Chapter C9

CHEMOKINES IN CORONAVIRUS-INDUCED DEMYELINATION

Matthew J. Trifilo[*], Michael T. Liu, William G. Glass, and Thomas E. Lane[*1]
*Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92697-3900

Abstract: Inflammation within the central nervous system (CNS) is critical in the development of the neuropathology associated with the human demyelinating disease multiple sclerosis (MS). Recent studies have identified a family of soluble proinflammatory molecules called chemokines that are able to direct leukocyte infiltration into the CNS in response to infection or injury. Identification of chemokines within and around demyelinating lesions in MS patients indicate a potential role for these molecules in contributing to the pathogenesis of MS. To address this issue, we have used mouse hepatitis virus (MHV) infection of the CNS to understand the dynamic interaction of chemokine expression as it relates to inflammation and neuropathology. Our results indicate that chemokine expression within the CNS results in persistent recruitment of both T lymphocytes and macrophages and results in subsequent myelin destruction. Herein, we demonstrate the complexity of the chemokine response to MHV infection of the CNS and the delicate balance that exists between host defense and development of disease.

Key words: Chemokines, T lymphocytes, Macrophages, Demyelination

1. INTRODUCTION

Mouse hepatitis virus (MHV) is a single strand, positive sense RNA virus that is a member of the Coronaviradae family. As with many viruses, the disease induced by MHV infection depends upon a variety of different factors such as host and viral genetics as well as the dose and route of

inoculation. For example, intracranial (i.c.) infection of susceptible strains of mice such as C57BL/6 (H-2^b background) with the neuroattenuated variant MHV strain J2.2v-1 results in infection of glial cells and oligodendrocytes resulting in an acute encephalomyelitis accompanied by white matter destruction. Type I interferons are expressed early following infection and this correlates with recruitment of neutrophils and natural killer (NK) cells into the CNS. Although not completely understood, it is thought that these components of the innate immune response control viral spread prior to the entry of virus-specific T cells as well as participate in amplifying chemokine expression within the CNS that results in the induction of the adaptive immune response. CD8$^+$ T cells are the primary effector component of the adaptive immune response and are able to eliminate infectious virus from the host through secretion of IFN-γ (oligodendrocytes) and perforin-mediated cytolysis (astrocytes and microglia) (1,2). CD4$^+$ T cells can also directly participate in reducing viral burden presumably through the release of antiviral cytokines such as IFN-γ. However, CD4$^+$ T cells also play an essential role in host defense by providing support for CD8$^+$ T cells (3,4). Although both CD4$^+$ and CD8$^+$ T cells are potent anti-viral effector cells within the CNS, sterile immunity is not achieved and viral RNA and protein persist primarily within white matter tracts in which astrocytes and oligodendrocytes are the predominant cellular reservoirs for virus.

Viral persistence in white matter tracts results in a chronic demyelinating disease in which foci of demyelination are associated with areas of viral RNA/antigen. Clinically, mice develop loss of tail tone and a partial to complete hind-limb paralysis. Recent reports have indicated that MHV-induced demyelination is complex and involves immunopathologic responses against viral antigens expressed in infected tissues. MHV infection of immunosuppressed or immunodeficient mice results in high titers of virus within the CNS and increased mortality but no detectable demyelination (5). Further, adoptive transfer of MHV-immune splenocytes restores demyelination to the infected recipients suggesting a role for immune cells in driving demyelination (5). We have recently demonstrated that CD4$^{-/-}$ mice displayed a significant reduction in the severity of demyelination as compared to CD8$^{-/-}$ and immunocompetent wildtype mice suggesting an important role for CD4$^+$ T cells in amplifying the severity of white matter destruction (6). Additional support for T cells in contributing to demyelination comes from a recent report by Wu *et al.* (3) that demonstrated that both CD4$^+$ and CD8$^+$ T cells are important in mediating myelin destruction following adoptive transfer into MHV-infected RAG1$^{-/-}$ mice. Although B cells and/or antibodies have been shown to play an important role in controlling the recrudescence of virus during the chronic stage of disease, they are not important in MHV-induced demyelination (7). We and

others have found that macrophages/microglia are also important in contributing to demyelination (3,6,8). Current evidence suggests that demyelination in MHV-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 (9). It is likely that T cells present within the CNS of MHV-infected mice are specific for viral antigens and these cells drive demyelination by producing and/or influencing the production of cytokines/chemokines that amplify and support inflammation. Therefore, collective evidence points to a role for inflammatory T cells in contributing to macrophage/microglia infiltration and activation that results in myelin destruction. Collectively, these studies illustrate the complexity of the MHV system as it relates to the immune response and demyelination.

Given the importance of both T cells and macrophages in amplifying the severity of myelin destruction in persistently infected mice, it is clear that maintenance of a chronic inflammatory response is important with regards to the neuropathology of this model system. Numerous cytokines including TNF-α, IL-1β, and IL-6 are produced by activated astrocytes within the CNS of persistently infected mice (10). Working in concert or individually, these molecules may contribute to disease and myelin destruction by attracting T cells and macrophages into the CNS. However, the soluble signals that regulate CNS invasion by inflammatory cells likely consist of additional factors. With this in mind, studies from our laboratory have focused on chemokines (*chemo*tactic cyto*kines*) and the role these molecules have in MHV-induced demyelination. This chapter will focus on recent studies evaluating the functional significance of chemokine expression as it relates to disease development following MHV infection of the CNS.

