Brain Pathology 11: 92-106(2001)

SYMPOSIUM: Pathobiology of the Oligodendrocyte

Viral Induced Demyelination

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Viral induced demyelination, in both humans and rodent models, has provided unique insights into the cell biology of oligodendroglia, their complex cell-cell interactions and mechanisms of myelin destruction. They illustrate mechanisms of viral persistence, including latent infections in which no infectious virus is readily evident, virus reactivation and viral-induced tissue damage. These studies have also provided excellent paradigms to study the interactions between the immune system and the central nervous system (CNS). Although of interest in their own right, an understanding of the diverse mechanisms used by viruses to induce demyelination may shed light into the etiology and pathogenesis of the common demyelinating disorder multiple sclerosis (MS). This notion is supported by the persistent view that a viral infection acquired during adolescence might initiate MS after a long period of quiescence.

Demyelination in both humans and rodents can be initiated by infection with a diverse group of enveloped and non-enveloped RNA and DNA viruses (Table 1). The mechanisms that ultimately result in the loss of CNS myelin appear to be equally diverse as the etiological agents capable of causing diseases which result in demyelination. Although demyelination can be a secondary result of axonal loss, in many examples of viral induced demyelination, myelin loss is primary and associated with axonal sparing. This suggests that demyelination induced by viral infections can result from: *1)* a direct viral infection of oligodendroglia resulting in cell death with degeneration of myelin and its subsequent removal; *2)* a persistent viral infection, in the presence or absence of infectious virus, resulting in the loss of normal cellular homeostasis and subsequent oligodendroglial death; 3) a vigorous virus-specific inflammatory response wherein the virus replicates in a cell type other than oligodendroglia, but cytokines and other immune mediators directly damage the oligodendroglia or the myelin sheath; or 4) infection initiates activation of an immune response specific for either oligodendroglia or myelin components. Virus-induced inflammation may be associated with the processing of myelin or oligodendroglial components and their presentation to the host's own T cell compartment. Alternatively, antigenic epitopes derived from the viral proteins may exhibit sufficient homology to host components that the immune response to the virus activates autoreactive T cells, *i.e.* molecular mimicry. Although it is not clear that each of these potential mechanisms participates in the pathogenesis of human demyelinating disease, analysis of the diverse demyelinating viral infections of both humans and rodents provides examples of many of these potential mechanisms.

Viral induced demyelination in humans

In the last century it became clear that under unusual circumstances, viruses were able to cause demyelination in humans. Viral induced demyelination in humans is most clearly associated with two uncommon chronic diseases, *i.e.*, progressive multifocal leukoencephalopathy (PML) and subacute sclerosing panencephalitis (SSPE). These demyelinating diseases are the result of infections by a papovavirus and measles virus, respectively. The fact that these disease states are both rare and are temporally remote from the acute infection fueled interest in the mechanisms of both virus persistence within the CNS, the immune response within the CNS and the mechanisms of viral induced demyelination. Demyelinating lesions in humans may also occur rarely following systemic, most likely viral upper respiratory infections. These are collectively grouped under the

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umbrella of "post-infectious encephalomyelitis" (PIE). Interestingly, a number of viral etiologies, but no other infectious etiologies, are associated with PIE. Viruses implicated include measles, mumps, varicella, and influenza virus. This syndrome was also a frequent complication following subcutaneous small pox vaccination. It is interesting that PIE can occur during acute infection, but it can also occur after the host's immune response appears to be in control of the infection. This fact, in addition to the usual inability to isolate the inciting virus directly from the CNS, suggests an autoimmune or autoimmune-like component. Indeed the pathological changes resemble some aspects of complications due to rabies virus vaccination and the autoimmune rodent model of multiple sclerosis, experimental allergic encephalomyelitis (EAE).

Subacute sclerosing panencephalitis

SSPE is an exceedingly rare but invariably fatal, chronic progressive panencephalitis occurring in less that 1 case per million children with measles. SSPE occurs 5-10 years after acute measles virus infection and is an example of a chronic defective CNS viral infection (69, 70). These are infections in which there is little evidence of infectious virus during disease, yet virus footprints can be detected. The original pathologic description of the disorder by Dawson (19) described the characteristic inclusions but not the demyelination or white matter astrogliosis; characteristic features that were later described in detail (122). The association of SSPE with a viral etiology initially came from ultrastructural studies showing typical paramyxovirus in the inclusions, but no viral budding from the plasma membrane (83). The intranuclear and cytoplasmic inclusions are found in the neurons and oligodendroglia of involved areas and vary considerably in size (3-10 microns). Direct association with measles virus was demonstrated by immunohistochemical studies showing measles antigens in both nuclear and cytoplasmic inclusions (49); a finding supported by the presence of high measles virus antibody concentrations in serum and cerebrospinal fluid (CSF) (17). As the name implies, there is a widespread chronic inflammatory infiltrate consisting predominantly of small lymphocytes, often in perivascular cuffs, and smaller numbers of plasma cells. Neuropathologically there is widespread involvement of the cerebrum and especially the occipital lobe. In contrast to the murine models of demyelination described below, the cerebellum and spinal cord are often not involved or only show focal infiltrates. The white matter shows focal demyelination and widespread astrogliosis.

Virus	Family	Host
Measles Virus	Paramyxovirus	Man
JC Virus	Papovavirus	Man
HTLV-1	Lentivirus	Man
HIV	Lentivirus	Man
SmallpoxVaccine	Pox Virus	Man
Herpes Simplex Virus	Herpes Virus	Man
Mouse Hepatitis Virus	Coronavirus	Mouse
Theiler's Virus	Picornavirus	Mouse
Semliki Forest Virus	Alphavirus	Mouse
Sindbis Virus	Alphavirus	Mouse
Canine Distemper Virus	Paramyxovirus	Dog
Visna Virus	Lentivirus	Sheep

Table 1. Viruses associated with demyelination.

