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CXCR2 signaling and host defense following coronavirus-induced encephalomyelitis

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Abstract

Inoculation of the neurotropic JHM strain of mouse hepatitis virus (JHMV) into the central nervous system (CNS) of susceptible strains of mice results in wide-spread replication within glial cells accompanied by infiltration of virus-specific T lymphocytes that control virus through cytokine secretion and cytolytic activity. Virus persists within white matter tracts of surviving mice resulting in demyelination that is amplified by inflammatory T cells and macrophages. In response to infection, numerous cytokines/chemokines are secreted by resident cells of the CNS and inflammatory leukocytes that participate in both host defense and disease. Among these are the ELR-positive chemokines that are able to signal through CXC chemokine receptors including CXCR2. Early following JHMV infection, ELR-positive chemokines contribute to host defense by attracting CXCR2-expressing cells including polymorphonuclear cells to the CNS that aid in host defense through increasing the permeability the blood-brain-barrier (BBB). During chronic disease, CXCR2 signaling on oligodendroglia protects these cells from apoptosis and restricts the severity of demyelination. This review covers aspects related to host defense and disease in response to JHMV infection and highlights the different roles of CXCR2 signaling in these processes.

Introduction

Mouse Hepatitis Virus (MHV) is a large (30–32kb), enveloped single-stranded RNA virus, which is a member of the Coronaviridae family in the Order Nidovirales [1]. These viruses include a variety of strains that can induce wide-ranging pathologies dependent on age, strain and route of infection. Examples include the hepatotropic MHV-2 strain which leads to fulminant hepatitis following intraperitoneal (i.p.) injection, but is weakly neurotropic, causing only mild acute meningitis following intracranial (i.c.) inoculation [2]. In contrast, i.c. infection of susceptible mice with the dual neurotropic and hepatotropic A59 strain results in infection of neurons and glial cells within the CNS, resulting in a mild encephalomyelitis and limited demyelination as well as hepatitis [3, 4]. The JHM strain of mouse hepatitis virus (JHMV), a well-characterized laboratory strain that was initially serially passaged in suckling mouse brains to produce a highly neurovirulent virus, can cause severe encephalomyelitis and demyelination in adult mice [5]. Infection of susceptible mice with neuroattenuated variants of JHMV have yielded useful surrogate mouse models for i) viral-induced encephalomyelitis, ii) defining molecular mechanisms governing neuroinflammation, iii) characterizing mechanisms associated with viral-induced immune-

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mediated demyelination, and iv) developing novel approaches to promote remyelination within the context of persistent viral infection of the CNS.

JHMOV-Induced Acute Encephalomyelitis

Following a sublethal i.c infection with the neuroadapted JHM strain of MHV (JHMOV), virus infects and replicates within ependymal cells lining the lateral ventricles [6]. Within 24-hours, JHMOV rapidly spreads and penetrates further into the parenchyma where astrocytes, oligodendrocytes, and microglia are targets of infection [6, 7]. Virus also disseminates to the spinal cord through cerebrospinal fluid targeting ependymal cells prior to spreading to glial cells of the white matter tracts [6]. Viral titers within the brain peak between days 5–7 post-infection (p.i.) and decline below levels of detection by plaque assay (~100 PFU/g tissue) between 10–14 days post-infection (p.i.) [8]. However, clearance is incomplete and viral antigen and RNA is capable of persisting within the CNS [9], and this is associated with chronic neuroinflammation leading to an immune-mediated demyelinating disease with clinical and histologic similarities to the human demyelinating disease multiple sclerosis (MS).

An innate antiviral response is triggered following JHMOV infection, characterized by increased expression of pro-inflammatory cytokines and chemokines, along with matrix-metalloproteinases (MMPs) [10, 11]. A cytokine milieu consisting of IL-1a, IL-1b, IL-6, IL-12 and TNF-a is detected within CNS during the first 48 hours following JHMOV infection [12, 13]. Type I IFN-a and IFN-b are also detected early in response to JHMOV infection suggesting an important role in host defense. Indeed, elegant studies by Bergmann and colleagues [14] showed an increase in viral spread and mortality in JHMOV infected *IFNAR*^{-/-} mice, while exogenous treatment of mice with either type I interferon limits viral replication and dissemination [15, 16]. In addition to directly inhibiting viral replication, type I interferons are believed to enhance antigen presentation via increased MHC class I expression, helping link innate and adaptive immune responses [17].