1.1 Chemokines and Chemokine Receptors

Chemokines are a family of small molecular weight, secreted proteins that have been shown to target the migration of specific populations of leukocytes (Reviewed in 11). The chemokines are currently divided into four subfamilies based upon the arrangement of conserved cysteine residues in the amino-terminus of the protein (structural criteria) and what leukocytes are targeted, e.g. monocytes, macrophages, granulocytes, neutrophils, and T lymphocytes (functional criteria)(11-13). A variety of lymphoid and nonlymphoid tissues can produce chemokines, often in connection with the initiation or progression of inflammation (11). However, recent evidence also identifies a role for chemokines in the regulation of normal leukocyte migration into, out of, and within lymphoid tissues and organs (14,15).

Much attention has focused on two distinctive groups of chemokines, the CXC and CC chemokines, because of their appearance in numerous disease models, including MS (11,16,17). The CXC family can be further subdivided into two groups based upon the presence of a glutamic acid-leucine-arginine (ELR) motif on the amino terminus. The appearance of the ELR motif encodes for chemotaxis of neutrophils, whereas its absence dictates the recruitment of lymphocytes and monocytes (11,13,18,19). In general, the CC chemokines share chemoattractant properties with non-ELR CXC chemokines, attracting monocytes, T lymphocytes, basophils, and eosinophils and have little or no effect on neutrophils (11,13).

As mentioned above, chemokines can be produced in the presence or absence of inflammation. However, much attention has focused on the beneficial and detrimental role of chemokines in leukocyte recruitment in response to infection (11,13, 20-28). A wide variety of cell types can produce chemokines in response to pathogen, including monocytes, macrophages, lymphocytes, neutrophils, endothelial cells, fibroblasts, and astrocytes. Although many of these cells can produce a diverse array of chemokines, specific chemokine production appears to be linked to the type and strength of the stimuli present. Chemokines are able to induce leukocyte migration into tissue by promoting the adherence and extravasation of leukocytes across the vascular endothelium. Once inside the inflamed tissue, leukocytes are able to migrate across chemokine gradients towards the highest localized concentration. These gradients are established through the binding of the highly basic chemokines to the acidic extracellular matrix on the luminal side of the endothelium. This interaction may also allow for reduced chemokine diffusion and help establish a fixed gradient towards the site of infection (29).

Although in vitro studies have shown extensive redundancy in the chemotactic properties of chemokines, in vivo studies utilizing knockout mice and neutralizing-antibodies have suggested that in response to viral infection of the CNS, chemokines are able to selectively attract distinct leukocyte populations (20-24). Importantly, accumulating evidence indicates that this non-redundant recruitment may influence a variety of CNS diseases by initiating events culminating in neuroinflammation (20,30,31). In addition to their prominent role in inflammation, chemokines may also directly contribute to disease progression by exerting a cytotoxic effect upon host tissue (32). Furthermore, there is increasing evidence that chemokines are able to influence the activation and differentiation (Th1 vs. Th2) of T cells (22,33).

Chemokines act through binding specific receptors present on the surface of numerous cell types. The majority of chemokine receptors belong to the seven-transmembrane G protein-coupled receptor superfamily. Unlike the

specificity observed between most ligand receptor pairs, chemokines exhibit a high level of promiscuity, capable of productively binding to multiple chemokine receptors and chemokine receptors can functionally bind multiple chemokines (34-37). Following chemokine binding to its cognate receptor, signaling events are initiated that result in various cellular processes including increases in intracellular calcium, production of cytokines and chemokines, adhesion to the endothelial matrix, and chemotaxis (38). Interestingly, numerous CXC and CC chemokines can also bind to a receptor identical to the Duffy blood group antigen that is present on the surface of erythrocytes (39-41). Binding of chemokines to these receptors does not result in intracellular signaling events, instead it appears to reduce the level of circulating chemokines that may decrease unwanted interactions between chemokines and leukocytes within the blood, maximizing the ability of circulating leukocytes to migrate to highly concentrated chemokine levels at the site of infection (40,42,43).

Leukocytes can limit chemokine signaling by effectively down-regulating chemokine receptor expression or by uncoupling the downstream signaling events from the chemokine receptor, allowing for immediate control of chemokine signaling. Additionally, leukocytes exposed to high concentrations of an individual chemokine can become desensitized via phosphorylation of chemokine receptors limiting leukocyte movement at the site of infection (34,44).

Chemokine receptors can be identified on nearly all lymphocyte populations. However, the expression pattern of certain receptors appears to correlate with the activation state of the cell. For this reason, specific chemokine receptors have been utilized to determine the activation state of specific sub-populations of T lymphocytes. For example, expression of the chemokine receptor CCR7, which is necessary for migration into secondary lymphoid tissues, has been localized to the surface of naïve T cells and a subpopulation of memory cells (45). In contrast, T cells expressing the chemokine receptors CXCR3, CCR1 and/or CCR5 selectively migrate into inflamed tertiary tissues, indicating an important role for these chemokine receptors and their respective ligands in promoting an inflammatory response (20,24,45-47).

