[Frontiers in Bioscience 13, 4393-4406, May 1, 2008]

Mouse hepatitis virus infection of the CNS: a model for defense, disease, and repair

Chris S. Schaumburg[1,2], Katherine S. Held[1], Thomas E. Lane[1,2,3]

[1]Department of Molecular Biology and Biochemistry, University of California, Irvine 92697-3900, [2]Sue and Bill Gross Stem Cell Research Center University of California, Irvine 92697-3900, [3]Center for Immunology, University of California, Irvine 92697-3900

TABLE OF CONTENTS

1. ABSTRACT

Viral infection of the central nervous system (CNS) results in varied outcomes ranging from encephalitis, paralytic poliomyelitis or other serious consequences. One of the principal factors that directs the outcome of infection is the localized innate immune response, which is proceeded by the adaptive immune response against the invading viral pathogen. The role of the immune system is to contain and control the spread of virus within the CNS, and paradoxically, this response may also be pathological. Studies with a neurotropic murine coronavirus, mouse hepatitis virus (MHV) have provided important insights into how the immune system combats neuroinvasive viruses, and have identified molecular and cellular mechanisms contributing to chronic disease in persistently infected mice.

2. INTRODUCTION

MHV is a member of the *Coronaviridae* family, which represents a ubiquitous group of positive-strand RNA viral pathogens of humans and animals associated with a wide-spectrum of respiratory, gastrointestinal, and neurological diseases (1-4). All coronaviruses are enveloped with the largest known RNA genome identified (27-31 kb). Human coronavirus (HCoV) infections cause acute enteritis and a significant percentage (up to 34%) of all common colds; and it is important to note that a new strain of HCoV also had dramatic impact on human disease as the etiological agent of severe acute respiratory syndrome (SARS) (4-6). In addition, previously unclassified human coronaviruses associated with respiratory disease have been identified (7-9). As a natural pathogen of mice, MHV primarily infects the liver and

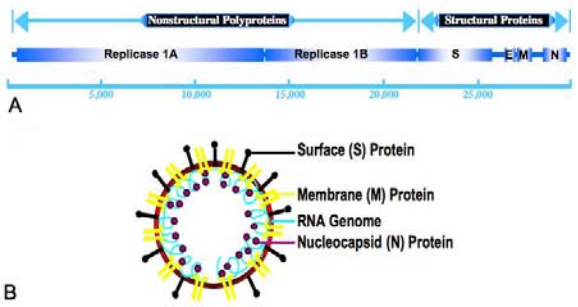

Figure 1. Genome organization and structural features of MHV. (A) Nonstructural and structural encoding ORFs are indicated. Upon infection of host cell, translation of the positive-strand RNA genome gives rise to a large polyprotein that undergoes proteolytic processing to generate an RNA-dependent RNA polymerase from which a full-length negative-strand template is generated. Sub-genomic mRNAs (monocistronic) are generated presumably from subgenomic negative-strand templates. (B) Overview of MHV structure with structural (S, M and N) indicated.

CNS resulting in a range of acute and chronic diseases, including hepatitis, encephalitis and encephalomyelitis associated with demyelination (1-3). Viral tropism and disease depends on a variety of factors, such as the strain of the virus, genetic background and age of mouse, as well as the route of infection (3).

The genome and structure of MHV is characteristic of other coronaviruses with similar organization of open reading frames (ORFs) encoding structural and non-structural proteins (Figure 1). Viral replication occurs exclusively in the cytoplasm of infected cells and is mediated at the genomic level by a virally encoded RNA-dependent RNA polymerase translated from ORF1 of the MHV genome. The three predominant MHV structural proteins are the nucleocapsid protein (N; 60 kDa), which complexes with the genome in a helical manner, the membrane protein (M; 25 kDa) that is important for envelope formation and viral budding, and the spike or surface protein (S; 180 kDa) necessary for receptor binding, cell fusion, and determination of viral tropism within the CNS (1, 2). The spike protein assembles into a trimeric peplomer extending from the surface of the virion resulting in the characteristic crown-like appearance from which the name coronavirus was derived. In addition, a hemaglutinin-esterase protein (HE; 65 kDa) is expressed by some strains of MHV. The genome and structural proteins are encapsulated and assembled into an intact virion. The transport vesicle containing the newly formed virus can fuse to the host cell plasma membrane for extracellular release, or fuse with neighboring cells for cell-to-cell passage.

The MHV spike protein is the main determinant of viral entry and cell-to-cell spread that together dictate cellular tropism within the CNS. Intracellular infection by MHV is initiated through the interaction between the S protein and the host cell receptor, carcinoembryonic antigen-cell adhesion molecule (CEACAM-1) (10, 11). CEACAM-1 is considered to be the predominant, and potentially the only receptor for MHV based on the observation that CEACAM-1-deficient mice are resistant to infection (12). However, cells that do not express CEACAM-1, such as B cells (13) are susceptible to MHV infection, highlighting the possibility that there may be alternative receptors or mechanisms that promote viral entry. For example, a pregnancy-specific glycoprotein expressed within the brain can support MHV binding and internalization (14). In addition, cellular tropism may be extended to non-CEACAM-1 bearing cells through cell-to-cell contact mediated by fusion of the S protein with adjacent cells (15-17). The biological relevance of alternative mechanisms of viral entry is also supported by low level expression of CEACAM-1 within the mouse CNS relative to other tissues, such as epithelial and endothelial cells lining the respiratory and digestive tracts (18), and the fact that expression has only been demonstrated on the endothelial cells of the cerebral blood vessels (19) and microglia (20). Despite the low-level and restricted expression of the CEACAM-1 receptor within the CNS, MHV is still capable of infecting and replicating within ependymal cells, astrocytes, oligodendrocytes and neurons *in vivo* (21), validating the concept that cellular tropism and viral replication is expanded to a variety of glial cells presumably in a CEACAM-1-independent manner.

3. A MODEL OF VIRAL-INDUCED CNS DISEASE

There are a number of different strains of MHV available that have been used to induce neurologic disease in susceptible strains of mice. In brief, these strains differ from extremely virulent (MHV-JHM, MHV-4), suitable for studies concentrated on acute encephalitis, to more attenuated strains (A59, J2.2v-1, OBLV-60, V5A13.1), in which acute disease as well as chronic demyelination can be studied (22-26). In general, differences in virulence relate to cellular tropism with the more neurovirulent strains *e.g.* MHV-4 exhibiting the ability to infect and replicate in both neurons and glia, while attenuated strains *e.g.* J2.2v-1 are restricted to glial cells. Differences in cellular tropism are dictated in part by variations in the S glycoprotein as it is the predominant viral surface protein mediating attachment to host cell receptor(s). Indeed, alterations and/or deletions in the ORF encoding the S glycoprotein are associated with muted virulence and cellular tropism as well as spread throughout the parenchyma (24).

Intracranial (i.c.) infection of susceptible strains of mice, such as C57BL/6 (H-2^b background) and BALB/c (H-2^d background) with neurotropic strains of MHV results in acute encephalomyelitis followed by a chronic demyelinating disease in surviving mice (Figure 2). MHV infection induces the localized expression of proinflammatory factors, including cytokines and chemokines that precedes and accompanies the activation and recruitment of immune cells into the CNS. Ultimately, virus-specific T cells are responsible for controlling viral replication through either secretion of IFN-gamma or cytolytic activity during the acute stage of disease.

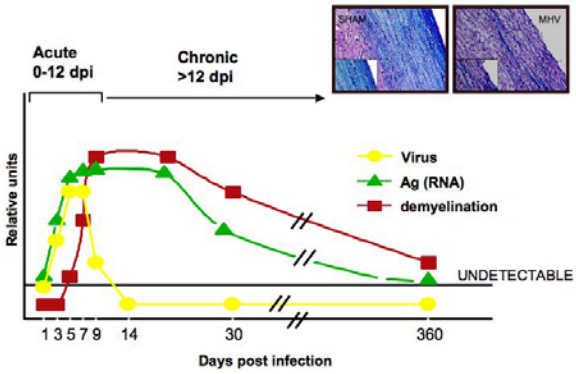

Figure 2. MHV infection results in viral persistence and demyelination. Intracranial infection with MHV results in rapid replication and spread of virus throughout the parenchyma during the acute stage of disease. Infiltrating virus-specific T cells control viral replication and reduce levels below detection. However, viral RNA and protein are detected up to 1 year following infection and animals develop an immune-mediated demyelinating disease. Photographs are representative spinal cords from uninfected (sham) and MHV-infected mice stained with luxol fast blue. Note the compact myelin sheath (stained blue) with linear organization of oligodendrocyte nuclei (inset) in the sham-infected animal. In contrast, the MHV-infected spinal cord (day 21 post-infection) shows destruction of the myelin sheath with numerous inflammatory cells (inset) present.

However, sterile immunity is not achieved and the majority of surviving mice will develop a chronic neurodegenerative disease characterized by viral persistence within white matter tracts that is associated with an immune-mediated demyelinating disease similar in pathology to the human demyelinating disease multiple sclerosis (MS). The biphasic progression of the immune response elicited by MHV infection has provided an excellent animal model to study the molecular and cellular mechanisms governing both host-defense and immune-mediated pathogenesis in response to viral infection of the CNS.