Cellular components of the innate immune response consist of neutrophils, natural killer (NK) cells and monocyte/macrophages that rapidly migrate to the CNS in response to JHMOV infection. Although these cells were initially not considered important in host defense, this opinion has been revised based upon recent studies that have more carefully examined their functional contributions. Both neutrophils and monocyte/macrophages are now thought to contribute to the permeabilization of the blood-brain-barrier (BBB) through secretion of MMPs, and this is critical for allowing access of virus-specific T cells into the CNS [18–22]. With regards to NK cells, Trifilo et al. [23] demonstrated that NK cells can contribute to control of viral replication following infection of immunodeficient mice with a recombinant JHMOV engineered to express the chemokine CXCL10. However, a more relevant study evaluating the functional contributions of NK cells in host defense in response to JHMOV infection was provided by Zuo et al., [24] in which IL-15-deficient mice which lack NK cells were shown to control JHMOV replication within the CNS as efficiently as wildtype mice and effectively argue that NK cells are dispensable with regards to effectively controlling JHMOV replication during acute disease.

JHMOV specific CD4⁺ and CD8⁺ T cells presumably expand to viral antigens presented within draining cervical lymph nodes [25] and traffic into the CNS through a permeable BBB in response to chemotactic signals including the chemokines CXCL9, CXCL10 and CCL5 [25–30]. Evidence for this is supported by chemokine neutralization studies where administration of anti-CXCL10 or anti-CCL5 blocking antibodies during acute JHM infection abrogates early CD4⁺ and CD8⁺ T cell infiltration to the CNS [26–29]. Administration of blocking antibodies specific to CXCL10 to JHMOV-infected mice during

acute disease impaired CD4⁺ and CD8⁺ T cell access to the CNS and inability to control viral replication [27]. Similarly, JHMV infection of CXCL10-deficient mice limited entry of CD4⁺ and CD8⁺ T cells into the CNS [31]. In contrast, treatment with anti-CXCL10 antisera in mice persistently infected with virus in which demyelination is established selectively inhibited virus-specific CD4⁺ T cells, but not virus-specific CD8⁺ T cell trafficking into the CNS resulting in a dramatic improvement in motor skills [32, 33]. Additionally, blocking CXCR3, the receptor for CXCL10, also negatively impacts CD4⁺ T cell recruitment to the CNS while trafficking of CD8⁺ T cells is not significantly impacted [26]. Follow up studies further support the role of CXCL10 in promoting T cell migration following JHMV infection but not in enhancing T cell antiviral effector functions [34]. These findings argue for differential roles for CXCL10 in mediating trafficking of CXCR3-bearing T cells into the CNS in response to JHMV infection and this is dictated, in part, by the stage of disease i.e. acute versus chronic. One potential explanation to these findings may lie in different trafficking patterns of T cells during chronic disease as well as evidence indicating that virus-specific CD8⁺ T cells are retained within the CNS during persistence [35]. Importantly, other chemokines exert a chemotactic effect on T cells, macrophages, as well as other inflammatory cells following JHMV infection. Adoptive transfer studies indicate that *CCR5* deficient CD4⁺ T cells, i.e., those that do not respond to the chemokine CCL5, transferred into JHMV-infected *RAG1*^{-/-} mice display muted ability to traffic to the CNS [36]. However, adoptive transfer of *CCR5*^{-/-} CD8⁺ T cells can infiltrate into the CNS in infected *RAG1* deficient mice, further demonstrating that differential signaling cues control CD4⁺ and CD8⁺ T cell migration during acute JHMV infection [37]. Genetic ablation of CCR2, but not the signaling ligand CCL2, results in increased mortality associated with impaired ability to control JHMV replication within the brain and this correlated with limited infiltration of virus-specific T cells into the CNS [38]. CCR2 has been shown to be critical in regulating BBB permeability by signaling through the receptor expressed on endothelial cells in other neuroinflammatory disease models suggesting that CCR2 signaling influences leukocyte access to the CNS by controlling BBB integrity in response to JHMV infection [39]. However, both CCL2 and CCR2 promote macrophage accumulation within the CNS of infected mice [38]. Follow up studies confirmed the importance of CCL2 in mediating monocyte/macrophage recruitment to the CNS of JHMV-infected mice [20, 40].