1.2   Chemokines and Multiple Sclerosis

Expression of chemokines has been associated with demyelinating plaque lesions present in MS patients (48,49). Hvas and colleagues (48) demonstrated expression of the CC chemokine CCL5 by infiltrating T-lymphocytes surrounding MS plaque lesions. Elevated levels of the non-

ELR CXC chemokines CXCL10, CXCL9 as well as CCL5 were found in the CSF of MS patients during periods of clinical attack (49). In addition, astrocyte expression of CXCL10 has been reported in plaque lesions present in MS patients (49). Recent work has demonstrated a direct correlation between clinical progression in the severity of MS with leukocyte infiltration into the CNS suggesting that production of CXCL10, CXCL9, and CCL5 may contribute to the pathogenesis of MS by recruiting T cells and macrophages into the CNS (49). Further, MS patients display increased expression of CXCR3 and CCR5, the cellular receptors for CXCL10, CXCL9, and CCL5, respectively, within the CNS as compared to control patients (49). T cells expressed both CXCR3 and CCR5 whereas macrophages/microglia were found to express CCR5 (49). Further support for chemokines and chemokine receptors in contributing to recruitment of activated leukocytes comes from a recent study by Baranzini et al. (50) that demonstrated increased expression of CCR1 (signaling receptor for CCL5 and CCL3) and CCR5 within the brains of MS patients. Collectively, these data emphasize the need for a better understanding the functional significance of chemokine and chemokine receptor expression as it relates to demyelination in MS patients.

1.3   MHV Infection and Chemokine Gene Expression

MHV infection of the CNS results in a well-orchestrated expression of chemokine genes which appears to be dictated, in part, by viral burden (51, Table I). CXCL10 mRNA transcripts are detected within 1 day following infection and co-localize with areas of MHV replication. At this time, the cellular source of CXCL10 transcripts are primarily ependymal cells lining the lateral ventricle (51). By day 6 p.i., virus has spread throughout the brain parenchyma and a robust inflammatory response, characterized primarily by $CD4^+$ and $CD8^+$ T cells and macrophages, is established within the brain. Chemokines expressed at this time include CXCL9, CXCL10, CCL2, CCL3, CCL5 and CCL7 (51). *In situ* double-labeling revealed that astrocytes are the predominant cellular source of mRNA transcripts for CXCL9 and CXCL10 during the acute stage of disease (21,51). In addition, MHV-infection of mouse cultured astrocytes results in the rapid induction of numerous chemokine genes including CXCL1, CXCL10, CCL2, CCL4, and

Table I. Summary of chemokine expression with clinical and pathological features following MHV infection of the CNS

	Days Postinfection			
	3	7	12	35
Clinical signs	None	Limp tail, ruffled fur,hunched	Limp tail, ruffled fur, hunched, awkward gait	Limp tail, ruffled fur, hunched, awkward gait
Chemokines[a]	CXCL10 CCL9 CCL5 CCL2 CCL3 CXCL11	CXCL10 CXCL9 CCL5 CCL2 CCL3 CXCL11	CXCL10 CCL5	CCL5 CXCL10
Viral Titer[c]	+++	++	BD[b]	BD
Viral RNA	+	++++	+++	++
Demyelination[d]	-	+	++	+++

[a]Brains and spinal cords were removed at indicated time points, and total RNA extracted and chemokine mRNA transcripts were evaluated by RPA. Chemokine mRNA transcripts are listed in order of abundance.

[b]BD= Below Detection.

[c]Viral RNA detectable by in situ hybridization.

[d]Demyelination was determined by Luxol fast blue staining of paraffin-embeded brain and spinal cord sections.

CCL7 (6). UV-inactivated MHV was sufficient to trigger low level chemokine gene expression indicating that viral replication is not necessary to induce expression of these genes (51). *In vivo* analysis of the chemokine receptor mRNA profile indicates increased expression of CCR1, CCR5, and CXCR3 during the acute stage of disease (20,24). Confocal microscopy revealed that macrophages (determined by F4/80 antigen expression) express CCR5 (24). In addition, studies have shown that both $CD4^+$ and $CD8^+$ T cells infiltrating into the brain express CXCR3 (20).

1.4 Functional Significance of Chemokine Expression during MHV-Induced Demyelination

CXCL10 and CCL5 are the predominant chemokines expressed in the CNS of persistently infected mice undergoing demyelination (51) (Table I). Moreover, CXCR3 and CCR5 expression (receptors for CXCL10 and CCL5, respectively) is also detected at this time (20,24). These findings have

parallels in human neurodegenerative diseases. Glial cell expression of CCL5 and CXCL10 as well as CXCR3-and CCR5-positive mononuclear cells have been reported within demyelinating lesions present in patients with MS (49,52). Moreover, increased levels of CXCL9, CXCL10 and CCL5 are present within the cerebral spinal fluid of MS patients during periods of clinical attack and their presence correlates with increased numbers of inflammatory cells (49). Therefore, the overlap in chemokine and chemokine receptor expression profiles within the demyelinating CNS of MHV-infected mice and MS patients provides an opportunity to assess the functional significance of these molecules as it relates to demyelination in the MHV model system.

