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Review

The chemokine receptor CXCR2 and coronavirus-induced neurologic disease

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ABSTRACT

Inoculation with the neurotropic JHM strain of mouse hepatitis virus (MHV) into the central nervous system (CNS) of susceptible strains of mice results in an acute encephalomyelitis in which virus preferentially replicates within glial cells while excluding neurons. Control of viral replication during acute disease is mediated by infiltrating virus-specific T cells via cytokine secretion and cytolytic activity, however sterile immunity is not achieved and virus persists resulting in chronic neuroinflammation associated with demyelination. CXCR2 is a chemokine receptor that upon binding to specific ligands promotes host defense through recruitment of myeloid cells to the CNS as well as protecting oligodendroglia from cytokine-mediated death in response to MHV infection. These findings highlight growing evidence of the diverse and important role of CXCR2 in regulating neuroinflammatory diseases.

Contents

Mouse hepatitis virus (MHV)

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 (Holmes and Lai, 1996; McIntosh, 1996; Perlman et al., 1999; Weiss and Navas-Martin, 2005). All coronaviruses are enveloped with, to date, 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) (Holmes, 2003; Masters, 2006; Weiss and Navas-Martin, 2005). In addition, previously unclassified human coronaviruses associated with respiratory disease have been identified (van der Hoek et al., 2006, 2004; Woo et al., 2005). As a natural pathogen of mice, MHV primarily infects the liver and CNS resulting in a range of acute and chronic diseases, including hepatitis, encephalitis and encephalomyelitis associated with demyelination (Holmes and Lai, 1996; McIntosh, 1996; Perlman et al., 1999). Viral tropism and disease depend 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 (Perlman et al., 1999).

Acute MHV-induced encephalomyelitis

Following intracranial infection, MHV replicates first within the ependymal cells of the lateral ventricles before spreading

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throughout the parenchyma primarily targeting astrocytes and oligodendrocytes (Wang et al., 1992). Neurons are spared within immunocompetent mice inoculated with neuroattenuated strains of MHV (Buchmeier et al., 1984; Fleming et al., 1983; Ireland et al., 2008). MHV infection of the CNS results in rapid upregulation of inflammatory cytokines, chemokines, and matrix-metalloproteinases, all of which serve to initiate, attract, and support a robust host anti-viral response (Glass et al., 2002; Lane et al., 1998; Parra et al., 1997; Pearce et al., 1994; Rempel et al., 2004, 2005; Sun et al., 1995; Zhou et al., 2005b, 2002).

Type I interferons (IFN-α and IFN-β), IL-1α, IL-1β, IL-6, IL-12, and TNFα are secreted following MHV infection (Parra et al., 1997; Pearce et al., 1994; Rempel et al., 2004, 2005; Sun et al., 1995). Protective roles for the type I interferons during MHV infection have been well described. Exogenous treatment of either IFN-α or IFN-β limits MHV replication and dissemination within the CNS (Minagawa et al., 1987; Smith et al., 1987), while mice deficient in IFN-α/β-receptor quickly succumb to MHV infection (Cervantes-Barragan et al., 2007). The mechanisms of type I IFN *in vivo* protection are however complicated since MHV is resistant to IFN-β treatment *in vitro* (Roth-Cross et al., 2007). Moreover, evidence suggests that MHV can shield their viral RNA genome from host pattern recognition receptors and therefore prevent IFN-β induction (Versteeg et al., 2007; Zhou and Perlman, 2007). Nevertheless, type I IFNs are clearly protective *in vivo*, and they may help to regulate innate and adaptive immune responses by enhancing MHC I expression (Akwa et al., 1998; Ireland et al., 2008).

Innate immune cells recruited into the CNS following MHV infection include neutrophils and macrophages (Templeton et al., 2008; Zuo et al., 2006). Neutrophils contribute to degradation of the blood brain barrier (BBB) by secreting matrix metalloproteinase (MMPs) that facilitate extracellular matrix and basement lamina degradation (Yong et al., 2001). Although neutrophils secrete MMP-9 (Zhou et al., 2002, 2003), they are not the sole source of matrix metalloproteinases within the CNS, as MMP-3 and MMP-12, derived from resident glia, may also have contribute to BBB breakdown (Savarin et al., 2010; Zhou et al., 2003). Nevertheless, neutrophils are important for enhanced anti-viral responses following MHV infection, as their depletion mutes leukocyte entry into the CNS, thus limiting effective control of viral replication and allowing viral spread (Zhou et al., 2003). Monocyte/macrophage infiltration is dependent upon numerous chemokine signaling pathways including CCR2/CCL2 (Chen et al., 2001; Held et al., 2004; Savarin et al., 2010), CCL3 (Trifilo et al., 2003), and CCL5/CCR5 (Glass et al., 2004, 2001; Lane et al., 2000). Macrophages do not appear to perform any direct anti-viral activity within the CNS, as depletion of macrophages or neutralization of CCL5 during acute MHV infection does not enhance viral burden (Lane et al., 2000; Xue et al., 1999). Both myeloid (CD11b+ CD11c+) and lymphoid (CD11b− CD11c+) derived dendritic cells (DC) are detectable within the CNS by day 2 p.i. (Trifilo and Lane, 2004), though the chemotactic signals controlling their infiltration has not been fully explored. Migration of myeloid DCs to the draining lymph nodes is dependent, in part, on CCL3 expression (Trifilo and Lane, 2004). Moreover, CCL3 deficiency reduces lymph node DC activation and skews T_H1 anti-MHV responses (Trifilo and Lane, 2004).

Virus-specific T cells are detectable within the local lymph nodes and spleen and subsequently migrate into the CNS early following CNS infection with MHV (Marten et al., 2003). Protective immunity and anti-viral responses conform to a T_H1 phenotype, broadly characterized by vigorous IFN-γ secretion and cytolytic activity (Bergmann et al., 2003; Lin et al., 1997; Parra et al., 1999). Virus-specific T cell generation is not strictly dependent on IL-12 and/or IL-23, as viral clearance is unaffected following antibody neutralization of IL-23 and IL-12/23 (Held et al., 2008) or genetic deletion of IL-12 (Kapil et al., 2009). T cells isolated from the CNS of MHV infected mice are CXCR3-reactive (Stiles et al., 2006) and their migration into the CNS is mediated by expression of the CXCR3 ligands CXCL9 and CXCL10 (Glass et al., 2001; Liu et al., 2000; Stiles et al., 2006; Walsh et al., 2007). Furthermore, CCL5 has been shown to differentially regulate T cell migration into the CNS. Neutralization of CCL5 during infection abrogates CD4+ and CD8+ T cell infiltration (Lane et al., 2000), however, CCR5 deficient CD8+ T cells adoptively transferred into MHV infected *RAG1*−/− recipients have no problem trafficking into the CNS (Glass and Lane, 2003a), while transferred CCR5 deficient CD4+ T cells do not efficiently enter the CNS (Glass and Lane, 2003b). Virus specific CD8+ T cells are the main cytolytic effector cell within the CNS and begin to accumulate by five days p.i. (Marten et al., 2000; Marten et al., 2003). CD8+ T cells are essential to controlling MHV replication (Bergmann et al., 2003); their accumulation within the CNS is concurrent with viral clearance from resident glia (Bergmann et al., 1999, 2006, 2003). CD8+ T cells isolated from the CNS are cytolytic *ex vivo* (Bergmann et al., 1999; Walsh et al., 2008), secreting IFN-γ and lytic molecules, including granzyme B and perforin (Ramakrishna et al., 2004). *In vivo,* perforin-mediated cytolysis eliminates MHV from astrocytes (Lin et al., 1997) and IFN-γ controls MHV replication within oligodendroglia (Gonzalez et al., 2006; Parra et al., 1999). Evidence has also demonstrated that NKG2D signaling within the CNS enhances anti-viral CD8+ cytotoxic activity (Walsh et al., 2008).

Virus specific CD4+ T cells function in a supporting role for CD8+ T cells, and they are also critical in controlling MHV replication (Phares et al., 2012). *In vivo* CD4+ T cells enhance immune cell activity within the CNS (Bergmann et al., 2003, 2004) by secreting IFN-γ, which facilitates viral clearance from oligodendroglia (Gonzalez et al., 2006; Parra et al., 1999), and upregulates MHC class II expression on microglia (Bergmann et al., 2003) and MHC class I expression on oligodendroglia (Malone et al., 2008). CD8+ cytotoxicity and survival within the CNS relies on the presence of CD4+ T cells (Phares et al., 2012; Stohlman et al., 1998; Zhou et al., 2005a). How CD4+ T cells support and enhance CD8+ T cell activity is unknown, however it is assumed to be a secreted factor, since CD4+ T cells are spatially restricted near the vasculature, instead of migrating throughout the parenchyma like CD8+ T cells, possibly as a result of CD4+ T cell TIMP-1 expression (Zhou et al., 2005b).