Direct evidence is lacking for CNS infection by measles virus during acute disease. However, indirect evidence, including electroencephalographic abnormalities and CSF pleiocytosis (38) in approximately 30% of patients with uncomplicated acute measles are consistent with virus gaining access to the CNS during the acute infection. Little is know about alterations in the homeostatic turnover of perivascular microglia during systemic infections; however, given that measles virus infects all populations of peripheral white blood cells, it is possible that the CNS is infected via either the normal (or altered) turnover of perivascular microglia. Rodent models have clearly shown that activated T cells enter the CNS parenchyma (44), although in the absence of cognate antigen these activated cells rapidly exit the CNS. In addition, B cells, both those secreting virus-specific and non viral-specific antibody are rapidly recruited into and retained within the rodent CNS (34, 52). Thus, a transient entry of measles virus-infected cells could provide an initial focus of CNS infection. The cellular site(s) of measles virus persistence during the latent phase, defined as the period between the acute infection and clinical onset of SSPE, is unknown. However, both immunohistochemical and ultrastructural analysis of SSPE brains show clearly that only neurons and oligodendroglia are infected (10, 41, 104). Large numbers of viral capsid structures are found, but there is a virtual absence of structures consistent with the budding of mature virions from the cell surface and the direct isolation of infectious virus is only possible by co-cultivation of infected brain cells with cells susceptible to measles virus infection (46, 79).

One hallmark of SSPE is the extremely high anti-

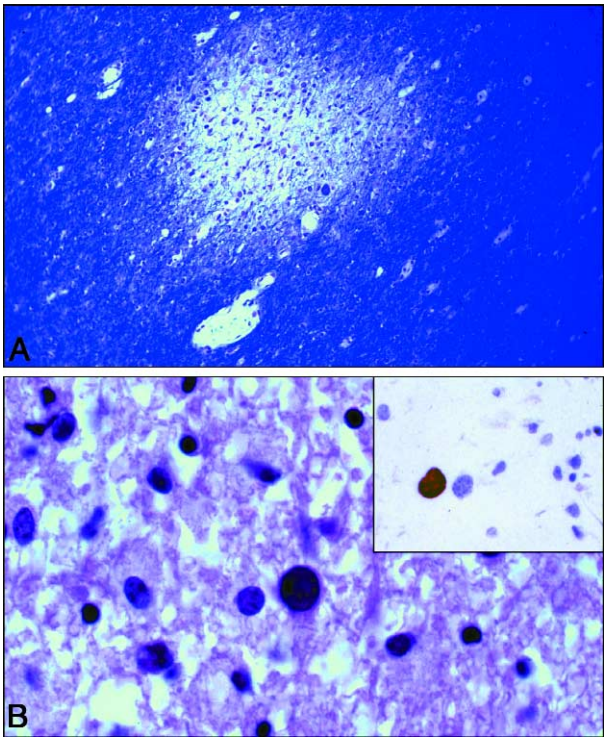

Figure 1. A demyelinating lesion due to progressive multifocal leukoencephalopathy in a patient with AIDS. The small, well-defined lesion lacks a significant inflammatory infiltrate (Panel **A**, Luxol fast blue ×50). The center of the PML lesion shows an enlarged oligodendroglial cell with a typical PML inclusion (Panel **B**, Hematoxylin and eosin stain ×775). An infected oligodendroglial cell expressing papovavirus antigen (Insert, immunoperoxidase stain [ABC Elite] with aminoethylcarbizole as chromogen ×400).

measles virus antibody concentrations found in the CSF (17). Initially, analysis of the CSF suggested the absence of antibodies specific for the M protein, which is important in viral assembly (35). Furthermore analysis of the brains from SSPE patients suggested the absence of immunoreactivity to the M protein (36, 37). These data suggested CNS persistence was related to a defect in M protein. However, using more sensitive techniques anti-M protein antibody is detected in CSF (22). Furthermore, sequence analysis of the recovered measles virus genomes showed not only the presence of mutations throughout the genome, but that infected CNS cells harbored full length genomes with mutations and truncated genomes containing deletions (12). As a result, these genomes lack components required for the assembly of infectious virus, consistent with the histological analysis. However, antibody does appear to be critical in the pathogenesis of SSPE. *In vitro* analysis suggests that the anti-measles antibody response aids in persistence by stripping viral envelope proteins from the cell surface (28). Furthermore, passive protection studied in rats infected with measles virus demonstrate that not only does antibody promote persistence (88, 89, 111) but it decreases viral replication at the transcriptional level (57).

The patchy demyelination associated with SSPE may occur as a result of several mechanisms. Although measles virus establishes an early infection primarily in neurons and oligodendroglia, the antibody response promotes viral persistence resulting in both the slow loss of infected cells and increasing CSF antibody levels. Indeed, Oldstone (74) showed that circulating antibodies from SSPE patients lysed brain cells cultured from a patient with SSPE. At the same time antibody holds infectious virus in check, it also enhances the accumulation of mutations within the viral genome, facilitating persistence. Current notions suggest a dynamic relationship in which infection is amplified within the CNS during the clinically latent period by cell-to-cell spread, while the virus continues to accumulate additional mutations. It appears to be the accumulation of viral products in neurons and oligodendroglia, as well as neurofibrillary tangles that result in cell death (48) and in neurons and glial fibrillary tangles in oligodendroglia in some cases, demyelination. Other studies have demonstrated the infiltration of $CD4^+$ and $CD8^+$ T cells as well as expression of inflammatory cytokines such as interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) suggesting that cell-mediated damage to infected cells may also play a role (45, 73). It is likely that the demyelination is primarily a result of the direct death of oligodendroglia, resulting in primary demyelination. Although secondary demyelination, as a result of direct neuronal loss may also occur, one quantitative study found that cortical thickness is not affected and there was no evidence for loss of neurons even though synaptic density was reduced (92).

Progressive multifocal leukoencephalopathy

PML is also a typically fatal demyelinating disease of the CNS. However, in contrast to SSPE, it predominantly affects immunocompromised individuals (47). In PML, multiple small foci and large regions of demyelination are present in the cerebrum, cerebellum and brain stem suggesting the evolution of smaller lesions into large confluent ones (Figure 1A). Cytologically, the lesions have characteristic features involving the oligodendroglia and astrocytes. The oligodendroglia are enlarged and show intranuclear, ground-glass inclusions (Figure 1B). The astrocytes within the lesions are often

large, reactive and atypical and may undergo mitosis. An inflammatory response is characteristically absent; however, activated microglia and lipid-containing macrophages may be seen. Large lesions may also show necrosis.