4. IMMUNOLOGY OF MOUSE HEPATITIS VIRUS INFECTION OF THE CNS

4.1. Innate response

Components of the innate immune response play key roles in shaping the subsequent adaptive immune response following CNS infection with MHV. Upon i.c. infection, virus replicates within ependymal cells lining the lateral ventricles within the brain and rapidly disseminates into the parenchyma by infecting glial cells that support viral replication, including macrophages/microglia, astrocytes and oligodendrocytes. As noted, neurons may also support viral replication depending on the strain of MHV. Both infected and uninfected astrocytes and microglia provide early inflammatory signals through regulated expression of proinflammatory cytokines, chemokines and matrix metalloproteinases (MMPs) that promote the anti-viral response. Cytokines expressed following infection include type I and type II interferons (IFNs), IL-1alpha, IL-1beta, IL-6 and IL-12 and TNF-alpha (25, 27-30). Expression of IFN-beta is detected within the CNS following MHV infection (25, 27, 28), as well as in mixed glial cultures *in vitro* (29, 31). There is strong evidence supporting an important role for type I IFNs in controlling MHV infection. For example, intraperitoneal (i.p.) treatment of mice with IFN-beta was protective against a lethal dose of MHV (32), and intranasal treatment with recombinant IFN-alpha or IFN-beta prevented local MHV infection within the brain, but did not limit viral emergence in other organs (33). Furthermore, inhibition of type I interferons resulted in enhanced virulence of MHV within the CNS (34), and type I interferon receptor-deficient mice exhibited high viral titers within brain, liver, spleen and lung, resulting in rapid mortality following i.p. injection of MHV (35). While these findings support a role for type I IFNs in controlling MHV replication, other studies indicate that viral proteins of MHV and SARS-CoV are type I IFN antagonists, indicating that coronavirus proteins can function in circumventing the innate immune response (36, 37). Type I IFNs also appear to regulate innate and adaptive immune function in response to MHV (29). For instance, the interferon response genes including IRF-7, STAT-1 and ISGF-3, in addition to numerous other factors involved in MHC class I antigen presentation are upregulated in MHV-infected glial cell cultures (29). Furthermore, MHC class I expression was similar on microglia from wild-type and IFN-gamma-deficient mice, suggesting that IFN-alpha/beta may functionally enhance lymphocyte function within the CNS of MHV-infected mice by promoting antigen presentation (38, 39).

Neutrophils are rapidly recruited to the CNS of MHV-infected mice and represent a critical component of the host-defense response (40). In support of this, mice depleted of neutrophils exhibited a dramatically impaired ability to control viral replication in the brain that correlated with increased mortality (41). One mechanism by which neutrophils may contribute to optimal host-defense is through the release of matrix metaloproteinases (MMPs) involved in tissue remodeling of the extracellular matrix, thus enabling infiltration of immune cells to sites of viral infection (42). Compared to other viral infections and autoimmune-mediated demyelinating diseases of the CNS, MHV induces mRNA expression of a limited number of potential MMPs, including MMP-3 and MMP-12 (43, 44). MMP-3 and -12 expression occurred primarily within astrocytes and oligodendrocytes respectively, and correlated with the severity of infection (43, 44); however, their roles in innate immunity to MHV are unclear. In contrast, it is apparent that MMP-9 plays an important role following MHV infection. MMP-9 expression levels correlated with the influx of neutrophils early during the host-response (43, 44). MMP-9 enzymatic activity was also detected in neutrophil lysates, but not in lysates prepared from a neutrophil depleted population (44), indicating that this cell type is the primary source of MMP-9. In addition, neutrophil-depleted mice showed diminished MMP-9 expression that was associated with significantly reduced mononuclear leukocyte infiltration into the CNS of MHV-infected mice (41). These findings suggest MMP-9 activity contributes to the loss of tight

junctions in the blood brain barrier, ultimately promoting inflammatory cell infiltration within the CNS (41). The tissue inhibitor of metalloproteinase 1 (TIMP-1) may be responsible for counteracting MMP-induced pathology during MHV infection. TIMP-1 mRNA followed a similar expression curve as the MMPs early following MHV infection, but TIMP-1 remained high as overall MMP expression decreased over time (43, 44). Infiltrating CD4+ T cells are a prominent cellular source of TIMP-1 (43). Therefore, it appears that neutrophil-dependent secretion of MMP-9 facilitates entry of inflammatory cells into the MHV-infected CNS early in the innate response, but TIMP-1 limits MMP-mediated tissue damage as CD4+ T cells accumulate during the acute stage of disease.

NK cells do not appear to play a vital role in anti-viral immunity to MHV as NK-deficient mice showed similar kinetics of class I and class II expression on microglia, and controlled MHV replication with similar efficiency with respect to wild-type mice (45). Further, evidence for NK cell-dependent anti-viral activity either by IFN-gamma or perforin secretion is lacking (39). Secretion of IFN-gamma by NK cells can be enhanced through ligation of the NK cell surface receptor, NKG2D, and this was demonstrated to control murine cytomegalovirus replication in infected tissues (46, 47). In contrast, NKG2D signaling through NK cells did not amplify IFN-gamma expression or lytic activity following MHV infection (Walsh and Lane, unpublished observation). Collectively, these findings argue for a diminished role for NK cells in controlling MHV replication early following infection of the CNS.

4.2. Cellular Response

Antigen-primed T cells are detected in lymphoid tissues early following i.c. MHV infection (48), and following expansion are quickly recruited into the CNS primarily in response to localized chemokine expression (40, 49). A protective immune response to MHV infection during acute disease is characteristic of a Th1 response and is associated with robust IFN-gamma secretion and cytolytic activity by virus-specific T cells (38, 50, 51). However, the mechanisms governing the evolution of IFN-gamma-secreting virus-specific T cells following MHV infection have not been clearly defined. For example, infection of mice deficient in either the IL-12p40 or p35 chain (IL-12-deficient mice) with hepatotropic strains of MHV resulted in a robust Th1 response characterized by high IFN-gamma levels and muted secretion of IL-4 (52). These findings suggest alternative pathways independent of IL-12 expression exist to provoke IFN-gamma production by virus-specific T cells. IL-23 is a heterodimeric protein that exhibits many similar structural and functional properties with IL-12 (53). Both IL-12 and IL-23 are expressed within the CNS in response to MHV infection and we have recently determined that blocking antibodies specific for IL-12 and IL-23 do not attenuate the severity of encephalomyelitis in mice infected i.c. with MHV (Held and Lane, unpublished observation). These findings highlight the possibility that alternative mechanisms exist that contribute to the generation of T cells necessary for a protective response.

We have demonstrated an important role for CCL3 signaling in tailoring T cell responses following CNS infection with MHV. Infection of mice deficient in CCL3 resulted in diminished ability for virus-specific T cells to undergo egress out of draining cervical lymph nodes and traffic into the CNS (54). Although generation of antigen-specific CD8+ T cells was not altered following MHV infection of *CCL3*-/- mice, a significant percentage of CD8+ T cells displayed an impaired ability to down-regulate lymph node homing receptors; and altered receptor expression coincided with the reduced chemokine receptor expression *e.g.* CXCR3 and CCR5 necessary for infiltration into the CNS of MHV-infected mice (54). Additionally, virus-specific T cells exhibited muted expression of IFN-gamma and diminished cytolytic activity demonstrating that virus-specific T cell effector responses were modulated in the absence of CCL3 signaling. To determine the underlying mechanism associated with attenuated T cell responses, we analyzed maturation of antigen-presenting cells within the CNS and lymphatic tissue in MHV-infected *CCL3*-/- mice. These findings revealed a diminished ability of dendritic cells (DCs) to undergo maturation within the CNS and this correlated with reduced numbers of DCs within the draining cervical lymph nodes of infected *CCL3*-/- mice (55). Moreover, DCs isolated from the cervical lymph nodes of *CCL3*-/- mice preferentially expressed IL-10, which may dampen T cell effector responses by virus-specific T cells. Collectively, these findings highlight a previously unappreciated role for CCL3 in host-defense following MHV infection of the CNS and supports a critical function for this chemokine in the generation of virus-specific effector T cells through enhanced maturation of dendritic-like cells present within the CNS (Figure 3).

In addition to CCL3, a number of other chemokines are produced within the CNS in response to MHV infection (Table 1). These include members of the CC chemokine family, such as CCL2, CCL4, CCL5 and the CXC chemokines CXCL9 and CXCL10 (49, 56). Chemokine expression precedes leukocyte invasion of the CNS indicating that resident cells are able to rapidly respond to viral infection; thus, cytokine secretion by infiltrating leukocytes is not necessary to trigger synthesis of chemokine transcripts. While CCL3 is important in modifying anti-viral T cell responses early following infection, the majority of chemokines function to attract immune cells into the CNS of MHV-infected mice. For example, treatment of MHV-infected mice with blocking antibodies specific for the T cell chemoattractant chemokines CXCL9 or CXCL10 resulted in increased mortality coinciding with reduced T cell infiltration into the CNS and inability to control viral replication in the brain (57, 58). Other reports have indicated that CXCL10 signaling is also important in regulating and/or imparting T cell effector functions (59-62). Using MHV infection of *CXCL10*-deficient animals, we have determined that CXCL10 signaling does not participate in elicitation of virus-specific T cells nor enhance cytokine secretion or cytolytic activity (63). Moreover, we determined that expression of the chemokine receptors CXCR3 and CCR5 is not altered on virus-specific T cells generated in MHV