MHV-induced demyelination

Mice that survive acute MHV infection develop a chronic immune-mediated demyelinating disease. Infected mice first demonstrate signs of ascending demyelination during acute infection that range from partial to complete hind limb paralysis. Analysis of the spinal cords of chronically-infected mice confirms that the loss of myelin integrity is associated with the continued presence of both viral antigen and inflammatory immune cells (Stohlman and Hinton, 2001) and not the apoptotic or necrotic death of myelinating oligodendrocytes (Wu and Perlman, 1999). No role for endogenous complement or antibody-mediated demyelination has been documented (Matthews et al., 2002a), although exogenous autoantibodies can exacerbate demyelination independent of complement during chronic infection (Burrer et al., 2007). Nevertheless, the immunopathology observed during chronic MHV infection resembles what is observed in the majority of active multiple sclerosis (MS) lesions (Houtman and Fleming, 1996; Matthews et al., 2002b), making chronic MHV infection an excellent surrogate model to study mechanisms

associated with the immunopathogenesis of MS and to develop novel treatments.

Concomitant with the absence of detectable infectious virus, neuroinflammation wanes yet virus-specific T cells and macrophages remain within the CNS for up to three months after infection (Castro et al., 1994; Liu et al., 2001; Marten et al., 2000; Ramakrishna et al., 2004). Unlike in other models of CNS demyelination (Katz-Levy et al., 2000; McMahon et al., 2005; Miller et al., 1997) and in MS (Goebels et al., 2000; Tuohy et al., 1997, 1999), autoreactive T cells specific to defined myelin epitopes are not considered important in contributing to disease, indicating that chronic demyelination is mainly driven by antiviral responses and not epitope spreading. While both CD4+ and CD8+ T cells remain CXCR3+ during chronic infection (Stiles et al., 2006), only CD4+ T cells appear to rely upon CXCL10 for antiviral trafficking into the CNS; CD8+ T cell infiltration remains relatively unaffected during CXCL10 neutralization (Liu et al., 2001). Notably, CCL5 neutralization abrogates both CD4+ and CD8+ T cell accumulation during chronic infection (Glass et al., 2004), indicating differential chemokine usage between the T cell subsets (Stiles et al., 2009). More recently, Bergmann and colleagues have provided compelling evidence that CXCR3 ligands CXCL9 and CXCL10 are crucial for allowing plasmablast migration into the CNS of MHV-infected mice via signaling through CXCR3 expressed on these cells (Marques et al., 2011; Phares et al., 2011; Tschen et al., 2006). These findings highlight a previously unappreciated role for these chemokines in host defense by attracting activated antibody secreting cells into the CNS of mice persistently infected with a neurotropic virus. Neutralizing antibody from B cells prevents viral recrudescence during chronic MHV infection (Lin et al., 1999; Ramakrishna et al., 2003, 2002).

The main effectors of demyelination during chronic MHV infection are T cells and macrophages. Both CD4+ and CD8+ T cells are important to the pathogenesis of chronic demyelination, although to differing degrees. Mice deficient in adaptive immune systems (Lane et al., 2000; Wu et al., 2000; Wu and Perlman, 1999) or CD4+ T cells (Lane et al., 2000) do not readily demyelinate, regardless of their ability to clear virus. Moreover, adoptive transfer of CD4+ T cells into MHV-infected *RAG1*−/− hosts is sufficient to initiate demyelination (Lane et al., 2000). CD4+ T cells also enhance demyelination, by attracting macrophages through CCL5 secretion (Lane et al., 2000). Although it was reported that *CD8*−/− mice exhibit muted demyelination during chronic MHV infection (Lane et al., 2000), IFN-γ dependent demyelination was observed following the transfer of CD8+ T cells into *RAG1*−/− mice (Pewe et al., 2002; Pewe and Perlman, 2002; Wu et al., 2000), providing evidence that CD8+ T cells are capable of initiating and amplifying demyelination.

As with other demyelinating diseases (Epstein et al., 1983; Field and Raine, 1966), ultrastructural analysis of MHV-induced demyelinating lesions reveal myelin laden macrophages stripping and engulfing myelin (Fleury et al., 1980). During chronic infection, macrophages are spatially associated within demyelinating white matter lesions of the spinal cord and are critical to demyelination. Neutralization of the potent macrophage chemokine CCL5 during chronic infection diminishes macrophage infiltration into the CNS and is associated with reduced demyelination (Glass et al., 2004; Lane et al., 2000). Moreover genetic silencing of CCR5, the chemokine receptor for CCL5, also prevents widespread demyelination, even in the absence of viral clearance (Glass et al., 2001). Adoptive transfer of MHV-immunized splenocytes into infected *RAG1*−/− recipients resulted in the rapid demyelination, and this was associated with the widespread recruitment of activated macrophages to regions of pathology (Wu and Perlman, 1999). These observations are consistent with other models of demyelination, including, EAE (Bauer et al., 1995; Tran et al., 1998) and cuprizone-induced demyelination (Hiremath et al., 1998); likewise, reactive macrophages have also been described within demyelinating MS plaques (Boyle and McGeer, 1990).

Although the main effectors of demyelination are certainly T cells and macrophages, this does not preclude the possibility that MHV may directly participate in damage, especially since oligodendrocytes are the main reservoir of MHV during chronic infection (Gonzalez et al., 2005, 2006). In some MS lesions, oligodendrocyte apoptosis has also been observed (Barnett and Prineas, 2004; Matute and Perez-Cerda, 2005), however the exact role of apoptosis in MS pathogenesis and pathology is unresolved (Frohman et al., 2006). *In vitro*, cultured murine oligodendrocytes are susceptible to MHV-induced apoptosis through FAS-spike glycoprotein interactions (Liu et al., 2003, 2006; Liu and Zhang, 2005, 2007). Moreover, the HIV protein Tat (Hauser et al., 2009) and the JC virus protein agnoprotein (Merabova et al., 2008) also enhance oligodendrocyte apoptosis *in vitro*. However, *in vivo* oligodendrocyte apoptosis during chronic MHV infection is not readily observed, and the presence of viral antigen does not appear to predispose an oligodendrocyte to apoptosis (Wu and Perlman, 1999). Therefore, it is likely that protective mechanisms exist during chronic infection that protect oligodendrocytes from MHV, IFN-γ, and other apoptotic inducers.

Neurobiology of CXCR2

CXCR2, a receptor for the ELR-positive CXC chemokines CXCL1 and CXCL2, which are defined by a glutamic acid-leucine-arginine (ELR) amino acid sequence preceding a group of conserved cysteine residues (CXC) at their amino termini is expressed by resident cells of the CNS including neurons, astrocytes, microglia, myelinating oligodendrocytes and oligodendrocyte progenitor cells (OPCs)(Cho and Miller, 2002; Coughlan et al., 2000; Danik et al., 2003; Filipovic et al., 2003; Flynn et al., 2003; Horuk et al., 1997; Nguyen and Stangel, 2001). In addition, CXCR2 has been shown to have a role in OPC differentiation and efficient myelination of axons by oligodendrocytes (Kerstetter et al., 2009). During CNS development, CXCR2 is necessary to obtain sufficient numbers of OPCs to ensure structural integrity and is also essential for positional migration of OPCs within the white matter of the mouse spinal cord (Robinson et al., 1998; Tsai et al., 2002). Acting in concert with the oligodendrocyte receptor ligand PDGF, signaling through the CXCR2-CXCL1 axis has also been shown to enhance OPC proliferation in the developing mouse spinal cord (Robinson et al., 1998).

Transgenic mice devoid of CXCR2 have insufficient numbers and misalignments of OPCs that persist into adulthood and result in reduced myelin and spinal cord white matter, and changes in expression of myelin-specific proteins such as PLP and MBP (Padovani-Claudio et al., 2006). In addition, OPCs derived from these *CXCR2*−/− mice have decreased numbers of mature oligodendrocytes when differentiated in culture, which demonstrates an important role for CXCR2 in OPC maturation (Padovani-Claudio et al., 2006). Studies of CXCR2 under pathologic conditions have yielded conflicting roles for the chemokine receptor in the CNS. There are numerous reports that CXCR2 is necessary for induction of EAE due to its ability to promote polymorphonuclear leukocyte (PMN) chemotaxis to the CNS yet the functional role of CXCR2 on resident cells of the CNS was not examined (Carlson et al., 2008; Glabinski et al., 2000; Kroenke et al., 2010). Omari et al. (2009) demonstrated that overexpression of CXCL1 from genetically-engineered mice exerted a protective effect within the CNS associated with a reduced severity in clinical disease and diminished neuropathology. More recently, a study by Liu et al.