PML was first identified as a viral infection in 1965 based on ultrastructural studies showing large accumulations of 30 nm papovavirus-like particles in the distended nuclei of oligodendroglia (123). Two antigenically related but distinct papoviruses were initially isolated from the brains of patients with PML. One was similar to the simian vacuolating virus 40 (SV40) (113). The other was less antigenically related to SV40 and was designated JC virus (75). Subsequently, all isolates have been similar to the original JC virus isolate. In contrast to SSPE, which occurs following acute measles virus infection of naïve populations, serological studies demonstrate that JC is a ubiquitous human virus acquired early in life with no definable acute syndrome (76). Also in contrast to SSPE, which is believed to establish residence within the CNS during acute infection, JC virus is not detectable in brains of normal individuals or in immunosuppressed individuals which have no symptoms of PML (14, 40, 93). This suggests that JC virus persists in a peripheral site.

The pathogenesis of PML appears to progress in the following way. Virus is acquired by the majority of humans during early life (76) and is maintained in an undetermined peripheral site, possibly the kidney (16), controlled by the host's immune response. The majority of adults are seropositive, but have no clinical evidence of infection. In those unfortunate individuals with underlying immunosuppressive diseases, JC virus cannot be held in check and replicates peripherally. A population-based study revealed that PML has a frequency of 5.1% in AIDS patients and 0.07% among patients with hematologic malignancies; however, similar clinical features of PML were found in each group (87). The isolation of JC virus from the lymphocyte populations of AIDS patients without evidence of PML, suggests either that HIV-1 induced immunosuppression is particularly destructive to the mechanisms effective in inhibiting JC virus (103), or even the possibility of a synergistic influence of these two viruses (23). Oligodendroglia which are not infected with HIV-1 may also contain soluble HIV-1 tat protein, a potent JC virus transactivator (108). It appears that infection of B cells may provide the vehicle for hematogenous spread into the CNS. Hematogenous spread is suspected to occur based on the widespread distribution of lesions. However, the lesions show no relationship to blood vessels, suggesting that infected cells must migrate into the parenchyma to establish foci of virus replication. Ultrastructural and immunohistochemical studies reveal that JC virus appears to preferentially infect oligodendroglia (Figure 1C); however, lesions also show infection of the enlarged astrocytes. Astrocytes may be semi-permissive to infection because only a small proportion of infected cells display evidence of viral capsid protein. Based on the frequency of mutations within the genome of virus isolated from the CNS versus virus isolated from the periphery of the same patients (103), it has been suggested that some alteration in viral replication may be required for CNS tropism. In support of a selective tropism for oligodendroglia, infected cells predominate around the edges of the lesions. The small size of many foci may correlate with selective oligodendroglia tropism and viral induced cell death. Although PML is considered to induce a primary demyelination, the center of advanced lesions show few intact axons and large lesions may become necrotic.

Murine models of viral induced demyelination

Murine models of viral induced demyelination highlight a number of aspects of CNS infection and mechanisms of acute and chronic demyelination. Two models in which the mouse is the natural viral host are discussed below. In both cases the virus was initially isolated from mice with spontaneous paralytic disease (2, 13, 105, 106). These viruses are from two different viral families, Theiler's murine encephalomyelitis virus (TMEV) is a non-enveloped positive stranded RNA virus of the picornaviridae family while mouse hepatitis virus (MHV) is an enveloped positive stranded RNA virus of the coronaviridae family (Table 1). These infections of the CNS provide examples of both the influence of the host's genetic background and regulation by the immune response. For example, the predominant mouse strain used for analysis of demyelination following TMEV infection is the SJL strain (3, 60, 65, 66, 67). This strain is also widely preferred for the study of EAE because CNS autoimmune disease can be induced with a variety of CNS antigens including myelin basic protein (MBP) and proteolipid protein (PLP). In addition, SJL undergo a relapsing and remitting form of autoimmune CNS disease. SJL mice are used for both TMEV pathogenesis and EAE due to the relative resistance of many other mouse strains to these disparate CNS diseases. By contrast, demyelination produced by the infection with MHV is mainly studied in C57/BL6 and BALB/c mice, but not the SJL strain (101). This is due to the absence of virion receptor expression in SJL mice

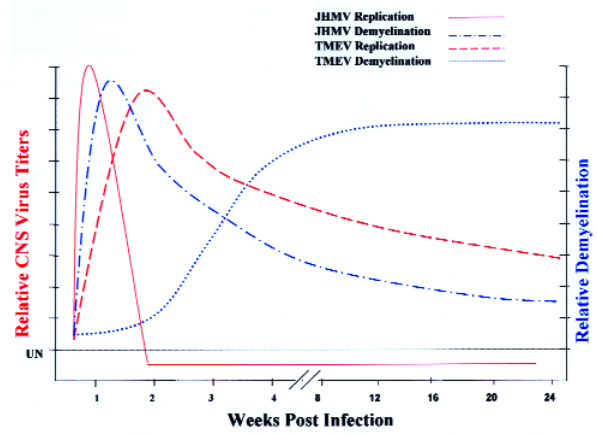

Figure 2. Comparison of the time course of CNS virus replication (Red) and relative demyelination scores (Blue) for JHMV and TMEV. Note: JHMV induces demyelination during the acute phase and infectious virus is cleared from the CNS. However, JHMV persists in a non-infectious form and although demyelination declines, new foci continue to appear for months following infection. By contrast, TMEV produces little or no demyelination during acute infection. Infectious virus is not eliminated from the CNS and increases with persistence concomitant with of an increase in demyelination.

(115), making this strain resistant to MHV infection. These examples of differential responses due to host genetic backgrounds, which only partially map to the major histocompatibility complex (MHC), provide vivid examples of the complexity of responses that can lead on the one hand to viral persistence within the CNS and on the other hand to very similar pathological changes, *i.e.*, demyelination (Figure 2). In both cases these viruses persist within the CNS following an acute episode, characterized globally as encephalomyelitis.

