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Review

Murine hepatitis virus—A model for virus-induced CNS demyelination

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Most murine hepatitis virus (MHV) strains, as their name suggests, infect the liver. However, several murine strains are tropic for the central nervous system (CNS) and cause encephalitis with subsequent CNS demyelination. The CNS demyelination shares pathological similarities with human CNS demyelinating diseases such as multiple sclerosis (MS). These viruses are, therefore, used to study the role of the immune system in viral clearance from the CNS, in CNS demyelination, and in remyelination. Nevertheless, it is still unclear exactly how MHV induces demyelination and to what extent the immune system plays a role in this pathology. Here we review this field in the context of the immune response to MHV in the liver and the CNS focusing on studies that have been published in the past 5 years. *Journal of NeuroVirology* (2002) **8,** 76–85.

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Murine hepatitis virus: The encephalitogenic murine coronavirus

Viral cell entry

Murine hepatitis virus (MHV) is a positive-strand RNA coronavirus with a genome of approximately 31 kilobases (Holmes and Lai, 1996). The two strains of MHV that have been used in the majority of studies of MHV infection in the CNS are MHV-A59 and JHM (MHV-4). These strains both infect mice; MHV-JHM can also infect rats and primates (Murray *et al*, 1992). MHV-A59 has approximately a 750-fold higher LD_{50} (3,000–5,000 pfu) (Lavi *et al*, 1986) than MHV-JHM (1–10 pfu) when given intracerebrally (i.c.). Some attenuated variants of MHV-JHM are less lethal and cause minimal encephalitis (Wang *et al*, 1992). Both viral strains express antigen primarily within specific areas of the brain, approximated by the limbic system, but MHV-JHM antigen is also found loosely scattered through the surrounding parenchyma (Lavi *et al*, 1990; Sun and Perlman, 1995). The age of mice at the time of MHV infection can affect the outcome with increased viral replication and demyelination in weanling mice compared to adult mice (Weiner, 1973). Most studies are performed on 4- to 6-week-old mice.

Both MHV-A59 and JHM infect, to some extent, oligodendrocytes, astrocytes (Lavi *et al*, 1987; Stohlman *et al*, 1995a), neurons (Parra *et al*, 1997; Lavi *et al*, 1999), hepatocytes (Weiner, 1973; Navas *et al*, 2001), macrophages, including Kuppfer cells (Even *et al*, 1995; Stohlman *et al*, 1995a), thymic epithelial cells (Knobler and Oldstone, 1987; Godfraind *et al*, 1995), and the endothelial cells which line blood vessels (Joseph *et al*, 1995; Lavi *et al*, 1999). MHV-A59 has also been shown to infect B cells (Coutelier *et al*, 1994), and MHV-JHM has been detected in ependymal cells (Stohlman *et al*, 1995a). Except for glial cells and neurons, these cell populations closely match those demonstrated to express the known MHV receptor Bgp1a (biliary glycoprotein 1a), a member of the carcinoembryonic antigen (CEA) family, which in turn is a member of the immunoglobulin superfamily (Williams *et al*, 1991). The Bgp1a receptor has been identified on hepatocytes, macrophages, epithelial cells (such as those in the intestine), endothelial cells, B cells, and a small proportion of thymic epithelial cells (Williams *et al*, 1991). A major determinant of tropism is the spike (S) protein (Phillips *et al*, 1999; Navas *et al*, 2001), suggesting that variations in MHV receptor (such as posttranslational modifications or expression of different isoforms) may affect recognition by different

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viruses. Expression of Bgp1a is very low in the CNS, and the question of how virus enters glial cells and neurons is still open.

Spread and kinetics of MHV infection

Infectious MHV can be found in astrocytes, oligodendrocytes, and neurons after acute infection (Sun and Perlman, 1995). Late after infection, virus is detectable only as low levels of antigen or RNA (Lavi *et al*, 1984b). In animals with viral persistence due to the absence of IFN-γ, antigen is detected mainly in oligodendrocytes (Parra *et al*, 1999). In mice infected in the presence of protective maternal antibody, antigen persists in astrocytes as well as other unidentified cells (Perlman and Ries, 1987). These studies demonstrate that virus can persist in glial cells.

After intracerebral (i.c.) or intranasal (i.n.) infection with MHV-A59, virus enters the brain and causes encephalitis (Lavi *et al*, 1986). Intranasal infection with MHV-JHM or -A59 leads to viral invasion through the olfactory bulb and along olfactory tracts, as well as a slower approach along the trigeminal nerve to the mesencephalic nucleus (Lavi *et al*, 1986; Perlman *et al*, 1989). Early spread of MHV-JHM and -A59 (up to 4 d.p.i.) appears to be along specific neural pathways in the CNS (Perlman *et al*, 1989) and, therefore, through neural connections. Later widespread infection can be blocked by passive transfer of antibody, suggesting spread through the blood, cerebrospinal fluid, and/or interstitial fluid (Perlman *et al*, 1989). Liver infection can occur after any route of infection (i.n., i.c., i.g., or i.p.) with MHV-A59 (Lavi *et al*, 1986). MHV-A59 liver titers peak 5 dpi after i.c. infection (Lavi *et al*, 1984b) and hepatitis develops during the first 1 to 2 weeks (Lavi *et al*, 1999). MHV-JHM replicates transiently in the lungs, blood, and cervical lymph nodes after i.n. infection, and occasionally is found in the thymus and bone marrow (Barthold and Smith, 1992).

As would be expected in an infection that comes under immunological control, viral titers peak around 5 d.p.i. in the brain (Parra *et al*, 1997), slightly later in the spinal cord (Matthews *et al*, 2001), and virus is cleared by 8–20 d.p.i. (Sutherland *et al*, 1997). The kinetics of viral antigen expression follow a pattern similar to that observed of viral titers during acute infection (Wang *et al*, 1992), but viral antigen is still detectable up to 30 d.p.i. (Lavi *et al*, 1984a). Viral RNA is detectable in the brain as late as 10–12 months postinfection, although the amount of RNA decreases with time (Lavi *et al*, 1984a). However, immunosuppression of cell-mediated immunity by drugs, radiation, or T-cell depletion at late time points after infection does not result in viral recrudescence from this RNA pool (Stohlman and Weiner, 1981).

The immune response to MHV in the CNS

The blood–brain barrier is a major factor of the CNS that limits immune function by preventing the easy entry of serum antibodies and infectious organisms in the absence of CNS inflammation (Cserr *et al*, 1992). Although it has been thought that the blood–brain barrier allows the entry of only activated T cells, a recent study demonstrated that naive T cells can enter the CNS if there are few activated T cells in circulation (Brabb *et al*, 2000).

