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Review

Pathogenesis of mouse hepatitis virus-induced demyelination

Jacqueline J Houtman[1] and John O Fleming[1,2,3]

Departments of [1]Medical Microbiology and Immunology and [2]Neurology, University of Wisconsin, Madison, WI, 53706; [3]William S Middleton Memorial Veterans Hospital, Madison, WI 53705, USA

Infection of rodents with neurotropic mouse hepatitis virus (MHV) may result in lethal encephalitis or paralytic demyelinating disease resembling the human disease multiple sclerosis. The outcome of MHV infection is dependent on a number of variables, including the passage history of the viral isolate, dose and route of inoculation, and the age and immune status of the host. Alterations in surface glycoproteins, especially the spike protein, can profoundly influence pathogenesis. Innate resistance to MHV infection may be related to the expression of cellular receptors or to immunological factors. The immune system plays a major role in MHV pathogenesis, affecting encephalitis, viral clearance, and demyelination. Antiviral antibodies, CD4$^+$ T lymphocytes, or CD8$^+$ T lymphocytes may protect infected animals from lethal encephalitis, but both CD4$^+$ and CD8$^+$ T lymphocytes are required for effective viral clearance. Demyelination in MHV-infected animals has been attributed to the cytolytic effects of viral infection on myelin-producing oligodendrocytes, but more recent evidence supports an immunopathological mechanism for demyelination. Immunopathological models for demyelination include autoimmunity, direct immune cytotoxicity, and indirect 'bystander' damage. Although evidence exists supporting all of these models, the authors favor the bystander demyelination model. Much remains to be revealed about the processes leading to demyelination in MHV-infected mice, and information gained from these investigations may aid in the study of demyelinating disease in humans.

Keywords: multiple sclerosis; immunopathology; coronavirus

Introduction

On 14 August 1947, two paralyzed mice were discovered in a stock colony of Swiss white mice at Harvard Medical School. Virus was isolated from the brains of these mice and subjected to repeated passage in mouse brains (Cheever *et al*, 1949). Early passages of the virus produced paralytic disease, but in later passages, a predominantly encephalitic disease occurred, with mortality occurring as soon as 36 h post-inoculation. This virus was named JHM virus (JHMV or MHV-4) after Harvard Professor J Howard Mueller (Pappenheimer, 1958; Weiner, 1987). Since that isolation, many related strains with differing tissue tropisms have been isolated and grouped together as mouse hepatitis virus (MHV), in the family Coronaviridae (Holmes, 1990; Siddell *et al*, 1982). MHV can cause hepatitis, enteritis, or encephalomyelitis in mice or rats depending on the strain of virus, route of inoculation, and background of the host (Bailey *et al*, 1949; Wege *et al*, 1982). MHV remains widespread in some mouse colonies and is of concern to those performing biomedical research with mice because of its potential confounding immunomodulatory effects (Compton *et al*, 1993; Cook-Mills *et al*, 1992; Cray *et al*, 1993; de Souza *et al*, 1991; de Souza and Smith, 1991; Smith *et al*, 1991b). Neurotropic strains of MHV (e.g., JHM and A59) are the subject of intensive study as models for the human demyelinating disease, multiple sclerosis (Dal Canto, 1990; Dal Canto and Rabinowitz, 1981; Fazakerley and Buchmeier, 1993; Martin and Nathanson, 1979; Shubin and Weiner, 1989). This review will focus on the pathogenesis of neurotropic MHV in mice and rats.

Correspondence: JO Fleming
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Pathogenesis

Neurovirulent strains of MHV, when inoculated intransally or intracerebrally into susceptible mice,

produce acute, usually fatal encephalomyelitis. Those mice that survive frequently develop chronic focal demyelinated lesions in the central nervous system (CNS) and paralysis (Kyuwa and Stohlman, 1990; Lavi and Weiss, 1989; Wege *et al*, 1982). JHMV is predominantly neurotropic, whereas A59 is both neurotropic and hepatotropic, but both can cause demyelination (Lavi and Weiss, 1989; Wege *et al*, 1982).

JHMV infection of suckling Lewis or Brown Norway rats leads to a fatal, acute encephalomyelitis. Infection of older rats, however, results in either acute encephalitis or subacute demyelinating encephalomyelitis in Lewis rats, and clinically silent subacute demyelinating encephalomyelitis in Brown Norway rats (Watanabe *et al*, 1987). In addition, mutant strains of JHM can cause relapsing demyelinating disease in rats (Wege *et al*, 1984b).

MHV-induced encephalomyelitis can result from intranasal or intracerebral inoculation of virus. After intranasal inoculation, virus spreads to the brain primarily via transneuronal routes (Barthold, 1988; Barthold and Smith, 1992; Jacobsen and Perlman, 1990; Perlman *et al*, 1990a). Hematogenous and lymphatic spread also occur after intranasal inoculation (Barthold and Smith, 1992). Viral antigen can be detected in the upper respiratory mucosa, lung, mesothelium, bone marrow, spleen, lymph nodes, and liver, but is mainly seen in the brain (Barthold and Smith, 1984). Within the CNS, virus may spread via neurons, astrocytes, or cerebrospinal fluid (Fazakerley *et al*, 1992; Sun and Perlman, 1995; Wang *et al*, 1992b). Virus replicates in neurons, astrocytes, and oligodendrocytes in the CNS (Kyuwa and Stohlman, 1990). The precise routes of viral spread and sites of viral replication are currently under active investigation.