(2010) used bone marrow chimeric mice to separate the contribution of CXCR2 expression on hematopoietic cells and CNS cells. Upon transfer of CXCR2-positive cells derived from the bone marrow of WT mice into *CXCR2−/−* mice, OPCs divided more rapidly and there was greater oligodendrocyte differentiation following demyelination in both EAE and cuprizone models of demyelination (Liu et al., 2010). This study suggests that CXCR2 expression on cells of the CNS is inhibitory for myelin repair following demyelination and therefore contributes to chronic disease. Other studies using CXCR2 antagonists in EAE and spinal cord injury showed that blocking CXCR2 induced oligodendrocyte differentiation and promoted recovery, further suggesting a pathologic role for CXCR2 under defined experimental conditions (Kerstetter et al., 2009; Marsh and Flemming, 2011). However, it should be noted that these CXCR2 antagonist treatments resulted in global reduction of CXCR2 and at least part of the benefits were due to decreased inflammatory cell infiltration into the CNS. Nevertheless, while CXCR2 is necessary for OPC proliferation during development it appears to be deleterious under some pathologic conditions. There may also be other factors involved in CXCR2 signaling on OPC proliferation and differentiation through CXCR2 expression on other cells of the CNS since the studies done in cuprizone and EAE did not specifically eliminate CXCR2 on OPCs.

Conversely, a pathologic condition in which CXCR2 expression has been suggested to be beneficial to oligodendroglia is following exposure to the cytokine IFN-γ, which is often highly expressed by CNS infiltrating activated T lymphocytes and NK cells under inflammatory conditions (Juedes et al., 2000; Traugott and Lebon, 1988a, b; Woodroofe and Cuzner, 1993). *In vitro,* IFN-γ-mediated death of mouse OPCs can be mitigated by expression and signaling through CXCR2 (Tirotta et al., 2011). OPCs, which are highly sensitive to IFN-γ-mediated apoptosis, constitutively express CXCR2 and treatment with CXCL1 facilitates protection from IFN-γ-mediated apoptosis (Tirotta et al., 2011). OPCs generated from *CXCR2−/−* mice are not protected from IFN-γ-induced apoptosis in the presence of CXCL1 (Tirotta et al., 2011). Mechanisms associated with CXCR2-mediated protection of OPC cell death are increased expression of anti-apoptotic Bcl2 and inhibition of pro-apoptotic caspase 3 activation (Tirotta et al., 2011). CXCR2 also guards OPCs from cell death mediated by the pro-inflammatory cytokine CXCL10, whose expression is induced by IFN-γ (Tirotta et al., 2011). These findings suggest that IFN-γ-induced expression of CXCL10 is one mechanism by which IFN-γ can induce OPC death. Follow up studies recently demonstrated that human OPCs generated from embryonic stem cells are also susceptible to IFN-γ-mediated apoptosis and CXCR2 signaling exerts a protective effect by inhibiting cleavage of caspase 3 (Tirotta et al., 2012). These data provides some mechanistic clarity on previous findings that CXCL10 is involved in cell death within the CNS during immunodeficiency virus-induced encephalitis, West Nile virus-induced encephalitis, and spinal cord injury (Glaser et al., 2006; Klein et al., 2005; Sui et al., 2004; Zhang et al., 2008, 2010).

Beyond blocking IFN-γ and CXCL10 mediated apoptosis of OPCs, CXCR2 can prevent β-amyloid accumulation-mediated neuronal death through CXCL1 and its other ligands CXCL8 and macrophage inflammatory protein 2 (MIP2) by signaling through the MEK1-ERK1/2 and PI3K-Akt signaling pathways (Raman et al., 2011; Watson and Fan, 2005). However, other groups' data show contrasting results; CXCR2 signaling results in enhanced γ-secretase activity and increased β-amyloid accumulation and specifically CXCR2 signaling through the ERK1/2 and Akt pathways results in more severe Alzheimer's pathology as a result of Tau hyperphosphorylation (Bakshi et al., 2008; Xia and Hyman, 2002). While the origin of neurons was different (hippocampal versus cortical, respectively), the reason for the discrepancy in the role of CXCR2 in neuron pathology is unclear and further studies are needed. Potential mechanisms to prevent neuronal death are of particular interest in Alzheimer's disease. Overall, CXCR2 is important for CNS development and has a role during CNS disease. Elucidation of the exact mechanisms behind the dichotomy of CXCR2 in its ability to protect oligodendrocytes and contribute to CNS damage is necessary to be able to use CXCR2 as a therapeutic target.

CXCR2 and MHV-induced neurologic disease

Chemokines are rapidly secreted within the CNS in response to MHV infection and contribute to host defense (Glass et al., 2001; Lane et al., 1998, 2000; Liu et al., 2000) and disease progression (Glass et al., 2004, 2001; Liu et al., 2001; Stiles et al., 2006). The chemokines CXCL1 and CXCL2 are up-regulated within the brains of MHV-infected mice (Lane et al., 1998; Scott et al., 2008; Zhou et al., 2002) and are potent chemoattractants for PMNs via binding and signaling through their receptor CXCR2 (Moser et al., 1990; Schumacher et al., 1992; Wolpe et al., 1989). Moreover, PMNs have been shown to enhance CNS inflammation by disrupting blood brain barrier (BBB) integrity in animal models of neuroinflammation (Carlson et al., 2008; Gorio et al., 2007; Tonai et al., 2001) as well as MHV-induced encephalomyelitis (Zhou et al., 2003). In addition, blocking or silencing of CXCR2 signaling mutes inflammation and tissue damage in mouse models in which PMN infiltration is critical to disease initiation (Belperio et al., 2005; Carlson et al., 2008; Gorio et al., 2007; Kielian et al., 2001; Londhe et al., 2005a,b; Strieter et al., 2005; Wareing et al., 2007). With regards to MHV infection, depletion of PMNs increases mortality due to abrogated BBB permeabilization and subsequent diminished T cell infiltration into the CNS (Zhou et al., 2003). We have shown that early following MHV infection, CXCR2-positive neutrophils are mobilized into the bloodstream and migrate to the CNS in response to elevated expression of the ELR+ chemokines CXCL1, CXCL2, and CXCL5 (Hosking et al., 2009). Neutrophil entry into the blood was not completely inhibited following CXCR2 neutralization, indicating that there may be additional signaling components that aid neutrophil release such as CXCL12 downregulation or G-CSF induction (Martin et al., 2003; Wengner et al., 2008). In addition, CXCR2 neutralization also reduces circulating levels of neutrophils within uninfected mice suggesting that CXCR2 ligands contribute to both normal neutrophil homeostasis and emergency release following infection with a neurotropic virus. These findings highlight a previously unappreciated functional role for ELR+ chemokines in host defense during viral-induced encephalomyelitis, rapidly recruiting PMNs into the blood with subsequent infiltration into the CNS. Administration of a blocking antibody specific for CXCR2 to MHV-infected mice reduced PMN migration to the CNS by $>95\%$, and this corresponded with increased mortality and uncontrolled viral replication. We determined that anti-CXCR2 treatment prevented PMN-mediated BBB permeabilization, associated with muted MMP-9 activity, and ultimately resulted in the impaired accumulation of virus-specific T cells within the CNS. These findings support and extend other studies highlighting the functional role of neutrophils in promoting vascular permeability in response to infection or injury to the CNS (Carlson et al., 2008; Kim et al., 2009; Zhou et al., 2003). Therefore, therapies targeting myeloid cell trafficking to the CNS during acute viral infection may offer a powerful approach to dampen neuroinflammation and decrease fatalities associated with viral encephalopathies.

How chemokine receptor signaling contributes to chronic neurologic diseases has largely been considered within the context of targeted leukocyte recruitment into the CNS (Hosking et al., 2009). Yet numerous resident cell types of the CNS express chemokine receptors under non-inflammatory and inflammatory conditions (Bajetto et al., 2001; Ubogu et al., 2006), indicating that these cells are capable of responding to specific chemokine ligands. As indicated above, CXCR2 is detected both *in vitro* and *in vivo* upon resident cells of the CNS, including OPCs (Dorf et al., 2000; Horuk et al., 1997; Omari et al., 2006, 2005; Popivanova et al., 2003; Tsai et al., 2002).

