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Functional Diversity of Chemokines and Chemokine Receptors in Response to Viral Infection of the Central Nervous System

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Abstract Encounters with neurotropic viruses result in varied outcomes ranging from encephalitis, paralytic poliomyelitis or other serious consequences to relatively benign infection. One of the principal factors that control the outcome of infection is the localized tissue response and subsequent immune response directed against the invading toxic agent. It is the role of the immune system to contain and control the spread of virus infection in the central nervous system (CNS), and paradoxically, this response may also be pathologic. Chemokines are potent proinflammatory molecules whose expression within virally infected tissues is often associated with protection and/or

pathology which correlates with migration and accumulation of immune cells. Indeed, studies with a neurotropic murine coronavirus, mouse hepatitis virus (MHV), have provided important insight into the functional roles of chemokines and chemokine receptors in participating in various aspects of host defense as well as disease development within the CNS. This chapter will highlight recent discoveries that have provided insight into the diverse biologic roles of chemokines and their receptors in coordinating immune responses following viral infection of the CNS.

1
Introduction

1.1
Biology and Biochemistry of *Coronaviridae*

Coronaviruses are classified on the basis of several fundamental characteristics, including nucleic acid type, a lipid envelope, and their distinctive morphology [42, 64, 79]. All members have characteristic petal-shaped proteins extending from the virion surface. Coronaviruses infect numerous vertebrate hosts including humans, chickens, pigs, and mice, causing a wide variety of disorders involving a number of different organ systems; however, there are specific tropisms for the CNS, lungs, gastrointestinal tract, and liver [42, 64, 79]. Receptor use among the varied coronaviruses is restricted to several well-defined proteins. Human coronavirus infections result in acute enteritis as well as 15% of common colds indistinguishable from those caused by other viruses [42, 64, 79]. More recently, a human coronavirus has been indicated to be the etiologic agent for severe acute respiratory syndrome (SARS). SARS is a potentially lethal disease and is recognized as a health threat internationally [43].

   The first murine coronavirus strain (mouse hepatitis virus, MHV), was isolated in 1949 [12]. MHV is a pathogen of wild mice, and natural infection is due to horizontal transmission, resulting in acute hepatitis with death in young animals and a variable course of persistent gastrointestinal tract infection in adults [79]. MHV is not an endemic mouse virus, but infects mouse colonies sporadically. It is very closely related to some human coronaviruses both at the genomic and protein levels. For example, human sera often contain antibody reactive to MHV. Therefore, characterizing the immune response to murine coronaviruses may provide important insight to mechanisms of control and elimination which may have important implications with regards to understanding the immune response to human coronaviruses such as the SARS coronavirus.

Coronavirus genomes are single-stranded positive-polarity RNA molecules, larger than the size of any other known stable RNA, ranging from 27 kb for the avian infectious bronchitis virus, to 31 kb for murine coronaviruses [50]. Genomic RNA is infectious, contains a cap structure at the 5′-end and poly(A) at the 3′-end. The genome is organized into seven or eight genes, each containing one or more open reading frames (ORF) separated by intergenic sequences that contain the signals for the initiation of transcription of the subgenomic viral messenger (m)RNA species. Upon entry, the viral RNA encodes an RNA polymerase that transcribes the genome into a negative-stranded RNA [50]. The latter serves as templates for positive-sensed genomic RNA and subgenomic mRNAs. Important viral structural proteins include the envelope glycoproteins (S) that bind to receptors on cell membranes [42, 64, 79]. Analysis of monoclonal antibody neutralization escape variants demonstrated that the viral S protein controls cellular tropism in vivo and the role of the S protein in tropism has recently been confirmed using stable recombinant viruses in which all genes except the S protein gene were held constant [9, 82].

1.2
Immunity to MHV Infection

The protective immune response to MHV infection is characterized predominantly by cell-mediated immunity during acute infection. A number of unique aspects of CNS viral infection have been described by analysis of the interactions between MHV and the immune response. Antibody, although protective if administered prior to infection, is not present in the serum of infected mice until after the vast majority of virus has been cleared from the CNS [56, 84]. Following infection, neutrophils, macrophages, and NK cells are rapidly recruited into the CNS, followed by T cells and B cells [104]. Inflammation is accompanied by a progressive loss of blood–brain barrier (BBB) integrity that is apparent as early as 4 days post-infection. The initial influx of innate effectors is important in facilitating T cell infiltration, as well as regulating viral replication [104]. However, the ability to survive MHV infection appears to be predominantly due to an effective T cell-mediated response [103]. Recent data have confirmed that cell-mediated immunity is critical during acute infection [53, 55, 74, 76, 92]; however, the ability to prevent viral recrudescence is associated with the continued presence of plasma cells in the CNS secreting neutralizing antibody [56, 84].