One characteristic of MHV infection is the ease with which persistence is established (Lavi and Weiss, 1989). In nonlethal JHMV infections, viral antigen can be detected by immunofluorescence in mice for at least a year (Kyuwa and Stohlman, 1990; Stohlman and Weiner, 1981). Viral RNA has been demonstrated by reverse transcription-polymerase chain reaction (RT-PCR) in the CNS 360 days post-inoculation (Fleming *et al*, 1994) and may persist for the life of the mouse (Fleming, unpublished). Viral RNA has been detected by cDNA hybridization to dot-blots from infected rat brains up to 5 months post-inoculation (PI) (Sorensen *et al*, 1984). Infectious virus can usually only be isolated from mice for about 15 days (Dalziel *et al*, 1986), but has been isolated as long as 1 year post-inoculation (Knobler *et al*, 1982a). The virus may spread to and persist in astrocytes in the anterior spinal cords of mice, although viral RNA can also be detected by *in situ* hybridization in oligodendrocytes (Perlman *et al*, 1990b; Perlman and Reis, 1987; Sun *et al*, 1995). In rats, virus may persist in neurons (Sorensen and Dales, 1985). Persistence *in vitro* is associated with reduced cytopathic effects and tropism for astrocytes (Massa *et al*, 1988).

The pathogenesis of demyelination in MHV-infected rodents has been the subject of much study and some controversy. The phenomenon is complex and depends on both viral and host factors. Viral factors associated with MHV pathogenesis include cell tropism and rate of replication, which may be altered by mutations in genes encoding structural or non-structural proteins. Host factors include innate resistance or susceptibility, as well as the immune status of the host. These factors can be manipulated genetically, through immunosuppression, or by transfer of immune cells or soluble factors to infected mice.

Viral factors associated with pathogenesis

Mouse hepatitis virus is classified among the coronaviruses, enveloped viruses with characteristic morphology and single stranded, positive-sense RNA genomes (Holmes, 1990; Siddell *et al*, 1982; Spaan *et al*, 1988). The replication strategy of coronaviruses is unique and involves a 3′ coterminal nested set of functionally monocistronic mRNAs (Compton *et al*, 1993; Holmes, 1990; Lai, 1990, 1995). MHV possesses genes for four non-structural and three or four structural proteins (Compton *et al*, 1993; Lai, 1990). Structural proteins of MHV include a nucleocapsid and two or three surface glycoproteins: the spike protein, the matrix protein and the optional hemagglutinin-esterase protein. An additional small membrane (sM) protein has been reported, but its function in viral replication and pathogenesis is currently unknown (Theil and Siddell, 1995; Yu *et al*, 1994). The 3′ end of the MHV genome, which codes for structural proteins, appears to play a major role in pathogenesis (Lavi *et al*, 1990). Little is known about the role of non-structural proteins or non-coding regions in pathogenesis, although some neuroattenuated mutants may have mutations in gene 1 (RNA polymerase) (Lai and Stohlman, 1992).

Spike protein

The spike glycoprotein (E2 or S) gives the virus its characteristic appearance and also seems to be the most important for viral interactions with the host cell. It is involved in both attachment and fusion, and antibodies directed against it are neutralizing (Kyuwa and Stohlman, 1990). Fusion can be inhibited by anti-spike protein antibodies (Sturman *et al*, 1985). Cleavage of the spike protein into S1 and S2 subunits by trypsin or cellular proteases enhances fusion, but is not an absolute requirement (Bos *et al*, 1995; Stauber *et al*, 1994; Sturman *et al*, 1985; Taguchi, 1993). The portion of the molecule

responsible for fusion is within the carboxyl (S2) subunit of the protein, whereas receptor binding function is localized to the amino-terminal (S1) subunit (Keck *et al*, 1988; Kubo *et al*, 1994; Taguchi, 1995). Different host cells may cleave the spike protein at different sites, suggesting a mechanism for host cell specificity and tissue tropism (Frana *et al*, 1985). It has also been reported that the spike glycoprotein can function as an IgG-specific Fc receptor (Oleszak *et al*, 1992b). A great deal of sequence variability has been noted in MHV. This variation seems especially pronounced in the spike protein (La Monica *et al*, 1991; Parker *et al*, 1989). The S1 region of the spike protein has been shown to be hypervariable (Banner *et al*, 1990; La Monica *et al*, 1991). In addition to its possible role in pathogenesis and immune evasion, this variability may be responsible for some of the varying results reported by different laboratories (Fazakerley *et al*, 1992; Lai and Stohlman, 1992).

Monoclonal antibodies directed at surface glycoproteins of MHV are very useful in elucidating the role of surface glycoproteins in neurovirulence. Anti-spike monoclonal antibodies can protect suckling rats, immunocompetent mice, and athymic nude mice from lethal encephalitis (Buchmeier *et al*, 1984; Talbot *et al*, 1987; Wege *et al*, 1984a). These antibodies can also be used to isolate neutralization-resistant escape variants which are often neuroattenuated. By analyzing the mutant viruses, researchers can identify crucial determinants of pathogenicity. For example, two monoclonal antibodies specific for different epitopes on the spike protein were used to select variants (Fleming *et al*, 1986, 1987; Wang *et al*, 1992a). Variants selected with one antibody retained the lethal encephalitic phenotype, whereas variants selected with the other antibody were neuroattenuated and caused demyelination with little encephalitis. An epitope on the S2 subunit of the spike protein recognized by the second antibody was concluded to be important in JHMV-induced encephalitis. A variant selected with both antibodies produced neither encephalitis nor demyelination and had mutations in both the S1 and S2 subunits, demonstrating that two distinct portions of the spike protein were involved in the pathogenesis of JHMV-induced disease.

Other workers have also reported neuroattenuated variants selected with anti-spike monoclonal antibodies. Several escape variants were tested *in vivo* and found to cause chronic demyelinating disease, but not fatal encephalitis (Dalziel *et al*, 1986). Sequence analysis of the spike glycoprotein gene of these variants revealed large deletions corresponding to the amino terminal, or S1 domain of the protein (Parker *et al*, 1989). Wege *et al*. (1988) also isolated multiple escape variants, one of which was neuroattenuated in mice and caused demyelination. Whereas the S1 domain of the spike protein is important for neurovirulence in both rats and mice, different portions of S1 may be critical for virulence in each host (Taguchi *et al*, 1995).

Neuroattenuated variants can also be isolated *in vivo*. Morris *et al*. (1989) reported on the isolation of a variant from JHMV-infected rats which had deletions in the gene encoding the spike protein and produced chronic demyelination, but no acute encephalitis. In another study, viral RNA isolated from rat brains 5 to 7 days post-inoculation encoded larger spike proteins than the parental strain (Taguchi *et al*, 1985). Analysis of RNA from persistently infected mice showed a diverse population of viral sequences in both the spike and nucleocapsid genes (Adami *et al*, 1995), suggesting that a heterogeneous population or quasispecies may exist in the CNS of infected mice.