In mice persistently infected with MHV, T cells and macrophages contribute to oligodendrocyte damage and demyelination that is associated with physical disability (Cheever et al., 1949; Perlman et al., 1999). Following MHV-induced immune-mediated apoptosis of oligodendrocytes, there is an increase of OPC proliferation and oligodendrocyte maturation followed by partial remyelination (Carbajal et al., 2011; Liu et al., 2006; Liu and Zhang, 2007; Wu and Perlman, 1999). Given the fact that viral RNA is found within spinal cord white matter tracts long past the acute encephalitis phase of disease there are likely intrinsic mechanisms in oligodendrocytes that help protect them from apoptosis and allow for differentiation and remyelination (Hosking et al., 2010; Marten et al., 2000). CXCL1 has been shown to protect against IFN-γ induced OPC death via signaling through CXCR2 (Tirotta et al., 2011), so CXCR2 is a likely mechanism to protect against viral specific activated T cell secretion of IFN-γ (Marten et al., 2001). Indeed, CXCL1 and CXCR2 are upregulated following MHV infection and remain elevated even after viral load has decreased, suggesting that this signaling pathways aids in continued survival and maturation of OPCs as well as remyelination (Hosking et al., 2010). In fact, CXCR2 was shown protect oligodendrocytes against apoptosis during the chronic phase of MHV infection as antibody mediated blockade of CXCR2 with neutralizing antiserum at the beginning of the chronic phase resulted in increased white matter demyelination and a more severe clinical course that was not associated with changes in infiltrating immune cells or viral titers (Hosking et al., 2010).

Within the CNS, astrocytes and microglia are reported sources of CXCL1 (Lu et al., 2005). Activation of astrocytes under pro-inflammatory conditions results in increased secretion of CXCL1 via signaling through the sphingosine kinase 1(SphK1)/sphingosine1-phosphate (S1P) receptor signaling pathway (Fischer et al., 2011). CXCL1 can induce cultured mouse and human OPC proliferation and promote their differentiation and myelination (Filipovic and Zecevic, 2008; Robinson et al., 1998; Turbic et al., 2011). A study using transgenic mice with inducible overexpression of CXCL1 at disease onset in EAE showed astrocyte-secreted CXCL1 led to an increased number of proliferating OPCs and increased remyelination (Omari et al. 2009). In addition to protective effects of CXCL1 signaling, CXCR2 signaling via CXCL8 on cultured astrocytes inhibits Fas-mediated apoptosis allowing for continual CXCL1 secretion, indicating another mechanism by which CXCR2 signaling aids in CNS recovery following MHV-mediated demyelination (Saas et al., 2002). In microglia, on the other hand, the switch from a pro-inflammatory to anti-inflammatory response includes suppression of CXCL1 and upregulation of the anti-inflammatory cytokine IFN-β through the interferon regulatory factor 3 (IRF3)/PI3K/Akt signaling pathway (Tarassishin et al., 2011). Following MHV infection, IFN-β is upregulated specifically on microglia and infiltrating macrophages and is necessary for viral control (Mazaleuskaya et al., 2012; Roth-Cross et al., 2008). These data suggest that the source of elevated CXCL1 during the chronic phase of MHV infection is astrocytes, which promote remyelination through CXCR2. In combination with the induction of anti-inflammatory-mediating microglia these mechanisms help protect the CNS from further demyelination and promote remyelination.

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Reference

Akwa, Y., Hassett, D.E., Eloranta, M.L., Sandberg, K., Masliah, E., Powell, H., Whitton, J.L., Bloom, F.E., Campbell, I.L., 1998. Transgenic expression of IFN-alpha in the central nervous system of mice protects against lethal neurotropic viral infection but induces inflammation and neurodegeneration. J. Immunol. 161, 5016–5026.

Bajetto, A., Bonavia, R., Barbero, S., Florio, T., Schettini, G., 2001. Chemokines and their receptors in the central nervous system. Front. Neuroendocrinol. 22, 147–184.

Bakshi, P., Margenthaler, E., Laporte, V., Crawford, F., Mullan, M., 2008. Novel role of CXCR2 in regulation of gamma-secretase activity. ACS Chem. Biol. 3, 777–789.

Barnett, M.H., Prineas, J.W., 2004. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Ann. Neurol. 55, 458–468.

Bauer, J., Huitinga, I., Zhao, W., Lassmann, H., Hickey, W.F., Dijkstra, C.D., 1995. The role of macrophages, perivascular cells, and microglial cells in the pathogenesis of experimental autoimmune encephalomyelitis. Glia 15, 437–446.

Belperio, J.A., Keane, M.P., Burdick, M.D., Gomperts, B.N., Xue, Y.Y., Hong, K., Mestas, J., Zisman, D., Ardehali, A., Saggar, R., Lynch 3rd, J.P., Ross, D.J., Strieter, R.M., 2005. CXCR2/CXCR2 ligand biology during lung transplant ischemia-reperfusion injury. J. Immunol. 175, 6931–6939.

Bergmann, C.C., Altman, J.D., Hinton, D., Stohlman, S.A., 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, C.C., Lane, T.E., Stohlman, S.A., 2006. Coronavirus infection of the central nervous system: host-virus stand-off. Nat. Rev. Microbiol. 4, 121–132.

Bergmann, C.C., Parra, B., Hinton, D.R., Chandran, R., Morrison, M., Stohlman, S.A., 2003. Perforin-mediated effector function within the central nervous system requires IFN-gamma-mediated MHC up-regulation. J. Immunol. 170, 3204–3213.

Bergmann, C.C., Parra, B., Hinton, D.R., Ramakrishna, C., Dowdell, K.C., Stohlman, S.A., 2004. 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, 1739–1750.

Boyle, E.A., McGeer, P.L., 1990. Cellular immune response in multiple sclerosis plaques. Am. J. Pathol. 137, 575–584.

Buchmeier, M.J., Lewicki, H.A., Talbot, P.J., Knobler, R.L., 1984. Murine hepatitis virus-4 (strain JHM)-induced neurologic disease is modulated *in vivo* by monoclonal antibody. Virology 132, 261–270.

Burrer, R., Buchmeier, M.J., Wolfe, T., Ting, J.P., Feuer, R., Iglesias, A., von Herrath, M.G., 2007. Exacerbated pathology of viral encephalitis in mice with central nervous system-specific autoantibodies. Am. J. Pathol. 170, 557–566.

Carbajal, K.S., Miranda, J.L., Tsukamoto, M.R., Lane, T.E., 2011. CXCR4 signaling regulates remyelination by endogenous oligodendrocyte progenitor cells in a viral model of demyelination. Glia 59, 1813–1821.

Carlson, T., Kroenke, M., Rao, P., Lane, T.E., Segal, B., 2008. The Th17-ELR+ CXC chemokine pathway is essential for the development of central nervous system autoimmune disease. J. Exp. Med. 205, 811–823.

Castro, R.F., Evans, G.D., Jaszewski, A., Perlman, S., 1994. Coronavirus-induced demyelination occurs in the presence of virus-specific cytotoxic T cells. Virology 200, 733–743.

Cervantes-Barragan, L., Zust, R., Weber, F., Spiegel, M., Lang, K.S., Akira, S., Thiel, V., Ludewig, B., 2007. Control of coronavirus infection through plasmacytoid dendritic-cell-derived type I interferon. Blood 109, 1131–1137.

Cheever, F.S., Daniels, J.B., et al., 1949. A murine virus (JHM) causing disseminated encephalomyelitis with extensive destruction of myelin. J. Exp. Med. 90, 181–210.

Chen, B.P., Kuziel, W.A., Lane, T.E., 2001. 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, 4585–4592.

Cho, C., Miller, R.J., 2002. Chemokine receptors and neural function. J. Neurovirol. 8, 573–584.

Coughlan, C.M., McManus, C.M., Sharron, M., Gao, Z., Murphy, D., Jaffer, S., Choe, W., Chen, W., Hesselgesser, J., Gaylord, H., Kalyuzhny, A., Lee, V.M., Wolf, B., Doms, R.W., Kolson, D.L., 2000. Expression of multiple functional chemokine receptors and monocyte chemoattractant protein-1 in human neurons. Neuroscience 97, 591–600.