It has been suggested that immunosuppressive cytokines such as transforming growth factor-beta, alpha-melanocyte stimulating hormone, and interleukin-10 (IL-10) may limit or alter immune function within the CNS (Gordon *et al*, 1998; Lipton and Gonzales-Scarano, 1978). Work with T cells responding to Sindbis virus infection of the CNS demonstrated that T cells lose their ability to proliferate and down-regulate IL-2 production after entering the brain, supporting the hypothesis that the environment of the CNS alters immune function (Irani *et al*, 1997). In addition, the brain fosters viral antigen or nucleic acid persistence, which is not observed in most peripheral organs and seems to be attributable at least in some instances to inhibited immune-mediated clearance (Kristensson and Norrby, 1986). The poor regenerative potential of neurons and the sensitivity of the CNS to small lesions may have contributed to the development of a CNS environment which downregulates the more destructive aspects of the immune response.

The cells of the immune system successfully enter the CNS after MHV infection. Mononuclear cells of bone marrow origin peak 7 d.p.i. They then decline, even in animals that do not control the infection (Williamson *et al*, 1991). In the absence of T cells in nude mice, infectious MHV-JHM is not cleared with normal kinetics (Houtman and Fleming, 1996), demonstrating that T cells are important for clearance of MHV.

$CD8^+$ T cell response to MHV

$CD8^+$ T cells are critical for the early control of MHV infection in the CNS and for prevention of mortality (Gombold *et al*, 1995; Stohlman *et al*, 1995b; Sutherland *et al*, 1997). Virus-specific $CD8^+$ T cells peak 7 days after MHV infection in the brain (Williamson *et al*, 1991). The ability to detect MHV specific $CD8^+$ T cells in the draining cervical lymph nodes (CLN) or the spleen varies (Bergmann *et al*, 1999; Parra *et al*, 1999). This variability may be due to differences in the specific injection sites. Virus delivered to the parenchyma results in a delayed peripheral immune response compared to virus delivered to the ventricles and cerebrospinal fluid (Stevenson *et al*, 1997).

$CD8^+$ T cells recognize peptides presented on cell surface MHC class I molecules. Although neurons do not express MHC class I and therefore are not anticipated targets of $CD8^+$ T cells, MHC class I is present on endothelial cells and microglia (Horwitz *et al*, 1999). In addition, class I expression can be induced in astrocytes and oligodendrocytes in culture if

exposed to factors secreted by cells infected with MHV-A59 (Suzumura *et al*, 1986). After MHV-A59 infection of $CD8^+$ T-cell-deficient mice, viral antigen clearance was delayed in microglia, lymphocytes, endothelial cells, and meningeal cells but not in neurons or glia (Lavi *et al*, 1999). In another study, transfer of $CD8^+$ T cells suppressed MHV-JHM antigen expression in astrocytes and microglia but not oligodendrocytes (Stohlman *et al*, 1995a).

$CD8^+$ T cells can function through the lytic molecule perforin, through apoptosis induced by Fas ligand (FasL), or by secreting cytokines such as IFN-gamma and TNF-alpha (Kajino *et al*, 1998). All of these mechanisms may be productively used in the immune response to MHV. In the CNS of mice deficient for perforin, viral clearance is delayed, thereby implicating a role for perforin in viral control (Lin *et al*, 1997). Efficient viral clearance in Fas-deficient mice demonstrated that FasL-mediated lysis is not required for viral clearance. Nonetheless, mice lacking both FasL-mediated apoptosis and perforin have delayed clearance compared to mice just lacking perforin, suggesting that Fas-FasL interactions can contribute to viral control although they may not be very important (Parra *et al*, 2000).

Both $CD4^+$ and $CD8^+$ T cells persist in the CNS for up to 34 d.p.i., with $CD8^+$ T cells being more prominent in the parenchyma both early and late after infection (Stohlman *et al*, 1998a). The number of $CD8^+$ T cells decreases with time and decreasing viral RNA (Bergmann *et al*, 1999; Marten *et al*, 2000). At 45 d.p.i., about 48% of these cells are still virus-specific, similar to the percentage of virus-specific T cells observed during acute infection. Many of these chronically present cells secrete IFN-gamma, but cytolytic activity is gradually lost (Bergmann *et al*, 1999).

$CD4^+$ T cell response to MHV

Although activated, MHV-specific, $CD8^+$ T cells transferred to infected mice can control viral replication, they are not sufficient to clear infectious virus from oligodendrocytes (Stohlman *et al*, 1998b). The presence of $CD4^+$ T cells is necessary for transferred $CD8^+$ T cell protection. Indeed, these cells appear to be important for $CD8^+$ T-cell lytic activity and survival in the CNS, perhaps through the secretion of IL-2 (Stohlman *et al*, 1998b). Virally activated $CD4^+$ T cells protect against lethal viral infections after transfer, but as with transferred $CD8^+$ T cells, they are not sufficient to clear the virus (Stohlman *et al*, 1986). Depletion of either $CD8^+$ or $CD4^+$ T cells results in the inability of mice to control acute viral titers in the brain (Williamson *et al*, 1990; Sutherland *et al*, 1997). Thus, both $CD4^+$ and $CD8^+$ T cells are important for control of acute viral infection in the CNS.

Unlike $CD8^+$ T cells, which accumulate in the parenchyma, $CD4^+$ T cells tend to accumulate perivascularly and in subarachnoid spaces (Stohlman *et al*, 1998a), locations rich in MHC class II positive macrophages and microglia (Hickey and Kimura, 1988; Braun *et al*, 1993). Interestingly, $CD4^+$ T cell numbers peak in the CNS at 9 d.p.i., a little later than $CD8^+$ T cells (Williamson *et al*, 1991), and persist in the CNS after viral clearance (Bergmann *et al*, 1999; Marten *et al*, 2000).

Cytokine response to MHV

The effector function of many lymphocytes involves the production and secretion of cytokines. mRNA from a wide number of inflammatory cytokines can be detected in the brain 3–7 days following i.c. infection with MHV-JHM. These include interferon (IFN) alpha and beta (Wang *et al*, 1998), interleukin (IL)-1 alpha, IL-1 beta, IL-6, IL-2, and TNF-alpha (Parra *et al*, 1997). Immunofluorescence of MHV-JHM infected spinal cords demonstrated the presence of TNF-alpha protein as well as IL-1 beta and IL-6 in cells with the morphology of macrophages early after infection and in uninfected astrocytes late after infection (Sun *et al*, 1995).

Both Th1 (IFN-gamma) and Th2 (IL-4, IL-5, and IL-10) cytokine mRNAs increase after infection with various strains of MHV, peaking 7 to 9 days after MHV-JHM infection (Stohlman *et al*, 1995a; Parra *et al*, 1997). In several studies MHV infection resulted in a predominantly IgG2a antibody isotype profile (Coutelier *et al*, 1987; Fleming *et al*, 1989) supporting the idea of a predominantly Th1 response, although in another study IgG2a did not predominate over IgG1 (Parra *et al*, 1997). These data, combined with the presence of both Th1 and Th2 cytokine mRNAs, suggest that neither T-helper cell type necessarily predominates, although a Th1 profile is slightly more common. MHV-JHM infection of mice deficient for IFN-gamma revealed that IFN-gamma is not required for optimal antibody production or for CTL responses. IFN-gamma is, however, necessary for clearance of virus from the oligodendrocytes (Parra *et al*, 1999). Clearance of an MHV strain that primarily infects neurons is delayed in the absence of IFN-gamma, suggesting that it may affect viral control in other cell types as well without being absolutely required for clearance (Lane *et al*, 1997). In mice deficient for IL-10, MHV-JHM causes a transient increase in viral titers 7 d.p.i. and greater mortality than in wild-type mice despite normal kinetics of viral clearance, which is thought to be due to an increase in inflammatory cytokines (Lin *et al*, 1998).