1.5   CCL5 and Disease

Intracranial infection of $CD4^{-/-}$ and $CD8^{-/-}$ mice with MHV resulted in increased mortality and delayed clearance of virus from the CNS demonstrating an important role for T lymphocytes in host defense against MHV-induced CNS disease (6). Interestingly, infected $CD4^{-/-}$ mice displayed significantly less severe inflammation and demyelination as compared to $CD8^{-/-}$ and wildtype C57BL/6 mice. FACS analysis of the cellular infiltrate present within the CNS of infected mice revealed that $CD4^{-/-}$ mice contain fewer activated macrophages and significantly lower levels of CCL5 mRNA transcripts and protein when compared to $CD8^{-/-}$ and C57BL/6 mice. These data suggested that $CD4^{+}$ T cells are the predominant source of CCL5 following MHV infection of the CNS, although it is also possible that $CD4^{+}$ T cells influence expression of CCL5 by other cell populations through the release of cytokines and/or chemokines. Additional cellular sources such as $CD8^{+}$ T cells, macrophage and glial cells must be considered due to the fact that CCL5 mRNA transcripts and protein are detected, albeit at lower levels, within the CNS of $CD4^{-/-}$ mice. In light of the fact that CCL5 exerts a potent chemotactic effect on both T cells and macrophages, these data suggest that the reduction in macrophage infiltration and the severity of demyelination into the CNS of $CD4^{-/-}$ mice is causally related to the reduced CCL5 levels observed.

To provide a direct test of CCL5 importance in contributing to MHV-induced CNS inflammation and demyelination, MHV-infected C57BL/6 mice were treated with anti-CCL5 antisera immediately following MHV infection and the severity of disease evaluated. Treatment lead to a disease in C57BL/6 mice similar to the phenotype observed in $CD4^{-/-}$ mice with mice displaying a significant reduction in the severity of demyelination. Decreased macrophage infiltration in anti-CCL5 treated mice correlated with the reduced severity of demyelination supporting the observations with

MHV-infected CD4$^{-/-}$ mice (6). These observations reinforce the functional significance of CCL5 expression during virus-induced CNS disease indicating that this chemokine has an important role in the recruitment of macrophages into the CNS following MHV infection. Moreover, administration of neutralizing anti-CCL5 antibodies to MHV-infected mice in which disease e.g. demyelination and paralysis is already established resulted in a marked improvement in neurologic disease that correlated with reduced T cell infiltration and a reduction in the severity of myelin destruction (Glass and Lane, unpublished observations). Collectively, these data highlight the important role of CCL5 signaling in both host defense and disease pathogenesis by regulating leukocyte accumulation within the CNS of MHV-infected mice.

Having established that CCL5, a major signaling ligand of CCR5, is important in contributing to demyelination in MHV-infected mice by attracting macrophages into the CNS, studies were performed to further characterize the contributions of CCR5 to neuroinflammation and demyelination in MHV-infected mice. Analysis of CCR5 expression within the CNS of MHV-infected mice revealed that the majority of cells expressing this receptor were macrophage/microglia (24). MHV infection of CCR5$^{-/-}$ mice did not result in an increase in either morbidity or mortality as compared to infected CCR5$^{+/+}$ mice. In addition, clearance of virus from the CNS of infected CCR5$^{-/-}$ mice was not impaired and this correlated with equivalent levels of infiltrating T cells into the CNS when compared to MHV-infected CCR5$^{+/+}$ mice. These data indicate that the expression of this receptor does not contribute to host defense. However, demyelination was reduced in MHV-infected CCR5$^{-/-}$ mice and this correlated with reduced levels of infiltrating macrophages as compared to CCR5$^{+/+}$ mice. These data suggest that CCL5 signaling through CCR5 is an important mechanism whereby macrophages are able to enter the CNS and contribute to demyelination. Additional support for the importance of CCR5 signaling in leukocyte migration comes from recent studies from our laboratory examining the outcome of MHV infection of CCL3 knock-out mice. In addition to CCL5, CCL3 is able to bind and signal through CCR5 and trigger T cell and macrophage activation and trafficking. Intracranial infection of CCL3$^{-/-}$ mice resulted in diminished T cell and macrophage accumulation within the CNS that correlated with a significant reduction in demyelination as compared to MHV-infected wild-type mice (22). Therefore, these data demonstrate that CCR5 signaling by either CCL3 or CCL5 enhances both T cell and macrophage trafficking and accumulation within the CNS of MHV-infected mice and this correlates with the severity of myelin destruction.