Danik, M., Puma, C., Quirion, R., Williams, S., 2003. Widely expressed transcripts for chemokine receptor CXCR1 in identified glutamatergic, gamma-aminobutyric acidergic, and cholinergic neurons and astrocytes of the rat brain: a single-cell reverse transcription-multiplex polymerase chain reaction study. J. Neurosci. Res. 74, 286–295.
Dorf, M.E., Berman, M.A., Tanabe, S., Heesen, M., Luo, Y., 2000. Astrocytes express functional chemokine receptors. J. Neuroimmunol. 111, 109–121.
Epstein, L.G., Prineas, J.W., Raine, C.S., 1983. Attachment of myelin to coated pits on macrophages in experimental allergic encephalomyelitis. J. Neurol. Sci. 61, 341–348.
Field, E.J., Raine, C.S., 1966. Experimental allergic encephalomyelitis. An electron microscopic study. Am. J. Pathol. 49, 537–553.
Filipovic, R., Jakovcevski, I., Zecevic, N., 2003. GRO-alpha and CXCR2 in the human fetal brain and multiple sclerosis lesions. Dev. Neurosci. 25, 279–290.
Filipovic, R., Zecevic, N., 2008. The effect of CXCL1 on human fetal oligodendrocyte progenitor cells. Glia 56, 1–15.
Fischer, I., Alliod, C., Martinier, N., Newcombe, J., Brana, C., Pouly, S., 2011. Sphingosine kinase 1 and sphingosine 1-phosphate receptor 3 are functionally upregulated on astrocytes under pro-inflammatory conditions. PLoS One 6, e23905.
Fleming, J.O., Stohlman, S.A., Harmon, R.C., Lai, M.M., Frelinger, J.A., Weiner, L.P., 1983. Antigenic relationships of murine coronaviruses: analysis using monoclonal antibodies to JHM (MHV-4) virus. Virology 131, 296–307.
Fleury, H.J., Sheppard, R.D., Bornstein, M.B., Raine, C.S., 1980. Further ultrastructural observations of virus morphogenesis and myelin pathology in JHM virus encephalomyelitis. Neuropathol. Appl. Neurobiol. 6, 165–179.
Flynn, G., Maru, S., Loughlin, J., Romero, I.A., Male, D., 2003. Regulation of chemokine receptor expression in human microglia and astrocytes. J. Neuroimmunol. 136, 84–93.
Frohman, E.M., Racke, M.K., Raine, C.S., 2006. Multiple sclerosis—the plaque and its pathogenesis. N. Engl. J. Med. 354, 942–955.
Glabinski, A.R., O'Bryant, S., Selmaj, K., Ransohoff, R.M., 2000. CXC chemokine receptors expression during chronic relapsing experimental autoimmune encephalomyelitis. Ann. N.Y. Acad. Sci. 917, 135–144.
Glaser, J., Gonzalez, R., Sadr, E., Keirstead, H.S., 2006. Neutralization of the chemokine CXCL10 reduces apoptosis and increases axon sprouting after spinal cord injury. J Neurosci. Res. 84, 724–734.
Glass, W.G., Chen, B.P., Liu, M.T., Lane, T.E., 2002. Mouse hepatitis virus infection of the central nervous system: chemokine-mediated regulation of host defense and disease. Viral. Immunol. 15, 261–272.
Glass, W.G., Hickey, M.J., Hardison, J.L., Liu, M.T., Manning, J.E., Lane, T.E., 2004. 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, 4018–4025.
Glass, W.G., Lane, T.E., 2003a. Functional analysis of the CC chemokine receptor 5 (CCR5) on virus-specific CD8+ T cells following coronavirus infection of the central nervous system. Virology 312, 407–414.
Glass, W.G., Lane, T.E., 2003b. Functional expression of chemokine receptor CCR5 on CD4(+) T cells during virus-induced central nervous system disease. J. Virol. 77, 191–198.
Glass, W.G., Liu, M.T., Kuziel, W.A., Lane, T.E., 2001. Reduced macrophage infiltration and demyelination in mice lacking the chemokine receptor CCR5 following infection with a neurotropic coronavirus. Virology 288, 8–17.
Goebels, N., Hofstetter, H., Schmidt, S., Brunner, C., Wekerle, H., Hohlfeld, R., 2000. Repertoire dynamics of autoreactive T cells in multiple sclerosis patients and healthy subjects: epitope spreading versus clonal persistence. Brain 123 (Pt 3), 508–518.
Gonzalez, J.M., Bergmann, C.C., Fuss, B., Hinton, D.R., Kangas, C., Macklin, W.B., Stohlman, S.A., 2005. Expression of a dominant negative IFN-gammareceptor on mouse oligodendrocytes. Glia 51, 22–34.
Gonzalez, J.M., Bergmann, C.C., Ramakrishna, C., Hinton, D.R., Atkinson, R., Hoskin, J., Macklin, W.B., Stohlman, S.A., 2006. Inhibition of interferon-gamma signaling in oligodendroglia delays coronavirus clearance without altering demyelination. Am. J. Pathol. 168, 796–804.
Gorio, A., Madaschi, L., Zadra, G., Marfia, G., Cavalieri, B., Bertini, R., Di Giulio, A.M., 2007. Reparixin, an inhibitor of CXCR2 function, attenuates inflammatory responses and promotes recovery of function after traumatic lesion to the spinal cord. J. Pharmacol. Exp. Ther. 322, 973–981.
Hauser, K.F., Hahn, Y.K., Adjan, V.V., Zou, S., Buch, S.K., Nath, A., Bruce-Keller, A.J., Knapp, P.E., 2009. HIV-1 Tat and morphine have interactive effects on oligodendrocyte survival and morphology. Glia 57, 194–206.
Held, K.S., Chen, B.P., Kuziel, W.A., Rollins, B.J., Lane, T.E., 2004. Differential roles of CCL2 and CCR2 in host defense to coronavirus infection. Virology 329, 251–260.
Held, K.S., Glass, W.G., Orlovsky, Y.I., Shamberger, K.A., Petley, T.D., Branigan, P.J., Carton, J.M., Beck, H.S., Cunningham, M.R., Benson, J.M., Lane, T.E., 2008. Generation of a protective T-cell response following coronavirus infection of the central nervous system is not dependent on IL-12/23 signaling. Viral. Immunol. 21, 173–188.
Hiremath, M.M., Saito, Y., Knapp, G.W., Ting, J.P., Suzuki, K., Matsushima, G.K., 1998. Microglial/macrophage accumulation during cuprizone-induced demyelination in C57BL/6 mice. J. Neuroimmunol. 92, 38–49.
Holmes, K., Lai, M., 1996. Fields Virology, in: al., B.F.e. (Ed.), Fields Virology, third ed. Lippincott-Raven Publishers, pp. 1075–1094.
Holmes, K.V., 2003. SARS-associated coronavirus. N. Engl. J. Med. 348, 1948–1951.