The major effectors of anti-viral immunity are virus-specific CD8^{+} T cells. Cytotoxic T lymphocyte (CTL) induction following MHV infection of the CNS has been shown to require CD4^{+} T cell help [92]. Although the pre-

cise mechanism or mechanisms by which CD4$^+$ T cells assist CD8$^+$ T cells have yet to be completely determined, recent studies have demonstrated that CD4$^+$ T cells are important in preventing apoptosis of CTL entering the CNS parenchyma [92]. In addition, the quality of the CTL response is CD4$^+$ T cell-dependent [92]. An important concept derived from analysis of MHV infection is that although CD8$^+$ T cells are the most prominent effectors for viral clearance during the acute infection, the mechanisms which control virus replication differ with the type of CNS cell infected. Cytolysis is important for the control of viral replication in microglia/macrophages and astrocytes while interferon (IFN)-γ is the critical effector responsible for control of virus replication in oligodendroglia [73]. The demonstration that CD8$^+$ CTL suppresses viral replication by two separate effector mechanisms, which function within the CNS in a cell type-specific manner, is an important new concept.

1.3
Viral Persistence and Immune-Mediated Demyelination

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 [51]. Clinically, mice develop loss of tail tone and a partial to complete hind-limb paralysis. As a result of the clinical and histologic similarities between MHV-induced demyelination and the human demyelinating disease multiple sclerosis (MS), the MHV system is considered a relevant model for studying the underlying immunopathologic mechanisms contributing to immune-mediated demyelinating diseases [51]. A variety of different mechanisms have been postulated to contribute to MHV-induced demyelination. Several studies suggest that MHV-induced demyelination involves immunopathologic responses against viral antigens expressed in infected tissues [30, 31, 37, 47]. Although virus-specific antibody is considered important in suppressing viral recrudescence [84, 85], it may also have a role in promoting demyelination [48]. MHV infection of immunosuppressed or immunodeficient mice results in high titers of virus within the CNS and death but not robust demyelination [53, 105]. Adoptive transfer of MHV-immune splenocytes results in demyelination to the infected recipients, suggesting a role for immune cells in amplifying demyelination [30, 31]. Additional evidence for T cells in contributing to demyelination is provided by Wu et al. [105] who demonstrated that both CD4$^+$ and CD8$^+$ T cells are important in mediating myelin destruction. In support of this are studies derived from our laboratory demonstrating that adoptive transfer of MHV-specific CD4$^+$ or CD8$^+$ T cells to MHV-infected RAG1$^{-/-}$ mice results in demyelination [30, 31]. However, demyelination was more severe in recipients of CD4$^+$ T cell

compared to CD8$^+$ T cell recipients, and this supports a more important role for CD4$^+$ T cells in amplifying demyelination in this model. Indeed, we have demonstrated that MHV-infected CD4$^{-/-}$ mice displayed a significant reduction in the severity of demyelination compared to CD8$^{-/-}$ and immuno-competent wildtype mice, suggesting an important role for CD4$^+$ T cells in amplifying the severity of white matter destruction [53].

While T cells are generally considered important in driving demyelination in mice persistently infected with MHV, the mechanisms by which these cells participate in disease may vary and depend upon various factors including the ability to secrete interferon (IFN)-γ [80, 81]. While conventional CD4 and CD8 αβ T cells are generally viewed as the primary T cell type important in disease, γδ T cells have also been shown to participate in demyelination in MHV-infected athymic mice [16]. In addition, we and others have found that macrophages/microglia are also important in contributing to demyeli-nation [29, 32, 53, 59, 105]. The collective evidence points to a role for in-flammatory T cells in contributing to macrophage/microglial infiltration and activation which ultimately results in myelin destruction. 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 demyeli-nation [69]. However, adoptive transfer of T cells from MHV-infected rats to naïve recipient's results in demyelination [100]. Whether a similar response occurs in MHV-infected mice and what the contributions are to demyelination is not clear at this time.

1.4
Chemokines and Chemokine Receptors

Chemokines represent a family of low molecular weight (7–17 kDa) proinflam-matory cytokines that are divided into four subfamilies based on structural and functional criteria [14, 60, 94]. The two major subfamilies are the CXC and CC chemokines. The CXC subfamily is structurally characterized by two conserved cysteine residues that are separated by an amino acid, while the CC subfamily is structurally characterized by conserved cysteine residues adjacent to one another. Lymphotactin, the sole member of the C family, is chemotactic for T cells [44]. The CX$_3$C chemokine, fractalkine, is unique in that it is expressed on the surface of cells as well as being secreted into the surrounding environment [5].