Antiviral effector mechanisms associated with viral clearance within the CNS include the upregulation of MHC class I and MHC class II on antigen presenting cells (APCs) following secretion of IFN- γ by both CD4⁺ and CD8⁺ T cells as well as perforin-mediated cytolysis of astrocytes and microglia by virus-specific CD8⁺ T cells [8, 41, 42]. Upon JHMV infection of the CNS, CD4⁺ T cells promote CD8⁺ T cell expansion within the periphery and aid in enhancing antiviral effector functions [43]. Depletion of CD4⁺ T cells diminishes CD8⁺ T cell-mediated control of viral replication within the CNS that is associated with reduced expression of IFN- γ and granzyme B and elevated CD8⁺ T cell apoptosis [43]. These results support and extend earlier studies [44, 45] that CD4⁺ T cells play a central role in both enhancing peripheral activation of CD8⁺ T cells and prolonging their antiviral function within the CNS and further suggest that IL-21 may be a critical factor in mediating these effects [43]. Infected oligodendrocytes appear to be resistant to the cytolytic effector functions of CD8⁺ T cells, but rather control JHMV replication through IFN- γ signaling generated from virus-specific T cells [41, 42, 46, 47]. The potent anti-viral response reduces viral replication to below detectable levels (as defined by plaque assay) between days 10–14 p.i., but sterile immunity does not occur; viral RNA and antigens persist and are sequestered primarily within white matter tracts, and this is accompanied by the presence of activated glial cells and retention of virus-specific CD4⁺ and CD8⁺ T cells [8, 9, 48]. Neutralizing JHMV-specific antibody is detected during chronic disease and is critical in preventing viral recrudescence [49–51].

More recently, Perlman and colleagues have provided important insight into the functional role of regulatory T cells (Tregs) during acute JHMV-induced CNS disease [52, 53]. Tregs are detected within the CNS at the same time as effector CD4⁺ T cells indicating the emergence and accumulation of both populations of cells is on a similar timeline following viral infection. Further, virus-specific Tregs express both IFN- γ and IL-10 suggesting immune regulatory capacities mediated through cytokines secreted following antigen stimulation. Indeed, virus-specific Tregs dampen proliferation of virus-specific effector CD4⁺ T cells and depletion of Tregs increases mortality [52, 53]. These data suggest that within the context of acute JHMV-induced neurologic disease, Tregs limit immunopathological disease without negatively impacting viral clearance [52].

JHMV-induced Demyelination

A hallmark feature of JHMV infection of the CNS occurs following the acute stage of disease in which virus has spread into the spinal cord where it infects astrocytes and oligodendroglia. As a result, virus persists as non-infectious RNA and animals develop demyelinating lesions within the brain and spinal cord that are associated with clinical manifestations including awkward gait and hindlimb paralysis. Current evidence suggests that demyelination in JHMV-infected mice is not the result of epitope spreading and induction of an immune response against neuroantigens as has recently been reported to occur during Theiler's virus-induced demyelination [54, 55]. However, adoptive transfer of T cells from JHMV-infected rats to naïve recipient's results in demyelination [56]. Whether a similar response occurs in MHV-infected mice and what the contributions are to demyelination are unknown at this time. A recent report has suggested that infection with mouse hepatitis virus strain A59 promotes activation of autoreactive T cells specific to myelin basic protein although the contributions of these cells to demyelination are defined [57].