Matrix protein

The matrix protein (E1 or M) is a 23 kDa glycoprotein which is responsible for viral budding from the rough endoplasmic reticulum and golgi complex. Antibodies to the matrix protein can protect mice from fatal encephalitis (Fleming *et al*, 1989). This protection does not correlate with the ability of the antibodies to neutralize virus *in vitro* or reduce virus titers *in vivo* and is independent of complement.

Hemagglutinin-esterase protein

The hemagglutinin-esterase protein (E3 or HE) is a 65 kDa surface glycoprotein which is only present on some coronaviruses and only on some strains of MHV and is thus sometimes regarded as nonessential. A59 lacks HE and different strains of JHMV possess various amounts of protein (Yokomori *et al*, 1991). HE may be involved in viral attachment, and the HE of bovine coronavirus has been shown to bind to and inactivate receptors on erythrocytes which possess 9-O-acetylated neuraminic acid residues (Holmes *et al*, 1989; Vlasak *et al*, 1988). The function of HE in viral replication is unclear, but it appears to play a significant role in pathogenesis. As with the spike and matrix proteins, monoclonal antibodies directed against the HE protein can protect infected mice from lethal encephalitis (Yokomori *et al*, 1992). There is a tendency for HE-defective mutants to accumulate during persistent JHMV infection (Yokomori *et al*, 1993b). Indeed, viruses with deletions in the HE gene have been isolated from a JHMV-infected rat 14 days PI (La Monica *et al*, 1991; Morris *et al*, 1989). Expression of the hemagglutinin-esterase protein seems to enhance neurovirulence by altering cell tropism, or by allowing an increased rate of spread within the CNS (Yokomori *et al*, 1995).

Temperature-sensitive and plaque morphology mutants

Temperature-sensitive (ts) and plaque morphology variants have also been isolated and have proven useful in elucidating the pathogenesis of MHV-induced disease. Knobler *et al.* (1982b) studied two temperature-sensitive mutants of JHM, ts8 and ts15. Whereas the parental virus caused fatal encephalitis, mice inoculated with ts8 survived. Viral spread was reduced, but the virus persisted and caused chronic demyelination. The second mutant, ts15, also caused a persistent nonlethal infection, but demyelination was rare. Another temperature sensitive mutant of JHM which produces demyelinating encephalomyelitis in rats with little acute encephalomyelitis shows reduced cytopathic effect in glial cultures when compared to the more neurovirulent parental strain (Massa *et al*, 1988). A ts mutant of A59 was shown to be neuroattenuated and caused demyelination in mice (Koolen *et al*, 1983, 1987). A small plaque variant of JHMV was also shown to be nonlethal and to induce demyelination in mice (Stohlman *et al*, 1982a).

The functional differences between the parental and neuroattenuated variant viruses are not well understood. One difference between encephalitis-causing and demyelinating viruses may be in their tissue tropism: neuronal infection is required for encephalitis, while glial infection results in demyelination (Knobler *et al*, 1981a). It has been reported, however, that a ts mutant can infect neurons of infected mice without killing neurons or causing severe encephalitis (Robb *et al*, 1979). Another possibility is that the parental virus spreads faster in the brain than the neuroattenuated variants (Fazakerley *et al*, 1992; Koolen *et al*, 1987). The viruses may also differ in their ability to induce cell fusion (Massa *et al*, 1988). The spike protein of some ts mutants may be glycosylated differently than that of parental strains (Oleszak *et al*, 1992a).

Analysis of neuroattenuated variants of this extremely mutable virus has revealed that viral proteins and the immune response to them play a critical role in the pathogenesis of demyelination. Changes in surface glycoproteins may affect the ability of the virus to attach to and infect a particular cell type, spread from cell to cell, or evade the immune system. The spike protein is a critical determinant in both viral replication and interactions with the host immune system. This protein is especially variable and a single point mutation may profoundly alter pathogenicity. The HE protein also appears to play an important role in pathogenesis, although the interactions between HE and host cells have yet to be fully elucidated.

Host factors associated with pathogenesis

Innate resistance

Most mice are susceptible to fatal JHMV infection. Mice 12 weeks of age or older of the SJL/J strain are resistant, but SJL/J mice less than 6 weeks old are susceptible to fatal infection (Stohlman *et al*, 1980). The age-related resistance in SJL/J mice is associated with maturation of an adherent cell population (Stohlman *et al*, 1982b). The strain-related resistance is associated with an intrinsic macrophage-mediated antiviral activity (Stohlman *et al*, 1982b) and the inability of the virus to replicate within SJL/J neurons (Knobler *et al*, 1981b). The genetics of susceptibility and resistance have been studied by several groups (reviewed by Buschman and Skamene, 1995). Knobler *et al.* (1981b) reported that resistance was a recessive trait controlled by a single autosomal gene which is not H-2 linked. In contrast, Stohlman and Frelinger (1978) found that resistance was mediated by two genes, one dominant and one recessive. Resistance to infection of macrophages has been mapped to a recessive gene on mouse chromosome 7 (Smith *et al*, 1984). Immunologic, as well as genetic, factors may play a role in SJL resistance, since immunosuppression of SJL/J mice with cyclosporin A can abrogate resistance (Pasick *et al*, 1992). Some aspects of the response to MHV infection may be H-2 linked (Castro *et al*, 1994). The genetic control of MHV resistance is likely under the control of multiple genes, some of which control immunological functions (Buschman and Skamene, 1995; Kyuwa *et al*, 1992).