Chemokines have been shown to selectively attract distinct leukocyte pop-ulations during periods of inflammation in various disease models. The CXC chemokines function primarily in attracting neutrophils, yet have a lim-ited effect on T lymphocytes and monocytes [14, 60, 94]. However, there

are exceptions to this rule in that CXC chemokines that lack the glutamic acid-leucine-arginine (ELR) motif on the amino terminus are chemotactic for T cells. For example, the non-ELR chemokine CXCL10 is a potent chemoattractant for activated T cells and NK cells and functions by binding to CXCR3 expressed on the surface of these cells [40, 83, 102, 106]. However, CXCL10 does not exert a chemotactic effect on neutrophils [19]. The CC chemokines are thought to attract T cells, monocytes, and macrophages, but not neutrophils [14, 60, 94]. The CC chemokine ligand 5 (CCL5) is able to attract both T cells and macrophages by binding to one of several CC chemokine receptors including CCR1 and CCR5 [14, 60, 94]. Furthermore, there is increasing evidence that chemokines, such as CCL3, influence other immune system activities including T_H1/T_H2 development and T cell proliferation [46, 95]. Chemokines function by binding to seven-transmembrane-spanning G protein-coupled receptors. The chemokine receptors are divided into those that preferentially bind CXC and CC chemokines. In addition, CC and CXC chemokine receptors are capable of binding more than one CC or CXC chemokine, respectively. A variety of cell types including lymphocytes and macrophages, as well as resident cells of the CNS such as neurons, astrocytes, and microglia, express chemokine receptors [60, 94].

2
Orchestrated Expression of Chemokines and Chemokine Receptors Within the CNS Following Infection with MHV

Instillation of MHV into the CNS of susceptible mice results in a well-orchestrated expression of chemokine genes, and the expression pattern correlates with the level of inflammation and disease [52]. Early (~1–3 days) following infection, transcripts for CXCL10 and CCL3 are detected within the CNS, suggesting an important role in initiation of immune responses (see following section; Table 1). By day 6 post-infection (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, CCL4, CCL5, and CCL7 (MIP-2) (Table 1). Analysis of chemokine receptor expression by both RNAse protection assay (RPA), immunostaining, and flow cytometry reveals that CCR1, CCR2, CCR5, and CXCR3 are the prominent receptors expressed within the CNS at various stages of disease (Table 2).

Chemokine transcripts are detected almost exclusively in areas in which virus is present, indicating a localized response to infection and subsequent

Table 1 Chemokine gene expression following MHV infection of the CNS

Days post infection	Chemokine	Function (cells attracted)	Reference(s)
1–3	CXCL10	NK cells	97
	CCL3	Dendritic cells	96
7 and 12	CCL2	Macrophage	39, 52
	CCL3	Dendritic cells, T cells	95, 96
	CCL4		52
	CCL5	T cells, macrophage	52, 53
	CXCL9	T cells	58
	CXCL10	T cells	18, 57
≥ 21	CXCL10	$CD4^+$ T cells	59
	CCL5	T cells, macrophages	32

Table 2 Chemokine receptors expressed within the CNS of MHV-infected mice

Days post infection	Receptor	Chemokine receptor expression	Reference(s)
1–3	CCR2	T cells, macrophages	13, 39
7 and 12	CCR2	T cells, macrophages	13, 39
	CCR5	T cells, macrophages	29, 30
	CXCR3	T cells	57
>21	CXCR3	T cells	59
	CCR5	T cells, macrophage	29

spread of the virus throughout the parenchyma. In situ hybridization indicates that astrocytes are the primary cellular source for many chemokines during the acute stage of disease [52]. Infection of primary cultures of mouse astrocytes with MHV and evaluating chemokine gene expression by RPA provide additional support for astrocytes as an important cellular source of chemokines in this model [52]. Moreover, viral replication appears to be a necessary prerequisite for inducing chemokine expression, as infection of astrocytes with inactivated virus results in a muted chemokine expression profile. Additional analysis revealed that both infected and noninfected astrocytes are capable of secreting chemokines following instillation of virus into the brain, indicating that viral infection is not required for chemokine gene synthesis by target cells. These data indicate that a factor or factors (possibly type I interferons) derived from infected cells are capable of functioning in both an autocrine and paracrine manner and regulate chemokine gene expression in response

to viral infection. Other cell types that may also secrete chemokines following MHV infection include resident microglia/inflammatory macrophages as well as neurons [52, 75].

