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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.

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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

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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

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-1 α , IL-1 β , IL-6, IL-12, and TNF α 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; Tschén 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; Woodroffe 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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