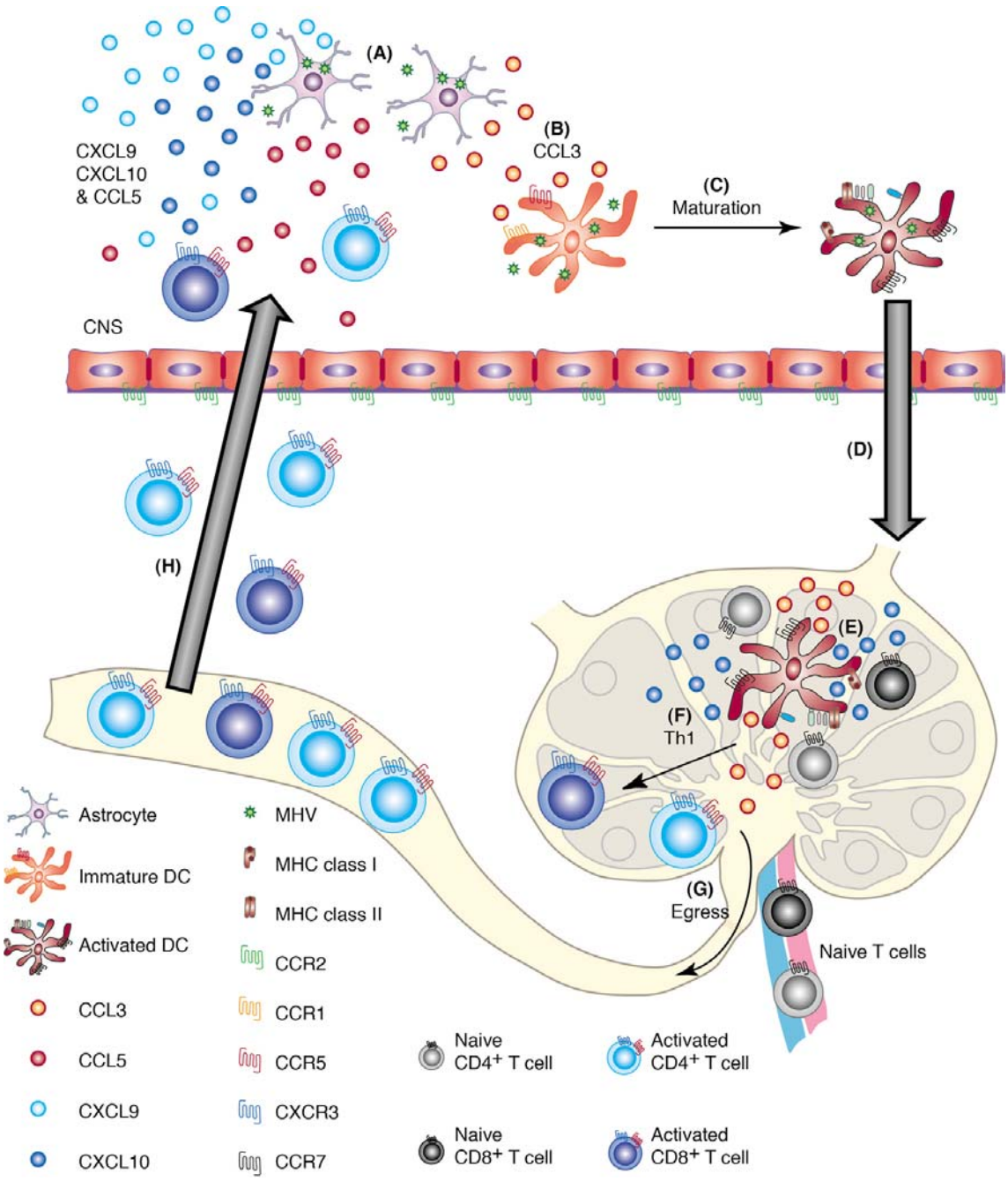

Figure 3. Chemokines and innate/adaptive immune response following MHV infection of the CNS. Instillation of MHV into the CNS of susceptible mice results in infection of astrocytes that are an important source of chemokines including CXCL9, CXCL10, CCL5, and CCL3 (a). In addition, immature DC-like cells may also be susceptible to infection and secrete CCL3 (b) that functions in a paracrine and autocrine manner to bind to CCR1 expressed on immature DC-like cells. As a result of CCL3 signaling and MHV infection, the DC-like cells undergo maturation and activation (c) resulting in a remodulation of the plasma membrane characterized by decreased expression of CCR1 accompanied by increased expression of CCR7 as well as major histocompatibility complex (MHC) class I and II. CCR7-expressing, activated DCs home to the draining cervical lymph node (d). Upon entry, activated DCs express a variety of soluble factors including CCL3 (e) that activate and enhance polarization of virus-specific T cells to a Th1 phenotype (f). Activated T cells exit the lymph node via the efferent lymph (g), enter the blood stream, and migrate to the CNS via expression of the chemokine receptors CXCR3 and CCR5 (h). Reprinted with permission from Springer Publishing.

Table 1. The function of chemokines and chemokine receptors expressed in the brain during MHV infection[1]

Disease Stage	Chemokine	Receptor	Target Cells	Function
Acute	CXCL9, CXCL10	CXCR3	Activated T cells (Th1), NK cells	T cell infiltration, viral clearance, IFN-gamma production
	CCL5	CCR1 CCR5	T cells, monocytes, macrophages, dendritic cells	Macrophage, T cell infiltration
	CCL2	CCR2	T cells, monocytes, macrophages, dendritic cells	T cell, macrophage infiltration
	CCL3	CCR1 CCR5	T cells, monocytes, macrophages, dendritic cells	CD8+ T cell infiltration, dendritic cell activation, viral clearance
Chronic	CXCL10	CXCR3	Activated T cells (Th1)	CD4+ T cell infiltration, demyelination
	CCL5	CCR5	T cells, monocytes, macrophages	T cell, macrophage infiltration, demyelination

[1]Determined by both RPA and ELISA

-infected *CXCL10*-/- mice and adoptive transfer of these cells into MHV-infected recombination-activating gene-1-deficient (*Rag1*-/-) mice resulted in efficient trafficking to sites of viral infection (63). In addition to providing protection from MHV-induced encephalitis, we have also demonstrated a previously undetermined protective role for CXCL10 in host-defense against MHV-induced hepatitis (64). Therefore, we conclude that CXCL10 functions primarily to attract activated virus-specific T cells to sites of MHV infection. This is supported by the fact that >90% of infiltrating virus-specific CD4+ and CD8+ T cells express CXCR3, which is the signaling receptor for CXCL9 and CXCL10, implying CXCR3 enhances accumulation of virus-specific T cells into the CNS of MHV-infected mice. Interestingly, administration of anti-CXCR3 blocking antibody to MHV-infected mice selectively reduced overall CD4+ T cells within the CNS while there were no differences in overall CD8+ T cell numbers (65). These findings suggest differential roles for CXCR3 in mediating T cell subset trafficking with CXCR3 necessary for CD4+ T cell accumulation, yet dispensable for CD8+ T cells (65). Alternatively, CXCR3 may not be required for CD8+ T cell trafficking into the CNS of MHV-infected mice, but necessary for undergoing egress from the perivascular space and entry into the parenchyma. Indeed, recent findings support a role for CXCR3 signaling in aiding exit from the microvasculature and positional migration of virus-specific T cells in response to i.c. infection with lymphocytic choriomeningitis virus (66).

In addition to CXCL9 and CXCL10, we have also attempted to define functional roles of other chemokines that are expressed within the CNS following MHV infection. For example, MHV infection of mice deficient in either CCL2 or it's signaling receptor CCR2 resulted in altered disease severity demonstrating an important role for this signaling axis in effective T cell responses and macrophage trafficking (67, 68). Additionally, CCL5 makes an important contribution to host-defense by recruiting macrophages and T cells expressing the chemokine receptors, CCR1 and CCR5 (69-71). MHV infection of *CCR5*-deficient mice resulted in an efficient ability to control viral replication within the CNS, but a reduction in virus-mediated pathology during chronic disease due to decreased macrophage influx (70). CD4+ T cells derived from *CCR5*-/- mice revealed reduced expression of CCR1, CCR2 and CXCR3, and therefore, CCR5 signaling may enhance expression of these receptors to ultimately facilitate CD4+ T cell homing into the CNS (72). By contrast, CCR5 signaling on CD8+ T cells modulates anti-viral activities, but is not essential for entry into the CNS (69). These findings highlight the non-redundancy in CCR5 signaling on different subsets of immune cells with respect to lymphocyte trafficking. Similar roles in chemokine signaling have been observed in MHV-infected *CCR1*-deficient mice. Like CCR5, CCR1 signaling did not significantly function in anti-MHV host-response (73). In addition, CCR1 signaling contributed to the infiltration of T cells during acute, but not chronic MHV infection of the CNS; and in contrast to CCR5, did not affect macrophage infiltration into the CNS nor the severity of demyelination when compared to *CCR1*+/+ mice (73).

CD4+ T cells are necessary for optimal viral clearance and protection from acute disease following MHV infection. In the case of MHV-induced hepatitis, MHC class II restricted CD4+ T cells have been reported to be cytotoxic and directly reduce viral burden within the liver and spleen (74, 75). Within the context of MHV infection of the CNS, CD4+ T cells may limit viral replication through secretion of IFN-gamma. Transfer of virus-specific CD4+ T cells into MHV-infected immunodeficient hosts limited anti-viral activity (76). More important is the demonstration that CD4+ T cells contributed to the survival and enhanced function of infiltrating cytotoxic T lymphocytes (CTLs), which are the primary anti-viral effectors against MHV (51, 77). In the absence of CD4+ T cells, MHV clearance is inhibited as a result of diminished CD8+ T cell infiltration and elevated apoptosis of lymphocytes is accompanied by unrestricted viral replication within the brain (51). Although the mechanism by which CD4+ T cells amplify CD8+ T cell-mediated protection is not defined, it is most likely through secretion of soluble factors, as evidence suggests that the majority of CD4+ T cells accumulate with the perivasculature and subarachnoid space rather than infiltrating into the parenchyma (51). The differential restriction of T cell subpopulations to access virally infected cells may be the result of TIMP-1 production by CD4+, but not CD8+ T cells (43). CD4+ T cells may also make an important contribution by facilitating inflammation and the overall success of the cellular immune response by regulating cytokine and chemokine production. For instance, CD4+ T cells are an early source of IFN-gamma important for a wide-array of anti-viral functions that fuel the adaptive response, such as antigen presentation. CD4+ T cells also modulate CCL5 expression, thereby attracting macrophages into the CNS, which promote demyelination during viral persistence (71). Collectively, the current data indicates that effective CTL function and MHV clearance is CD4-dependent, although

whether or not CD4+ T cells provide direct or indirect support to CD8+ T cells and by what factors is currently unknown.