Horuk, R., Martin, A.W., Wang, Z., Schweitzer, L., Gerassimides, A., Guo, H., Lu, Z., Hesselgesser, J., Perez, H.D., Kim, J., Parker, J., Hadley, T.J., Peiper, S.C., 1997. Expression of chemokine receptors by subsets of neurons in the central nervous system. J. Immunol. 158, 2882–2890.
Hosking, M.P., Liu, L., Ransohoff, R.M., Lane, T.E., 2009. A protective role for ELR+ chemokines during acute viral encephalomyelitis. PLoS Pathog. 5, e1000648.
Hosking, M.P., Tirotta, E., Ransohoff, R.M., Lane, T.E., 2010. CXCR2 signaling protects oligodendrocytes and restricts demyelination in a mouse model of viral-induced demyelination. PLoS One 5, 12.
Houtman, J.J., Fleming, J.O., 1996. Pathogenesis of mouse hepatitis virus-induced demyelination. J. Neurovirol. 2, 361–376.
Ireland, D.D., Stohlman, S.A., Hinton, D.R., Atkinson, R., Bergmann, C.C., 2008. Type I interferons are essential in controlling neurotropic coronavirus infection irrespective of functional CD8 T cells. J. Virol. 82, 300–310.
Juedes, A.E., Hjelmstrom, P., Bergman, C.M., Neild, A.L., Ruddle, N.H., 2000. Kinetics and cellular origin of cytokines in the central nervous system: insight into mechanisms of myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis. J. Immunol. 164, 419–426.
Kapil, P., Atkinson, R., Ramakrishna, C., Cua, D.J., Bergmann, C.C., Stohlman, S.A., 2009. Interleukin-12 (IL-12), but not IL-23, deficiency ameliorates viral encephalitis without affecting viral control. J. Virol. 83, 5978–5986.
Katz-Levy, Y., Neville, K.L., Padilla, J., Rahbe, S., Begolka, W.S., Girvin, A.M., Olson, J.K., Vanderlugt, C.L., Miller, S.D., 2000. Temporal development of autoreactive Th1 responses and endogenous presentation of self myelin epitopes by central nervous system-resident APCs in Theiler's virus-infected mice. J. Immunol. 165, 5304–5314.
Kerstetter, A.E., Padovani-Claudio, D.A., Bai, L., Miller, R.H., 2009. Inhibition of CXCR2 signaling promotes recovery in models of multiple sclerosis. Exp. Neurol. 220, 44–56.
Kielian, T., Barry, B., Hickey, W.F., 2001. CXC chemokine receptor-2 ligands are required for neutrophil-mediated host defense in experimental brain abscesses. J. Immunol. 166, 4634–4643.
Kim, J.V., Kang, S.S., Dustin, M.L., McGavern, D.B., 2009. Myelomonocytic cell recruitment causes fatal CNS vascular injury during acute viral meningitis. Nature 457, 191–195.
Klein, R.S., Lin, E., Zhang, B., Luster, A.D., Tollett, J., Samuel, M.A., Engle, M., Diamond, M.S., 2005. Neuronal CXCL10 directs CD8(+) T-cell recruitment and control of West Nile virus encephalitis. J. Virol. 79, 11457–11466.
Kroenke, M.A., Chensue, S.W., Segal, B.M., 2010. EAE mediated by a non-IFN-gamma/non-IL-17 pathway. Eur. J. Immunol. 40, 2340–2348.
Lane, T.E., Asensio, V.C., Yu, N., Paoletti, A.D., Campbell, I.L., Buchmeier, M.J., 1998. Dynamic regulation of alpha- and beta-chemokine expression in the central nervous system during mouse hepatitis virus-induced demyelinating disease. J. Immunol. 160, 970–978.
Lane, T.E., Liu, M.T., Chen, B.P., Asensio, V.C., Samawi, R.M., Paoletti, A.D., Campbell, I.L., Kunkel, S.L., Fox, H.S., Buchmeier, M.J., 2000. A central role for CD4(+) T cells and RANTES in virus-induced central nervous system inflammation and demyelination. J. Virol. 74, 1415–1424.
Lin, M.T., Hinton, D.R., Marten, N.W., Bergmann, C.C., Stohlman, S.A., 1999. Antibody prevents virus reactivation within the central nervous system. J. Immunol. 162, 7358–7368.
Lin, M.T., Stohlman, S.A., Hinton, D.R., 1997. Mouse hepatitis virus is cleared from the central nervous systems of mice lacking perforin-mediated cytolysis. J. Virol. 71, 383–391.
Liu, L., Darnall, L., Hu, T., Choi, K., Lane, T.E., Ransohoff, R.M., 2010. Myelin repair is accelerated by inactivating CXCR2 on nonhematopoietic cells. J. Neurosci. 30, 9074–9083.
Liu, M.T., Chen, B.P., Oertel, P., Buchmeier, M.J., Armstrong, D., Hamilton, T.A., Lane, T.E., 2000. The T cell chemoattractant IFN-inducible protein 10 is essential in host defense against viral-induced neurologic disease. J. Immunol. 165, 2327–2330.
Liu, M.T., Keirstead, H.S., Lane, T.E., 2001. 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, 4091–4097.
Liu, Y., Cai, Y., Zhang, X., 2003. Induction of caspase-dependent apoptosis in cultured rat oligodendrocytes by murine coronavirus is mediated during cell entry and does not require virus replication. J. Virol. 77, 11952–11963.
Liu, Y., Pu, Y.H., Zhang, X.M., 2006. Role of the mitochondrial signaling pathway in murine coronavirus-induced oligodendrocyte apoptosis. J Virol. 80, 395–403.
Liu, Y., Zhang, X., 2005. Expression of cellular oncogene Bcl-xL prevents coronavirus-induced cell death and converts acute infection to persistent infection in progenitor rat oligodendrocytes. J. Virol. 79, 47–56.
Liu, Y., Zhang, X.M., 2007. Murine coronavirus-induced oligodendrocyte apoptosis is mediated through the activation of the Fas signaling pathway. Virology 360, 364–375.
Londhe, V.A., Belperio, J.A., Keane, M.P., Burdick, M.D., Xue, Y.Y., Strieter, R.M., 2005a. CXCR2 is critical for dsRNA-induced lung injury: relevance to viral lung infection. J. Inflamm. (Lond) 2, 4.
Londhe, V.A., Belperio, J.A., Keane, M.P., Burdick, M.D., Xue, Y.Y., Strieter, R.M., 2005b. CXCR2/CXCR2 ligand biological axis impairs alveologenesis during dsRNA-induced lung inflammation in mice. Pediatr. Res. 58, 919–926.
Lu, W., Maheshwari, A., Misiuta, I., Fox, S.E., Chen, N., Zigova, T., Christensen, R.D., Calhoun, D.A., 2005. Neutrophil-specific chemokines are produced by astrocytic cells but not by neuronal cells. Brain Res. Dev. Brain Res. 155, 127–134.