By day 12 p.i., MHV-infected mice that have survived the acute stage of disease develop an immune-mediated demyelinating disease. Mice have cleared infectious virus (as determined by plaque assay) by 12 days, yet viral RNA and protein can be detected within white matter tracts for months after infection. As the level of CNS infiltration subsides following reduction of viral burden there is a corollary reduction in the expression of chemokine transcripts. Analysis of chemokine message expression within the brains and spinal cords of MHV-infected mice during the demyelinating phase of disease (days 12 and onward) indicates that CXCL10 and CCL5 are the two prominent chemokines expressed [52]. In situ hybridization for chemokine transcripts indicated expression was limited primarily to areas of viral persistence within white matter tracts undergoing active demyelination [52]. Similar to what was found during acute disease, astrocytes were determined to be the cellular source of CXCL10 at this stage of disease whereas inflammatory cells, presumably CD4$^+$ T lymphocytes, expressed CCL5. More recent data now indicate that MHV-infected astrocytes treated with IFN-γ can also express CCL5 mRNA transcripts and protein (T.E. Lane, unpublished observations). Chemokine receptors expressed during chronic demyelination include CXCR3 and CCR5, which are capable of binding CXCL10 and CCL5, respectively. Indeed, we have recently determined that the majority (~90%) of infiltrating virus-specific CD4$^+$ and CD8$^+$ T cells express CXCR3 (T.E. Lane, unpublished observations).

3
Chemokines, Innate Immune Response, and MHV-Infection of the CNS

The presence of dendritic cells (DCs) within the CNS has been debated for quite some time. However, a series of recent studies clearly indicates that during induction of an autoimmune demyelinating disease, there exists the presence of cell types within the brain that clearly have characteristics of DCs [34, 65]. In addition, emerging evidence points to a previously unappreciated role for chemokines in activating and inducing the migration of differing populations of DCs in response to microbial infection of the CNS [22, 23]. These cells may be important in initiation and/or maintenance of disease by participating in the activation of T cells. Given the potential importance of this population of cells with regards to linking innate and adaptive immune responses following viral infection of the CNS, we investigated whether DC-

like cells were present within the CNS in response to MHV infection. In brief, our findings clearly indicate that a DC-like population of cells is detectable within the CNS as early as day 2 p.i. with MHV [96]. The activation/maturation of these cells as well as the ability to accumulate within the draining cervical lymph node (CLN) appeared to be dictated by localized expression of CCL3 [96]. Moreover, the ability of cultured DCs to secrete cytokines associated with the development of a T_H1 response such as interleukin (IL)-12 was profoundly altered in the absence of CCL3 [96]. The importance of CCL3 signaling and the evolution of an effective T cell response was further confirmed by the demonstration that in the absence of CCL3 signaling, robust anti-viral effector responses, e.g., cytokine production and CTL activity, were dramatically compromised following MHV infection of CCL3$^{-/-}$ mice [95, 96]. Collectively, these studies highlight a previously unappreciated role for the importance of chemokine signaling and DC maturation/activation following MHV infection of the CNS. Moreover, these studies demonstrate that generation of effective T cell responses relies upon CCL3 signaling to successfully combat MHV infection.

4
Chemokines and Chemokine Receptors and Their Role in Acute Viral-Induced Encephalomyelitis

4.1
CCL3

CCL3 is a chemoattractant for both T cells and macrophages and has been implicated in host defense following infection with a wide variety of microbial pathogens. Mice deficient in CCL3 production exhibit increased susceptibility to disease following infection with paramyxovirus [17], influenza virus [15], and coxsackievirus, as well as other microbial pathogens [67, 72]. In all cases, alterations in an effective host response correlated with a paucity in leukocyte accumulation at sites of infection. Although originally thought to participate in defense by attracting effector cells to infected tissue, recent reports also suggest that CCL3 expression is important in coordinating a T_H1 response [46]. Numerous studies now indicate that DCs are capable of expressing various chemokines including CCL3 [21, 66, 77, 78]. Moreover, DC precursors express the CCL3 receptors CCR1 and CCR5 and are capable of responding to CCL3 in vivo and in vitro resulting in both mobilization and maturation [24, 108]. Indeed, Flesch and colleagues have demonstrated an important role for CCL3 in DC-dependent priming of CTL to viral antigens [24].

Using CCL3$^{-/-}$ mice, we have demonstrated a role for CCL3 in regulating trafficking as well as antiviral effector functions following MHV infection of the CNS [95]. Specifically, our experiments revealed an important role for CCL3 signaling in tailoring T cell responses that allowed for egress out of draining cervical lymph nodes and trafficking into the CNS. Although generation of antigen-specific CD8$^+$ T cells was not impaired following MHV infection of CCL3$^{-/-}$ mice, a significant percentage of CD8$^+$ T cells retained expression of lymph-node homing receptors CD62L (L-selectin) and the CC chemokine receptor 7 (CCR7) and did not display a dramatic increase in mRNA transcripts for either CXCR3 or CCR5, two receptors which are important in allowing MHV-specific T cells access to the CNS [95]. Moreover, adoptive transfer of CCL3$^{-/-}$ CD8$^+$ T cells into MHV-infected RAG1$^{-/-}$ mice (which express CCL3 following MHV infection) resulted in homing back to secondary lymphoid organs, suggesting that lack of CCL3 imprinted on these cells carries an inability to remodulate surface tissue homing receptors. Analysis of antiviral effector functions also revealed that CCL3$^{-/-}$ CD8$^+$ T cells displayed overall muted cytolytic activity as well as expression of IFN-γ when compared to CCL3$^{+/+}$ CD8$^+$ T cells [95]. Collectively, these studies highlight that, in addition to chemotactic function, chemokines influence specific lymphocyte responses and ultimately effector functions that are required for optimal host defense against microbial pathogens.