Much attention has been directed toward the study of cellular receptors for MHV as possible mediators of strain-specific or species-specific susceptibility to infection. MHV receptors are 110 to 120 kDa biliary glycoproteins (Bgp) related to the carcinoembryonic antigen, a member of the immunoglobulin gene superfamily (Dveksler *et al*, 1991, 1993). The prototype Bgp receptor was shown to be expressed on the plasma membranes of hepatocytes and brush border enterocytes of susceptible mice, but not of resistant SJL/J mice or other species (Boyle *et al*, 1987; Compton *et al*, 1992; Dveksler *et al*, 1991). This protein was not, however, detected in the brains of susceptible mice, and so could not account for viral infection of the CNS (Williams *et al*, 1991). It has been subsequently discovered that multiple isoforms of the *Bgp1* gene, as well as an additional gene, *Bgp2*, code for functional MHV receptors and are expressed in different tissues, including brain (Dveksler *et al*, 1993; Godfraind *et al*, 1995; Nédellec *et al*, 1994; Yokomori and Lai, 1992a). This may account for the wide variety of tissue tropisms exhibited by MHV. Bgp receptors appear to be required for viral

infection, regardless of the presence of the HE protein (Gagneten *et al*, 1995). It has also been reported, however, that resistant SJL/J mice possess functional MHV receptors and that additional cellular factors are required for viral infection (Asanaka and Lai, 1993; Yokomori and Lai, 1992b; Yokomori *et al*, 1993a). Thus, MHV receptors are necessary, but not sufficient for viral infection. Once infection is established, however, expression of MHV receptors does not appear to be necessary for direct cell to cell spread of virus (Gallagher *et al*, 1992).

Both Lewis and Brown Norway rats are susceptible to lethal JHMV infection at a very young age. Older Lewis rats, however, are resistant to clinical infection, whereas older Brown Norway rats may develop either acute encephalitis or a subacute demyelinating encephalomyelitis (Watanabe *et al*, 1987). Wistar Lewis and Long Evans rats are susceptible up to 10 days of age, but develop complete resistance after that (Sorensen *et al*, 1987b). Wistar-Furth rats are susceptible up to 3 weeks of age, and resistance in these rats is expressed as a homozygous recessive trait (Sorensen *et al*, 1987a). Resistance to disease in Brown Norway and Wistar Lewis rats appears related to immunological factors (Hein *et al*, 1995; Sorensen *et al*, 1987a). In addition, infection of rat oligodendrocytes appears to be limited to a distinct developmental stage, suggesting a mechanism for age-related resistance (Pasick and Dales, 1991).

Role of the immune system in encephalitis, viral clearance and demyelination

Encephalitis, viral clearance, and demyelination are three important and conceptually distinct outcomes of MHV pathogenesis (Figure 1). Encephalitis is usually assessed by the observation of destructive cerebral lesions and mortality in experimental animals. Viral clearance usually refers to the reduction of infectious virus on a macroscopic level as determined by assay of brain homogenates. Alternatively, clearance may refer to localized elimination of viral antigen or RNA as revealed by immunohistochemistry or *in situ* hybridization. Demyelination is evaluated by myelin-specific histochemical stains or ultrastructural evidence of myelin loss. Demyelination may be subclinical or may be accompanied by clinical manifestations such as paralysis. These three aspects of MHV pathogenesis have been shown to be influenced by the activity of the host immune system, and intense infiltrates of lymphocytes and macrophages are prominent features of MHV-induced pathology (Dörries *et al*, 1991; Nagashima *et al*, 1978; Sedgwick *et al*, 1991; Wang *et al*, 1992b; Williamson, 1992; Williamson *et al*, 1991). The host immune system may influence MHV-induced encephalitis, viral clearance, or demyelination individually or in concert. Thus, these three outcomes

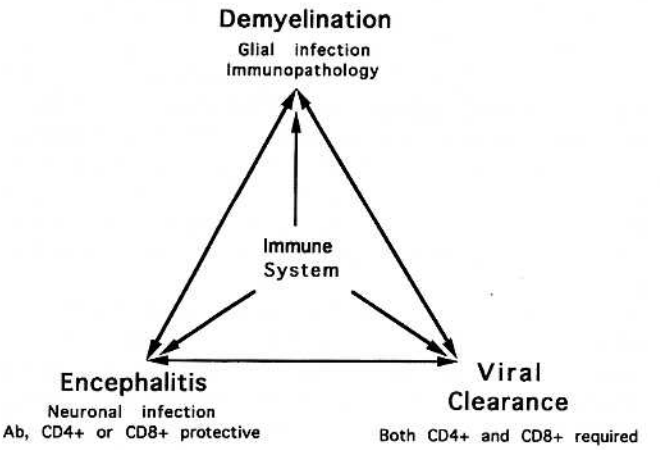

Figure 1 Three outcomes of MHV pathogenesis are encephalitis, viral clearance, and demyelination. These essentially distinct phenomena may be influenced individually or in concert by the immune system, or they may influence each other (see text).

of MHV infection are likely to involve immunological pathways which are inherently distinct but nonetheless interact under certain conditions.

The occurrence of encephalitis may sometimes interfere with the study of demyelination. For example, highly virulent MHV strains may cause an acute, fatal encephalitis which can mask potential demyelination, i.e., animals may die of encephalitis before they can fully develop demyelination. In this case, the intensity of encephalitis appears to depend on rapid viral spread to neurons which occurs before the immune system can eliminate the virus. By using less virulent or slower spreading forms of MHV, however, investigators can study the pathogenesis of the virus under conditions which allow the immune system to more effectively control viral replication. Neuroattenuated variant viruses may allow for development of demyelination in the relative absence of encephalitis, and some variants produce neither encephalitis nor demyelination (Dalziel *et al*, 1986; Erlich *et al*, 1987; Fleming *et al*, 1986; Knobler *et al*, 1982b; Stohlman *et al*, 1982a). Host factors can also be manipulated to allow for the development of demyelination in the absence of lethal encephalitis. Depending on their age, rats may show lethal encephalitis or subacute demyelination (Watanabe *et al*, 1987). Mice protected from lethal encephalitis by transfer of antibodies or T lymphocytes but in which viral replication is not suppressed appear to be more susceptible to chronic demyelination (Buchmeier *et al*, 1984; Stohlman *et al*, 1986, 1992). Thus, under certain experimental conditions, encephalitis and demyelination can be separated, and the role of the immune response in each studied separately.