Signaling through CCR5 appears to selectively regulate the trafficking of subsets of T cells into the CNS of MHV-infected mice. Adoptive transfer of

virus-specific CD4$^+$ T cells derived from MHV-immunized CCR5$^{+/+}$ mice and expanded to the immunodominant epitope present in the matrix protein spanning residues 133-147 into MHV-infected RAG1$^{-/-}$ mice resulted in T cell accumulation within the CNS and increased CCL5 expression that correlated with macrophage infiltration and demyelination (53). In contrast, adoptive transfer of virus-specific CD4$^+$ T cells obtained from immunized CCR5$^{-/-}$ mice resulted in only limited numbers of T cells present within the CNS and diminished CCL5 expression that correlated with a reduction in macrophage infiltration and demyelination. Examination of chemokine receptor expression on virus-specific CD4$^+$ T cells derived from CCR5$^{+/+}$ and CCR5$^{-/-}$ mice indicated a marked decrease in mRNA transcripts for several receptors. Notably, CXCR3, which is the signaling receptor for CXCL10, was significantly reduced. These data imply that CCR5 signaling may regulate expression of additional chemokine receptors which enhance T cell trafficking into target tissues. In contrast, CCR5 signaling is not required for trafficking of virus-specific CD8$^+$ T cells into the CNS (Glass and Lane, unpublished observations). However, CCR5 does appear to contribute to effector functions of CD8$^+$ T cells as CD8$^+$ T cells lacking CCR5 exhibit increased production of IFN-γ and increased cytolytic activity as compared to wildtype mice. These data highlight the importance of chemokines and chemokine receptors with regards to the trafficking and activation of specific subsets of T cells as it relates to host defense and disease progression in the MHV model system.

1.6   CXCL10 and Disease

As mentioned above, the pattern of chemokine expression as well as the appearance of the respective chemokine receptors on the surface of activated T cells suggested that these molecules may control the migration of lymphocytes following viral infection of the CNS. Although many chemokines are expressed at specific times during the course of MHV infection, one of the most prominent chemokines expressed is CXCL10 (51). Analysis of the functional contributions of CXCL10 during the acute stage of disease indicated a direct role in activated CD4$^+$ and CD8$^+$ T cell chemotaxis into the CNS. The correlation between T cell infiltration and demyelination following MHV infection as well as the appearance of CXCL10 during the chronic stage of MHV disease suggested that this chemokine may also contribute to T cell mediated myelin destruction. To address the functional contributions of CXCL10 to MHV induced demyelination, mice were treated with anti-CXCL10 neutralizing antibodies 12 days following i.c. infection. Strikingly, anti-CXCL10 treatment not only stopped further progression of myelin destruction, but decreased the severity

of clinical symptoms as compared to mice treated with control antibody (31). Histological analysis revealed that reduced clinical symptoms correlated with a dramatic reduction in the level of demyelination within anti-CXCL10-treated mice. Furthermore, this decrease in behavioral deficits correlated with the presence of increased levels of remyelination within the CNS as assessed by electron microscopy. Analysis of T cell infiltration following anti-CXCL10 treatment indicated a dramatic reduction in CD4$^+$ T cells while CD8$^+$ T cell levels remained similar to control. The preferential effect on CD4$^+$ T cells was in contrast to effects observed following anti-CXCL10 treatment during the acute stage of disease, where both CD4$^+$ and CD8$^+$ T cell migration was compromised (20). However, analysis of CXCR3 expression on infiltrating lymphocytes during both the acute and chronic stage of MHV infection indicated that while both CD4$^+$ and CD8$^+$ T cells express abundant CXCR3 expression during the acute disease, CD4$^+$ T cells preferentially express increased levels of the CXCL10 receptor during chronic disease, suggesting that receptor expression is responsible for selective CD4$^+$ T cell migration (31). Regardless, these results indicate that CD4$^+$ T cell infiltration can contribute not only to myelin destruction, but also inhibit remyelination following MHV infection. These results indicate that one mechanism by which CXCL10 can contribute to demyelination is by recruiting activated CD4$^+$ T cells into the CNS that can participate in disease pathogenesis.

1.7   Conclusion

MHV infection of the CNS provides a consistent, reliable model in which to study not only host response to viral infection but also to determine the contributions of chemokines to neuroinflammation and demyelination. Although the studies mentioned above indicate a complex web of chemokines and chemokine receptor expression, analysis of individual chemokines utilizing knockout mice and neutralizing antibodies has indicated specific and selective roles for these molecules within the CNS. As depicted in Figure 1, chemokines are expressed within the CNS throughout MHV infection, and have now been shown to participate in the recruitment and/or activation of cells based on their temporal expression. Indeed, early expression of CXCL10 contributes to the development of the innate response that can both control viral replication and further amplify chemokine production aiding in the establishment of the adaptive immune response (Trifilo and Lane, unpublished observations) (Figure 1, 1). By day 7 p.i., robust expression of numerous CXC and CC chemokines (see Table I) are detected within the CNS. Highlighted is CXCL10 and CCL5 expression that are critical for the infiltration of T cells (CD4$^+$ and CD8$^+$) and activated