4.2
CXCL9 and CXCL10

CXCL9 and CXCL10 attract activated T lymphocytes following binding to CXCR3. Analysis of CXCL9 and CXCL10 mRNA expression within the CNS of MHV-infected mice revealed that CXCL10 was clearly detectable by day 1 p.i. and was prominently expressed at days 7, 12, and 35 p.i. [52]. In contrast, CXCL9 transcripts were only detected at days 7 and 12 p.i. [58]. These data suggested that both CXCL9 and CXCL10 might be important in host defense by attracting antiviral T lymphocytes into the CNS. In support of this is the observation that administration of neutralizing antibodies specific for either CXCL9 or CXCL10 to MHV-infected mice during the acute stage of disease results in a dramatic increase in mortality [57, 58]. Additionally, this treatment also resulted in a significant decrease in numbers of CD4$^+$ and CD8$^+$ T lymphocyte infiltrating into the CNS which correlated with decreased expression of IFN-γ and increased levels of virus [57, 58]. MHV infection of CXCL10$^{-/-}$ mice supported and extended our previous work on antibody-mediated neutralization of CXCL10 in that MHV-infected CXCL10$^{-/-}$ mice display reduced T cell infiltration into the CNS accompanied by reduced IFN-γ

secretion and increased viral burden [18]. Therefore, the collective evidence points to pivotal roles for both CXCL9 and CXCL10 as important sentinel molecules in promoting a protective response following MHV infection of the CNS by attracting T cells into the CNS that participate in elimination of virus.

4.3
CCL5

CCL5 is a T cell and macrophage chemoattractant that has been shown to influence leukocyte migration during periods of inflammation. Upon MHV infection of the CNS of mice, CCL5 transcripts and protein are readily detected within the brain [52]. Initial studies in which $CD4^{-/-}$ or $CD8^{-/-}$ mice were infected with MHV indicated an overall reduction in CCL5 mRNA transcripts within the brains of $CD4^{-/-}$ mice, suggesting that $CD4^+$ T cells were either a primary cellular source for CCL5 and/or influenced the expression of CCL5 by resident and inflammatory cells [53]. We now know that both inflammatory $CD4^+$ T cells as well as astrocytes are capable of expressing CCL5 following instillation of MHV into the CNS [32, 53]. Furthermore, treatment with neutralizing anti-CCL5 antisera results in diminished T cell and macrophage accumulation within the CNS, suggesting that in this model CCL5 is capable of regulating trafficking of these two populations of cells [32].

4.4
CCR5

CCR5 is a member of the CC chemokine receptor family that is expressed on various hematopoietic cells including lymphocytes and macrophages [86]. Chemokines that are capable of binding to CCR5 include CCL3, CCL4, and CCL5 [7, 68, 86]. Recent studies have clearly indicated that CCR5 expression correlates with leukocyte trafficking to sites of inflammation as well as regulating the immune response following microbial infection. For example, mice deficient in CCR5 ($CCR5^{-/-}$) exhibit altered T cell activity and impaired macrophage function [88, 109]. Furthermore, macrophage trafficking in response to antigen is impaired in $CCR5^{-/-}$ mice, indicating that CCR5 is required for migration of this population of cells [45]. Given that both T cells and macrophages express CCR5 following MHV infection of the CNS and these cells clearly influence outcome in response to infection, we have defined the contributions of CCR5 to both host defense and disease in response to MHV infection. Using an adoptive transfer model in which virus-expanded T cells are transferred into MHV-infected $RAG1^{-/-}$ mice, we have been able to examine how CCR5 expression influences trafficking of T cells into the CNS. Transfer of $CCR5^{+/+}$-derived $CD4^+$ T cells to MHV-infected $RAG1^{-/-}$ mice

resulted in CD4$^+$ T cell entry into the CNS and a reduction in viral titers within the brain [30]. These mice also displayed robust demyelination correlating with macrophage accumulation within the CNS. Conversely, CD4$^+$ T cells from CCR5$^{-/-}$ mice displayed an impaired ability to traffic into the CNS of MHV-infected RAG1$^{-/-}$ recipients, which correlated with increased viral titers, diminished macrophage accumulation, and limited demyelination. Analysis of chemokine receptor mRNA expression by M133–147-expanded CCR5$^{-/-}$-derived CD4$^+$ T cells revealed reduced expression of CCR1, CCR2, and CXCR3, indicating that CCR5 signaling is important in increased expression of these receptors which aid in trafficking of CD4$^+$ T cells into the CNS. Collectively these results demonstrate that CCR5 signaling is important to migration of CD4$^+$ T cells to the CNS following MHV infection.