Theiler's murine encephalomyelitis virus (TMEV)

TMEV was a relatively common enteric pathogen of laboratory mice; however, naturally occurring CNS infection was rare (105, 106). Experimental CNS infection with the reduced neurovirulent BeAn or DA strains results in a biphasic CNS disease (60). By contrast, infection with more neurovirulent TMEV strains, *i.e.*, GDVII and FA, generally results in a monophasic fatal disease (105, 106). Infection by all four strains results in an acute encephalomyelitis characterized by loss of anterior horn cells; however, only the BeAn and DA strains induce a flacid paralysis. Infection of mice with the reduced neurovirulent strains results in virus replication that reaches a peak followed by subsequent viral clearance from the CNS (Figure 2). In mice genetically resistant to the chronic phase, infectious virus is completely cleared. Therefore the host response is able to achieve a sterile immunity. TMEV is a member of the picornavirus family, a viral group that includes poliovirus and is generally considered to be primarily controlled by the humoral immune response. Genetic analysis however, suggests that MHC class 1 molecules are important in resistance to TMEV persistence suggesting that $CD8^+$ T cells, possibly those cytotoxic T lymphocytes (CTL) that mediate the lysis of infected CNS cells during the acute phase of infection, may play a critical role in preventing virus reactivation. Indeed, CTL specific for TMEV have been described, although their role in the individual mouse strains susceptible and/or resistant to TMEV chronic demyelination is controversial (50). Analysis of CTL responses in the early phase of virus replication shows that resistant mice make excellent CTL responses (21). By contrast, the CTL response in strains that progress to persistent infection and demyelination make relatively poor CTL responses. It has been suggested that $CD8^+$ T cell responses to TMEV contribute to demyelination during the late chronic phase of viral persistence (90). These data suggest that TMEV persistence is a direct consequence of a genetically influenced immune response unable to completely clear virus from the CNS during the acute infection.

Histopathological findings following TMEV infection of mice susceptible to chronic disease are consistent with a biphasic disease (20) (Figure 2). In the first or neuronal phase, virus replicates rapidly within the CNS, infecting cells of the thalamus, hypothalamus, and brain stem and anterior horn cells in the spinal cord. Infection of white matter, meninges, choroid plexus or ependyma are not found. Little demyelination or parenchymal inflammation are found during the first phase, even in mice susceptible to the late phase. Therefore, the early phase of TMEV CNS infection resembles acute polio virus-induced encephalomyelitis with paralysis due to cytolytic infection of motor neurons resulting in the transient loss of hind limb function. The absence of inflammation is interesting because high titers of infectious virus remain at the end of the acute phase in mice that progress to the late phase of infection (Figure 2). In this second phase, inflammation and demyelination increase in the spinal cord and correlate with the persistence of infectious virus. Lesions are most common in the lateral columns of the thoracic region and the largest may encompass the majority of the white matter. Lesions are characterized by the presence of infected macrophages; however, occasionally neurons and astrocytes may also be infected. Interestingly, only rarely is

there infection of oligodendroglia, even though the disease is primarily one of an inflammatory demyelination. Therefore, in the early lesions of the second chronic phase of TMEV infection, myelin is destroyed although the oligodendroglia are not infected with virus. Inflammatory infiltrates consist predominantly of macrophages and $CD4^+$ T cells. The $CD4^+$ T cells express the phenotype of highly activated cells, *i.e.*, expression of the high affinity IL-2R (85). Analysis of infection in $CD8^+$-depleted mice (9), and β-2 microglobulin deficient mice that lack MHC class I expression (25), indicate little support for a role of $CD8^+$ T cells during this second phase. As disease progresses in this phase, recent data have implicated a perforin-dependent $CD8^+$ T cell mechanism for the neurological deficits (70). MHC class I deficient mice show decreased neurological deficits compared to wild type mice, possibly due to preservation of axons coincident with increased expression of sodium channel density (90). Consistent with an effect of $CD8^+$ T cells on neuronal function late in infection, increased axonal damage has also been observed during the late phase of TMEV persistent CNS infection (62, 63, 107). Axonal damage has also been implicated in the pathogenesis of lesions in MS patients (7), possibly via an indirect effect of activated T cells on microglia (32). Although activated microglia and macrophages are abundant during chronic TMEV induced demyelination, it is not clear if axonal damage contributes to TMEV pathogenesis or reflects the loss of axonal function due to the extensive loss of myelin. In addition to the presence of activated $CD4^+$ T cells there is a preponderance of pro-inflammatory cytokines in the CNS during chronic TMEV infection (4). Based on the paucity of $CD8^+$ T cells within the inflamed CNS, it appears that these cytokines are either derived from the activated $CD4^+$ T cells or are secreted by the infiltrating macrophages or activated microglia, or both.

Four potential mechanisms have been proposed to explain demyelination in TMEV-infected mice. First, virus infection of oligodendroglia, although rare, results in sufficient loss of cells to produce demyelination. Although plausible, there appears to be too few oligodendroglia infected at any time to account for the extensive demyelination. However, it remains possible that TMEV infection of oligodendroglia does contribute to the overall loss of myelin. Second, the predominant anti-viral inflammatory Th1 type $CD4^+$ T cell response suggests that myelin, or the oligodendroglia itself, is damaged by the sustained presence of pro-inflammatory cytokines. This issue has been difficult to address, because most attempts to eliminate the host immune response have resulted in death due to overwhelming virus infection rather than alterations in demyelination. Third, the continued secretion of IFN-γ could maintain macrophage/microglial activation, resulting in a direct attack on myelin. This CNS antigen-nonspecific induction of demyelination is termed "bystander" demyelination (118). Finally, it has also been proposed that $CD8^+$ T cell mediated cytolysis of TMEV-infected oligodendroglia could result in cell death and ultimately myelin loss. However, the small number of infected oligodendroglia along with little evidence for sustained $CD8^+$ T cell activity in the CNS during TMEV persistence, suggests that this is not a predominant mechanism.