Oligodendrocytes are an important viral reservoir during chronic disease [6, 7], however viral-induced lysis of oligodendrocytes is not considered a primary mechanism contributing to demyelination, as JHMV-infection of immunodeficient mice, *e.g.* those lacking thymically-educated T and B lymphocytes, results in wide-spread viral replication within oligodendrocytes with very limited demyelination [58]. Moreover, adoptive transfer of splenocytes from MHV-immunized immunocompetent mice into JHMV infected immunodeficient mice results in robust demyelination, implicating T cells as mediators of white matter damage [58–60]. Additionally, JHMV infected *CD4*^{-/-} or *CD8*^{-/-} mice develop demyelination demonstrating the importance of both T cell subsets in contributing to neuropathology, although JHMV-infected *CD4*^{-/-} mice exhibited a diminished severity of myelin loss in the white matter tracts of the spinal cord compared to infected *CD8*^{-/-} mice, suggesting an important role for CD4⁺ T cells in amplifying disease progression [29]. One possible mechanism by which CD4⁺ T cells accelerate demyelination is through secretion of the chemokine CCL5, a potent chemoattractant for inflammatory macrophages [29]. Macrophages have been shown to be important in development of demyelinating lesions within spinal cord white matter during chronic JHMV-infection [58, 61]. Furthermore, CCL5 neutralization or genetic ablation of *CCR5* is associated with reduced macrophage infiltration correlating to a reduction in demyelination [62, 63]. An alternate view is the CD8⁺ T cells also enhance demyelination through release of IFN- γ and this promotes migration and accumulation of activated macrophages/microglia within white matter tracts of JHMV-infected mice [64]. Other studies argue for a more protective role for CD4⁺ T cells through IFN- γ -mediated control of viral replication and/or additional undefined mechanisms [65, 66]. Bystander CD4⁺ T cells *e.g.* activated yet not specific for viral antigens are also thought to not be involved in white matter damage in JHMV-infected mice [67]. Adding additional insight into how T cells contribute to either disease or defense

are studies from Trandem et al. [68] showing that adoptive transfer of Tregs to JHMV-infected mice attenuates clinical disease severity and this is associated with dampened neuroinflammation and demyelination. Clearly, T cell infiltration into the CNS of mice persistently infected with JHMV is important in the pathogenesis of disease although a unifying mechanism(s) attributed to how these cells contribute to disease progression as well as protection remains elusive and, most likely, reflects different model systems used to evaluate functional roles for T cell subsets.

Although infectious virus is cleared by day 12 p.i., IFN- γ secretion from activated T-lymphocytes can be detected within the brain for up to four weeks p.i. and likely contributes to the deleterious effects on oligodendrocytes during the chronic stage of the disease [12]. Early studies have shown adoptive transfer of enriched CD8 $^{+}$ T cells obtained from JHMV-sensitized IFN- γ -deficient mice into JHMV-infected *RAG1* $^{-/-}$ mice exhibited less severe demyelination compared to recipients of IFN- γ -expressing CD8 $^{+}$ T cells, implicating CD8 $^{+}$ T cell-derived IFN- γ as an important contributor to demyelination via destruction/damage to oligodendroglia [64]. Numerous groups have demonstrated increased sensitivity of oligodendrocyte progenitor cells (OPCs) to IFN- γ -induced apoptosis when compared to mature oligodendrocytes illustrating that susceptibility to IFN- γ -induced death is controlled, in part, by the maturation state of the cell [69–74]. Interestingly, demyelination is observed within JHMV-infected mice in which the IFN- γ receptor is selectively ablated in oligodendrocyte lineage cells suggesting that demyelination is multifaceted and additional mechanisms, including the local release of chemokines such as CXCL10 from activated resident glia, may directly contribute to dysregulation of oligodendrocyte function and/or death [46, 75, 76]. Early studies also implicated the release of nitric oxide (NO) from activated inflammatory macrophages and resident microglia as a factor involved in inducing demyelination [76]. Treatment of JHMV-infected mice with aminoguanidine (AG), a selective inhibitor of nitric oxide synthase 2 (NOS2), diminished clinical disease severity and this was associated with reduced neuroinflammation and demyelination arguing for a potential role of NOS2-derived NO in regulating proinflammatory gene expression within the CNS of infected mice [76]. However, genetic ablation of NOS2 did not dampen demyelination in response to JHMV infection when compared to wildtype control mice indicating that NO does not contribute to myelin damage [77, 78]. Differences may relate to off-target effects of AG that, in addition to muting NOS2 activity, also alter gene expression of specific chemokine genes within resident glial cells.