The relationship between encephalitis and viral clearance is illustrated by studies which

demonstrate protection from encephalitis. Antiviral antibodies or virus-specific $CD4^+$ or $CD8^+$ T lymphocytes can protect mice from a lethal challenge with MHV, but do not produce effective viral clearance (Buchmeier *et al*, 1984; Fleming *et al*, 1989; Jacobsen and Perlman, 1990; Perlman *et al*, 1987; Stohlman *et al*, 1986, 1988, 1995a; Yamaguchi *et al*, 1991; Yokomori *et al*, 1992). Both $CD4^+$ and $CD8^+$ lymphocytes, together, however, can protect mice and effectively clear infectious virus from the CNS (Pearce *et al*, 1994; Sussman *et al*, 1989; Williamson and Stohlman, 1990). Protection from lethal encephalitis, therefore, seems to be independent of the ability to clear virus, even though some experimental treatments (e.g., transfer of both $CD4^+$ and $CD8^+$ T lymphocytes) may influence both encephalitis and clearance.

Demyelination and viral clearance have been difficult to study independently and often appear to be linked. For example, an early effective immune response can prevent demyelination by clearing infectious virus before it becomes widespread, thus influencing both demyelination and viral clearance (Dörries *et al*, 1994; Stohlman *et al*, 1995a). Recent work with immunodeficient mice, however, suggests that demyelination and viral clearance may be separable. Profoundly immunosuppressed mice (irradiated mice and mice with severe combined immunodeficiency (SCID) mutation) can neither clear virus nor undergo demyelination, whereas immunocompetent controls effectively clear virus and undergo robust demyelination (Fleming *et al*, 1993; Houtman and Fleming, 1996; Wang *et al*, 1990). In contrast, partially immunodeficient mice (athymic nude mice and mice deficient in $CD4^+$ or $CD8^+$ T lymphocytes) may undergo demyelination, yet show impaired ability to clear infectious virus (Gombold *et al*, 1995; Houtman and Fleming, 1996). Thus, demyelination can occur whether or not virus is effectively cleared, and impaired ability to clear virus may or may not abrogate demyelination. It may be possible, then, to study demyelination independently of viral clearance.

Antibody Passively administered antibodies (either antiviral monoclonal antibodies injected into mice or maternally derived antibodies from immune dams) can protect mice infected with MHV from lethal encephalitis, but are not sufficient for effective viral clearance (Buchmeier *et al*, 1984; Fleming *et al*, 1989; Jacobsen and Perlman, 1990; Perlman *et al*, 1987; Pickel *et al*, 1985; Yokomori *et al*, 1992). In these experiments, antibodies are not able to prevent viral replication or clear infectious virus, nor are they able to inhibit the subsequent development of demyelination. Furthermore, protection seems to be mediated by an Fc-independent mechanism (Lamarre and Talbot, 1995). The mechanism by which antibodies protect from encephalitis is currently unknown, but antibodies might alter the effective cell tropism of the virus, preventing infection of neurons.

The role of antibodies in pathogenesis of JHMV in rats has also been studied. A virus-specific antibody response in the CNS has been shown to limit viral spread (Dörries *et al*, 1994). In addition, an earlier, more robust CNS antiviral antibody response was demonstrated in JHMV-resistant Brown Norway rats compared to susceptible Lewis rats. As with mice, antibodies alone are insufficient for complete viral clearance from the CNS (Schwender *et al*, 1991). Antibodies may also play a pathological role in demyelination in rats through a cytotoxic mechanism (Zimprich *et al*, 1991).

T lymphocytes A virus-specific delayed-type hypersensitivity response mediated by $CD4^+$ T lymphocytes can protect mice from lethal encephalitis, but has no effect on viral replication (Stohlman *et al*, 1986, 1988). Transfer of virus-specific $CD4^+$ T lymphocytes which secrete gamma interferon (IFN-γ) and interleukin-2 (IL-2) protects mice from lethal challenge and increases incidence of demyelination, but does not suppress viral replication (Erlich *et al*, 1989). $CD4^+$ T lymphocytes responsive to the matrix and spike proteins have been isolated from infected mice (Mobley *et al*, 1992).

Antiviral $CD8^+$ T lymphocytes, like $CD4^+$ T lymphocytes, can protect infected mice from lethal encephalitis, but both $CD4^+$ and $CD8^+$ T lymphocytes are required for viral clearance (Stohlman *et al*, 1995a; Sussman *et al*, 1989; Williamson and Stohlman, 1990; Yamaguchi *et al*, 1991). The nucleocapsid protein appears to be immunodominant for $CD8^+$ cytotoxic T lymphocytes (CTLs) (Bergmann *et al*, 1993; Stohlman *et al*, 1992, 1993, 1994). Nucleocapsid-specific CTLs can protect mice from lethal encephalitis and may be partially able to prevent the onset of demyelination, but cannot clear virus from the CNS (Castro *et al*, 1994; Stohlman *et al*, 1995a). Anti-spike $CD8^+$ CTLs are also unable to prevent the onset of demyelination, but the epitopes of the spike proteins which they recognize are in portions of the spike which are often deleted upon *in vivo* passage, suggesting a role for anti-spike CTLs in the establishment of persistent infection (Castro and Perlman, 1995). CTL clones have been shown *in vitro* to induce apoptosis in infected cells (Shibata *et al*, 1994).

Infiltrating T lymphocytes from susceptible Lewis rats proliferate *in vitro* in response to myelin basic protein and JHMV, but those from resistant Brown Norway rats do not (Watanabe *et al*, 1987). $CD4^+$ T lymphocytes specific for nucleocapsid or spike proteins can protect infected rats from lethal encephalitis (Körner *et al*, 1991; Wege *et al*, 1993). $CD8^+$ T lymphocytes also appear to have a protective effect (Flory *et al*, 1993).

In addition to classical T lymphocyte cells with natural killer (NK) phenotype or function have been demonstrated after JHMV infection (Stohlman *et al*, 1983; Williamson *et al*, 1991).