With regards to the role of CCR5 in CD8$^+$ T cell trafficking, comparable numbers of virus-specific CD8$^+$ T cells derived from immunized CCR5$^{+/+}$ or CCR5$^{-/-}$ mice were present within the CNS of MHV-infected RAG1$^{-/-}$ mice following adoptive transfer, indicating that CCR5 is not required for trafficking of these cells into the CNS [30]. RAG1$^{-/-}$ recipients of CCR5$^{-/-}$-derived CD8$^+$ T cells exhibited a modest yet significant ($p \leq 0.05$) reduction in viral burden within the brain that correlated with increased cytolytic activity and IFN-γ expression. Histologic analysis of RAG1$^{-/-}$ recipients of either CCR5$^{+/+}$ or CCR5$^{-/-}$-derived CD8$^+$ T cells revealed only focal areas of demyelination with no significant differences in white matter destruction. These data indicate that CCR5 signaling on virus-specific CD8$^+$ T cells modulates antiviral activities but is not essential for entry into the CNS.

Finally, MHV infection of CCR5$^{-/-}$ mice resulted in a dramatic reduction in macrophage (defined as CD45^{high}F4/80$^+$ dual-positive cells) accumulation within the brains, and this correlated with a significant reduction in the severity of demyelination compared to CCR5$^{+/+}$ mice. Collectively, these data suggest that ligand binding, e.g., CCL5 and/or CCL3, and signaling via CCR5 results in macrophage migration and infiltration into the CNS. However, we have previously demonstrated that CCL3 is expressed only at low levels during acute disease and is not detectable during chronic demyelination, whereas robust expression of CCL5 is detected during both phases of disease, and this suggests that CCL5 is the primary CCR5 signaling chemokine in this model. This is supported by earlier studies that showed an important role for CCL5 in attracting macrophages into the CNS following MHV infection [53]. Therefore, the data presented in this study suggest that one mechanism by which CCL5 contributes to demyelination is via attracting macrophages into the CNS through CCR5-mediated signaling pathways. Additional evidence supporting this is provided by the observation that even in the presence of increased CCL5 expression at day 12 p.i., demyelination is reduced in CCR5$^{-/-}$ mice.

4.5
CCL2 and CCR2

CCL2 is capable of regulating the pathobiology of various inflammatory diseases including MS and atherosclerosis [1, 8, 28, 33, 35, 61]. In addition to its potent chemoattractant effect on monocytes and macrophages, CCL2 also influences T_H2 polarization in response to certain antigenic challenge [36, 41, 46, 99]. The influence of CCL2 on T cell polarization may be due to the fact that CCL2 is constitutively expressed within secondary lymphoid tissue and would be capable of affecting cellular responses following exposure to antigen [36]. Thus, available evidence indicates that expression of CCL2 is capable of influencing both innate as well as adaptive immune responses by regulating monocyte and T cell responses, respectively.

Analysis of chemokine receptor expression following MHV infection reveals that CCR2 is expressed by endogenous cells of the CNS as well as by inflammatory T cells and macrophages, indicating a role for these receptors in regulating both the immune response and disease development [13, 31]. Indeed, MHV-infection of CCR2$^{-/-}$ mice resulted in a dramatic increase in mortality and enhanced viral recovery from the brain that correlated with reduced T cell and macrophage entry into the CNS compared to viral infection of CCR2$^{+/+}$ mice [13].

MHV infection of CCL2$^{-/-}$ mice does not result in a similar disease phenotype as observed in CCR2$^{-/-}$ mice. This was somewhat surprising as CCR2 is currently the only known functional receptor for CCL2. Specifically, CCL2$^{-/-}$ mice were able to clear virus from the brain in a similar time frame as wildtype mice, and this correlated with the ability to generate antigen-specific T cells [39]. The deficiency in CCR2$^{-/-}$ mice to clear virus from the brain is not the result of an inherent inability to generate an effective adaptive immune response to virus, as CCR2$^{-/-}$ mice had a similar frequency of antigen-presenting cells (APC) and virus-specific T cells present within draining CLN compared to either CCL2$^{-/-}$ or wildtype mice. Our findings from MHV infection of CCL2$^{-/-}$ mice indicated that while CCL2 does influence leukocyte migration into the CNS in response to viral infection, CCR2 is clearly more influential in directing T cell trafficking into the CNS. In support of the role for CCL2 in promoting leukocyte migration into the CNS of MHV-infected mice are recent studies by Perlman and colleagues demonstrating that localized CCL2 expression within the CNS promotes macrophage infiltration [47]. These data highlight the possibility that ligand(s) other than CCL2 are important in signaling through the CCR2 receptor. Alternatively, it is possible that CCR2 signaling by either endothelial cells and/or astrocytes regulates the permeability of the BBB, as recently suggested by Stamatovic and colleagues [91].