CD8+ T cells are the principle anti-viral effectors following MHV infection and are critical for controlling viral replication during acute disease (48, 78). Following i.c. infection, virus-specific CD8+ T cells accumulate to high frequencies (~50%) within the CNS (79-81), and to significantly higher numbers (~10-fold) compared to the peripheral lymphoid tissues (79). The presence of CD8+ T cells correlated with a significant reduction of replicating MHV within astrocytes, microglia and oligodendrocytes (38, 40, 78). Memory CD8+ T cells transferred into immunodeficient hosts controlled viral replication within the CNS (38, 78), indicating that they elicit direct CTL activity against MHV-infected cells. Owing to their role as potent anti-viral effectors, MHV-specific CD8+ T cells isolated from the CNS express and secrete key anti-viral factors, including IFN-gamma and the protease granzyme B, a component of perforin-mediated cytolysis (78, 81). CD8+ T cell effector mechanisms are cell-type-specific within the MHV-infected CNS. Control of viral replication in astrocytes and macrophage/microglia is directed by perforin-mediated cytolysis, whereas in oligodendrocytes the anti-viral mechanism is non-cytolytic and IFN-gamma-dependent (38, 50, 78, 82, 83). In perforin-deficient mice the rate of MHV clearance was delayed in astrocytes and microglia, but remained unchanged within oligodendrocytes (83). By contrast, IFN-gamma-deficient mice harboring the capacity for perforin-mediated cytolysis controlled viral replication within astrocytes and microglia, but not in oligodendrocytes (50). Effective perforin-mediated cytolysis within the CNS may be IFN-gamma-dependent as *IFN-gamma-/-* mice display suboptimal MHC class I expression (38) and as a result CD8+ T cell cytotoxic function may be impaired. Nonetheless, mice deficient in both IFN-gamma and perforin-mediated cytolysis showed uncontrolled viral replication and increased mortality (38), supporting the findings that both mechanisms act together to limit infection. Fas/FasL interaction between infected target cells and activated CD8+ T cells is also an important cytolytic mechanism following viral infections (84), but in the context of MHV it is not necessary for promoting viral clearance from the CNS (85). Nevertheless, a recent *in vitro* study demonstrated that MHV cell-entry triggered oligodendrocyte apoptosis through activation of the Fas signaling pathway (86). Finally, we have recently determined that NKG2D receptor activation selectively enhanced CTL activity of infiltrating virus-specific CD8+ T cells during host-defense against acute MHV-induced encephalitis (Walsh and Lane, unpublished observation).

4.3. Humoral response.

A protective role for antibody (Ab) has been known for quite some time as early studies by Buchmeier and colleagues (87) demonstrated that administration of neutralizing Ab to mice protected against neurologic disease following MHV infection. Further, virus-specific antibody secreting cells (ASCs) and serum Ab are crucial in regulating viral persistence (87-92). ASCs are present at high frequencies in the CNS during viral persistence and are detected up to 3 months p.i. (78, 93). Retention of ASCs and sustained Ab secretion may be facilitated by upregulation of the B cell-activating factor (BAFF), during viral persistence (94). In mice deficient in generating an Ab response, infection is controlled with similar efficiency compared to wild-type (89, 92), confirming the central role of T cells in eliminating infectious MHV by inhibiting viral replication. However, mice that cannot secrete Ab showed increased mortality subsequent to acute disease that correlated with viral reemergence within the CNS during chronic infection (89, 92). One way in which antibodies may provide protection during chronic MHV infection is by neutralizing cell-free virus or inhibiting cell-to-cell spread. Indeed, passive transfer of MHV-specific neutralizing Ab into B cell knock-out mice prevented viral recrudescence during persistence, but infectious virus was reactivated as Ab levels waned (91). Protection via transfer of neutralizing Ab from nursing dams to MHV-infected suckling mice has also been demonstrated (95, 96). Further, immunization with serum Ab containing both neutralizing and non-neutralizing antibodies provided protection against subsequent MHV infection (26, 97); however, passive transfer of non-neutralizing antibodies during persistence did not (91). The mechanisms that provide protection beyond neutralization are unclear and it appears that complement does not influence the function of non-neutralizing Abs and therefore does not regulate the efficiency of the humoral response (88). Collectively, these results confirm that ASCs and neutralizing antibodies are vital for inhibiting MHV recrudescence and providing lasting protection during viral persistence (89).

5. DEMYELINATION IN MICE PERSISTENTLY INFECTED WITH MHV

The mechanisms contributing to MHV-induced pathogenesis are complex and are likely mediated by immunopathologic responses against viral antigens expressed in infected tissues (71, 76, 98). To date, the mechanisms governing the demyelinating disease induced during MHV infection have not been clearly defined. Evidence shows that the disease process involves the loss and/or damage of oligodendrocytes, the glial cells that generate the myelin sheath insulating axons, and axonal damage, which begins during the acute phase and continues throughout persistent infection (99). Indeed, both necrotic and apoptotic death of oligodendrocytes can result in demyelination, and although death is limited to apoptosis during chronic demyelination, it not believed to make a major contribution to myelin loss (100). As stated, components of the host-defense necessary to control viral infection also facilitate the demyelinating disease. By day 12 p.i., replicating virus cannot be detected within the CNS; however, signs of persistent infection exist with the presence of viral antigen and RNA (101). Throughout the course of acute and chronic infection mice develop ascending paralysis, which is evaluated using a well-described clinical scoring method (102, 103). Symptomatic mice first show signs of loss of tail tone and difficulty in walking, followed by partial-to-complete hind-limb paralysis. Histological assessment of CNS tissues from

infected mice confirmed myelin loss that correlated with presence of viral antigen and inflammatory cells (3, 104).

The immunopathology found within the CNS during chronic MHV infection is similar both clinically and histologically to the human demyelinating disease MS (105, 106). Indeed, there are several similarities between MHV-induced demyelination and MS that make this an excellent laboratory model for studying the underlying mechanisms contributing to demyelination (107). Genetic susceptibility appears to play a prominent role in development of MS in humans while the genetic background of rodents, which determines susceptibility and immune response, is of crucial importance in MHV-induced demyelination (107). Furthermore, MS patients often experience cyclic periods of exacerbation followed by remission. In a similar manner, MHV-infected rodents go through progressive stages of acute and chronic disease accompanied by demyelination and remyelination (106). In terms of neuropathology, MS patients and MHV-infected mice display multi-focal white matter lesions accompanied by myelin stripping. Finally, in both MS and MHV-induced CNS disease, immune mechanisms are thought to participate in the disease (106, 108).

Demyelination during MHV infection begins in the acute phase and continues throughout chronic stages of persistent infection. As noted, the chronic phase of MHV infection is defined by on-going demyelination, persistence of viral antigen and retention of T cells. The contribution of immune-mediated responses to MHV pathogenesis is clearly demonstrated in studies using a non-persistent neurotropic MHV strain. The lack of viral antigen following clearance of replicating virus was accompanied by the absence of both CD4+ and CD8+ T cells from the CNS and the absence of demyelination (109). Indeed, CD4+ T cells amplified the severity of white matter destruction and *CD4*-/- mice displayed less severe demyelination compared to *CD8*-/- mice and immunocompetent wild-type mice (71). Moreover, the induction of demyelination in MHV infected *Rag1*-/- mice occurred only upon adoptive transfer of MHV-primed effector T cells (102, 110). While B cells and/or antibodies are not important in MHV-induced demyelination, macrophages/microglia are thought to be critical in contributing to myelin destruction (71, 76, 89), although their mechanism of function in the development of demyelination has not been defined. Nonetheless, it is evident that T cell-mediated demyelination is related to IFN-gamma expression and likely functions via activation of macrophages/microglia (111, 112). Moreover, expression of IFN-gamma by infiltrating CD8+ T cells, but not CD4+ T cells, is considered important in promoting white matter damage (110, 111). Although evidence is lacking to support direct killing of oligodendrocytes by T cells and macrophages, the individual contributions of cytolytic molecules such as iNOS (inducible nitric oxide synthase) and perforin have been shown to be dispensable for MHV-induced demyelination, suggesting that a more complex interplay of immune-mediated damage (50, 83, 113, 114).

The expression of the chemokines during MHV infection promotes infiltration and retention of T cells and macrophages and influences the degree of myelin destruction (115) highlighting the role of CNS inflammation as a requisite for demyelination. Systematically, the contributions of chemokines to cell-mediated pathology during MHV infection have been elucidated. For example, studies by Liu *et al.* (58) demonstrated anti-CXCL10 neutralizing antibody administration to mice with established demyelination resulted in diminished CD4+ T cell infiltration and a reduction in the extent of myelin destruction, which was accompanied with increased remyelination of damaged axons. The selective decrease in CD4+ T cell recruitment into the CNS of anti-CXCL10-treated mice suggests the possibility of differential chemotactic signaling requirements between CD4+ and CD8+ T cells that are either recruited or retained within the CNS of persistently infected mice. Indeed, treatment with an anti-CXCR3 blocking antibody selectively affected CD4+ T cell infiltration into the CNS while CD8+ trafficking was relatively undisturbed (65). We believe one possible explanation for the differential T cell responses in antibody-treated mice may be the result of selective retention of CD8+ T cells within the CNS as CD4+ T cells continue to percolate throughout the mouse during chronic disease (48, 109). Alternatively, we have shown that blocking CCL5 signaling in MHV-infected mice with established demyelination impacted both CD4+ and CD8+ T cell recruitment indicating that both CXCL10 and CCL5 contribute to inflammation during the chronic stage of disease (69). Macrophage trafficking appears to rely on expression of CCR5; inhibition of CCL5 or infection of *CCR5*-/- mice resulted in diminished macrophage accumulation within the CNS of MHV-infected mice and a marked decrease in the severity of myelin damage (69, 70). Reduced macrophage numbers within the CNS of anti-CXCL10 treated mice related to the overall reduction in CCL5 protein levels and this correlated with our previous demonstration that infiltrating CD4+ T cells expressed CCL5 during chronic disease (49). Collectively, our findings demonstrate that MHV persistence within the CNS results in chronic expression of CXCL10 and CCL5 that together contribute to the maintenance of a chronic inflammatory disease by attracting both T cells and macrophages. Clearly, these observations show that chemokine signaling is an integral component involved in eliciting protective immunity in response to viral infection of the CNS. Conversely, our studies also demonstrate that chronic localized secretion of select chemokines ultimately amplifies disease severity by maintaining inflammation within the CNS. Importantly, studies derived from the MHV system demonstrate that antibody targeting of select chemokines offers a powerful approach towards delineating the functional contributions of these molecules in a model of immune-mediated demyelination. Further, these studies highlight the relevancy of such an approach in treating human neuroinflammatory and demyelinating diseases such as MS.