The presence of demyelinating lesions in mice chronically infected with TMEV correlates with high levels of $CD4^+$ T cell mediating a virus-specific delayed type hypersensitivity reaction (15). Consistent with the role of virus specific $CD4^+$ T cells secreting pro-inflammatory cytokines in the demyelinating process, transfer of virus specific $CD4^+$ T cells secreting Th1 cytokines into infected mice increases the severity of disease (31). The majority of data support a virus-specific but indirect role of cell mediated immunity in the demyelination. Initial attempts to correlate the chronic demyelinating phase of TMEV infection with induction of autoimmune T cells were unsuccessful. Demyelination could not be transferred with either T cells or sera from infected mice (3). Neuroantigen specific T cells responses could not be detected following TMEV infection, including during the chronic demyelinating phase of infection (65). Importantly, tolerance to neuroantigen induced via the transfer of antigen-coupled syngeneic spleen cells also failed to affect the ability of TMEV to induce a chronic demyelinating disease (66). In contrast to these studies, recent data have demonstrated a progressive activation of neuroantigen specific T cells during TMEV chronic demyelination (67). There has been no evidence presented to suggest that these neuroantigen specific T cells are induced via the recognition of viral elements which cross react with host determinants (molecular mimicry) (29). Although the relevance of these neuroantigen specific T cells to the progression of chronic demyelination is still not clear, the data clearly demonstrate activation of immunity to host antigens during inflammation induced by a persistent viral infection.

TMEV induced demyelination is predominantly a consequence of the viral specific $CD4^+$ T cell-mediated chronic inflammatory response. Infectious virus preferentially replicates in macrophages and microglia within the spinal cord. The susceptible host not only mounts a vigorous anti-viral $CD4^+$ T cell response but also neu-

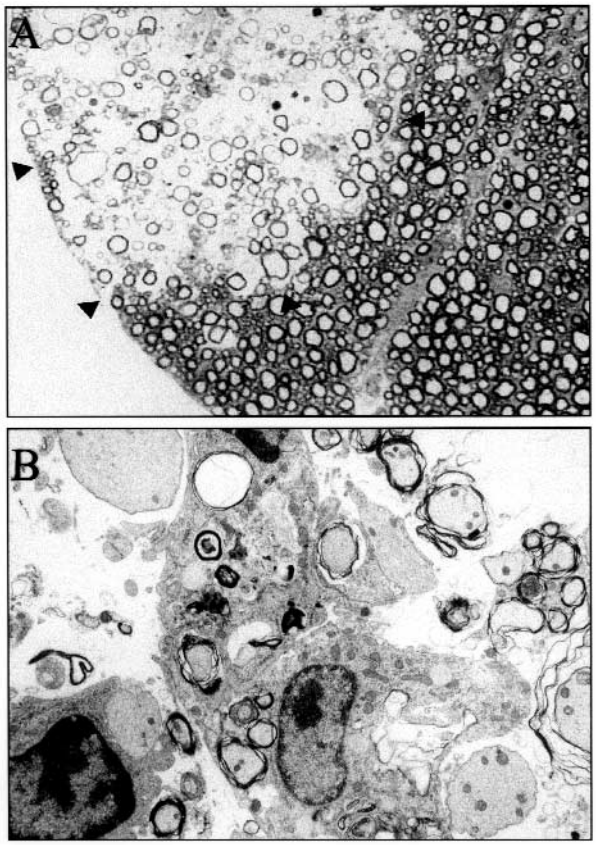

Figure 3. Cross sections of thoracic spinal cord of a JHMV infected mouse. **A**. a well-defined area of demyelination (arrow heads) in the anterior funiculus. A one micron plastic section stained with toluidine blue (×1840). **B**. An electron micrograph from a similar region of cord showing demyelinated and demyelinating axons closely associated with macrophages (×4900).

tralizing antibody. It is the presence of infectious virus that induces the inflammatory response which on the one hand is unable to eliminate infectious virus from the CNS; but on the other hand leads to a progressive increase in T cells which recognize potentially encephalitogenic host neuroantigen epitopes. These host antigen specific T cells do not appear to contribute significantly to the demyelinating process. Why the antiviral immune effectors are unable to either eliminate infectious virus from the CNS during the acute neuronal phase or control replication of infectious virus during the chronic phase of infection is not clear. However, analysis of TMEV induced demyelination has provided critically important support for the concept that a chronic inflammatory response within the CNS can result in the activation of autoreactive T cells (67). In addition, analysis of this model has clearly shown that the fine antigen specificity recognized by these inflammation-induced autoreactive T cells changes with time. This suggests that broad-based immunotherapeutic approaches to inhibit chronic human autoimmune CNS disease may hold greater promise than specifically targeted approaches.

Mouse hepatitis virus

MHV also used to be a common enteric pathogen of laboratory mice and, similar to TMEV, dissemination to the CNS was a rare event. The most studied strain that causes demyelination, the JHM strain (JHMV) or MHV-4 serotype, was isolated from a mouse with spontaneous hind limb paralysis (2). It produces an acute encephalomyelitis accompanied by primary demyelination in mice, rats and non-human primates with little or no evidence of hepatitis (13, 55, 71, 86, 95, 114). Pathogenesis is dependent upon viral dose, route of infection, the host's age and genetic background; however, prominent CNS infections can be induced with the neurotropic MHV strains either via the intranasal route or by direct inoculation into the CNS. The parental JHMV strain infects astrocytes, oligodendroglia, microglia and neurons. To increase the number of survivors, thereby allowing a more careful study of its pathogenesis, a number of JHMV variants, which have limited or no ability to infect neurons (*i.e.* the small plaque mutants, ds [24]; the temperature sensitive mutant, ts8 [39]; and the neutralizing monoclonal antibody resistant 2.2v-1 variant [26]), have been examined in detail. In general, both the more neuronotropic parental virus as well as the strains with limited or no tropism for neurons produce an acute encephalitis accompanied by acute primary demyelination. Low dose infection of adult mice with the parental virus results in chronic demyelination; however, its virulence and rapid induction of death has limited its usefulness. In addition, virus replication in the CNS does not appear to be compromised in the variant strains; however, the initial cellular sites of virus replication are altered. The viral surface (S) envelop glycoprotein, that contains not only the sites of binding by neutralizing antibody but also has the domain which interacts with the viral receptor, is believed to also regulate which CNS cells are infected. This is based on both the analysis of neutralizing monoclonal antibody resistant variants (2.2v-1) and recombinant MHV in which the JHMV S protein was inserted into the genetic background of the more hepatotropic A59 strain (84). However, recent data testing a different set of recombinant MHV suggest that a component other than the S protein might contribute to CNS