Cytokines Cytokines play a crucial role in the immune response to viruses, and several workers have investigated cytokine induction in MHV infections. Infection of immunocompetent mice with the MHV variant OBLV60 results in upregulation of mRNA for IL-1, IL-6, tumor necrosis factor-α (TNF-α) and IFN-γ, whereas infection of athymic nude mice results in upregulation of IL-1, IL-6, and TFN-α, but not IFN-γ mRNA (Pearce *et al*, 1994). Using immunohistochemical techniques, Sun *et al.* (1995) detected production of TNF-α, IL-1β, IL-6, and type 2 nitric oxide synthase (iNOS) by astrocytes in spinal cords of mice chronically infected with JHMV and production of TNF-α, IL-6, and iNOS by mononuclear cells in acutely infected spinal cords. Both induction of IL-6 mRNA and secretion of biologically active IL-6 were observed when murine astrocytes were exposed to infectious or inactivated JHMV (Joseph *et al*, 1993). Although TNF-α mRNA is upregulated on JHMV infection, TNF-α is not secreted and TNF-α does not appear to play a significant role in JHMV-induced encephalitis or demyelination (Stohlman *et al*, 1995b). IFN-γ, on the other hand, appears to be important for viral clearance and protection from lethal encephalitis (Smith *et al*, 1991a).

MHC expression Some of the pathology caused by MHV may involve modulation of the expression of major histocompatibility complex (MHC) molecules in infected neural tissues. Upregulation of MHC class I or II molecules may enhance the role of $CD8^+$ or $CD4^+$ T lymphocytes, respectively. Conversely, downregulation of these molecules may allow the virus to evade the immune response. MHV A59 can induce the expression of MHC class I antigens on the surface of cultured mouse astrocytes and oligodendrocytes, cells which do not normally express these antigens (Suzumura *et al*, 1986, 1988). This induction requires infectious virus and is mediated by a soluble factor (which is not interferon) derived from infected astrocytes (Lavi *et al*, 1989; Suzumura *et al*, 1986, 1988). Upregulation of class I mRNA in both infected and uninfected cells in the brains of A59-infected mice has also been demonstrated, supporting a role for a soluble factor *in vivo* (Gombold and Weiss, 1992). Induction of MHC class I molecules has been detected by immunohistochemistry in the brains of immunocompetent and athymic nuce BALB/c mice infected with MHV variant OBLV60 (Pearce *et al*, 1994). Upregulation of class I molecules may contribute to $CD8^+$ T lymphocyte-mediated lysis of infected cells, leading to CNS damage. In contrast to A59 infection, it was reported that JHMV infection did not induce class I expression on acutely infected astrocytes, and inhibited class I expression on persistently infected astrocytes (Correale *et al*, 1995; Gilmore *et al*, 1994). This downregulation of class I expression was hypothesized to contribute to the establishment of persistent JHMV infections. *In vitro* infection with JHMV also decreases surface expression of class I molecules (Kyuwa *et al*, 1994). In agreement with these studies, Sun *et al.* (1995) detected neither class I nor class II molecules on astrocytes in the spinal cords of mice chronically infected with JHMV, although both class I and II molecules were observed on macrophages or microglia by immunohistochemistry. MHC class I and II antigens can be expressed on the surface of cultured astrocytes and oligodendrocytes in response to IFN-γ (Correale *et al*, 1995; Gilmore *et al*, 1990; Massa *et al*, 1986). In this way, infiltrating T cells may contribute to bystander damage to the CNS by IFN-γ-mediated upregulation of MHC molecules on uninfected cells (Correale *et al*, 1995). In the absence of IFN-γ, infectious or inactivated JHMV can induce expression of class II antigens on cultured rat astrocytes (Massa *et al*, 1986). MHV has also been shown to modulate expression of MHC molecules on cultured cerebral endothelial cells, suggesting that infection may alter the traffic of immune cells or virus into the CNS (Joseph *et al*, 1990, 1991).

The complex host response to infection with MHV is a critical determinant in the development of disease. The genetics and immune status of the host, as well as the distribution of cellular receptors for MHV, can profoundly affect susceptibility to infection and demyelinating disease. The cells, antibodies, and cytokines of the immune system are intimately involved in protection from lethal encephalitis, clearance of infectious virus, and the development of demyelination. Protection from lethal encephalitis can be mediated by antibodies or antiviral $CD4^+$ or $CD8^+$ T lymphocytes, but does not require viral neutralization or clearance (Buchmeier *et al*, 1984; Fleming *et al*, 1989; Jacobsen and Perlman, 1990; Perlman *et al*, 1987; Pickel *et al*, 1985; Stohlman *et al*, 1986, 1988, 1995a; Yamaguchi *et al*, 1991; Yokomori *et al*, 1992). Effective clearance of infectious virus requires both $CD4^+$ and $CD8^+$ T lymphocytes (Pearce *et al*, 1994; Sussman *et al*, 1989; Williamson and Stohlman, 1990). The cellular and molecular requirements for demyelination, however, remain unclear.

Mechanisms of demyelination

We have seen that many factors contribute to the pathogenesis of MHV infection, including viral genetics, innate resistance, and the immune status of the host. Experimental factors such as the passage history of the virus, dose and route of

inoculation, as well as the age of the host can affect the occurrence of CNS demyelination and the interpretation of experimental findings. For example, subacute and chronic demyelination are associated with different histopathological changes. Subacute demyelination is associated with the presence of infectious virus and a considerable inflammatory infiltrate, often with concomitant neuronal infection and encephalitis (Lavi and Weiss, 1989). Chronic demyelination, on the other hand, usually occurs in the relative absence of viral replication and inflammation. In addition, the immune systems of newborn mice are immature, and experimental models which use newborns may produce results which appear to differ from those of models using older mice. Different strains of mice may respond to infection in different ways and different rodent species (Castro *et al*, 1994, Taguchi *et al*, 1995).