Malone, K.E., Stohlman, S.A., Ramakrishna, C., Macklin, W., Bergmann, C.C., 2008. Induction of class I antigen processing components in oligodendroglia and microglia during viral encephalomyelitis. Glia 56, 426–435.
Marques, C.P., Kapil, P., Hinton, D.R., Hindinger, C., Nutt, S.L., Ransohoff, R.M., Phares, T.W., Stohlman, S.A., Bergmann, C.C., 2011. CXCR3-dependent plasma blast migration to the central nervous system during viral encephalomyelitis. J. Virol. 85, 6136–6147.
Marsh, D.R., Flemming, J.M., 2011. Inhibition of CXCR1 and CXCR2 chemokine receptors attenuates acute inflammation, preserves gray matter and diminishes autonomic dysreflexia after spinal cord injury. Spinal Cordoba 49, 337–344.
Marten, N.W., Stohlman, S.A., Bergmann, C.C., 2000. Role of viral persistence in retaining CD8(+) T cells within the central nervous system. J. Virol. 74, 7903–7910.
Marten, N.W., Stohlman, S.A., Bergmann, C.C., 2001. MHV infection of the CNS: mechanisms of immune-mediated control. Viral Immunol. 14, 1–18.
Marten, N.W., Stohlman, S.A., Zhou, J., Bergmann, C.C., 2003. Kinetics of virus-specific CD8+ -T-cell expansion and trafficking following central nervous system infection. J. Virol. 77, 2775–2778.
Martin, C., Burdon, P.C., Bridger, G., Gutierrez-Ramos, J.C., Williams, T.J., Rankin, S.M., 2003. Chemokines acting via CXCR2 and CXCR4 control the release of neutrophils from the bone marrow and their return following senescence. Immunity 19 (583-593).
Masters, P.S., 2006. The molecular biology of coronaviruses. Adv. Virus Res. 66, 193–292.
Matthews, A.E., Lavi, E., Weiss, S.R., Paterson, Y., 2002a. Neither B cells nor T cells are required for CNS demyelination in mice persistently infected with MHV-A59. J. Neurovirol. 8, 257–264.
Matthews, A.E., Weiss, S.R., Paterson, Y., 2002b. Murine hepatitis virus—a model for virus-induced CNS demyelination. J. Neurovirol. 8, 76–85.
Matute, C., Perez-Cerda, F., 2005. Multiple sclerosis: novel perspectives on newly forming lesions. Trends Neurosci. 28, 173–175.
Mazaleuskaya, L., Veltrop, R., Ikpeze, N., Martin-Garcia, J., Navas-Martin, S., 2012. Protective role of toll-like receptor 3-induced type I interferon in murine coronavirus infection of macrophages. Viruses 4, 901–923.
McIntosh, K., 1996. Fields Virology, in: al., B.F.e. (Ed.), Fields Virology, third ed. Lippincott-Raven Publishers, pp. 401–430.
McMahon, E.J., Bailey, S.L., Castenada, C.V., Waldner, H., Miller, S.D., 2005. Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis. Nat. Med. 11, 335–339.
Merabova, N., Kaniowska, D., Kaminski, R., Deshmane, S.L., White, M.K., Amini, S., Darbinyan, A., Khalili, K., 2008. JC virus agnoprotein inhibits *in vitro* differentiation of oligodendrocytes and promotes apoptosis. J. Virol. 82, 1558–1569.
Miller, S.D., Vanderlugt, C.L., Begolka, W.S., Pao, W., Yauch, R.L., Neville, K.L., Katz-Levy, Y., Carrizosa, A., Kim, B.S., 1997. Persistent infection with Theiler's virus leads to CNS autoimmunity via epitope spreading. Nat. Med. 3, 1133–1136.
Minagawa, H., Takenaka, A., Mohri, S., Mori, R., 1987. Protective effect of recombinant murine interferon beta against mouse hepatitis virus infection. Antiviral. Res. 8, 85–95.
Moser, B., Clark-Lewis, I., Zwahlen, R., Baggiolini, M., 1990. Neutrophil-activating properties of the melanoma growth-stimulatory activity. J. Exp. Med. 171, 1797–1802.
Nguyen, D., Stangel, M., 2001. Expression of the chemokine receptors CXCR1 and CXCR2 in rat oligodendroglial cells. Brain Res. Dev. Brain Res. 128, 77–81.
Omari, K.M., John, G., Lango, R., Raine, C.S., 2006. Role for CXCR2 and CXCL1 on glia in multiple sclerosis. Glia 53, 24–31.
Omari, K.M., John, G.R., Sealfon, S.C., Raine, C.S., 2005. CXC chemokine receptors on human oligodendrocytes: implications for multiple sclerosis. Brain 128, 1003–1015.
Omari, K.M., Lutz, S.E., Santambrogio, L., Lira, S.A., Raine, C.S., 2009. Neuroprotection and remyelination after autoimmune demyelination in mice that inducibly overexpress CXCL1. Am. J. Pathol. 174, 164–176.
Padovani-Claudio, D.A., Liu, L.P., Ransohoff, R.M., Miller, R.H., 2006. Alterations in the oligodendrocyte lineage, myelin, and white matter in adult mice lacking the chemokine receptor CXCR2. Glia 54, 471–483.
Parra, B., Hinton, D.R., Lin, M.T., Cua, D.J., Stohlman, S.A., 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, D.R., Marten, N.W., Bergmann, C.C., Lin, M.T., Yang, C.S., Stohlman, S.A., 1999. IFN-gamma is required for viral clearance from central nervous system oligodendroglia. J. Immunol. 162, 1641–1647.
Pearce, B.D., Hobbs, M.V., McGraw, T.S., Buchmeier, M.J., 1994. Cytokine induction during T-cell-mediated clearance of mouse hepatitis virus from neurons *in vivo*. J. Virol. 68, 5483–5495.
Perlman, S.R., Lane, T.E., Buchmeier, M.J., 1999. Cornoaviruses: hepatitis, peritonitis, and central nervous system disease. In: Cunningham, M.W., Fujinami, R.S. (Eds.), Effects of Microbes on the Immune System, pp. 331–348.
Pewe, L., Haring, J., Perlman, S., 2002. CD4 T-cell-mediated demyelination is increased in the absence of gamma interferon in mice infected with mouse hepatitis virus. J. Virol. 76, 7329–7333.
Pewe, L., Perlman, S., 2002. Cutting edge: CD8 T cell-mediated demyelination is IFN-gamma dependent in mice infected with a neurotropic coronavirus. J. Immunol. 168, 1547–1551.
Phares, T.W., Marques, C.P., Stohlman, S.A., Hinton, D.R., Bergmann, C.C., 2011. Factors supporting intrathecal humoral responses following viral encephalomyelitis. J. Virol. 85, 2589–2598.
Phares, T.W., Stohlman, S.A., Hwang, M., Min, B., Hinton, D.R., Bergmann, C.C., 2012. CD4 T cells promote CD8 T cell immunity at the priming and effector site during viral encephalitis. J. Virol. 86, 2416–2427.
Popivanova, B.K., Koike, K., Tonchev, A.B., Ishida, Y., Kondo, T., Ogawa, S., Mukaida, N., Inoue, M., Yamashima, T., 2003. Accumulation of microglial cells expressing ELR motif-positive CXC chemokines and their receptor CXCR2 in monkey hippocampus after ischemia-reperfusion. Brain Res. 970, 195–204.
Ramakrishna, C., Bergmann, C.C., Atkinson, R., Stohlman, S.A., 2003. Control of central nervous system viral persistence by neutralizing antibody. J. Virol. 77, 4670–4678.
Ramakrishna, C., Stohlman, S.A., Atkinson, R.A., Hinton, D.R., Bergmann, C.C., 2004. Differential regulation of primary and secondary CD8+ T cells in the central nervous system. J. Immunol. 173, 6265–6273.
Ramakrishna, C., Stohlman, S.A., Atkinson, R.D., Shlomchik, M.J., Bergmann, C.C., 2002. Mechanisms of central nervous system viral persistence: the critical role of antibody and B cells. J. Immunol. 168, 1204–1211.
Raman, D., Milatovic, S.Z., Milatovic, D., Splittgerber, R., Fan, G.H., Richmond, A., 2011. Chemokines, macrophage inflammatory protein-2 and stromal cell-derived factor-1alpha, suppress amyloid beta-induced neurotoxicity. Toxicol Appl. Pharmacol. 256, 300–313.
Rempel, J.D., Murray, S.J., Meisner, J., Buchmeier, M.J., 2004. Differential regulation of innate and adaptive immune responses in viral encephalitis. Virology 318, 381–392.
Rempel, J.D., Quina, L.A., Blakely-Gonzales, P.K., Buchmeier, M.J., Gruol, D.L., 2005. Viral induction of central nervous system innate immune responses. J. Virol. 79, 4369–4381.
Robinson, S., Tani, M., Strieter, R.M., Ransohoff, R.M., Miller, R.H., 1998. The chemokine growth-regulated oncogene-alpha promotes spinal cord oligodendrocyte precursor proliferation. J. Neurosci. 18, 10457–10463.
Roth-Cross, J.K., Bender, S.J., Weiss, S.R., 2008. Murine coronavirus mouse hepatitis virus is recognized by MDA5 and induces type I interferon in brain macrophages/microglia. J. Virol. 82, 9829–9838.
Roth-Cross, J.K., Martinez-Sobrido, L., Scott, E.P., Garcia-Sastre, A., Weiss, S.R., 2007. Inhibition of the alpha/beta interferon response by mouse hepatitis virus at multiple levels. J. Virol. 81, 7189–7199.
Saas, P., Walker, P.R., Quiquerez, A.L., Chalmers, D.E., Arrighi, J.F., Lienard, A., Boucraut, J., Dietrich, P.Y., 2002. A self-defence mechanism of astrocytes against Fas-mediated death involving interleukin-8 and CXCR2. Neuroreport 13, 1921–1924.
Savarin, C., Stohlman, S.A., Atkinson, R., Ransohoff, R.M., Bergmann, C.C., 2010. Monocytes regulate T cell migration through the glia limitans during acute viral encephalitis. J. Virol. 84, 4878–4888.
Schumacher, C., Clark-Lewis, I., Baggiolini, M., Moser, B., 1992. High- and low-affinity binding of GRO alpha and neutrophil-activating peptide 2 to interleukin 8 receptors on human neutrophils. Proc. Nat. Acad. Sci. U.S.A. 89, 10542–10546.
Scott, E.P., Branigan, P.J., Del Vecchio, A.M., Weiss, S.R., 2008. Chemokine expression during mouse-hepatitis-virus-induced encephalitis: contributions of the spike and background genes. J. Neurovirol. 14, 5–16.
Smith, A.L., Barthold, S.W., Beck, D.S., 1987. Intranasally administered alpha/beta interferon prevents extension of mouse hepatitis virus, strain JHM, into the brains of BALB/cByJ mice. Antiviral Res. 8, 239–245.
Stiles, L.N., Hosking, M.P., Edwards, R.A., Strieter, R.M., Lane, T.E., 2006. Differential roles for CXCR3 in CD4+ and CD8+ T cell trafficking following viral infection of the CNS. Eur. J. Immunol. 36, 613–622.
Stiles, L.N., Liu, M.T., Kane, J.A.C., Lane, T.E., 2009. CXCL10 and trafficking of virus-specific T cells during coronavirus-induced demyelination. Autoimmunity 42, 484–491.
Stohlman, S.A., Bergmann, C.C., Lin, M.T., Cua, D.J., Hinton, D.R., 1998. CTL effector function within the central nervous system requires CD4+ T cells. J. Immunol. 160, 2896–2904.
Stohlman, S.A., Hinton, David R., 2001. Viral induced demyelination. Brain Pathol. 11, 92–106.
Strieter, R.M., Keane, M.P., Burdick, M.D., Sakkour, A., Murray, L.A., Belperio, J.A., 2005. The role of CXCR2/CXCR2 ligands in acute lung injury. Curr. Drug. Targets Inflamm. Allergy 4, 299–303.
Sui, Y.J., Potula, R., Dhillon, N., Pinson, D., Li, S.P., Nath, A., Anderson, C., Turchan, J., Kolson, D., Narayan, O., Buch, S., 2004. Neuronal apoptosis is mediated by CXCL10 overexpression in simian human immunodeficiency virus encephalitis. Am. J. Pathol. 164, 1557–1566.
Sun, N., Grzybicki, D., Castro, R.F., Murphy, S., Perlman, S., 1995. Activation of astrocytes in the spinal cord of mice chronically infected with a neurotropic coronavirus. Virology 213, 482–493.
Tarassishin, L., Loudig, O., Bauman, A., Shafit-Zagardo, B., Suh, H.S., Lee, S.C., 2011. Interferon regulatory factor 3 inhibits astrocyte inflammatory gene expression through suppression of the proinflammatory miR-155 and miR-155*. Glia 59, 1911–1922.
Templeton, S.P., Kim, T.S., O'Malley, K., Perlman, S., 2008. Maturation and localization of macrophages and microglia during infection with a neurotropic murine coronavirus. Brain Pathol. 18, 40–51.
Tirotta, E., Kirby, L.A., Hatch, M.N., Lane, T.E., 2012. IFN-gamma-induced apoptosis of human embryonic stem cell derived oligodendrocyte progenitor cells is restricted by CXCR2 signaling. Stem Cell Res. 9, 208–217.
Tirotta, E., Ransohoff, R.M., Lane, T.E., 2011. CXCR2 signaling protects oligodendrocyte progenitor cells from IFN-gamma/CXCL10-mediated apoptosis. Glia 59, 1518–1528.