B cell response to MHV

The role of B cells in the control of MHV has been studied in rats and mice. In Lewis rats, the transfer of B cells into irradiated recipients decreased MHV-JHM titers in the CNS (Schwender *et al*, 1999). IgM, the first detectable antibody isotype, first appears 6 d.p.i. with MHV-JHM i.c. in mice (Stohlman *et al*, 1986; Williamson *et al*, 1991). Neutralizing antibodies are detected in the serum by 9 d.p.i. with MHV-JHM given i.c., but were not sufficient to control viral titers within 11 d.p.i. in the absence of $CD8^+$ T

cells (Lin *et al*, 1997). Similarly, after MHV-A59 infection, serum antibody is detectable 7 d.p.i. and reaches maximal levels 14–56 d.p.i. (Lavi *et al*, 1984b). By electron microscopy, plasma cells can be observed in the CNS as late as 90 d.p.i. with MHV-JHM (Stohlman and Weiner, 1981). Antibody mostly of isotype IgG2a is detected after infection (Lardans *et al*, 1996), although sometimes IgG2a and IgG1 eventually reach equivalent levels (Parra *et al*, 1997). Transfer of virus-specific antibodies increases survival, presumably by decreasing peak viral levels, delaying neuronal infection, and/or decreasing neuronal infection (Fleming *et al*, 1989; Yokomori *et al*, 1992).

The contribution of humoral immunity to viral clearance and persistent infection in the CNS has been investigated using mice deficient in secreted antibodies or B cells (Lin *et al*, 1999; Bergmann *et al*, 2001; Matthews *et al*, 2001). Mice homozygous for disruption of the Ig mu gene (muMT mice) lack B cells and develop acute disease in the CNS with similar kinetics and severity to wild-type C57BL/6 mice after MHV-JHM infection (Lin *et al*, 1999) or MHV-A59 infection (Matthews *et al*, 2001). Viral clearance during acute infection is similar in both groups. However, although virus is quickly cleared from the CNS of wild-type mice, it reemerges and persists in the CNS of muMT mice. B-cell-deficient mice have been shown to have impaired T cell immunity against a number of viral infections including MHV-JHM (Bergmann *et al*, 2001), possibly due to their absence as antigen-presenting cells in these mice. To clarify how B cells mediate viral clearance in the CNS, we compared the role of B cells as antigen-presenting cells, using mice with B cells that are unable to secrete antibody, with that of secreted antibody, using muMT mice reconstituted with MHV-A59 immune immunoglobulin (Matthews *et al*, 2001). muMT mice that received A59-specific antibody had decreased virus in the CNS, whereas mice with B cells deficient in antibody secretion did not clear virus from the CNS. These data suggest a major role for immune antibody in controlling virus replication in the CNS.

Nonlymphocytic immune response to MHV

Natural killer (NK) cells (asialoGM1$^+$ cells) peak in the CNS at 7 days after i.c. inoculation with MHV-JHM. The 7 d.p.i. CNS mononuclear cells are actively cytotoxic on the classic NK target cell line YAC-1 when assayed *ex vivo* (Williamson *et al*, 1991). In the absence of T cells, a transient decrease in viral titers 5 to 7 d.p.i. has been attributed to the NK cell response (Williamson *et al*, 1990). After immunosuppression by cyclophosphamide, in mice infected with a high dose of MHV-JHM, death occurs more quickly (3 d.p.i.) than in untreated mice (6–7 d.p.i.) (Weiner, 1973). This observation suggests that the innate immune response that expands in response to infection, such as the NK cells, can be important in the early control of viral infection.

Macrophages are susceptible to infection by MHV (Wijburg *et al*, 1996), although only a subset is infected initially. It has been proposed that CD4$^+$ T cells are necessary as a source of RANTES to attract monocyte/macrophage infiltration of the CNS (Lane *et al*, 2000). The role of macrophages in the immune response is difficult to pinpoint. Depletion of blood-borne macrophages had little effect on CNS demyelination (Xue *et al*, 1999). On the other hand, depletion of macrophages before i.v. infection with a highly lethal isolate of MHV-A59 resulted in earlier mortality (3–5 d.p.i.) associated with increased viral titers in the spleen and liver (Wijburg *et al*, 1997). Macrophage depletion before i.n. infection with MHV-JHM also resulted in rapid death (Xue *et al*, 1999). The early time point of mortality suggests that macrophages help control viral titers before the T and B cell populations are fully activated and effective.

Immune response to MHV in the liver

The immune response that controls MHV infection in the liver is similar in some ways to that important in the CNS. Pre-existing antibody can protect from lethal liver infections and decrease liver lesions, so antibody is capable of controlling liver viral titers (Buchmeier *et al*, 1984). However in contrast to the importance of antibody in controlling MHV-A59 replication in the CNS, antibody is not required for viral clearance from the liver. In muMT mice, which lack mature B lymphocytes, and in IgM-Tg mice with B cells that do not secrete antibody, infectious virus was cleared from the livers with similar kinetics to wild-type mice after i.c. or i.h. infection (Matthews *et al*, 2001).

Cell-mediated immunity is required for the control of infection in the liver. Depletion of CD4$^+$ or CD8$^+$ T cells has been shown to result in increased MHV-JHM titers in the liver and prolonged accumulation of infectious virus to 5 d.p.i. When both T cell populations were depleted, viral titers were not controlled within 7 d.p.i., although the mice did subsequently recover (Kyuwa *et al*, 1996). These data suggest that T cells play a role in controlling viral titers, even before 5 d.p.i. Beta2-microglobulin-deficient mice, which lack CD8$^+$ T cells, have delayed clearance of virus from the liver (Lavi *et al*, 1999). IFN-gamma-deficient mice experience greater mortality than do wild-type mice after i.p. infection with MHV-JHM or MHV-A59 (Kyuwa *et al*, 1998; Schijns *et al*, 1996). Virus persists up to 48 d.p.i. in the liver and large lesions develop in these mice, although there is no chronic increase in liver enzyme activity indicative of loss of liver function. Therefore, IFN-gamma is critical for viral control in the liver. In the absence of IFN-gamma, CD8$^+$ T cell depletion further increased the severity of disease (Kyuwa *et al*, 1998). Therefore, CD8$^+$ T cell functions other than

IFN-gamma production are also important for viral control.