The JHMV and A59 genomes share 60–74% of their sequences, yet appear to differ in subtle, but potentially important ways (Correale *et al*, 1995; Lai and Stohlman, 1981; Lavi *et al*, 1990; Weiss and Leibowitz, 1981; Yokomori *et al*, 1991). The hypervariability of the virus must also be considered: each virus stock likely consists of a unique population. This very variable virus is subject to inter-laboratory differences which, along with experimental variables, have hampered efforts to come to a consensus on the mechanism of demyelination.

Demyelination caused by viral infection of the CNS may be due to at least four general processes, as depicted in Figure 2 (Fazakerley and Buchmeier, 1993; Shubin and Weiner, 1989; Wisniewski, 1977). (a) *Viral cytolytic model.* The virus may destroy those cells in which it replicates through a cytolytic infection. The immune system plays no part in the demyelinating process. (b) *Autoimmune model.* The infection may stimulate the immune system to react with self antigens, possibly through molecular mimicry. Once autoimmunity is established, cells or myelin need not express viral proteins to be destroyed by the immune system. (c) *Direct immune response model.* The immune system may respond to viral infection by destroying infected myelin-producing oligodendrocytes, thus causing demyelination by direct cytotoxicity. (d) *'Bystander' immune response model.* An indirect nonspecific 'bystander' immune response may result in demyelination in the immediate vicinity of a specific immune response to infected cells or cells presenting viral antigens.

Demyelination caused by JHMV infection has often been attributed to the direct lytic effects of the virus on myelin-producing oligodendrocytes (Figure 2a) (Kyuwa and Stohlman 1990; Lampert *et al*, 1973; Sorensen *et al*, 1982, 1987b; Weiner, 1973; Zimmer and Dales, 1989). Recent studies, however, suggest that the mechanism may instead be immunopathological in nature. SCID mice or mice immunosuppressed by irradiation do not undergo consistent demyelination, although virus replicates in the CNS to high titers, demonstrating a role for the immune system in the pathology of demyelination (Fleming *et al*, 1990; Houtman and Fleming, 1996; Wang *et al*, 1990). In addition, adoptive transfer of spleen cells to SCID mice or irradiated infected mice can restore demyelination (Fleming *et al*, 1993; Houtman and Fleming, 1996; Wang *et al*, 1990). Demyelination also appears to be immunologically mediated in Lewis rats since paralysis can be abrogated by irradiation and restored by the adoptive transfer of T lymphocytes (Schwender *et al*, 1994).

The precise immunopathological mechanism for demyelination is poorly understood, but may involve autoimmunity (Figure 2b), direct viral antigen-specific cytotoxicity (Figure 2c) or nonspecific bystander effects (Figure 2d) (Fazakerley and Buchmeier, 1993; Shubin and Weiner, 1989; Wisniewski, 1977). There is some evidence for autoimmune mechanisms in mice and rats (Kyuwa *et al*, 1988, 1991; Watanabe *et al*, 1983, 1987). Molecular mimicry, which may contribute to autoimmunity, has been demonstrated between the spike protein of MHV and Fc receptors for IgG, between the HE protein and MHC class I, and between the nucleocapsid protein and the microtubule-associated protein tau (Dales, 1995; Fujinami and Oldstone, 1985; Kalicharran and Dales, 1995a,b; Luytjes *et al*, 1988; Oleszak *et al*, 1995; Wucherpfennig and Strominger, 1995). The evidence does not appear strong enough at this time, however, to prove that autoimmunity contributes significantly to demyelination in MHV-infected rodents.

Infection of the CNS may result in damage to myelin or to myelin-producing oligodendrocytes through an antiviral immune response. This damage may be direct or indirect. Direct damage due to an antiviral immune response may be caused by antibody-mediated cytotoxicity (Zimprich *et al*, 1991) or through cytotoxic T lymphocytes (Dörries *et al*, 1991). These cytotoxic effector mechanisms occur in response to infected cells; widespread or persistent viral antigen may serve as a target, resulting in widespread or chronic demyelination. In order for T lymphocytes to participate in antiviral cytotoxicity, viral antigens must be presented on the surface of infected cells by MHC molecules. As we have seen, MHV infection can affect the expression of MHC molecules on CNS cells, allowing the virus to play an active role in presentation of its own antigens. Although direct cytolytic destruction of infected oligodendrocytes likely occurs during the process of viral clearance, we do not believe this process is sufficient to account for widespread demyelination, especially where oligodendrocytes are not themselves infected or in the absence of viral