Neutrophils, BBB breakdown and CXCR2 Signaling During Acute JHMV-Induced Disease

Polymorphonuclear (PMN) neutrophils can infiltrate into the CNS within 24-hours p.i. following i.c. inoculation with JHMV where are thought to carry out critical effector functions in host defense including the release of matrix metalloproteinase-9 (MMP-9) from stored granules [22, 79]. Their rapid migration to the luminal side of the perivascular space correlates with degradation of the basal lamina and extracellular matrix of the blood-brain-barrier (BBB), thus enhancing inflammation by facilitating the extravasation of monocytes and leukocytes into the parenchyma [19, 21, 80–82]. The importance of neutrophils in host-defense following JHMV infection was initially highlighted by Stohlman and colleagues [22] demonstrating that there is an increase in viral burden and mortality of neutropenic mice infected with a lethal variant of JHMV. This correlated with a lack of detectable MMP-9 within CNS extracts and only minor disruption of the BBB, indicating a protective role for neutrophils during the early stages of lethal JHMV infection [22].

The ELR-positive chemokines CXCL1 and CXCL2 in mice (and CXCL8 in humans), delineated by a glutamic acid-leucine-arginine (ELR) amino acid sequence preceding a group of conserved cysteine residues (CXC) at the amino termini of the molecules, are potent chemoattractants for neutrophils which express the cognate chemokine receptor

CXCR2. Indeed, CXCL1 overexpression from oligodendrocytes within the CNS of naive transgenic mice results in rapid neutrophil accumulation into the perivascular, meningeal and parenchymal areas of the brain [83]. CXCL1 and CXCL2 are up-regulated early within the brain and spinal cord of JHMV-infected mice with CXCL1 protein expression appearing to colocalize with reactive astrocytes [19, 82]. Similar to the results of neutropenic mice infected with JHMV, blocking neutrophil recruitment to the CNS through antibody mediated neutralization of the ligand domain of CXCR2 during sublethal JHMV-infection leads to a significant increase in mortality and the inability to control virus replication [19]. This is associated with over a 50% reduction in leukocytes infiltrating into the CNS that correlated with an intact BBB and demonstrates a critical role of ELR-positive chemokines in shaping the adaptive immune response to JHMV [19].

Curiously, host-defense to JHMV infection in *CXCR2*-deficient mice contradicts the CXCR2 neutralization studies as *CXCR2*-deficient mice infected with JHMV have no deficits in survival, viral clearance or BBB degradation [19]. Although reduced in numbers, neutrophils were still detected within the CNS of JHMV infected *CXCR2*^{-/-} mice indicating that their migration may be the result of induction of compensatory receptors that may promote their chemotaxis, such as CXCR1 [19, 84]. Furthermore, recent evidence has shown that MMP secretion from neutrophils is not essential for promoting inflammation since JHMV-infected *MMP9*^{-/-} mice show similar BBB permeability and subsequent leukocyte infiltration compared to infected *MMP9*^{+/+} controls, indicating that MMP9 is dispensable for BBB disruption and compensatory mechanisms to promote BBB permeability exist, such as enhanced MMP-3 secretion from resident glia [85]. In addition to MMP-9 secretion by neutrophils, it is also feasible that these cells can also influence MMP production by resident cells of the CNS. Finally, Bergmann and colleagues [20] have shown that by impairing monocyte recruitment to the CNS through genetic ablation of *CCR2* there is the retention of leukocytes within the perivascular space of the CNS, corresponding to a reduction in neuroinflammation. This occurs regardless of neutrophil activity at the BBB and further illustrates the complexity of early events leading to breakdown of the BBB following JHMV infection [20].

Neutrophil effector activity has also been shown to have critical roles in other CNS-based viral infections. Depletion of PMNs following infection of susceptible mice with West Nile Virus (WNV) leads to higher viremia and reduced survival arguing for a protective role for these cells [86]. Indeed, Bai et al. [86] demonstrated that infection of CXCR2-deficient mice with WNV results in higher viremia at D5 p.i. However, one caveat to this study is that neutrophils may be a viral reservoir for WNV, and the lack of neutrophil accumulation within the brains of *CXCR2*^{-/-} results in lower viremia at early timepoints post-inoculation with WNV. Nonetheless, neutrophils were shown to be important in host-defense to WNV. Conversely, McGavern and colleagues [87] showed a detrimental effect of neutrophil activity following infection with the mouse adapted Armstrong strain of lymphocytic choriomeningitis virus (LCMV), where it was demonstrated that cytotoxic CD8⁺ T cells promote neutrophil accumulation at the BBB, leading to a loss of vascular integrity that results in fatal encephalitis. Moreover, silencing of CXCR2 through antibody blockade or genetic ablation has been shown to dampen inflammation and tissue injury in models in which neutrophil infiltration correlates with disease initiation, such as inflammatory mediated injury to the lung [88–90] and during experimental bacterial infections within the brain [91].