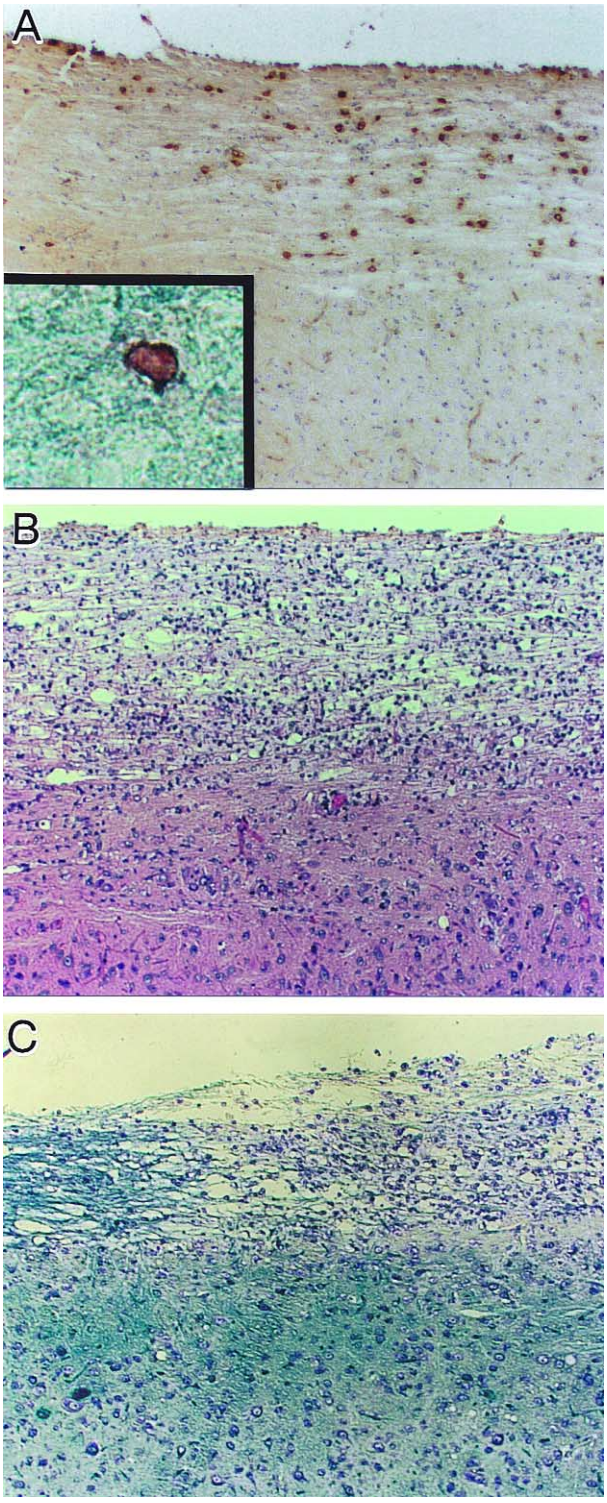

Figure 4. Spinal cord of mice infected with the neurotropic strain of the mouse hepatitis virus (JHMV). **A**. Infected cells are confined to the white matter where numerous virus antigen-positive cells with the morphologic appearance of oligodendroglial cells are seen. (virus antigen detected using virus specific monoclonal antibody). Inset confirms the localization of virus antigen in oligodendroglial cells by double label immunoperoxidase staining. The oligodendroglial-specific antibody Rip stains the membrane blue. JHMV-specific antibody stains the cytoplasm red. Magnification ×110 (inset ×440).
B. Extensive demyelination in the spinal cord associated with a diffuse infiltrate of T cells and macrophages. Hematoxylin and eosin (×110).
C. The edge of a large plaque of demyelination is shown demonstrating a relatively discrete plaque border. Luxol fast blue (×110).

cellular tropism (18).

MHV induces a monophasic CNS infection in contrast to TMEV. The most complete analysis of the early phases of JHMV pathogenesis was described using the 2.2v-1 variant (109). Following intracerebral inoculation, virus replication was initiated in the ependymal cells lining the cerebral ventricles. Inflammatory changes were noted first on day 4 post infection as the virus replication extended to the periventricular white matter. Virus replication extended to the spinal cord canal and with time moved through the central gray matter into the white matter. Demyelination with axonal sparing was initially noted on day 5 post infection and extended rapidly into the anterior funiculi (Figure 3A). These data contrast dramatically with infection by TMEV in which no demyelination is detected during the acute phase of infection. The rapid control of gray matter infection by day 7 post JHMV infection correlates with the increasing mononuclear cell infiltrate with contained both lymphocytes and macrophages. Demyelination increases until day 19 post infection, the last time point studied and then begins a slow decline exemplified by repair of existing lesions and initiation of new focal areas of demyelination, probably for the life of the mouse (24) (Figure 4). Following initial infection, oligodendroglia undergo both necrotic and apoptotic death; however, death is limited to apoptosis during chronic demyelination (5). Interestingly, lesions contain predominantly macrophages and microglia but there is very little viral antigen (Figure 3B). Infectious virus is usually eliminated from the CNS at approximately day 14 post infection (58, 59, 77, 78), although ts8 can be recovered during chronic infection (51). Following clearance of infectious virus, the number of viral antigen positive cells declines. Therefore, JHMV appears to preferentially infect ependymal cells during

the initial phase of infection. Replication then proceeds down to the spinal cord and peripherally into the gray and white matter, with the virus infecting astrocytes, microglia and oligodendroglia. In contrast to TMEV, only rarely has infectious JHMV been isolated from the CNS following immune mediated clearance (51). The virus appears to establish a latent infection in astrocytes and microglia (102) but cannot be recovered following either immunosuppression or explantation of CNS tissues (95, 101). However, rare viral antigen positive cells can be detected for months within the white matter tracks, and viral specific RNA can be detected in the CNS for at least 1 year post infection (1). This persistent, but noninfectious virus, correlates with the presence of ongoing foci of demyelination and remyelination.