6. CELL REPLACEMENT STRATEGIES FOR PROMOTING REMYELINATION

While MHV infection of the CNS of mice provides an excellent model for studying the underlying molecular and cellular mechanisms contributing to both acute and chronic disease, we have also

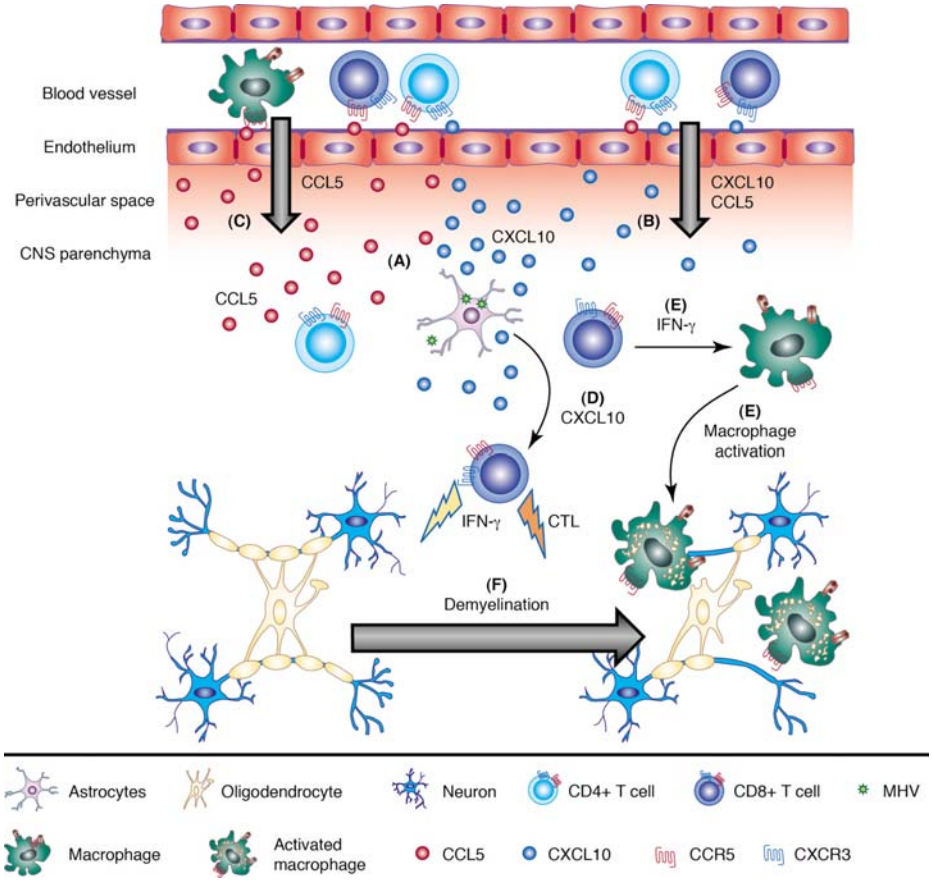

Figure 4. Progenitor cell-induced remyelination. Persistent MHV infection of astrocytes during chronic disease results in secretion of CXCL10 and CCL5 (a) that serves to recruit activated macrophages and T cells via signaling through chemokine receptors expressed on the cell surface (b) into the CNS. Implanted progenitor cells (PC) express chemokine receptors that enable them to migrate (c) to areas of demyelination and promote remyelination (d). In addition, our contention is that reducing T cell and macrophage accumulation within the CNS following progenitor cell implantation will enhance remyelination. Reprinted with permission from Springer Publishing.

utilized this system to assess novel therapeutic approaches to promote remyelination through cell replacement strategies. Stem cells and neural precursor cells represent attractive sources for the generation of remyelination-competent cells, as they can be readily amplified and differentiated to the oligodendrocyte lineage (116, 117). Studies in animal models have proven invaluable for identifying new methods for inducing remyelination in animals with established demyelination. Many studies have shown that transplantation of stem cells into animal models of acute demyelination results in remyelination (118). Transplant of rodent embryonic stem cells into myelin-deficient shiverer mice resulted in cellular migration in the spinal cord, differentiation into oligodendrocytes and astrocytes, and myelination of axons (117). Similarly, transplant of human embryonic stem cell-derived oligodendrocyte progenitor cells (OPCs) into myelin-deficient shiverer mice resulted in oligodendrocyte differentiation and remyelination (119). Other models of demyelination have also reported reduced demyelination following transplantation of stem cells. For example, injection of adult neuronal precursor cells into mice with experimental autoimmune encephalomyelitis promoted recovery from disease and a significant decrease in the level of demyelination (120).

In order to determine whether transplantation represents a viable strategy for treating demyelination, it is necessary to better understand the range of environmental conditions that support transplantation-mediated remyelination. We have previously determined that intraspinal transplantation of OPCs into the mice persistently infected with MHV resulted in extensive migration of transplanted cells, robust remyelination, axonal sparing, and behavioral improvement (121). Moreover, implantation of OPCs did not modulate the severity of inflammation, indicating that remyelination can occur in a pathogenic environment (122). These results show that transplant-mediated remyelination is possible following intraspinal transplantation into an environment of ongoing pathogenesis resembling MS. We believe that the transplanted progenitor cells are likely using specific chemokine receptors to allow for positional migration into areas of demyelination and are directly responsible for the remyelination of demyelinated axons (Figure 4).

7. PERSPECTIVES

The use of MHV infection of the CNS as a model of viral-induced encephalomyelitis has been critical in understanding the relationships between cellular components of the innate and adaptive immune response and the cytokines and chemokines responsible for their recruitment, activation and anti-viral activity. This interplay of cellular inflammation of the CNS can also be applied to other viral models, including Theiler's murine encephalomyelitis virus and human immunodeficiency virus (123, 124). Importantly, the cell-mediated immunological damage induced by a persistent MHV infection is helping to elucidate the underlining mechanisms involved in human demyelinating diseases such as MS. Moreover, novel therapeutic strategies for promoting remyelination may be developed using the MHV model of demyelinating disease.

8. ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grants NS41249, NS18146, and National Multiple Sclerosis Society grants 3278 and 3857A5 to T.E.L. C.S.S. is supported by a post-doctoral fellowship T1-00008 from the California Institute of Regenerative Medicine (CIRM).

9. REFERENCES

1. Holmes, K. & M. Lai, Fields Virology - Coronaviruses and their replication. Philadelphia: *Lippincott-Raven Publishers*, 1996.
2. McIntosh, K.: Fields Virology - Coronavirues. Philadelphia: *Lippincott-Raven Publishers*, 1996.
3. Perlman, S. & M. J. Buchmeier: Effects of Microbes on the Immune System. Philadelphia: *Lippincott WIlliams and Wilkins*, 1999.
4. Weiss, S. R. & S. Navas-Martin: Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiol Mol Biol Rev, 69, 635-64 (2005)
5. Holmes, K. V.: SARS-associated coronavirus. N Engl J Med, 348, 1948-51 (2003)
6. Masters, P. S.: The molecular biology of coronaviruses. Adv Virus Res, 66, 193-292 (2006)
7. van der Hoek, L., K. Pyrc, M. F. Jebbink, W. Vermeulen-Oost, R. J. Berkhout, K. C. Wolthers, P. M. Wertheim-van Dillen, J. Kaandorp, J. Spaargaren & B. Berkhout: Identification of a new human coronavirus. Nat Med, 10, 368-73 (2004)
8. van der Hoek, L., K. Pyrc & B. Berkhout: Human coronavirus NL63, a new respiratory virus. FEMS Microbiol Rev, 30, 760-73 (2006)
9. Woo, P. C., S. K. Lau, C. M. Chu, K. H. Chan, H. W. Tsoi, Y. Huang, B. H. Wong, R. W. Poon, J. J. Cai, W. K. Luk, L. L. Poon, S. S. Wong, Y. Guan, J. S. Peiris & K. Y. Yuen: Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. J Virol, 79, 884-95 (2005)
10. Compton, S. R., S. W. Barthold & A. L. Smith: The cellular and molecular pathogenesis of coronaviruses. *Lab Anim Sci*, 43, 15-28 (1993)
11. Williams, R. K., G. S. Jiang, S. W. Snyder, M. F. Frana & K. V. Holmes: Purification of the 110-kilodalton glycoprotein receptor for mouse hepatitis virus (MHV)-A59 from mouse liver and identification of a nonfunctional, homologous protein in MHV-resistant SJL/J mice. *J Virol*, 64, 3817-23 (1990)
12. Hemmila, E., C. Turbide, M. Olson, S. Jothy, K. V. Holmes & N. Beauchemin: Ceacam1a-/- mice are completely resistant to infection by murine coronavirus mouse hepatitis virus A59. *J Virol*, 78, 10156-65 (2004)
13. Morales, S., B. Parra, C. Ramakrishna, D. M. Blau & S. A. Stohlman: B-cell-mediated lysis of cells infected with the neurotropic JHM strain of mouse hepatitis virus. *Virology*, 286, 160-7 (2001)
14. Chen, D. S., M. Asanaka, K. Yokomori, F. Wang, S. B. Hwang, H. P. Li & M. M. Lai: A pregnancy-specific glycoprotein is expressed in the brain and serves as a receptor for mouse hepatitis virus. *Proc Natl Acad Sci U S A*, 92, 12095-9 (1995)
15. Gallagher, T. M., M. J. Buchmeier & S. Perlman: Cell receptor-independent infection by a neurotropic murine coronavirus. *Virology*, 191, 517-22 (1992)
16. Nakagaki, K., K. Nakagaki & F. Taguchi: Receptor-independent spread of a highly neurotropic murine coronavirus JHMV strain from initially infected microglial cells in mixed neural cultures. *J Virol*, 79, 6102-10 (2005)
17. Ontiveros, E., T. S. Kim, T. M. Gallagher & S. Perlman: Enhanced virulence mediated by the murine coronavirus, mouse hepatitis virus strain JHM, is associated with a glycine at residue 310 of the spike glycoprotein. *J Virol*, 77, 10260-9 (2003)
18. Godfraind, C., S. G. Langreth, C. B. Cardellichio, R. Knobler, J. P. Coutelier, M. Dubois-Dalcq & K. V. Holmes: Tissue and cellular distribution of an adhesion molecule in the carcinoembryonic antigen family that serves as a receptor for mouse hepatitis virus. *Lab Invest*, 73, 615-27 (1995)
19. Godfraind, C., N. Havaux, K. V. Holmes & J. P. Coutelier: Role of virus receptor-bearing endothelial cells of the blood-brain barrier in preventing the spread of mouse hepatitis virus-A59 into the central nervous system. *J Neurovirol*, 3, 428-34 (1997)
20. Ramakrishna, C., C. C. Bergmann, K. V. Holmes & S. A. Stohlman: Expression of the mouse hepatitis virus receptor by central nervous system microglia. *J Virol*, 78, 7828-32 (2004)
21. Wang, F. I., D. R. Hinton, W. Gilmore, M. D. Trousdale & J. O. Fleming: Sequential infection of glial cells by the murine hepatitis virus JHM strain (MHV-4) leads to a characteristic distribution of demyelination. *Lab Invest*, 66, 744-54 (1992)
22. Dalziel, R. G., P. W. Lampert, P. J. Talbot & M. J. Buchmeier: Site-specific alteration of murine hepatitis virus type 4 peplomer glycoprotein E2 results in reduced neurovirulence. *J Virol*, 59, 463-71 (1986)
23. Fleming, J. O., M. D. Trousdale, F. A. el-Zaatari, S. A. Stohlman & L. P. Weiner: Pathogenicity of antigenic variants of murine coronavirus JHM selected with monoclonal antibodies. *J Virol*, 58, 869-75 (1986)

