaccinia (pVV-20) were found to have no obvious lesions. In another experiment mice primed with VVplp and challenged with MCMV showed marked weight loss and had righting reflex disturbances, compared with mice injected with PBS or VVsc11 followed by MCMV challenge (Tsunoda I and Fujinami RS, manuscript in preparation). These experiments are important in that they provide a working model mirroring what may be occurring in human patients with MS.

Conclusions

Recently, polymicrobial infection has been demonstrated to play an important role in the pathogenesis of immune-mediated diseases [117]. Multiple different infections may be involved, first in priming the immune system for autoimmunity and then in triggering the actual disease. The role of multiple infections in the development of autoimmune disease may explain why no one virus has been implicated in MS. Based on the above observations, we hypothesize how molecular mimicry and bystander activation associate with each other and lead to CNS disease (Figure 3). Virus infection can prime CNS specific T cells if virus has molecular mimicry with CNS proteins, as in VVplp. However, a single viral infection alone might not activate enough autoreactive T cells for induction of inflammation in the CNS. A second, irrelevant virus infection can suppress or augment preexisting autoimmune responses. Infection with a virus such as LCMV induces IFN-α/β production and suppresses autoimmunity. On the other hand, infection with a virus such as MCMV can activate NK, NKT and dendritic cells, resulting in IL-12 and IFN-γ

production. These cytokines can cause the bystander activation of autoreactive T cells, leading to CNS inflammatory diseases, such as MS. Among viruses, some virus may not only induce proinflammatory cytokines but also share molecular mimicry with self-protein, leading to efficient activation of autoreactive T cells, without help from a second virus infection.

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