In addition to JHMV, a number of MHV-induced demyelination studies have been carried out with the A59 strain which was initially isolated from a mouse with acute hepatitis. MHV-A59 exhibits both hepatotropism as well as neurotropism. At low doses of infectious virus, MHV- A59 causes an acute hepatitis and a meningoencephalomyelitis with small foci of demyelination. Immunity clears infectious virus from the CNS by 10 days. Demyelination becomes predominant after virus is cleared (119). At high doses, MHV-A59 produces an infection similar to the biphasic infection induced by TMEV with some notable differences, including a more predominant hepatitis. However virus is cleared from the blood and liver by approximately one week post infection and the hepatitis begins to resolve. Virus replicates to high titers in the brain and spinal cord but is partially controlled. Infectious virus persists in the spinal cord and brain for 4 weeks post infection before complete clearance. This delayed clearance may be related to the absence of the immunodominant CTL epitope (11, 30). Although no infectious virus is recovered, viral antigen can be detected in survivors for 4 months post infection. Primary demyelination with axonal sparing is the predominant finding following infection with both doses of MHV-A59, although a number of mice injected with the high dose also exhibit non-inflammatory, non-obstructive hydrocephalus (56). In contrast to TMEV, there are few inflammatory cells associated with these demyelinating lesions, although similar to both TMEV and the demyelination induced by JHMV, macrophages laden with intracellular myelin debris are present.

MHV infection of the CNS results in the induction of a vigorous local immune response confined primarily to the CNS (6, 81, 97) which protect the host from the lethal effects of viral CNS infection. However, similar to TMEV infection of the CNS, sterile immunity is not achieved. In the case of MHV this results in persistence of non-infectious virus within the CNS. Two potential mechanisms which limit T cell reactivity in the periphery were examined to determine if they contributed to limiting the response before sterile immunity could be achieved; however, neither IL-10 nor Fas/FasL interactions participated in limiting JHMV immunity (58, 79). Therefore, MHV infection is characterized by demyelination during the acute phase of replication associated with infectious virus. Following the clearance of infectious virus from the CNS, the second phase is best described as a latent infection. During this phase persistent virus is associated with continuing foci of demyelination. It must be noted that the absence of detectable infectious virus during this phase does not preclude that the foci of demyelination are initiated by limited amounts of local infectious virus, which are undetectable by conventional means. The observation that the amount of detectable viral footprints, *i.e.*, antigen or viral RNA, diminish with time, as do the new foci of demyelination, lends credence to the possibility that as new infectious virus is produced it is rapidly eliminated by the immune response.

It is the inability of the immune response to produce a sterile immunity that has led to an examination of the mechanisms that control virus replication within the CNS. The acute inflammatory response is characterized by the influx of all types of immune effectors into the murine CNS, *i.e.*, natural killer (NK) cells; $CD4^+$ T cells, $CD8^+$ T cells, B cells and macrophages (117). Neither NK cells, B cells, nor the antibody response, play a role in initial viral control. Both the $CD4^+$ and $CD8^+$ T cell populations appear to be the critical elements (116). The $CD4^+$ T cells localize to the perivascular areas and play an as yet undefined role in the maintenance of $CD8^+$ T cells (100). The $CD8^+$ T cells are the major effectors of anti-viral activity (99). Virus-specific, and apparently non-viral-specific $CD8^+$ T cells (6), are rapidly recruited to the CNS. In contrast to $CD4^+$ T cells, they enter the parenchyma and are therefore close to the sites of virus replication. Interestingly, analysis of JHMV pathogenesis has shown that $CD8^+$ T cells use two separate antiviral effector mechanisms, dependant upon the CNS cell type infected. Perforin-mediated cytotoxicity controls infection of microglia and astrocytes (59). By contrast, IFN-γ is the antiviral mechanism responsible for controlling the infection of oligodendroglia (77). Therefore, immunity to JHMV infection of the CNS is predominantly mediated by the antiviral $CD8^+$ T cell response; however, their ability to control CNS replication is high-

ly dependent upon both the $CD4^+$ T cell response and the individual CNS cell types infected. TNF-α, implicated in the progression of autoimmune demyelination (91, 94), plays no role in either the accumulation of inflammatory cells within the CNS nor in JHMV induced demyelination (98). In addition to providing an example of cell type effector mechanisms, these data have raised a number of interesting questions concerning the interaction of the immune system with the CNS. For example, why are oligodendroglia apparently refractory to MHC class I mediated cytotoxicity? If they express MHC class I molecules are they deficient in processing of the appropriate peptide? Are they unable to express sufficient MHC class I molecules to initiate a cytolytic attack? Do the intrinsic properties of the membrane prevent immune mediated cytotoxic attack? Future analysis should shed light on these possibilities.

JHMV infection has also provided insights into the interactions of the immune system and the CNS in regulating viral persistence. Analysis of the pathogenesis of JHMV infection in mice deficient in B cells, and therefore unable to mount an anti-viral antibody response, showed that virus replication is controlled during the acute infection, similar to the control in wild type mice (59). However, following initial control of infectious virus, virus re-emerged within the CNS of these B cell-deficient mice. Again, these data demonstrate that a functional T-cell immune response is unable to provide a sterile immunity following JHMV infection. Importantly, the passive transfer of antiviral antibody following initial clearance completely prevented virus reactivation. These data suggest that although cell mediated immunity, predominantly the antiviral $CD8^+$ T cell response, fails to provide sterile immunity, humoral immunity compensates by preventing the re-emergence of infectious virus during persistence.