24. Fazakerley, J. K., S. E. Parker, F. Bloom & M. J. Buchmeier: The V5A13.1 envelope glycoprotein deletion mutant of mouse hepatitis virus type-4 is neuroattenuated by its reduced rate of spread in the central nervous system. *Virology*, 187, 178-88 (1992)
25. Pearce, B. D., M. V. Hobbs, T. S. McGraw & M. J. Buchmeier: Cytokine induction during T-cell-mediated clearance of mouse hepatitis virus from neurons *in vivo*. *J Virol*, 68, 5483-95 (1994)
26. Perlman, S.: Pathogenesis of coronavirus-induced infections. Review of pathological and immunological aspects. *Adv Exp Med Biol*, 440, 503-13 (1998)
27. Parra, B., D. R. Hinton, M. T. Lin, D. J. Cua & S. A. Stohlman: Kinetics of cytokine mRNA expression in the central nervous system following lethal and nonlethal coronavirus-induced acute encephalomyelitis. *Virology*, 233, 260-70 (1997)
28. Rempel, J. D., S. J. Murray, J. Meisner & M. J. Buchmeier: Differential regulation of innate and adaptive immune responses in viral encephalitis. *Virology*, 318, 381-92 (2004)
29. Rempel, J. D., L. A. Quina, P. K. Blakely-Gonzales, M. J. Buchmeier & D. L. Gruol: Viral induction of central nervous system innate immune responses. *J Virol*, 79, 4369-81 (2005)
30. Sun, N., D. Grzybicki, R. F. Castro, S. Murphy & S. Perlman: Activation of astrocytes in the spinal cord of mice chronically infected with a neurotropic coronavirus. *Virology*, 213, 482-93 (1995)
31. Li, Y., L. Fu, D. M. Gonzales & E. Lavi: Coronavirus neurovirulence correlates with the ability of the virus to induce proinflammatory cytokine signals from astrocytes and microglia. *J Virol*, 78, 3398-406 (2004)
32. Minagawa, H., A. Takenaka, S. Mohri & R. Mori: Protective effect of recombinant murine interferon beta against mouse hepatitis virus infection. *Antiviral Res*, 8, 85-95 (1987)
33. Smith, A. L., S. W. Barthold & D. S. Beck: Intranasally administered alpha/beta interferon prevents extension of mouse hepatitis virus, strain JHM, into the brains of BALB/cByJ mice. *Antiviral Res*, 8, 239-45 (1987)
34. Lavi, E. & Q. Wang: The protective role of cytotoxic T cells and interferon against coronavirus invasion of the brain. *Adv Exp Med Biol*, 380, 145-9 (1995)
35. Cervantes-Barragan, L., R. Zust, F. Weber, M. Spiegel, K. S. Lang, S. Akira, V. Thiel & B. Ludewig: Control of coronavirus infection through plasmacytoid dendritic cell-derived type I interferon. *Blood*, 109, 1131-1137 (2006)
36. Frieman, M., B. Yount, M. Heise, S. A. Kopecky-Bromberg, P. Palese & R. S. Baric: Severe acute respiratory syndrome coronavirus ORF6 antagonizes STAT1 function by sequestering nuclear import factors on the rough endoplasmic reticulum/Golgi membrane. *J Virol*, 81, 9812-24 (2007)
37. Ye, Y., K. Hauns, J. O. Langland, B. L. Jacobs & B. G. Hogue: Mouse hepatitis coronavirus A59 nucleocapsid protein is a type I interferon antagonist. *J Virol*, 81, 2554-63 (2007)
38. Bergmann, C. C., B. Parra, D. R. Hinton, R. Chandran, M. Morrison & S. A. Stohlman: Perforin-mediated effector function within the central nervous system requires IFN-gamma-mediated MHC up-regulation. *J Immunol*, 170, 3204-13 (2003)
39. Bergmann, C. C., B. Parra, D. R. Hinton, C. Ramakrishna, K. C. Dowdell & S. A. Stohlman: Perforin and gamma interferon-mediated control of coronavirus central nervous system infection by CD8 T cells in the absence of CD4 T cells. *J Virol*, 78, 1739-50 (2004)
40. Bergmann, C. C., T. E. Lane & S. A. Stohlman: Coronavirus infection of the central nervous system: host-virus stand-off. *Nat Rev Microbiol*, 4, 121-32 (2006)
41. Zhou, J., S. A. Stohlman, D. R. Hinton & N. W. Marten: Neutrophils promote mononuclear cell infiltration during viral-induced encephalitis. *J Immunol*, 170, 3331-6 (2003)
42. Yong, V. W., C. Power, P. Forsyth & D. R. Edwards: Metalloproteinases in biology and pathology of the nervous system. *Nat Rev Neurosci*, 2, 502-11 (2001)
43. Zhou, J., N. W. Marten, C. C. Bergmann, W. B. Macklin, D. R. Hinton & S. A. Stohlman: Expression of matrix metalloproteinases and their tissue inhibitor during viral encephalitis. *J Virol*, 79, 4764-73 (2005)
44. Zhou, J., S. A. Stohlman, R. Atkinson, D. R. Hinton & N. W. Marten: Matrix metalloproteinase expression correlates with virulence following neurotropic mouse hepatitis virus infection. *J Virol*, 76, 7374-84 (2002)
45. Zuo, J., S. A. Stohlman, J. B. Hoskin, D. R. Hinton, R. Atkinson & C. C. Bergmann: Mouse hepatitis virus pathogenesis in the central nervous system is independent of IL-15 and natural killer cells. *Virology*, 350, 206-15 (2006)
46. Lodoen, M., K. Ogasawara, J. A. Hamerman, H. Arase, J. P. Houchins, E. S. Mocarski & L. L. Lanier: NKG2D-mediated natural killer cell protection against cytomegalovirus is impaired by viral gp40 modulation of retinoic acid early inducible 1 gene molecules. *J Exp Med*, 197, 1245-53 (2003)
47. Lodoen, M. B., G. Abenes, S. Umamoto, J. P. Houchins, F. Liu & L. L. Lanier: The cytomegalovirus m155 gene product subverts natural killer cell antiviral protection by disruption of H60-NKG2D interactions. *J Exp Med*, 200, 1075-81 (2004)
48. Marten, N. W., S. A. Stohlman, J. Zhou & C. C. Bergmann: Kinetics of virus-specific CD8+ -T-cell expansion and trafficking following central nervous system infection. *J Virol*, 77, 2775-8 (2003)
49. Lane, T. E., V. C. Asensio, N. Yu, A. D. Paoletti, I. L. Campbell & M. J. Buchmeier: Dynamic regulation of alpha- and beta-chemokine expression in the central nervous system during mouse hepatitis virus-induced demyelinating disease. *J Immunol*, 160, 970-8 (1998)
50. Parra, B., D. R. Hinton, N. W. Marten, C. C. Bergmann, M. T. Lin, C. S. Yang & S. A. Stohlman: IFN-gamma is required for viral clearance from central nervous system oligodendroglia. *J Immunol*, 162, 1641-7 (1999)
51. Stohlman, S. A., C. C. Bergmann, M. T. Lin, D. J. Cua & D. R. Hinton: CTL effector function within the central nervous system requires CD4+ T cells. *J Immunol*, 160, 2896-904 (1998)
52. Schijns, V. E., B. L. Haagmans, C. M. Wierda, B. Kruithof, I. A. Heijnen, G. Alber & M. C. Horzinek: Mice lacking IL-12 develop polarized Th1 cells during viral infection. *J Immunol*, 160, 3958-64 (1998)