MHV-induced CNS demyelination

CNS demyelination develops as active MHV-A59 or MHV-JHM infection resolves. These lesions are histologically very similar to those observed in MS patients. CNS lesions are predominantly created by primary demyelination as evidenced by the intact, naked axons present in these lesions, although axonal damage is also detected (Lavi *et al*, 1986). The peripheral nervous system is not affected (Lampert *et al*, 1973). Lesions are scattered randomly throughout the spinal cord (Lampert, 1978). Chronic lesions are associated with lipid-laden macrophages (presumably full of myelin), scattered lymphocytes, and naked axons. Viral particles are not detected. Astrocytic reactions and perivascular cuffing are also sometimes found in lesions (Weiner, 1973; Stohlman and Weiner, 1981). Chronic lesions can persist as late as 90 d.p.i. (Stohlman and Weiner, 1981), and scattered demyelinated axons can be detected even 16 months after infection (Herndon *et al*, 1975). Early demyelination after high-dose, lethal MHV-JHM infection is associated with polymorphonuclear cells, mononuclear cells, and extracellular myelin debris. Degenerating axons are sometimes detectable (Lampert *et al*, 1973). Nevertheless, even in necrotic lesions formed during lethal infections, some axonal preservation is observed (Weiner, 1973).

Chronic disease in MHV-infected animals is characterized by a single major episode of demyelination associated with the development of ataxia, hind limb paresis, and paralysis (Lavi *et al*, 1984b), following which animals usually recover. Recovery is mediated by CNS remyelination, sometimes accompanied by peripheral nervous system remyelination occurring in the CNS (Takahashi *et al*, 1987). Remyelination has been reported to begin anywhere from 14 to 70 d.p.i. (Kristensson and Norrby, 1986; Takahashi *et al*, 1987). As early as 2 to 3 weeks postinfection, there is a dramatic increase in MBP mRNA at the edges of lesions suggestive of early remyelination before histological detection (Kristensson and Norrby, 1986). At 28 d.p.i., replicating oligodendrocytes were detectable near lesions (Herndon *et al*, 1977). It is thought that remyelination involves oligodendrocyte precursor O-2A cell proliferation (Godfraind *et al*, 1989).

How does CNS demyelination occur? This is perhaps the major question posed with the MHV model of MS. Neither acute encephalitis nor RNA persistence is sufficient to cause demyelination, as demonstrated with two mutants of MHV-A59 (Leparc-Goffart *et al*, 1998; Das Sarma *et al*, 2000). Infectious virus does not have to be successfully cleared for demyelination to occur (Houtman and Fleming, 1996). Transfer of $CD8^+$ T cells during acute infection results in no subsequent demyelination, although whether this treatment changes viral RNA persistence is unclear (Stohlman *et al*, 1995a). Early MHV-JHM-induced demyelination is rare in immunodeficient transgenic SCID and RAG1 knockout mice or irradiated mice, demonstrating a strong if not absolute requirement for lymphocytes (Houtman and Fleming, 1996; Wu and Perlman, 1999).

Electron micrographs of demyelinating lesions demonstrate that macrophage processes slip between layers in the myelin sheath, suggesting that macrophages could be the direct mediators of demyelination (Powell and Lampert, 1975). Macrophages localize to demyelinating lesions, and their appearance correlates with the development of lesions. They do not appear in great numbers in the absence of lymphocytes, consistent with the observation that lymphocytes are important for demyelination (Wu and Perlman, 1999; Lane *et al*, 2000). Despite the visual evidence that macrophages invade myelin sheaths, it is possible that macrophages could be localizing to areas of demyelination to clean up the damaged myelin caused by a nonmacrophage dependent mechanism. Depletion of blood-borne macrophages does not affect the severity of demyelination, although similar techniques do affect demyelination in EAE and Theiler's virus-induced demyelination (Huitinga *et al*, 1995; Rossi *et al*, 1997; Xue *et al*, 1999). However, macrophages that are assumed to be derived from microglia or perivascular macrophages are present in the CNS of these depleted mice and may still play a role beyond that of scavenging for disintegrating myelin (Xue *et al*, 1999).

T cells could mediate demyelination through direct lysis of oligodendrocytes or through inflammation mediated by $CD4^+$ T cell-dependent inflammation as demonstrated in experimental autoimmune encephalomyelitis. Certainly T cells persist in the CNS after virus is cleared and during active demyelination (Marten *et al*, 2000). There is a significant amount of data demonstrating that neither $CD4^+$ nor $CD8^+$ T cells are specifically required for demyelination. Demyelination occurs during acute infection of T cell-deficient nude mice. Beta-2-microglobulin-deficient mice cannot express MHC class I and therefore do not develop mature $CD8^+$ T cells. I-A^b-deficient mice lack MHC class II and therefore do not develop mature $CD4^+$ T cells. Both of these strains of T cell-deficient mice demonstrate early MHV-JHM induced demyelination (Houtman and Fleming, 1996). Infection with MHV-A59 of beta-2 microglobulin-deficient mice and mice efficiently depleted of CD8+ T cells also results in demyelination, although less frequently than in wild-type mice (Gombold *et al*, 1995; Sutherland *et al*, 1997; Lavi *et al*, 1999).

Partial depletion of $CD4^+$ and $CD8^+$ T cells does not inhibit chronic demyelination either, although in this study T cells were not depleted until 7 d.p.i. (Sutherland *et al*, 1997). A few studies suggested that the absence of $CD4^+$ or $CD8^+$ T cells resulted in deficient demyelination. $CD4^+$ or $CD8^+$ T cell-deficient

mice infected with MHV-JHM were less likely to develop acute demyelination, but the mice that did develop demyelination had similar severity to that observed in wild-type mice (Houtman and Fleming, 1996). $CD4^+$ T cell-deficient mice infected with MHV-JHM developed acute and chronic demyelination, but it was significantly less severe than that seen in CD8 deficient or wild-type mice (Lane *et al*, 2000). When splenocytes were transferred to immunodeficient RAG1 knockout mice, either $CD4^+$ or $CD8^+$ T cells were sufficient to generate significant levels of acute demyelination, although $CD4^+$ T cells were less efficient at inducing demyelination than $CD8^+$ cells (Wu *et al*, 2000). These data suggest that each of these T-cell subpopulations has the capability to mediate CNS demyelination after MHV infection and may induce slightly different levels or pathways of demyelination. On the other hand we have observed (Matthews *et al*, 2002) moderately sized, typical demyelinating lesions as well as atypical small round necrotic lesions in MHV-A59 infected RAG1 knockout mice, suggesting that, although T cells may be required for robust demyelination, other mechanisms must also be at work.