5
Chemokines and Chronic Viral-Induced Demyelination

Expression of chemokines has been associated with demyelinating plaque lesions present in MS patients [3, 4, 26, 27]. Elevated levels of chemokines, notably CXCL10, were found in the cerebral spinal fluid (CSF) of MS patients during periods of clinical attack [25, 89]. Indeed, the concentration of CXCL10 within the CSF of MS patients correlated with numbers of inflammatory cells and the severity of clinical disease [2, 89, 90]. Moreover, when CXCL10 levels decreased, there was a corresponding decrease in inflammation and disease severity [89]. Astrocyte expression of CXCL10 has been reported in active plaque lesions present in MS patients, and the majority of T cells infiltrating into the CNS of MS patients express the CXCL10 receptor, CXCR3. Collectively, these studies highlight a potentially important role for CXCL10 in the pathogenesis of demyelinating diseases such as MS by attracting CXCR3-expressing T cells into the CNS and support targeting chemokines and their receptors for therapeutic intervention in the treatment of MS [10, 54, 70, 90].

Studies from animal models of MS support this notion by demonstrating that blocking of CXCL10 often results in diminished disease severity accompanied by a marked reduction in neuroinflammation. For example, several recent reports indicate that treatment with anti-CXCL10 neutralizing antibodies resulted in delayed disease onset and diminished neuroinflammation in mice with the autoimmune demyelinating disease experimental autoimmune encephalomyelitis (EAE) [20]. These studies support the idea that localized expression of CXCL10 within the CNS amplifies disease severity by attracting CXCR3-expressing T cells into the CNS. Once present, these cells enhance neuroinflammation by secreting additional chemokines as well as cytokines that can activate resident glia cells. Importantly, these studies also implicate CXCL10 as a potential therapeutic target and suggest that alternative CXCR3 ligands, e.g., CXCL9 and CXCL11, do not exert a prominent effect on T cell infiltration into the CNS. However, the role of CXCL10 in contributing to neurologic disease in EAE has been questioned by results indicating that CXCL10 may actually exert a protective effect in mice with EAE [49, 71]. Antibody-mediated neutralization following induction of EAE in rats resulted in increased disease severity, and this was associated with smaller draining lymph nodes and increased numbers of CD4$^+$ T cells infiltrating into the CNS [71]. In addition, CXCL10$^{-/-}$ mice exhibited increased clinical disease severity following immunization with myelin peptides, and this correlated with diminished lymph node sizes although T cell infiltration into the CNS was not dramatically altered when compared to wildtype mice [49]. In these

particular EAE models in which mice are immunized peripherally with anti-gen, CXCL10 expression within secondary lymphoid tissue is considered important in dictating disease outcome by serving to retain lymphocytes and tailoring T cell responses. Moreover, these findings highlight the different roles of CXCL10 in regulating cellular immune responses in different models of neuroinflammation and emphasize the need for a better understanding of how signaling by this chemokine regulates inflammation and disease.

As indicated, we have determined that MHV infection of the CNS results in an orchestrated expression of chemokine and chemokine receptor genes that are regulated, in large part, by the viral burden. Similar to MS patients, CXCL10 is expressed primarily by astrocytes in areas undergoing demyelination, suggesting an important role in the pathogenesis of demyelination by attracting CXCR3-expressing T cells into the CNS [52, 59]. Indeed, our laboratory was the first to demonstrate that treatment of mice with established demyelination and paralysis with anti-CXCL10 neutralizing antibody resulted in a significant reduction in CD4+—but not CD8+—T cells present within the CNS, and this correlated with improved motor skills and a reduction in the severity of demyelination [59]. Moreover, the dramatic regain of movement in anti-CXCL10-treated mice corresponded with more than 80% of previously demyelinated axons undergoing remyelination, indicating that removal of CXCL10 promoted an environment capable of remyelination. In addition to reduced numbers of CD4+ T cells within the CNS, there was a paucity of macrophage infiltration into the CNS of anti-CXCL10-treated mice that correlated with a dramatic reduction in the levels of the macrophage-chemoattractant CCL5. These data were consistent with previous studies indicating that CD4+ T cells were the major source for CCL5 in MHV-infected mice undergoing demyelination [53, 59]. The influence of CXCL10 in contributing to T cell responses was also examined. T cells isolated from secondary lymphoid tissue of mice treated with anti-CXCL10 displayed muted expression of IFN-γ in response to viral antigen when compared to T cells isolated from control mice, suggesting that CXCL10 also serves to influence T cell effector functions during chronic disease (T.E. Lane, unpublished observations).