Emerging evidence also suggests that CXCR2 bearing neutrophils are necessary to promote CNS-based autoimmune disorders in mice [92, 93]. Willenborg and colleagues [94] first demonstrated that antibody depletion of PMNs could dampen clinical severity of early effector phases of experimental autoimmune encephalomyelitis (EAE) following

immunization with encephalitogenic myelin peptides. Furthermore, CXCL1 and CXCL2 are up-regulated within the CNS during chronic EAE disease and genetic ablation or antibody blockade of CXCR2 within mice resulted in the failure to develop EAE, while adoptive transfer of CXCR2 expressing PMNs into *CXCR2*^{-/-} mice reestablished EAE disease, although with slightly less severity [92, 93]. Using a cuprizone-induced demyelinating disease model, Liu et al. [95] discovered that neutrophils are necessary to induce demyelination following cuprizone treatment, but *CXCR2*-deficient mice were resistant to demyelination even though these cells accumulated within the CNS, implicating aberrant effector functions in *CXCR2*^{-/-}-neutrophils. Together, these findings highlight a role of ELR⁺ chemokines in initiating and amplifying inflammation within the CNS in response to infection with a neurotropic virus and sensitization to encephalitogenic myelin peptides by enhancing migration, accumulation and effector functions of neutrophils.

Neuroprotective Effects of ELR⁺ Chemokine Signaling During Chronic JHMV-Induced Disease

Oligodendrocytes appear to be refractory to JHMV-induced apoptosis *in vivo* [58], suggesting that a signaling pathway could be triggered in oligodendrocytes that can act to protect them against JHMV-induced death and limit the severity of demyelination. Recent studies have shown that activation of the PI3K/Akt signaling pathway through stimulation of the tyrosine kinase family of receptors on oligodendrocytes enhances their survival when treated pro-apoptotic TNF α [96, 97]. In addition, CXCR2 signaling may serve beneficial roles that extend beyond its influence on promoting chemotaxis towards ELR-positive chemokine gradients. CXCR2 is expressed on a variety of CNS cell subsets including neurons [98, 99], astrocytes [100], microglia [101], and cells of the oligodendrocyte lineage [75, 82, 102]. Human CXCL8, a ligand for CXCR2, provides a neurotrophic effect on cultured hippocampal and cerebellar granule neurons during in-vitro viability assays [103, 104], and also blocks Fas-mediated apoptosis of cultured astrocytes [105]. Furthermore, cultured mouse OPCs proliferate in response to CXCL1 secreted from spinal cord astrocytes [106], while the CXCL1/CXCR2 signaling system is associated with the proliferation of human fetal OPCs [107] and OPC positioning and proliferation within the developing mouse spinal cord [108]. Finally, Omari et al. [109] have described a potential protective effect of CXCL1 overexpression during EAE. Using a doxycycline-inducible transgenic mouse system, the authors revealed that astrocyte-specific CXCL1 overexpression following the induction of EAE reduces clinical severity and is associated with diminished demyelination and enhanced remyelination [64]. However, the results of this study remain enigmatic due to the difficulty in defining the exact mechanism by which CXCL1 leads to the observed effects as overexpression of CXCL1 could desensitize CXCR2 signaling on neutrophils and negatively impact their migration to the CNS [110].