Knockout mice have been used to demonstrate that the mechanism by which demyelination takes place requires neither perforin nor Fas-FasL interactions (Lin *et al*, 1997; Parra *et al*, 2000). Furthermore, removing TNF-alpha, IL-10, or IFN-gamma through depletion or the use of genetically deficient mice did not decrease demyelination (Stohlman *et al*, 1995b; Lin *et al*, 1998; Parra *et al*, 1999). Chemokines, however, may play a role in demyelination. Gene expression for a number of chemokines has been found in the CNS of mice undergoing chronic demyelination (Lane *et al*, 1998). Of these the C-C chemokine RANTES has been shown to possibly play a role in MHV-induced demyelination (Lane *et al*, 2000). Systemic depletion of RANTES with RANTES-specific antisera resulted in a significant reduction in macrophage infiltration and demyelination in C57BL/6 mice infected with MHV-4. RANTES is a pro-inflammatory chemokine produced by T cells, platelets, and endothelia that acts as a chemoattractant for a variety of lymphocytic and myeloid cell types including monocytes and granulocytes. Lane and colleagues believe that the reduced demyelination observed in MHV-infected $CD4^{-/-}$ mice may be due to the absence of $CD4^+$ T cell-derived RANTES (Lane *et al*, 2000). Thus, RANTES produced by $CD4^+$ T cells may attract macrophages into the CNS during viral infection. Activated macrophages are a potent source of toxic nitric oxide intermediates produced by inducible nitric oxide synthases (iNOS). Inhibition of one of these, NOS-2, has been shown to reduce MHV-induced demyelination (Lane and Buchmeier, 1999), providing further evidence for a role for pro-inflammatory innate immune mechanisms in CNS demyelination in this mouse model. Among the CXC chemokines expressed in the CNS during MHV-induced demyelinating disease is IFN-inducible protein 10 (IP-10), suggesting a possible role for this chemokine in demyelination (Lane *et al*, 1998). However, IP-10 has also been shown to be crucial for control of MHV viral replication and recovery from acute encephalitis in the C57BL/6 mouse (Liu *et al*, 2000). Administration of anti-IP-10 antibody led to a significant reduction in T cells infiltrating the CNS of MHV-infected mice, decreased levels of IFN-γ in the CNS and increased mortality of infected mice (Liu *et al*, 2000). Thus, although chemokine-mediated inflammatory responses in the CNS may contribute to demyelination, as with all immune response elements examined, they also play a vital role in viral clearance.

B cells and the antibodies they secrete could potentially also mediate demyelination. However, no correlation has been found in MHV-infected mice between antibody titers and severity of demyelination (Koolen *et al*, 1987). In some animal models of CNS demyelination, antibodies against self-antigens in the brain induce or exacerbate demyelination (Brehm *et al*, 1999; Genain *et al*, 1999) but there is no evidence for the induction of antibodies that recognize host proteins after MHV infection of the CNS. We have examined the role of B cells in demyelination using B cell-deficient muMT mice (Matthews *et al*, 2002). In infected B cell-deficient mice, robust demyelination not only occurred but was also statistically more severe than in wild-type animals 30 and 60 d.p.i. This increase in demyelination could be associated with the persistence of infectious virus in the absence of B cells. In mice lacking antibody Fc receptors or complement pathway activity, thereby lacking the ability to utilize antibody effector functions, virus did not persist yet demyelination was similar to that observed in wild-type mice. Antibody has also been suggested to facilitate remyelination. However, in muMT mice, remyelination was still detected. Therefore, we find no evidence that B cells are important for CNS demyelination, nor are they required for remyelination.

Another hypothesis that has been proposed is that MHV invades the oligodendrocytes or cells that provide critical functions to the oligodendrocyte and damages them directly, thereby causing oligodendrocyte death or retraction of the myelin sheath. Early work on MHV-JHM using electron microscopy and immunoflourescence demonstrated that the virus infects oligodendrocytes early and possibly late after infection, and thereby supported this hypothesis (Lampert *et al*, 1973; Powell and Lampert, 1975; Wu and Perlman, 1999). However, the degenerating oligodendrocytes detected during early paralysis rarely actually contained viral particles (Powell and Lampert, 1975). Viral particles were also detected in astrocytes 7 d.p.i., and virus could potentially damage oligodendrocytes by disrupting the function of these cells (Sun and Perlman, 1995). Interestingly, mice immunosuppressed using cyclophosphamide

with resultant loss of antibody production and decreased perivascular inflammation still displayed small demyelinating lesions (Weiner, 1973). Likewise, lipid-laden macrophages and occasional small lesions were detected in RAG1 knockout mice (Wu and Perlman, 1999), and SCID mice can develop demyelination, if rarely (Houtman and Fleming, 1996). Direct virally mediated demyelination is unlikely to be the sole cause of demyelination, however, because virus in the presence of T cells more reliably induces demyelinating lesions (Wang *et al*, 1992).

Conclusions

It is clear that demyelination after MHV infection is a complex issue. It is possible that both direct viral destruction and immune mediated destruction contribute to the development of demyelinating lesions. Past research has focused on dissecting the immune response to MHV in order to determine the role of single components in the demyelinating phase of the disease. However, in contrast to other mouse models of demyelination, there does not appear to be a clear role for any one particular lymphocytic or monocytic subset that mediates the demyelination. Rather, it appears that a balance of immune components may be required for viral clearance and that various immune and nonimmune pathways may mediate the subsequent demyelinating events. It is this balance that determines the level of encephalitis in acute infection and sets up the conditions that lead to demyelination. One model that would account for the involvement of a variety of immune elements in demyelination would associate CNS pathology with free radical production by inflammatory cells of the myeloid lineage. These may be attracted into the CNS by chemokines secreted by viral specific $CD4^+$ T cells. In this model cytokines secreted by $CD8^+$ T cells would assist in the state of activation of macrophages and astrocytes and therefore influence the level of CNS disease. Such a model for CNS pathology has been implicated for EAE (Koprowski *et al*, 1993; Lin *et al*, 1993) and Borna disease virus-induced CNS pathology (Hooper *et al*, 2001). Future studies are required to determine if this mechanism also prevails in MHV-induced demyelination.

In this review we have described studies in which modulating the immune response has resulted in perturbation of the balance between replication/spread of the virus and clearance with consequential changes in acute disease and demyelination. Knowledge of the contribution of different components of the disease would be useful in manipulating this balance so that robust viral clearance does not result in chronic CNS demyelination.

References

Barthold SW, Smith AL (1992). Viremic dissemination of mouse hepatitis virus-JHM following intranasal inoculation of mice. *Arch Virol* **122:** 35–44.

Bergmann CC, Altman JD, Hinton D, Stohlman SA (1999). Inverted immunodominance and impaired cytolytic function of $CD8^+$ T cells during viral persistence in the central nervous system. *J Immunol* **163:** 3379–3387.

Bergmann CC, Ramakrishna C, Kornacki M, Stohlman SA (2001). Impaired T cell immunity in B cell-deficient mice following viral central nervous system infection. *J Immunol* **167:** 1575–1583.

Brabb T, von Dassow P, Ordonez N, Schnabel B, Duke B, Goverman J (2000). *In situ* tolerance within the central nervous system as a mechanism for preventing autoimmunity. *J Exp Med* **192:** 871–880.

Braun JS, Kaissling B, Le Hir M, Zenker W (1993). Cellular components of the immune barrier in the spinal meninges and dorsal root ganglia of the normal rat: Immunohistochemical (MHC class II) and electron-microscopic observations. *Cell Tissue Res* **273:** 209–217.