The mechanism of demyelination during JHMV infection has been controversial. Initial studies showed that virus actively replicates in oligodendroglia. This suggested that oligodendroglial death due to infection resulted in primary demyelination (55, 86). Indeed, electron microscopic studies of JHMV infected CNS have shown marginated nuclear chromatin in infected oligodendroglia (26), consistent with the suggestion that oligodendroglia undergo apoptotic death following infection (5). Interestingly, apoptosis of oligodendroglia has also not been detected in the CNS of mice with EAE (8). It is clear however, that an immune component is critical for JHMV induced demyelination. Immunosuppressed mice that succumb to overwhelming neuronal infection, have no detectable demyelination. Reconstitution of these mice with immune cells results in demyelination (27), supporting a role for an immune component in JHMV induced demyelination. However, analysis of a mice deficient in a variety of immune components, especially those lacking both T cell populations, exhibit viral-induced demyelination (33). It appears that an inflammatory response, along with preventing rapid death due to overwhelming viral infection, is required to initiate macrophage/microglial removal of myelin (24, 42, 43, 120). Interestingly, it has recently been suggested that infiltrating macrophages are not required for demyelination, *i.e.*, that activated microglia can remove sufficient myelin for demyelination to be evident (121). In contrast to TMEV infection, no evidence has been reported for the activation of immunity to CNS self-antigens in mice infected with JHMV, although infection does induce the activation of T cells specific for self-antigen in the periphery (54). However, self-reactive T cells capable of mediating an EAE-like disease upon adoptive transfer into naïve recipients have been isolated from rats during JHMV induced subacute demyelinating encephalomyelitis (110). Whether JHMV infection of mice is capable of inducing autoreactive T cells, and what their potential contributions are to chronic demyelination in mice, remain open questions.

CNS infection by JHMV induces an acute encephalomyelitis with virus replication in astrocytes, microglia, oligodendroglia and rarely in neurons. The host controls infectious virus via a vigorous anti-viral immune response that is predominantly localized to the CNS. This results in protection from death; however, immunity is unable to completely eliminate all traces of the virus. The result is in an acute infection characterized by immune infiltrates and primary demyelination. The demyelination most likely results from virus infection of oligodendroglia coupled with the massive influx of macrophages and activation of microglia which strip the myelin sheaths from axons leading to primary demyelination. This scenario is supported by the data demonstrating infection of oligodendroglia (26, 42, 55, 86), the absence of demyelination following MHV infections of the CNS in which oligodendroglia are not infected (26, 61), and the absence of demyelination in mice which are immunosuppressed sufficiently to prevent macrophage infiltration (27). Immunity also plays a critical role in the establishment and maintenance of viral persistence in what appears to be a latent state. The mechanism(s) of chronic demyelination are less well understood, but may relate to activation of virus replication resulting in the new foci of demyelination. The limited focal nature of the demyelination and the reduced

frequency of new lesions with time during the "latent" phase are consistent with local events, possibly at the single oligodendroglial level, producing foci of new demyelination. This interpretation is consistent with temporal reductions in both viral antigen positive cells and viral RNA (1, 95). However, virus infection in CNS cells types other than oligodendroglia have been noted during the latent phase (103). These data have been interpreted to suggest that the health of the oligodendroglial cell may be affected adversely by infection of adjacent CNS cell types.

Summary

In addition to the pathological outcome of demyelination associated with viral infections of both humans and rodents, analysis of these viral infections have highlighted a number of common aspects. First, both human infections we describe which result in demyelination result from peripheral viral infections. SSPE follows an acute measles virus infection in a naïve host while PML results from the inability of the immune response to control a persistent peripheral infection leading to dissemination to the CNS. Both rodent models described are based on viruses derived from a rare occurrence of dissemination into the CNS, most probably from an enteric site of infection. Although the immune status of the initial mice which exhibited viral mediated CNS disease was not examined in either case, it is likely that these rare events were either: *1)* the result of an underlying immunosuppression similar to PML or; *2)* the natural selection of variants with increased neurotropism, similar to the events contributing to the evolution of both PML and SSPE. In terms of the antiviral immune response, TMEV infection appears to have some aspects in common with SSPE. During both acute phases of infections there is little or no evidence of demyelination. Although it is not clear what events predispose to SSPE, TMEV persists in the CNS even though both $CD4^+$ T cells and antibody responses are mounted during the acute phase. It is interesting that during SSPE and TMEV, neutralizing antibody is present during the virus-induced demyelination. In contrast to these viral diseases, JHMV produces demyelination during the acute infection and at least the infectious virus is controlled by the cellular arm of the immune response. However, it is the anti-viral antibody response which inhibits the JHMV reactivation following acute infection. These data suggest that either the specificity or affinity of the antibody may indeed be a critical factor in the progression of all three of these demyelinating diseases. Indeed, nursing JHMV infected pups on dams immunized with JHMV prevents acute death. However a significant proportion of these mice undergo temporally distinct spontaneous virus reactivation characterized by both the presence of infectious JHMV in the CNS and demyelination (82). Direct viral infection of oligodendroglia is clearly capable of producing demyelination (SSPE, PML, JHMV). However, the contribution of infection or persistence in other CNS cell types to the pathological processes associated with demyelination may play a substantial role, although the mechanisms are not clear. For example, analysis of the JHMV model has shown not only that specific subsets of immune effectors are critical in controlling virus infection of the major CNS cell types and viral persistence, but that demyelination is absent without the immune response associated with encephalomyelitis. In addition, there is controversy concerning the infection of oligodendroglia by TMEV; however, even the most optimistic estimates suggest that it is primarily the infection of myelomonocytic lineage cells which predominates. Finally, advances in immunological techniques have provided evidence that during persistent TMEV infection the inflammatory response, directed predominantly toward viral components, results in the eventual activation of cell mediated immunity specific for components of myelin. Although it is not clear how these self reactive components contribute to TMEV pathogenesis, these data have again raised the distinct possibility that an initiating viral infection of the CNS could result eventually in a demyelinating disorder whose major characteristics would be consistent with an autoimmune disease.

Acknowledgements

Supported by NIH grant NS18146. The authors wish to thank Ernesto Barron for excellent technical assistance.

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