53. Oppmann, B., R. Lesley, B. Blom, J. C. Timans, Y. Xu, B. Hunte, F. Vega, N. Yu, J. Wang, K. Singh, F. Zonin, E. Vaisberg, T. Churakova, M. Liu, D. Gorman, J. Wagner, S. Zurawski, Y. Liu, J. S. Abrams, K. W. Moore, D. Rennick, R. de Waal-Malefyt, C. Hannum, J. F. Bazan & R. A. Kastelein: Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity*, 13, 715-25 (2000)
54. Trifilo, M. J., C. C. Bergmann, W. A. Kuziel & T. E. Lane: CC chemokine ligand 3 (CCL3) regulates CD8 (+)-T-cell effector function and migration following viral infection. *J Virol*, 77, 4004-14 (2003)
55. Trifilo, M. J. & T. E. Lane: The CC chemokine ligand 3 regulates CD11c+CD11b+CD8alpha- dendritic cell maturation and activation following viral infection of the central nervous system: implications for a role in T cell activation. *Virology*, 327, 8-15 (2004)
56. Liu, M. T., B. P. Chen, P. Oertel, M. J. Buchmeier, T. A. Hamilton, D. A. Armstrong & T. E. Lane: The CXC chemokines IP-10 and Mig are essential in host defense following infection with a neurotropic coronavirus. *Adv Exp Med Biol*, 494, 323-7 (2001)
57. Liu, M. T., D. Armstrong, T. A. Hamilton & T. E. Lane: Expression of Mig (monokine induced by interferon-gamma) is important in T lymphocyte recruitment and host defense following viral infection of the central nervous system. *J Immunol*, 166, 1790-5 (2001)
58. Liu, M. T., H. S. Keirstead & T. E. Lane: Neutralization of the chemokine CXCL10 reduces inflammatory cell invasion and demyelination and improves neurological function in a viral model of multiple sclerosis. *J Immunol*, 167, 4091-7 (2001)
59. Dufour, J. H., M. Dziejman, M. T. Liu, J. H. Leung, T. E. Lane & A. D. Luster: IFN-gamma-inducible protein 10 (IP-10; CXCL10)-deficient mice reveal a role for IP-10 in effector T cell generation and trafficking. *J Immunol*, 168, 3195-204 (2002)
60. Khan, I. A., J. A. MacLean, F. S. Lee, L. Casciotti, E. DeHaan, J. D. Schwartzman & A. D. Luster: IP-10 is critical for effector T cell trafficking and host survival in Toxoplasma gondii infection. *Immunity*, 12, 483-94 (2000)
61. Liu, L., D. Huang, M. Matsui, T. T. He, T. Hu, J. Demartino, B. Lu, C. Gerard & R. M. Ransohoff: Severe disease, unaltered leukocyte migration, and reduced IFN-gamma production in CXCR3-/- mice with experimental autoimmune encephalomyelitis. *J Immunol*, 176, 4399-409 (2006)
62. Thomas, M. S., S. L. Kunkel & N. W. Lukacs: Differential role of IFN-gamma-inducible protein 10 kDa in a cockroach antigen-induced model of allergic airway hyperreactivity: systemic versus local effects. *J Immunol*, 169, 7045-53 (2002)
63. Stiles, L. N., J. L. Hardison, C. S. Schuamburg, L. M. Whitman & T. E. Lane: T cell anti-viral effector function is not dependent on CXCL10 following murine coronavirus infection. *J Immunol*, 177, 8372-8380 (2006)
64. Walsh, K. B., R. A. Edwards, K. M. Romero, M. V. Kotlajich, S. A. Stohlman & T. E. Lane: Expression of CXC chemokine ligand 10 from the mouse hepatitis virus genome results in protection from viral-induced neurological and liver disease. *J Immunol*, 179, 1155-65 (2007)
65. Stiles, L. N., M. P. Hosking, R. A. Edwards, R. M. Strieter & T. E. Lane: Differential roles for CXCR3 in CD4+ and CD8+ T cell trafficking following viral infection of the CNS. *Eur J Immunol*, 36, 613-22 (2006)
66. Christensen, J. E., A. Nansen, T. Moos, B. Lu, C. Gerard, J. P. Christensen & A. R. Thomsen: Efficient T-cell surveillance of the CNS requires expression of the CXC chemokine receptor 3. *J Neurosci*, 24, 4849-58 (2004)
67. Chen, B. P., W. A. Kuziel & T. E. Lane: Lack of CCR2 results in increased mortality and impaired leukocyte activation and trafficking following infection of the central nervous system with a neurotropic coronavirus. *J Immunol*, 167, 4585-92 (2001)
68. Held, K. S., B. P. Chen, W. A. Kuziel, B. J. Rollins & T. E. Lane: Differential roles of CCL2 and CCR2 in host defense to coronavirus infection. *Virology*, 329, 251-60 (2004)
69. Glass, W. G., M. J. Hickey, J. L. Hardison, M. T. Liu, J. E. Manning & T. E. Lane: Antibody targeting of the CC chemokine ligand 5 results in diminished leukocyte infiltration into the central nervous system and reduced neurologic disease in a viral model of multiple sclerosis. *J Immunol*, 172, 4018-25 (2004)
70. Glass, W. G., M. T. Liu, W. A. Kuziel & T. E. Lane: Reduced macrophage infiltration and demyelination in mice lacking the chemokine receptor CCR5 following infection with a neurotropic coronavirus. *Virology*, 288, 8-17 (2001)
71. Lane, T. E., M. T. Liu, B. P. Chen, V. C. Asensio, R. M. Samawi, A. D. Paoletti, I. L. Campbell, S. L. Kunkel, H. S. Fox & M. J. Buchmeier: A central role for CD4 (+) T cells and RANTES in virus-induced central nervous system inflammation and demyelination. *J Virol*, 74, 1415-24 (2000)
72. Glass, W. G. & T. E. Lane: Functional expression of chemokine receptor CCR5 on CD4 (+) T cells during virus-induced central nervous system disease. *J Virol*, 77, 191-8 (2003)
73. Hickey, M. J., K. S. Held, E. Baum, J. L. Gao, P. M. Murphy & T. E. Lane: CCR1 deficiency increases susceptibility to fatal coronavirus infection of the central nervous system. *Viral Immunol* (2007)
74. Heemskerk, M. H., H. M. Schoemaker, W. J. Spaan & C. J. Boog: Predominance of MHC class II-restricted CD4+ cytotoxic T cells against mouse hepatitis virus A59. *Immunology*, 84, 521-7 (1995)
75. Wijburg, O. L., M. H. Heemskerk, A. Sanders, C. J. Boog & N. Van Rooijen: Role of virus-specific CD4+ cytotoxic T cells in recovery from mouse hepatitis virus infection. *Immunology*, 87, 34-41 (1996)
76. Wu, G. F., A. A. Dandekar, L. Pewe & S. Perlman: CD4 and CD8 T cells have redundant but not identical roles in virus-induced demyelination. *J Immunol*, 165, 2278-86 (2000)
77. Zhou, J., D. R. Hinton, S. A. Stohlman, C. P. Liu, L. Zhong & N. W. Marten: Maintenance of CD8+ T cells during acute viral infection of the central nervous system requires CD4+ T cells but not interleukin-2. *Viral Immunol*, 18, 162-9 (2005)
78. Bergmann, C. C., J. D. Altman, D. Hinton & S. A. Stohlman: Inverted immunodominance and impaired cytolytic function of CD8+ T cells during viral persistence