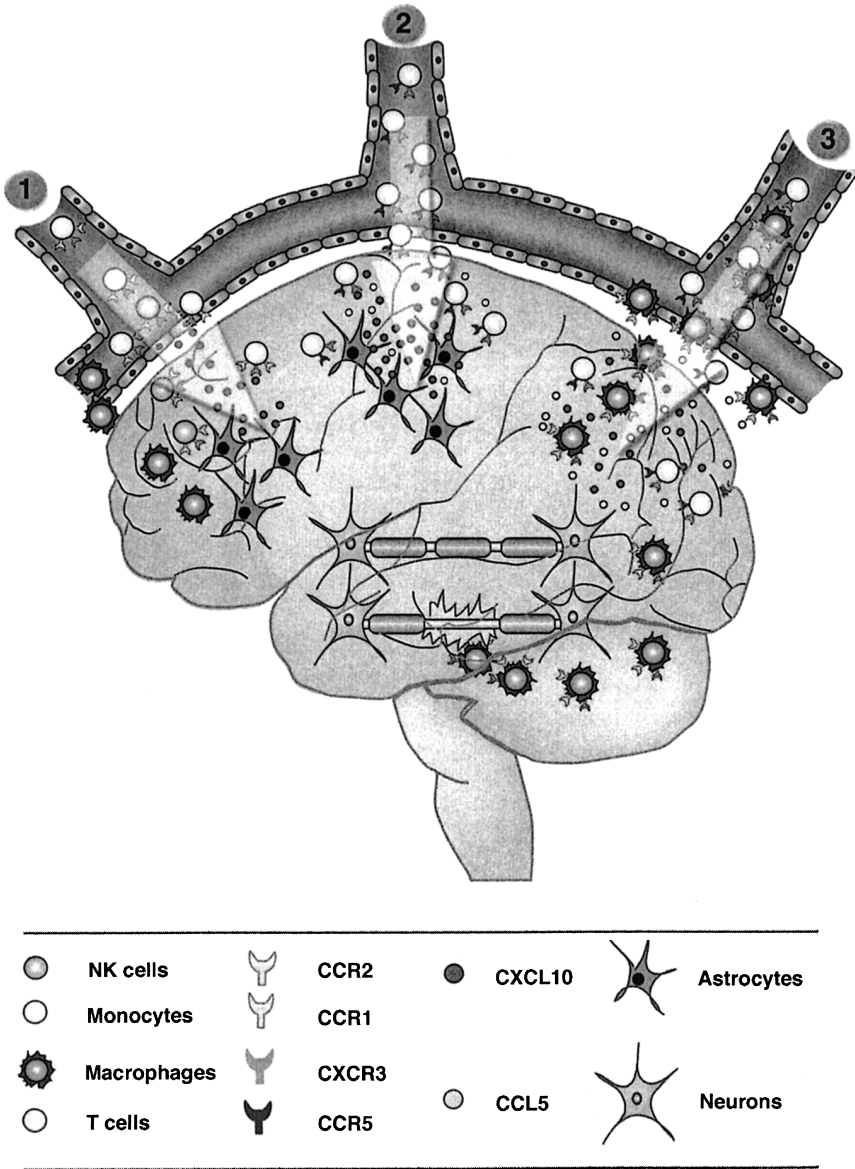|						
◐	NK cells	⋎	CCR2	●	CXCL10	✶ Astrocytes
○	Monocytes	⋎	CCR1			
✹	Macrophages	⋎	CXCR3	○	CCL5	✳ Neurons
○	T cells	⋎	CCR5			

Figure C9-1. MHV infection of the CNS

macrophages (20,24) (Figure 1, **2**). T cell infiltration mediates clearance of replicating virus from the CNS by 12 days p.i. through the release of cytokines (ie. IFN-γ) and cell mediated cytolysis (CTL). Although

replicating virus can no longer be detected, viral protein and RNA persist after 12 days p.i., resulting in chronic expression of both CXCL10 and CCL5 (Figure 1, **3**). These chemokines induce the chronic recruitment of activated T cells and macrophages that subsequently participate in demyelination. This model reveals the protective and destructive properties chemokines posses based on both the level and time of expression.

Collectively, our data indicate that chemokines can play specific and selective roles in T lymphocyte and macrophage recruitment within the CNS (CXCL10 and CCL5) as well as contribute to the activation of virus-specific T cells (CCL3). More importantly, although numerous chemokines are detected within the CNS following MHV infection, it is clear that these molecules function in a nonredundant manner, thus meriting further studies on chemokines with regards to the role of these molecules in viral-induced CNS disease. Finally, the data clearly demonstrate that chemokines and their receptors may represent viable targets in modulating the severity of human nueroinflammatory diseases including MS.