With regards to a protective role for CXCR2 in preventing cells from an oligodendrocyte lineage from death during JHMV infection, we have recently demonstrated that administration of a blocking antibody specific for CXCR2 to JHMV-infected mice increases clinical disease severity, and this correlates with a significant increase in oligodendrocyte apoptosis and demyelination [82]. Furthermore, JHMV infection of cultured OPCs derived from mouse neural progenitor cells (NPCs) results in apoptosis and this is blocked following inclusion of the CXCL1 [82]. The protective effect of CXCR2 signaling also extends to IFN- γ -induced apoptosis of cultured OPCs as addition of CXCL1 to IFN- γ treated cultures restricts cell death [75]. Our findings suggest that one mechanism by which IFN- γ induces OPC death is through induction of CXCL10 that subsequently signals through CXCR3 [75]. Highlighting the importance of CXCL10 in enhancing IFN- γ -mediated cell death is the demonstration of reduced apoptosis in IFN- γ -treated OPC cultures derived from *CXCR3*^{-/-} mice [75]. This observation supports and extends other studies demonstrating CXCL10

promotes neuronal apoptosis during simian immunodeficiency virus-induced encephalitis [111] and WNV-induced encephalitis [112–114]. Furthermore, the significance of CXCR2 signaling to OPC protection from IFN- γ and CXCL10-mediated cell death is further underscored by the finding that CXCR2-deficient OPCs are resistant to the tonic effects of CXCL1 in response to treatment with either IFN- γ or CXCL10. Mechanisms associated with CXCR2-mediated protection in these findings [75] include reduced activation of the proapoptotic caspase 3 and increased expression of the anti-apoptotic Bcl2 in CXCL1-treated cultures.

Conclusion

The JHMV-induced model of encephalomyelitis provides an important tool for interrogating mechanisms associated with the initiation and persistence of neuroinflammation and demyelination in response to viral infection of the CNS. Ongoing research in our laboratory continues to focus on the role of ELR-positive chemokine signaling on oligodendroglia during JHMV-induced neuroinflammation. It will be important to analyze the effects of selectively ablating CXCR2 on oligodendroglia during JHMV-induced demyelination and also identifying and manipulating the cellular sources of ELR-positive chemokines in the CNS that may contribute to neuroprotection during chronic JHMV-induced disease.

Future Perspectives

Explicit mechanisms by which PMNs and MMPs govern the breakdown of the BBB to promote the infiltration of inflammatory leukocytes in response to viral infection of the CNS remain to be characterized. Answers to these questions will provide further insights into pathological mechanisms that will allow a better understanding of inflammatory events surrounding viral encephalitis in humans. Moreover, uncovering potential endogenous neuroprotective mechanisms such as the CXCR2 signaling axis has potential to limit myelin loss and potentiate axonal sparing and remyelination in human demyelinating diseases such as MS [102, 115].

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Executive summary

Initiation of an acute anti-viral cellular response to JHMV-infection occurs following efficient breakdown of the BBB

- Neutrophil specific MMP-9 secretion on the luminal side of the BBB and astrocyte-specific MMP-3 secretion on the parenchymal side of the BBB degrade the basement membrane and extracellular matrix, facilitating leukocyte migration into the CNS.
- The specific enzymatic role of each MMP is still unclear due to compensatory mechanisms that arise following genetic ablation of individual MMPs
- It remains to be determined if neutrophils can execute other pro-inflammatory effector functions in promoting host-defense to JHMV

JHMV-induced chronic demyelinated disease is amplified by activated virus-specific T-cells and macrophages/microglia

- IFN- γ secretion by activated virus-specific CD4+ and CD8+ T-cells contributes damage to cells of an oligodendrocyte lineage and enhances proinflammatory cytokine/chemokines secretion from inflammatory macrophages as well as resident glia.

The CXCR2-CXCL1 signaling chemokine system may provide neuroprotective effects on cells of the oligodendrocyte lineage following JHMV-induced Chronic Disease

- Astrocyte-specific expression of CXCL1 may promote anti-apoptotic effects on oligodendroglia as CXCR2 neutralization during chronic JHMV-induced disease results in increased disease severity correlating with increased numbers of apoptotic oligodendrocytes and oligodendrocyte progenitor cells.
- IFN- γ -induced expression of CXCL10 promotes apoptosis of cultured OPCs through signaling via the CXCR3 receptor. This can be blocked through tonic effect of CXCL1 by preventing cleavage of caspase-3 and increasing expression of Bcl-2.
- Oligodendrocyte-specific ablation of CXCR2 during chronic JHMV-induced disease will provide further insights into potential endogenous protective mechanisms of ELR+ chemokine signaling.