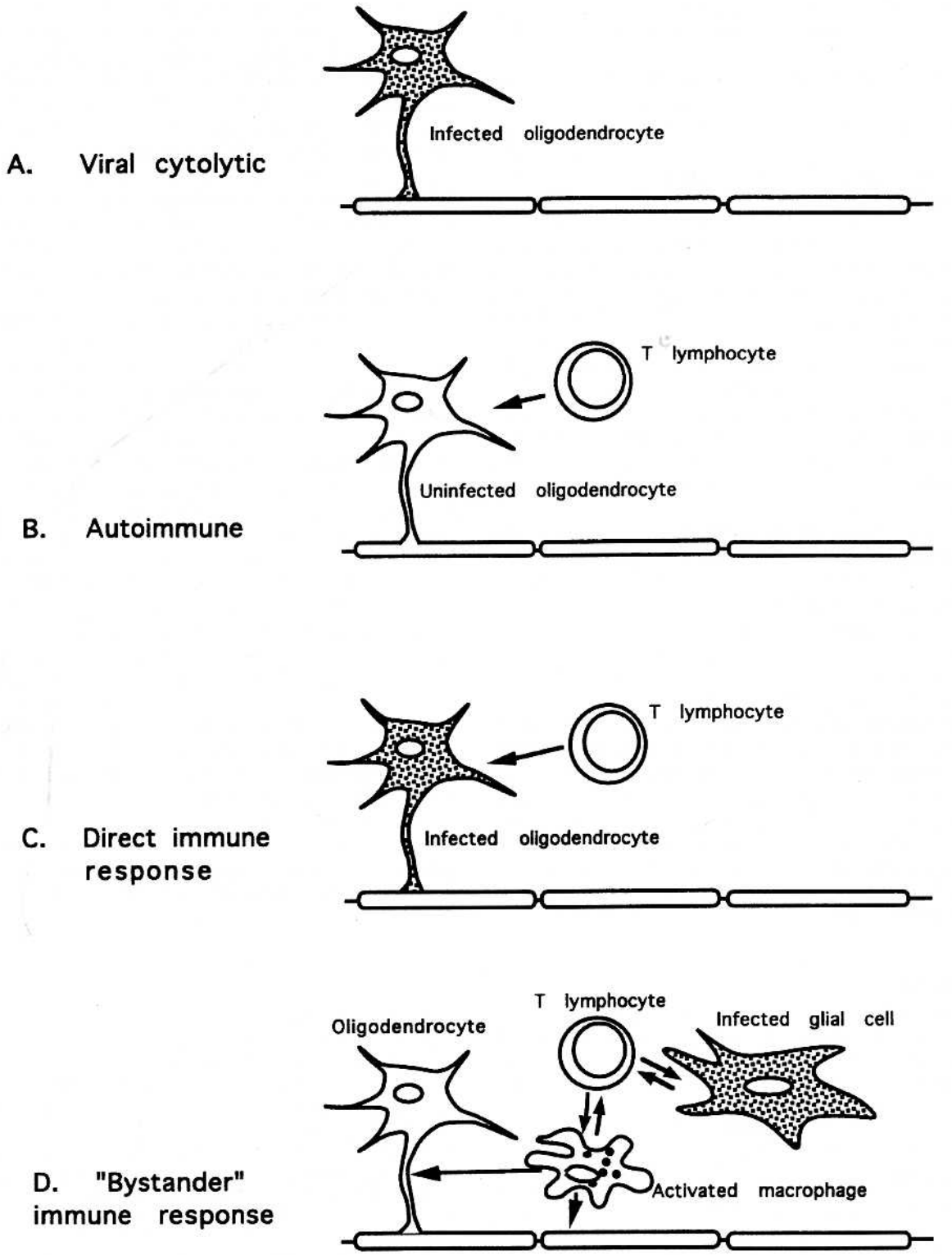

Figure 2 Four mechanisms proposed for virally-induced pathology directed against the oligodendrocyte-myelin unit of the CNS. Infected cells are indicated by stippling. (a) In the viral cytolytic model, virus destroys infected cells through a cytolytic infection. (b) In the autoimmune model, viral infection stimulates the immune system to react with self antigens, possibly through molecular mimicry. (c) In the direct immune response model, the immune system responds to viral infection by destroying infected oligodendrocytes. (d) In the 'bystander' immune response model, an indirect nonspecific 'bystander' immune response results in demyelination in the vicinity of a specific immune response.

clearance. For example, athymic nude mice and mice deficient in $CD4^+$ or $CD8^+$ T lymphocytes are unable to effectively clear virus, yet nonetheless develop demyelination (Houtman and Fleming, 1996).

Indirect damage to myelin through an antiviral immune response may take the form of 'bystander' demyelinatin (Figure 2d). A specific immune response to viral antigen may occur, with nonspecific damage occurring in the adjacent area. For example, a delayed-type hypersensitivity (DTH) response results in the influx of $CD4^+$ T lymphocytes and monocytes/macrophages (Erlich *et al*, 1989). The T lymphocytes may respond to antigen presented by infected cells, activating nearby macrophages in the process. The cytokines and toxic products of these activated macrophages are then the ultimate effectors for demyelination, causing damage to nearby oligodendrocytes or myelin, whether or not they are infected. Oligodendrocytes and myelin are especially sensitive to the products of activated macrophages, such as reactive oxygen species, TNF-α, and proteases (Brosnan *et al*, 1988; Bürge *et al*, 1989; Griot *et al*, 1989; Liuzzi *et al*, 1995; Selmaj and Raine, 1988). Thus the damage caused by these inflammatory cells is itself nonspecific, but it occurs adjacent to, and as a result of, a specific antiviral immune response.

In our view, the preponderance of current evidence favors the bystander or indirect immune-mediated mechanism for MHV-induced demyelination. As mentioned above, the other proposed mechanisms do not appear to explain the occurrence of demyelination in immunodeficient mice which are unable to clear virus (Fleming *et al*, 1990; Houtman and Fleming, 1996; Schwender *et al*, 1994; Wang *et al*, 1990). In addition, demyelination can be enhanced by the adoptive transfer of DTH-inducing $CD4^+$ T cells (Erlich *et al*, 1989). The bystander model does not require the myelin-producing cells themselves to be infected in order for them to be damaged. We have shown that intense inflammatory infiltrates occur within fully-developed plaques of demyelination, but that viral antigen cannot be detected within these plaques (Houtman and Fleming, 1996). These findings suggest that successful local clearance of virus may come at a price, the loss of nearby myelin. Bystander demyelination also appears to play an important role in demyelination induced by Theiler's murine encephalomyelitis virus (Clatch *et al*, 1986) and canine distemper virus (Bürge *et al*, 1989; Griot *et al*, 1989), and in mycobacterial models of demyelination (Matyszak and Perry, 1995; Wisniewski and Bloom, 1975).

In conclusion, the CNS demyelination caused by MHV infection of rats and mice is an extremely complex phenomenon which is most likely controlled by a wide range of factors. It has been nearly 50 years since JHMV was first isolated, and much remains to be learned about the mechanisms of demyelination caused by mouse hepatitis virus. Viral proteins clearly play an important role in cell tropism and viral spread through the CNS, but much remains to be learned about the molecular mechanisms involved. The role of the immune system and inflammatory mediators in the pathogenesis of demyelination is still the subject of much controversy, which may be resolved with the aid of recent technological advances in molecular immunology such as the development of new reagents and mouse strains with defined immunological defects. Improved understanding of MHV pathogenesis will prove invaluable to our comprehension of host-virus interactions in the unique microenvironment of the CNS. As the processes involved in demyelination in MHV-infected rodents come into focus, they may provide insights into the pathogenesis and treatment of human demyelinating diseases, including multiple sclerosis.

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