ACKNOWLEDGEMENTS

REFERENCES

1. Lin MT, Hinton DR, Stohlman SA. Mouse hepatitis virus is cleared from the central nervous system in mice lacking perforin-mediated cytolysis. *J Virol* 1997 **71**:383-391.
2. Parra B, Hinton DR, Marten NW, Bergmann CC, Ln MT, Yang CS, Stohlman SA. IFN-gamma is required for viral clearance from central nervous system oligodendroglia. *J Immunol* 1999 **162**:1641-1647.
3. Wu GF, Dandekar AA, Pewe L, Perlman S. CD4 and CD8 T cells have redundant but not identical roles in virus-induced demyelination. *J Immunol* 2000 **165**:2278-2286.
4. Stohlman SA, Bergmann CC, Lin MT, Cua DJ, Hinton DR. CTL effector function within the central nervous system requires CD4+ T cells. *J Immunol* 1998 **160**:2896-2904.
5. Wang F, Stohlman SA, Fleming JO. Demyelination induced by murine hepatitis virus JHM (MHV-4) is immunologically mediated. *J Neuroimmunol* 1990 **30**:31-41.

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

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

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

9. Miller SD, Vanderlugt CL, Begolka WS, Pao W, Yaunch RL, Neville KL, Katz-Levy Y, Carrizosa A, Kim BS. Persistent infection with Theiler's virus leads to CNS autoimmunity via epitope spreading. *Nat Med* 1997 **3**:1133-1136.

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

11. Luster AD. Chemokines-chemotactic cytokines that mediate inflammation. *New Engl J Med* 1998 **338**:436-445.

12. Clark-Lewis I, Kim K, Rajarathan K, Gong J, Dewald B, Moser B, Baggiolini M, Sykes B. Structure activity relationships of chemokines. *J Leuk Biol* 1995 **57**:703-711.

13. Taub DD, Oppenheim JJ. Chemokines, inflammation and the immune system. *Ther Immunol* 1994 **1**:229-246.

14. Cyster JG. Chemokines and cell migration in secondary lymphoid organs. *Science* 1999 **286**:2098-2102.

15. Cyster JG. Leukocyte migration: scent of the T zone. *Cur Biol* 2000 **10**:R30-33.

16. Huang D, Han Y, Rani MR, Glabinski A, Trebst C, Sorensen T, Tani M, Wang J, Chien P, O'Bryan S, Bielecki B, Zhou ZL, Majumder S, Ransohoff RM. Chemokines and chemokine receptors in inflammation of the nervous system: manifold role and exquisite regulation. *Immunol Rev* 2000 **177**:52-67.

17. Glabinski AR, Ransohoff RM. Chemokines and chemokine receptors in CNS pathology. *J Neurovirol* 1999 **5**:3-12.

18. Farber JM. Mig and IP-10: CXC chemokines that target lymphocytes. *J Leukoc Biol* 1997 **61**:246-257.

19. Piali L, Weber C, LaRosa G, Mackay CR, Springer TA, Clark-Lewis I, Moser B. The chemokine receptor CXCR3 mediates rapid and shear-resistant adhesion-induction of effector T lymphocytes by the chemokines IP-10 and Mig. *Eur J Immunol* 1998 **28**:961-972.

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

21. Liu MT, Armstrong D, Hamilton TA, Lane TE. Expression of Mig (monokine induced by interferon-g) is important in T lymphocyte recruitment and host defense following viral infection of the central nervous system. *J Immunol* 2001 **166**:1790-1795.

22. Trifilo MJ, Bergmann CC, Kuziel WA, Lane TE. The CC chemokine ligand 3 (CCL3) regulates CD8$^+$ T cell effector function and migration following viral infection. *J Virol* 2003 in press.

23. Chen BP, Kuziel WA, Lane TE. Lack of CCR2 results in increased mortality and impaired leukocte activation and trafficking following infection of the central nervous system with a neurotropic coronavirus. *J Immunol* 2001 **167**:4585-4592.

24. Glass WG, Liu MT, Kuziel WA, Lane TE. Reduced macrophage infiltration and demyelination in mice lacking the chemokine receptor CCR5 following infection with a neurotropic coronavirus. *Virology* 2001 **288**:8-17.

25. Murray PD, Krivacic K, Chernosky A, Wei T, Ransohoff RM, Rodriquez M. Biphasic and regionally-restricted chemokine expression in the central nervous system in the Theiler's virus model of multiple sclerosis. *J Neurovirol* 2000 **6**:S44-52.

26. Ransohoff RM, Wei T, Pavelko KD, Lee JC, Murray PD, Rodriguez, M. Chemokine expression in the central nervous system of mice with a viral disease resembling multiple sclerosis: roles of CD4+ and CD8+ T cells and viral persistence. *J Virol* 2002 **76**:2217-2224.

27. Olszewski MA, Huffnagle GB, McDonald RA, Lindell DM, Moore BB, Cook DN, Toews GB. The role of macrophage inflammatory protein-1 alpha/CCL3 in regulation of T cell-mediated immunity to Cryptococcus neoformans infection. *J Immunol* 2000 **165**:6429-6436.

28. Huffnagle GB, Strieter RM, Standiford TJ, McDonald RA, Burdick MD, Kunkel SL, Toews GB. The role of monocytes chemotactic protein-1 (MCP-1) in the recruitment of monocytes and CD4+ T cells during a pulmonary Cryptococcus neoformans infection. *J Immunol* 1995 **155**:4790-4997.