Brehm U, Piddlesden SJ, Gardinier MV, Linington C (1999). Epitope specificity of demyelinating monoclonal autoantibodies directed against the human myelin oligodendrocyte glycoprotein (MOG). *J Neuroimmunol* **97:** 9–15.

Buchmeier MJ, Lewicki HA, Talbot PJ, Knobler RL (1984). Murine hepatitis virus-4 (strain JHM)-induced neurologic disease is modulated *in vivo* by monoclonal antibody. *Virology* **132:** 261–270.

Coutelier JP, Godfraind C, Dveksler GS, Wysocka M, Cardellichio CB, Noel H, Holmes KV (1994). B lymphocyte and macrophage expression of carcinoembryonic antigen-related adhesion molecules that serve as receptors for murine coronavirus. *Eur J Immunol* **24:** 1383–1390.

Coutelier JP, van der Logt JT, Heessen FW, Warnier G, Van Snick J (1987). IgG2a restriction of murine antibodies elicited by viral infections. *J Exp Med* **165:** 64–69.

Cserr HF, DePasquale M, Harling-Berg CJ, Park JT, Knopf PM (1992). Afferent and efferent arms of the humoral immune response to CSF-administered albumins in a rat model with normal blood–brain barrier permeability. *J Neuroimmunol* **41:** 195–202.

Das Sarma J, Fu L, Tsai JC, Weiss SR, Lavi E (2000). Demyelination determinants map to the spike glycoprotein gene of coronavirus mouse hepatitis virus. *J Virol* **74:** 9206–9213.

Even C, Rowland RR, Plagemann PG (1995). Mouse hepatitis virus infection of mice causes long-term depletion of lactate dehydrogenase-elevating virus-permissive macrophages and T lymphocyte alterations. *Virus Res* **39:** 355–364.

Fleming JO, Shubin RA, Sussman MA, Casteel N, Stohlman SA (1989). Monoclonal antibodies to the matrix (E1) glycoprotein of mouse hepatitis virus protect mice from encephalitis. *Virology* **168:** 162–167.

Genain CP, Cannella B, Hauser S, Raine CS (1999). Identification of autoantibodies associated with myelin damage in multiple sclerosis. *Nature Med* **5:** 170–175.

Godfraind C, Friedrich VL, Holmes KV, Dubois-Dalcq M (1989). *In vivo* analysis of glial cell phenotypes during a viral demyelinating disease in mice. *J Cell Biol* **109:** 2405–2416.

Godfraind C, Holmes KV, Coutelier JP (1995). Thymus involution induced by mouse hepatitis virus A59 in BALB/c mice. *J Virol* **69:** 6541–6547.

Gombold JL, Sutherland RM, Lavi E, Paterson Y, Weiss SR (1995). Mouse hepatitis virus A59-induced demyelination can occur in the absence of $CD8^+$ T cells. *Microb Pathog* **18:** 211–221.

Gordon LB, Nolan SC, Ksander BR, Knopf PM, Harling-Berg CJ (1998). Normal cerebrospinal fluid suppresses the in vitro development of cytotoxic T cells: Role of the brain microenvironment in CNS immune regulation. *J Neuroimmunol* **88:** 77–84.

Herndon RM, Griffin DE, McCormick U, Weiner LP (1975). Mouse hepatitis virus-induced recurrent demyelination. A preliminary report. *Arch Neurol* **32:** 32–35.

Herndon RM, Price DL, Weiner LP (1977). Regeneration of oligodendroglia during recovery from demyelinating disease. *Science* **195:** 693–694.

Hickey WF, Kimura H (1988). Perivascular microglial cells of the CNS are bone marrow-derived and present antigen *in vivo*. *Science* **239:** 290–292.

Holmes KV, Lai MCC (1996). *Coronaviridae*: The viruses and their replication. In *Fields virology*, V1. Fields BN, Knipe DM, Howley PM (eds). Lippincott-Raven: Philadelphia, pp 1075–1093.

Hooper DC, Kean RB, Scott GS, Spitsin SV, Mikheeva T, Morimoto K, Bette M, Rohrenbeck AM, Dietzschold B, Weihe E (2001). The central nervous system inflammatory response to neurotropic virus infection is peroxynitrite dependent. *J Immunol* **167:** 3470–3477.

Horwitz MS, Evans CF, Klier FG, Oldstone MB (1999). Detailed *in vivo* analysis of interferon-gamma induced major histocompatibility complex expression in the central nervous system: Astrocytes fail to express major histocompatibility complex class I and II molecules. *Lab Invest* **79:** 235–242.

Houtman JJ, Fleming JO (1996). Dissociation of demyelination and viral clearance in congenitally immunodeficient mice infected with murine coronavirus JHM. *J NeuroVirol* **2:** 101–110.

Huitinga I, Ruuls SR, Jung S, Van Rooijen N, Hartung HP, Dijkstra CD (1995). Macrophages in T cell line-mediated, demyelinating, and chronic relapsing experimental autoimmune encephalomyelitis in Lewis rats. *Clin Exp Immunol* **100:** 344–351.

Irani DN, Lin KI, Griffin DE (1997). Regulation of brain-derived T cells during acute central nervous system inflammation. *J Immunol* **158:** 2318–2326.

Joseph J, Kim R, Siebert K, Lublin FD, Offenbach C, Knobler RL (1995). Organ specific endothelial cell heterogeneity influences differential replication and cytopathogenicity of MHV-3 and MHV-4. Implications in viral tropism. *Adv Exp Med Biol* **380:** 43–50.

Kajino K, Kajino Y, Greene MI (1998). Fas- and perforin-independent mechanisms of cytotoxic T lymphocyte. *Immunol Res* **17:** 89–93.

Knobler RL, Lampert PW, Oldstone MB (1982). Virus persistence and recurring demyelination produced by a temperature-sensitive mutant of MHV-4. *Nature* **298:** 279–280.

Knobler RL, Oldstone, MB (1987). Infection and involution of mouse thymus by MHV-4. *Adv Exp Med Biol* **218:** 451–453.

Koolen MJ, Love S, Wouda W, Calafat J, Horzinek MC, van der Zeijst BA (1987). Induction of demyelination by a temperature-sensitive mutant of the coronavirus MHV-A59 is associated with restriction of viral replication in the brain. *J Gen Virol* **68:** 703–714.

Koprowski H, Zheng YM, Heber-Katz E, Fraser N, Rorke L, Fu ZF, Hanlon C, Dietzschold B (1993). *In vivo* expression of inducible nitric oxide synthase in experimentally induced neurologic diseases. *Proc Natl Acad Sci USA* **90:** 3024–3027.

Korner H, Schliephake A, Winter J, Zimprich F, Lassmann H, Sedgwick J, Siddell S, Wege H (1991). Nucleocapsid or spike protein-specific $CD4^+$ T lymphocytes protect against coronavirus-induced encephalomyelitis in the absence of $CD8^+$ T cells. *J Immunol* **147:** 2317–2323.