in the central nervous system. *J Immunol*, 163, 3379-87 (1999)
79. Bergmann, C., E. Dimacali, S. Stohl, W. Wei, M. M. Lai, S. Tahara & N. Marten: Variability of persisting MHV RNA sequences constituting immune and replication-relevant domains. *Virology*, 244, 563-72 (1998)
80. Marten, N. W., S. A. Stohlman, R. D. Atkinson, D. R. Hinton, J. O. Fleming & C. C. Bergmann: Contributions of CD8+ T cells and viral spread to demyelinating disease. *J Immunol*, 164, 4080-8 (2000)
81. Ramakrishna, C., S. A. Stohlman, R. A. Atkinson, D. R. Hinton & C. C. Bergmann: Differential regulation of primary and secondary CD8+ T cells in the central nervous system. *J Immunol*, 173, 6265-73 (2004)
82. Gonzalez, J. M., C. C. Bergmann, C. Ramakrishna, D. R. Hinton, R. Atkinson, J. Hoskin, W. B. Macklin & S. A. Stohlman: Inhibition of interferon-gamma signaling in oligodendroglia delays coronavirus clearance without altering demyelination. *Am J Pathol*, 168, 796-804 (2006)
83. Lin, M. T., S. A. Stohlman & D. R. Hinton: Mouse hepatitis virus is cleared from the central nervous systems of mice lacking perforin-mediated cytolysis. *J Virol*, 71, 383-91 (1997)
84. Russell, J. H. & T. J. Ley: Lymphocyte-mediated cytotoxicity. *Annu Rev Immunol*, 20, 323-70 (2002)
85. Parra, B., M. T. Lin, S. A. Stohlman, C. C. Bergmann, R. Atkinson & D. R. Hinton: Contributions of Fas-Fas ligand interactions to the pathogenesis of mouse hepatitis virus in the central nervous system. *J Virol*, 74, 2447-50 (2000)
86. Liu, Y. & X. Zhang: Murine coronavirus-induced oligodendrocyte apoptosis is mediated through the activation of the Fas signaling pathway. *Virology*, 360, 364-375 (2007)
87. Buchmeier, M. J., H. A. Lewicki, P. J. Talbot & R. L. Knobler: Murine hepatitis virus-4 (strain JHM)-induced neurologic disease is modulated *in vivo* by monoclonal antibody. *Virology*, 132, 261-70 (1984)
88. Fleming, J. O., R. A. Shubin, M. A. Sussman, N. Casteel & S. A. Stohlman: Monoclonal antibodies to the matrix (E1) glycoprotein of mouse hepatitis virus protect mice from encephalitis. *Virology*, 168, 162-7 (1989)
89. Lin, M. T., D. R. Hinton, N. W. Marten, C. C. Bergmann & S. A. Stohlman: Antibody prevents virus reactivation within the central nervous system. *J Immunol*, 162, 7358-68 (1999)
90. Perlman, S., R. Schelper, E. Bolger & D. Ries: Late onset, symptomatic, demyelinating encephalomyelitis in mice infected with MHV-JHM in the presence of maternal antibody. *Microb Pathog*, 2, 185-94 (1987)
91. Ramakrishna, C., C. C. Bergmann, R. Atkinson & S. A. Stohlman: Control of central nervous system viral persistence by neutralizing antibody. *J Virol*, 77, 4670-8 (2003)
92. Ramakrishna, C., S. A. Stohlman, R. D. Atkinson, M. J. Shlomchik & C. C. Bergmann: Mechanisms of central nervous system viral persistence: the critical role of antibody and B cells. *J Immunol*, 168, 1204-11 (2002)
93. Tschen, S. I., C. C. Bergmann, C. Ramakrishna, S. Morales, R. Atkinson & S. A. Stohlman: Recruitment kinetics and composition of antibody-secreting cells within the central nervous system following viral encephalomyelitis. *J Immunol*, 168, 2922-9 (2002)
94. Tschen, S. I., S. A. Stohlman, C. Ramakrishna, D. R. Hinton, R. D. Atkinson & C. C. Bergmann: CNS viral infection diverts homing of antibody-secreting cells from lymphoid organs to the CNS. *Eur J Immunol*, 36, 603-12 (2006)
95. Kolb, A. F., L. Pewe, J. Webster, S. Perlman, C. B. Whitelaw & S. G. Siddell: Virus-neutralizing monoclonal antibody expressed in milk of transgenic mice provides full protection against virus-induced encephalitis. *J Virol*, 75, 2803-9 (2001)
96. Perlman, S., R. Schelper & D. Ries: Maternal antibody-modulated MHV-JHM infection in C57BL/6 and BALB/c mice. *Adv Exp Med Biol*, 218, 297-305 (1987)
97. Stohlman, S. A.: Persistent Viral Infection. John Wiley, New York (1999)
98. Wang, F. I., S. A. Stohlman & J. O. Fleming: Demyelination induced by murine hepatitis virus JHM strain (MHV-4) is immunologically mediated. *J Neuroimmunol*, 30, 31-41 (1990)
99. Dandekar, A. A., G. F. Wu, L. Pewe & S. Perlman: Axonal damage is T cell mediated and occurs concomitantly with demyelination in mice infected with a neurotropic coronavirus. *J Virol*, 75, 6115-20 (2001)
100. Wu, G. F. & S. Perlman: Macrophage infiltration, but not apoptosis, is correlated with immune-mediated demyelination following murine infection with a neurotropic coronavirus. *J Virol*, 73, 8771-80 (1999)
101. Perlman, S. R., T. E. Lane & M. J. Buchmeier: Coronaviruses: Hepatitis, peritonitis, and central nervous system disease. In: Effects of Microbes on the Immune System. Eds: M. W. Cunningham&R. S. Fujinami. Lippincott Williams & Wilkins, Philadelphia (1999)
102. Houtman, J. J. & J. O. Fleming: Dissociation of demyelination and viral clearance in congenitally immunodeficient mice infected with murine coronavirus JHM. *J Neurovirol*, 2, 101-10 (1996)
103. Lane, T. E., H. S. Fox & M. J. Buchmeier: 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 (1999)
104. Stohlman, S. A. & D. R. Hinton: Viral induced demyelination. *Brain Pathol*, 11, 92-106 (2001)
105. Houtman, J. J. & J. O. Fleming: Pathogenesis of mouse hepatitis virus-induced demyelination. *J Neurovirol*, 2, 361-76 (1996)
106. Matthews, A. E., S. R. Weiss & Y. Paterson: Murine hepatitis virus--a model for virus-induced CNS demyelination. *J Neurovirol*, 8, 76-85 (2002)
107. Lane, T. E. & M. J. Buchmeier: Murine coronavirus infection: a paradigm for virus-induced demyelinating disease. *Trends Microbiol*, 5, 9-14 (1997)
108. Buchmeier, M. J. & T. E. Lane: Viral-induced neurodegenerative disease. *Curr Opin Microbiol*, 2, 398-402 (1999)
109. Marten, N. W., S. A. Stohlman & C. C. Bergmann: Role of viral persistence in retaining CD8 (+) T cells within the central nervous system. *J Virol*, 74, 7903-10 (2000)
110. Pewe, L. & S. Perlman: Cutting edge: CD8 T cell-mediated demyelination is IFN-gamma dependent in mice

infected with a neurotropic coronavirus. *J Immunol*, 168, 1547-51 (2002)
111. Dandekar, A. A., D. Anghelina & S. Perlman: Bystander CD8 T-cell-mediated demyelination is interferon-gamma-dependent in a coronavirus model of multiple sclerosis. *Am J Pathol*, 164, 363-9 (2004)
112. Dandekar, A. A., K. O'Malley & S. Perlman: Important roles for gamma interferon and NKG2D in gammadelta T-cell-induced demyelination in T-cell receptor beta-deficient mice infected with a coronavirus. *J Virol*, 79, 9388-96 (2005)
113. Chen, B. P. & T. E. Lane: Lack of nitric oxide synthase type 2 (NOS2) results in reduced neuronal apoptosis and mortality following mouse hepatitis virus infection of the central nervous system. *J Neurovirol*, 8, 58-63 (2002)
114. Stohlman, S. A., D. R. Hinton, D. Cua, E. Dimacali, J. Sensintaffar, F. M. Hofman, S. M. Tahara & Q. Yao: Tumor necrosis factor expression during mouse hepatitis virus-induced demyelinating encephalomyelitis. *J Virol*, 69, 5898-903 (1995)
115. Lane, T. E., J. L. Hardison & K. B. Walsh: Functional diversity of chemokines and chemokine receptors in response to viral infection of the central nervous system. *Curr Top Microbiol Immunol*, 303, 1-27 (2006)
116. Ben-Hur, T., B. Rogister, K. Murray, G. Rougon & M. Dubois-Dalcq: Growth and fate of PSA-NCAM+ precursors of the postnatal brain. *J Neurosci*, 18, 5777-88 (1998)
117. Brustle, O., K. N. Jones, R. D. Learish, K. Karram, K. Choudhary, O. D. Wiestler, I. D. Duncan & R. D. McKay: Embryonic stem cell-derived glial precursors: a source of myelinating transplants. *Science*, 285, 754-6 (1999)
118. Cao, Q., R. L. Benton & S. R. Whittemore: Stem cell repair of central nervous system injury. *J Neurosci Res*, 68, 501-10 (2002)
119. Nistor, G. I., M. O. Totoiu, N. Haque, M. K. Carpenter & H. S. Keirstead: Human embryonic stem cells differentiate into oligodendrocytes in high purity and myelinate after spinal cord transplantation. *Glia*, 49, 385-96 (2005)
120. Pluchino, S., A. Quattrini, E. Brambilla, A. Gritti, G. Salani, G. Dina, R. Galli, U. Del Carro, S. Amadio, A. Bergami, R. Furlan, G. Comi, A. L. Vescovi & G. Martino: Injection of adult neurospheres induces recovery in a chronic model of multiple sclerosis. *Nature*, 422, 688-94 (2003)
121. Totoiu, M. O., G. I. Nistor, T. E. Lane & H. S. Keirstead: Remyelination, axonal sparing, and locomotor recovery following transplantation of glial-committed progenitor cells into the MHV model of multiple sclerosis. *Exp Neurol*, 187, 254-65 (2004)
122. Hardison, J. L., G. Nistor, R. Gonzalez, H. S. Keirstead & T. E. Lane: Transplantation of glial-committed progenitor cells into a viral model of multiple sclerosis induces remyelination in the absence of an attenuated inflammatory response. *Exp Neurol*, 197, 420-9 (2006)
123. Binder, G. K. & D. E. Griffin: Immune-mediated clearance of virus from the central nervous system. *Microbes Infect*, 5, 439-48 (2003)
124. Buchmeier, M. J. & A. J. Zajac, Lymphocytic Choriomeningitis Virus. New Jersey: *John Wiley and Sons*, (1999)

Abbreviations: mouse hepatitis virus (MHV), human coronavirus (HCoV), open reading frames (ORFs), multiple sclerosis (MS), severe acute respiratory syndrome (SARS), carcinoembryonic antigen-cell adhesion molecule (CEACAM-1), interferons (IFNs), tumor necrosis factor alpha (TNF-alpha), matrix metalloproteinases (MMPs), tissue inhibitor of metalloproteinase 1 (TIMP-1), recombination-activating gene-1 deficient (*Rag1-/-),* dendritic cells (DCs), cytotoxic T lymphocytes (CTLs), antibody secreting cells (ASCs), oligodendrocyte progenitor cells (OPCs),

Key Words: Virus, Central Nervous System, Host-Defense, Disease, Chemokines, Cytokines, Review

Send correspondence to: Dr. Thomas E. Lane, Department of Molecular Biology and Biochemistry, 3205 McGaugh Hall, University of California, Irvine, Irvine, CA 92697-3900, Tel: 949-824-5878, Fax: 949-825-8551, E-mail: tlane@uci.edu

http://www.bioscience.org/current/vol13.htm