Tonai, T., Shiba, K., Taketani, Y., Ohmoto, Y., Murata, K., Muraguchi, M., Ohsaki, H., Takeda, E., Nishisho, T., 2001. A neutrophil elastase inhibitor (ONO-5046) reduces neurologic damage after spinal cord injury in rats. J. Neurochem. 78, 1064–1072.

Tran, E.H., Hoekstra, K., van Rooijen, N., Dijkstra, C.D., Owens, T., 1998. Immune invasion of the central nervous system parenchyma and experimental allergic encephalomyelitis, but not leukocyte extravasation from blood, are prevented in macrophage-depleted mice. J. Immunol. 161, 3767–3775.

Traugott, U., Lebon, P., 1988a. Demonstration of alpha, beta, and gamma interferon in active chronic multiple sclerosis lesions. Ann. N.Y. Acad. Sci. 540, 309–311.

Traugott, U., Lebon, P., 1988b. Interferon-gamma and Ia antigen are present on astrocytes in active chronic multiple sclerosis lesions. J. Neurol. Sci. 84, 257–264.

Trifilo, M.J., Bergmann, C.C., Kuziel, W.A., Lane, T.E., 2003. CC chemokine ligand 3 (CCL3) regulates CD8(+)-T-cell effector function and migration following viral infection. J. Virol. 77, 4004–4014.

Trifilo, M.J., Lane, T.E., 2004. 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.

Tsai, H.H., Frost, E., Robinson, V., Ffrench-Constant, S., Geertman, C., Ransohoff, R., Miller, R.H., R.M., 2002. The chemokine receptor CXCR2 controls positioning of oligodendrocyte precursors in developing spinal cord by arresting their migration. Cell 110, 373–383.

Tschen, S.I., Stohlman, S.A., Ramakrishna, C., Hinton, D.R., Atkinson, R.D., Bergmann, C.C., 2006. CNS viral infection diverts homing of antibody-secreting cells from lymphoid organs to the CNS. Eur. J. Immunol. 36, 603–612.

Tuohy, V.K., Yu, M., Weinstock-Guttman, B., Kinkel, R.P., 1997. Diversity and plasticity of self recognition during the development of multiple sclerosis. J. Clin. Invest. 99, 1682–1690.

Tuohy, V.K., Yu, M., Yin, L., Kawczak, J.A., Kinkel, R.P., 1999. Spontaneous regression of primary autoreactivity during chronic progression of experimental autoimmune encephalomyelitis and multiple sclerosis. J. Exp. Med. 189, 1033–1042.

Turbic, A., Leong, S.Y., Turnley, A.M., 2011. Chemokines and inflammatory mediators interact to regulate adult murine neural precursor cell proliferation, survival and differentiation. PLoS One 6, e25406.

Ubogu, E.E., Cossoy, M.B., Ransohoff, R.M., 2006. The expression and function of chemokines involved in CNS inflammation. Trends Pharmacol. Sci. 27, 48–55.

van der Hoek, L., Pyrc, K., Berkhout, B., 2006. Human coronavirus NL63, a new respiratory virus. FEMS Microbiol. Rev. 30, 760–773.

van der Hoek, L., Pyrc, K., Jebbink, M.F., Vermeulen-Oost, W., Berkhout, R.J., Wolthers, K.C., Wertheim-van Dillen, P.M., Kaandorp, J., Spaargaren, J., Berkhout, B., 2004. Identification of a new human coronavirus. Nat. Med. 10, 368–373.

Versteeg, G.A., Bredenbeek, P.J., van den Worm, S.H., Spaan, W.J., 2007. Group 2 coronaviruses prevent immediate early interferon induction by protection of viral RNA from host cell recognition. Virology 361, 18–26.

Walsh, K.B., Edwards, R.A., Romero, K.M., Kotlajich, M.V., Stohlman, S.A., Lane, T.E., 2007. 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, 1155–1165.

Walsh, K.B., Lanier, L.L., Lane, T.E., 2008. NKG2D receptor signaling enhances cytolytic activity by virus-specific CD8+ T cells: evidence for a protective role in virus-induced encephalitis. J. Virol. 82, 3031–3044.

Wang, F.I., Hinton, D.R., Gilmore, W., Trousdale, M.D., Fleming, J.O., 1992. Sequential infection of glial cells by the murine hepatitis virus JHM strain (MHV-4) leads to a characteristic distribution of demyelination. Lab. Invest.; J. Tech. Meth. Pathol. 66, 744–754.

Wareing, M.D., Shea, A.L., Inglis, C.A., Dias, P.B., Sarawar, S.R., 2007. CXCR2 is required for neutrophil recruitment to the lung during influenza virus infection, but is not essential for viral clearance. Viral Immunol. 20, 369–378.

Watson, K., Fan, G.H., 2005. Macrophage inflammatory protein 2 inhibits beta-amyloid peptide (1-42)-mediated hippocampal neuronal apoptosis through activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase signaling pathways. Mol. Pharmacol. 67, 757–765.

Weiss, S.R., Navas-Martin, S., 2005. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiol. Mol. Biol. Rev. 69, 635–664.

Wengner, A.M., Pitchford, S.C., Furze, R.C., Rankin, S.M., 2008. The coordinated action of G-CSF and ELR+CXC chemokines in neutrophil mobilization during acute inflammation. Blood 111, 42–49.

Wolpe, S.D., Sherry, B., Juers, D., Davatelis, G., Yurt, R.W., Cerami, A., 1989. Identification and characterization of macrophage inflammatory protein 2. Proc. Nat. Acad. Sci U.S.A. 86, 612–616.

Woo, P.C., Lau, S.K., Chu, C.M., Chan, K.H., Tsoi, H.W., Huang, Y., Wong, B.H., Poon, R.W., Cai, J.J., Luk, W.K., Poon, L.L., Wong, S.S., Guan, Y., Peiris, J.S., Yuen, K.Y., 2005. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. J. Virol. 79, 884–895.

Woodroofe, M.N., Cuzner, M.L., 1993. Cytokine mRNA expression in inflammatory multiple sclerosis lesions: detection by non-radioactive *in situ* hybridization. Cytokine 5, 583–588.

Wu, G.F., Dandekar, A.A., 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, G.F., 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.

Xia, M., Hyman, B.T., 2002. GROalpha/KC, a chemokine receptor CXCR2 ligand, can be a potent trigger for neuronal ERK1/2 and PI-3 kinase pathways and for tau hyperphosphorylation-a role in Alzheimer's disease? J. Neuroimmunol. 122, 55–64.

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.

Yong, V.W., Power, C., Forsyth, P., Edwards, D.R., 2001. Metalloproteinases in biology and pathology of the nervous system. Nat. Rev. Neurosci. 2, 502–511.

Zhang, B., Chan, Y.K., Lu, B., Diamond, M.S., Klein, R.S., 2008. CXCR3 mediates region-specific antiviral T cell trafficking within the central nervous system during west nile virus encephalitis. J. Immunol. 180, 2641–2649.

Zhang, B., Patel, J., Croyle, M., Diamond, M.S., Klein, R.S., 2010. TNF-alpha-dependent regulation of CXCR3 expression modulates neuronal survival during West Nile virus encephalitis. J. Neuroimmunol. 224, 28–38.

Zhou, H., Perlman, S., 2007. Mouse hepatitis virus does not induce Beta interferon synthesis and does not inhibit its induction by double-stranded RNA. J. Virol. 81, 568–574.

Zhou, J., Hinton, D.R., Stohlman, S.A., Liu, C.P., Zhong, L., Marten, N.W., 2005a. 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, 162–169.

Zhou, J., Marten, N.W., Bergmann, C.C., Macklin, W.B., Hinton, D.R., Stohlman, S.A., 2005b. Expression of matrix metalloproteinases and their tissue inhibitor during viral encephalitis. J. Virol. 79, 4764–4773.

Zhou, J., Stohlman, S.A., Atkinson, R., Hinton, D.R., Marten, N.W., 2002. Matrix metalloproteinase expression correlates with virulence following neurotropic mouse hepatitis virus infection. J. Virol. 76, 7374–7384.

Zhou, J., Stohlman, S.A., Hinton, D.R., Marten, N.W., 2003. Neutrophils promote mononuclear cell infiltration during viral-induced encephalitis. J. Immunol. 170, 3331–3336.

Zuo, J., Stohlman, S.A., Hoskin, J.B., Hinton, D.R., Atkinson, R., Bergmann, C.C., 2006. Mouse hepatitis virus pathogenesis in the central nervous system is independent of IL-15 and natural killer cells. Virology 350, 206–215.