Kristensson K, Norrby E (1986). Persistence of RNA viruses in the central nervous system. *Annu Rev Microbiol* **40:** 159–184.

Kyuwa S, Machii K, Shibata S (1996). Role of $CD4^+$ and $CD8^+$ T cells in mouse hepatitis virus infection in mice. *Exp Anim* **45:** 81–83.

Lampert PW (1978). Autoimmune and virus induced demyelinating diseases. A review. *Am J Pathol* **91:** 176–208.

Lampert PW, Sims JK, Kniazeff AJ (1973). Mechanism of demyelination in JHM virus encephalomyelitis. Electron microscopic studies. *Acta Neuropathol (Berl)* **24:** 76–85.

Lane TE, Asensio VC, Yu N, Paoletti AD, Campbell IL, Buchmeier MJ (1998). Dynamic regulation of α- and β-chemokine expression in the central nervous system during mouse hepatitis virus-induced demyelinating disease. *J Immunol* **160:** 970–978.

Lane TE, Buchmeier MJ (1999). Inhibition of nitric oxide synthase-2 reduces the severity of mouse hepatitis virus-induced demyelination: Implications for NOS2/NO regulation of chemokine expression and inflammation. *J NeuroVirol* **5:** 48–54.

Lane TE, Liu MT, Chen BP, Asensio VC, Samawi RM, Paoletti AD, Campbell IL, Kunkel SL, Fox HS, Buchmeier MJ (2000). A central role for $CD4^+$ T cells and RANTES in virus-induced central nervous system inflammation and demyelination. *J Virol* **74:** 1415–1424.

Lane TE, Paoletti AD, Buchmeier MJ (1997). Disassociation between the *in vitro* and *in vivo* effects of nitric oxide on a neurotropic murine coronavirus. *J Virol* **71:** 2202–2210.

Lardans V, Godfraind C, van der Logt JT, Heessen WA, Gonzalez MD, Coutelier JP (1996). Polyclonal B lymphocyte activation induced by mouse hepatitis virus A59 infection. *J Gen Virol* **77:** 1005–1009.

Lavi E, Das Sarma J, Weiss SR (1999). Cellular reservoirs for coronavirus infection of the brain in beta2-microglobulin knockout mice. *Pathobiology* **67:** 75–83.

Lavi E, Gilden DH, Highkin MK, Weiss SR (1984a). Detection of MHV-A59 RNA by *in situ* hybridization. *Adv Exp Med Biol* **173:** 247–257.

Lavi E, Gilden DH, Highkin MK, Weiss SR (1986). The organ tropism of mouse hepatitis virus A59 in mice is dependent on dose and route of inoculation. *Lab Anim Sci* **36:** 130–135.

Lavi E, Gilden DH, Wroblewska Z, Rorke RB, Weiss SR (1984b). Experimental demyelination produced by the

A59 strain of mouse hepatitis virus. *Neurology* **34:** 597–603.

Lavi E, Murray EM, Makino S, Stohlman SA, Lai MM, Weiss SR (1990). Determinants of coronavirus MHV pathogenesis are localized to 3′ portions of the genome as determined by ribonucleic acid-ribonucleic acid recombination. *Lab Invest* **62:** 570–578.

Lavi E, Suzumura A, Hirayama M, Highkin MK, Dambach DM, Silberberg DH, Weiss SR (1987). Coronavirus mouse hepatitis virus (MHV)-A59 causes a persistent, productive infection in primary glial cell cultures. *Microb Pathog* **3:** 79–86.

Leparc-Goffart I, Hingley ST, Chua MM, Phillips J, Lavi E, Weiss SR (1998). Targeted recombination within the spike gene of murine coronavirus mouse hepatitis virus-A59: Q159 is a determinant of hepatotropism. *J Virol* **72:** 9628–9636.

Lin MT, Hinton DR, Marten NW, Bergmann CC, Stohlman SA (1999). Antibody prevents virus reactivation within the central nervous system. *J Immunol* **162:** 7358–7368.

Lin MT, Hinton DR, Parra B, Stohlman SA, van der Veen RC (1998). The role of IL-10 in mouse hepatitis virus-induced demyelinating encephalomyelitis. *Virology* **245:** 270–280.

Lin MT, Stohlman SA, Hinton DR (1997). Mouse hepatitis virus is cleared from the central nervous systems of mice lacking perforin-mediated cytolysis. *J Virol* **71:** 383–391.

Lin RF, Lin T-S, Tilton RG, Cross (1993). Nitric oxide localized in the spinal cords of mice with experimental allergic encephalomyelitis: An electroparamagnetic study. *J Exp Med* **178:** 643–648.

Lipton HL, Gonzalez-Scarano F (1978). Central nervous system immunity in mice infected with Theiler's virus. I. Local neutralizing antibody response. *J Infect Dis* **137:** 145–151.

Liu MT, Chen BP, Oertel P, Buchmeier MJ, Armstrong D, Hamilton TA, Lane TE (2000). Cutting edge: The T cell chemoattractant IFN-inducible protein 10 is essential in host defense against viral-induced neurologic disease. *J Immunol* **165:** 2327–2330.

Marten NW, Stohlman SA, Atkinson RD, Hinton DR, Fleming JO, Bergmann CC (2000). Contributions of $CD8^+$ T cells and viral spread to demyelinating disease. *J Immunol* **164:** 4080–4088.

Matthews AE, Lavi E, Weiss SR, Paterson Y (2002). Neither B cells nor T cells are required for CNS demyelination in mice persistently infected with MHV-A59. *J NeuroVirol*, in press.

Matthews AE, Weiss SR, Shlomchik M, Hannum LG, Gombold JL, Paterson Y (2001). Antibody is required for clearance of infectious murine hepatitis virus A59 from the CNS but not the liver. *J Immunol*, in press.

Murray RS, Cai GY, Hoel K, Zhang JY, Soike KF, Cabirac GF (1992). Coronavirus infects and causes demyelination in primate central nervous system. *Virology* **188:** 274–284.

Navas S, Seo SH, Chua MM, Sarma JD, Lavi E, Hingley ST, Weiss SR (2001). Murine coronavirus spike protein determines the ability of the virus to replicate in the liver and cause hepatitis. *J Virol* **75:** 2452–2457.

Pachuk CJ, Bredenbeek PJ, Zoltick PW, Spaan WJ, Weiss SR (1989). Molecular cloning of the gene encoding the putative polymerase of mouse hepatitis coronavirus, strain A59. *Virology* **171:** 141–148.

Parra B, Hinton DR, Lin MT, Cua DJ, Stohlman SA (1997). Kinetics of cytokine mRNA expression in the central nervous system following lethal and nonlethal coronavirus-induced acute encephalomyelitis. *Virology* **233:** 260–270.

Parra B, Hinton DR, Marten NW, Bergmann CC, Lin MT, Yang CS, Stohlman SA (1999). IFN-gamma is required for viral clearance from central nervous system oligodendroglia. *J Immunol* **162:** 1641–1647.