29. Tanaka Y, Adams DH, Hubscher S, Hirano H, Siebenlist U, Shaw S. T-cell adhesion induced by proteoglcan-imobilized cytokine MIP-1 beta. *Nature* 1993 **361**:79-82.

30. Karpus WJ. Chemokines and central nervous system disorders. *J Neurovirol* 2001 **7**:493-500.

31. Liu MT, Keirstead HS, Lane TE. Neutralization of the chemokine CXCL10 reduces inflammatory cell invasion and demyelination and improves neurological function in a viral model of multiple sclerosis. *J Immunol* 2001 **167**:4091-4097.

32. Furie MB, Randolph GJ. Chemokines and tissue injury. *Am J Pathol* 1995 **146**:1287-1301.

33. Karpus WJ, Lukacs NW, Kennedy KJ, Smith WS, Hurst SD, Barrett TA. Differential CC chemokine-induced enhancement of T helper cell cycle cytokine production. *J Immunol* 1997 **158**:4129-4136.

34. Murphy P. The molecular biology of leukocyte chemoattractant receptors. *Ann Rev Immunol* 1994 **12**:593-633.

35. Kelvin D, Michiel D, Johnston J, Lloyd A, Sprenger H, Oppenheim J, Wang JM. Chemokines and serpentines: the molecular biology of chemokine receptors. *J Leukocyte Biol* 1993 **54**:604-612.

36. Horuk R. Molecular properties of the chemokine receptor family. *Trends Pharmacol Sci* 1994 **15**:159-165.

37. Horuk R. The interleukin-8-receptor family: from chemokines to malaria. *Immunol Today* 1994 **15**:169-174.

38. Ward SG, Bacon K, Westwick J. Chemokines and T lymphocytes: more than an attraction. *Immunity* 1998 **9**:1-11.

39. Horuk R, Chitnis CE, Darbonne WC, Colby TJ, Rybicki A, Hadley TJ, Miller H. A receptor for the malarial parasite Plasmodium vivax: the erythrocyte chemokine receptor. *Science* 1993 **261**:1182-1184.

40. Chaudhuri A, Polyakove J, Zbrzezna V, Williams K, Gulati S, Pogo A. Cloning of glycoprotein D cDNA, which encodes the major subunit of the Duffy blood group system and the receptor for Plasmodium vivax malaria parasite. *Proc Nat Acad Sci USA* 1993 **90**:10793-10797.

41. Chaudhuri A, Zbrzezna V, Polyakova J, Pogo A, Hesselgesser J, Horuk R. Expression of the Duffy antigen in K562 cells: evidence that it is the human chemokine erythrocyte receptor. *J Biol Chem* 1994 **269**:7835-7838.

42. Neote K, Darbonne W, Ogez J, Horuk R, Schall T. Identification of a promiscuous inflammatory peptide receptor on the surface of red blood cells. *J Biol Chem* 1993 **268**:12247-12249.

43. Horuk R, Colby T, Darbonne W, Schall T, Neote K. The human erythrocyte inflammatory peptide (chemokine) receptor. Biochemical characterization, solubilization, and development of a binding assay for the soluble receptor. *Biochemistry* 1993 **32**:5733-5738.

44. Ben-Baruch A, Michiel DF, Oppenheim JJ. Signals and receptors involved in recruitment of inflammatory cells. *J Biol Chem* 1995 **270**:11703-11706.

45. Sallusto F, Mackay CR, Lanzavecchia A. The role of chemokine receptors in primary, effector, and memory immune responses. *Annu Rev Immunol* 2000 **18**:593-620.

46. Hamann A, Klugewitz K, Austrup F, Jablonski-Westrich D. Activation induces rapid and profound alterations in the trafficking of T cells. *Eur J Immunol* 2000 **30**:3207-3218.

47. Potsch C, Vohringer D, Pircher H. Distinct migration patterns of naïve and effector CD8 T cells in the spleen: correlation with CCR7 receptor expression and chemokine reactivity. *Eur J Immunol* 1999 **29**:3562-3570.

48. Hvas J, Bernard CC. Molecular detection and quantitation of the chemokine RANTES mRNA in neurological brain. *APMIS* 1998 **106**:598-604.

49. Sorensen TL, Tani M, Jensen J, Pierce V, Lucchinetti C, Folcik VA, Qin S, Rottman J, Sellebjerg F, Strieter RM, Frederiksen JL, Ransohoff RM. Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. *J Clin Invest* 1999 **103**:807-815.

50. Baranzini SE, Elfstrom C, Chang SY, Butunoi C, Murray R, Higuchi R, Oksenberg JR. Transcriptional analysis of multiple sclerosis brain lesions reveals a complex pattern of cytokine expression. *J Immunol* 2000 **165**: 6576-6582.

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

52. Balashov KE, Rottman JB, Weiner HL, Hancock WW. CCR5(+) and CCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1-alpha and IP-10 are expressed in demyelinating brain lesions. *Proc Natl Acad Sci USA* 1999 **96**:6873-6878.

53. Glass WG, Lane TE. Functional expression of chemokine receptor CCR5 on CD4(+) T cells during virus-induced central nervous system disease. *J Virol* 2003 **77**: 191-198.