Parra, Lin MT, Stohlman SA, Bergmann CC, Atkinson R, Hinton DR (2000). Contributions of Fas-Fas ligand interactions to the pathogenesis of mouse hepatitis virus in the central nervous system. *J Virol* **74:** 2447–2450.

Perlman S, Jacobsen G, Afifi A (1989). Spread of a neurotropic murine coronavirus into the CNS via the trigeminal and olfactory nerves. *Virology* **170:** 556–560.

Perlman S, Ries D (1987). The astrocyte is a target cell in mice persistently infected with mouse hepatitis virus, strain JHM. *Microb Pathog* **3:** 309–314.

Phillips JJ, Chua MM, Lavi E, Weiss SR (1999). Pathogenesis of chimeric MHV-4/MHV-A59 recombinant viruses: The murine coronavirus spike protein is a major determinant of neurovirulence. *J Virol* **73:** 7752–7760.

Powell HC, Lampert PW (1975). Oligodendrocytes and their myelin-plasma membrane connections in JHM mouse hepatitis virus encephalomyelitis. *Lab Invest* **33:** 440–445.

Rossi CP, Delcroix M, Huitinga I, McAllister A, van Rooijen N, Claassen E, Brahic M (1997). Role of macrophages during Theiler's virus infection. *J Virol* **71:** 3336–3340.

Schijns VE, Wierda CM, van Hoeij M, Horzinek MC (1996). Exacerbated viral hepatitis in IFN-gamma receptor-deficient mice is not suppressed by IL-12. *J Immunol* **157:** 815–821.

Schwender S, Hein A, Imrich H, Czub S, Dorries R (1999). Modulation of acute coronavirus-induced encephalomyelitis in gamma-irradiated rats by transfer of naïve lymphocyte subsets before infection. *J NeuroVirol* **5:** 249–257.

Stevenson PG, Freeman S, Bangham CR, Hawke S (1997). Virus dissemination through the brain parenchyma without immunologic control. *J Immunol* **159:** 1876–1884.

Stohlman SA, Bergmann CC, Cua DJ, Lin MT, Ho S, Wei W, Hinton DR (1998a). Apoptosis of JHMV-specific CTL in the CNS in the absence of $CD4^+$ T cells. *Adv Exp Med Biol* **440:** 425–430.

Stohlman SA, Bergmann CC, Lin MT, Cua DJ, Hinton DR (1998b). CTL effector function within the central nervous system requires $CD4^+$ T cells. *J Immunol* **160:** 2896–2904.

Stohlman SA, Bergmann CC, van der Veen RC, Hinton DR (1995a). Mouse hepatitis virus-specific cytotoxic T lymphocytes protect from lethal infection without eliminating virus from the central nervous system. *J Virol* **69:** 684–694.

Stohlman SA, Hinton DR, Cua D, Dimacali E, Sensintaffar J, Hofman FM, Tahara SM, Yao Q (1995b). Tumor necrosis factor expression during mouse hepatitis virus-induced demyelinating encephalomyelitis. *J Virol* **69:** 5898–5903.

Stohlman SA, Matsushima GK, Casteel N, Weiner LP (1986). *In vivo* effects of coronavirus-specific T cell clones: DTH inducer cells prevent a lethal infection but do not inhibit virus replication. *J Immunol* **136:** 3052–3056.

Stohlman SA, Weiner LP (1981). Chronic central nervous system demyelination in mice after JHM virus infection. *Neurology* **31:** 38–44.

Sun N, Grzybicki D, Castro RF, Murphy S, Perlman S (1995). Activation of astrocytes in the spinal cord of mice chronically infected with a neurotropic coronavirus. *Virology* **213:** 482–493.

Sun N, Perlman S (1995). Spread of a neurotropic coronavirus to spinal cord white matter via neurons and astrocytes. *J Virol* **69:** 633–641.

Sutherland RM, Chua MM, Lavi E, Weiss SR, Paterson Y (1997). $CD4^+$ and $CD8^+$ T cells are not major effectors of mouse hepatitis virus A59-induced demyelinating disease. *J NeuroVirol* **3:** 225–228.

Suzumura A, Lavi E, Weiss SR, Silberberg DH (1986). Coronavirus infection induces H-2 antigen expression on oligodendrocytes and astrocytes. *Science* **232:** 991–993.

Takahashi K, Goto N, Matsubara Y, Fujiwara K (1987). Post inflammatory remyelination in the spinal cord of mice infected with mouse hepatitis virus, JHM strain. *Jpn J Exp Med* **57:** 145–151.

Wang FI, Hinton DR, Gilmore W, Trousdale MD, Fleming JO (1992). Sequential infection of glial cells by the murine hepatitis virus JHM strain (MHV-4) leads to a characteristic distribution of demyelination. *Lab Invest* **66:** 744–754.

Wang Q, Haluskey JA, Lavi E (1998). Coronavirus MHV-A59 causes upregulation of interferon-beta RNA in primary glial cell cultures. *Adv Exper Med Biol* **440:** 451–454.

Weiner LP (1973). Pathogenesis of demyelination induced by a mouse hepatitis. *Arch Neurol* **28:** 298–303.

Wijburg OL, Heemskerk MH, Boog CJ, Van Rooijen N (1997). Role of spleen macrophages in innate and acquired immune responses against mouse hepatitis virus strain A59. *Immunology* **92:** 252–258.

Wijburg OL, Heemskerk MH, Sanders A, Boog CJ, Van Rooijen N (1996). Role of virus-specific $CD4^+$ cytotoxic T cells in recovery from mouse hepatitis virus infection. *Immunology* **87:** 34–41.

Williams RK, Jiang GS, Holmes KV (1991). Receptor for mouse hepatitis virus is a member of the carcinoembryonic antigen family of glycoproteins. *Proc Natl Acad Sci USA* **88:** 5533–5536.

Williamson JSP, Stohlman SA (1990). Effective clearance of mouse hepatitis virus from the central nervous system requires both $CD4^+$ and $CD8^+$ T cells. *J Virol* **64:** 4589–4592.

Williamson JSP, Sykes KC, Stohlman SA (1991). Characterization of brain-infiltrating mononuclear cells during infection with mouse hepatitis virus strain JHM. *J Neuroimmunol* **32:** 199–207.

Wu, GF, Dandekar AA, Pewe L, Perlman S (2000). CD4 and CD8 T cells have redundant but not identical roles in virus-induced demyelination. *J Immunol* **165:** 2278–2286.

Wu GF, Perlman S (1999). Macrophage infiltration, but not apoptosis, is correlated with immune-mediated demyelination following murine infection with a neurotropic coronavirus. *J Virol* **73:** 8771–8780.

Xue S, Sun N, Van Rooijen N, Perlman S (1999). Depletion of blood-borne macrophages does not reduce demyelination in mice infected with a neurotropic coronavirus. *J Virol* **73:** 6327–6334.

Yokomori K, Baker SC, Stohlman SA, Lai MM (1992). Hemagglutinin-esterase-specific monoclonal antibodies alter the neuropathogenicity of mouse hepatitis virus. *J Virol* **66:** 2865–2874.