We have previously determined that CCL5 mRNA transcripts and protein are present within the CNS of MHV-infected mice during chronic demyelination, indicating a potentially important role for this chemokine in promoting inflammation [52, 53]. In order to assess the functional role of CCL5 in participating in viral-induced immune-mediated demyelination, MHV-infected mice were treated via intraperitoneal (i.p.) injection with anti-CCL5 monoclonal antibody (mAb) following onset of clinical disease and demyelination. Such treatment resulted in a significant ($p \leq 0.05$) reduction in the severity of

clinical disease compared to mice treated with an isotype (IgG$_1$)-matched antibody [32]. Upon removal of anti-CCL5 treatment, clinical disease returned to mice such that there was no difference between the two experimental groups of mice. Immunophenotyping the cellular infiltrate of mice treated with anti-CCL5 revealed reduced T cell and macrophage infiltration into the CNS that is consistent with our earlier studies that CCL5 attracts these cells into the CNS of mice with chronic demyelination. Further, analysis of the severity of demyelination in experimental groups of mice indicated that anti-CCL5 treatment resulted in a significant ($p<0.05$) reduction in the severity of demyelination compared to control-treated mice.

A picture is slowly evolving from our experiments designed to test the functional contributions of CXCL10 and CCL5 to chronic demyelination within MHV-infected mice. Antibody targeting of the T cell chemoattractant CXCL10 in MHV-infected mice selectively affects CD4$^+$ T cell accumulation within the CNS accompanied by improved motor skills and a reduction in the severity of demyelination [59]. In contrast, CCL5 is capable of attracting both CD4$^+$ and CD8$^+$ T cells into the CNS. It is also important to emphasize that our data on CCL5 and CXCL10 inhibition with regards to T cell and macrophage trafficking are corollary and it is possible that alternative scenarios exist. For example, studies by Bergmann and colleagues suggest that during persistent MHV infection there is limited to no trafficking of T cells from the periphery into the CNS. Rather, upon entry during acute encephalomyelitis a certain percentage of CD4$^+$ and CD8$^+$ T cells is retained and participate in disease [62, 93]. In this instance, CXCL10 expression would not be functioning as a T cell chemoattractant but rather to influence specific biologic functions of T cells as well as potentiating the retention of T cells within the CNS. In support of this, it is possible that CXCL10 serves to enhance CD4$^+$ T cell proliferation, as several recent studies indicate that CXCL10 is important in contributing to T cell proliferation [18, 71, 101].

It is unlikely that CXCL10 contributes to T cell survival, as CXCL10$^{-/-}$ mice do not display any abnormalities with regards to T cell half-life nor do we see any increase in numbers of apoptotic T cells following anti-CXCL10 treatment. In addition, Narumi et al. [71] speculate that CXCL10 actually serves to retain CXCR3$^+$ T cells within tissues and this influences disease severity. Therefore, the selective reduction in CD4$^+$ T cells within the CNS of MHV-infected mice may not be the result of impaired trafficking. Rather, either CD4$^+$ T cells are not undergoing a steady-state turnover or are actually migrating out of the CNS in the absence of signals specifying their retention.

In addition, recent studies indicate an important role for CXCL10 in imparting effector functions to T cells. For example, Salomon and colleagues demonstrated that anti-CXCL10 treatment improved joint swelling in a rodent

model of arthritis and this correlated in part with an altered T_H1/T_H2 balance, suggesting that CXCL10 expression promotes and maintains a T_H1 state in T cells in this model [87].

Similarly, we have shown that MHV-infection of CXCL10$^{-/-}$ mice results in diminished IFN-γ expression by virus-specific T cells, supporting the idea that CXCL10 expression serves to maintain a T_H1-like state in T cells [18] (T.E. Lane, unpublished observations). CCL5 signaling also modulates cytokine production by T cells following antigenic challenge. In support of this is our demonstration that inhibition of CCL5 signaling results in enhanced IFN-γ expression by virus-specific T cells, supporting the idea that CCL5 expression serves to regulate a T_H1-like state in T cells [32]. Moreover, ablation of CCL5 signaling also modifies the cytolytic activity of MHV-specific CD8$^+$ T cells [30].

6
Perspectives

This chapter highlights mechanisms by which chemokines participate in both host defense and disease progression in response to MHV infection of the CNS. An overview of the potential functional role for select chemokines in linking innate and adaptive immune responses in response to viral infection of the CNS is provided in Fig. 1. In brief, following MHV infection there is robust expression of chemokines by infected astrocytes including CCL3 that contribute to the maturation/activation of local DCs, which ultimately enables migration to draining cervical lymph nodes. Activated DCs present antigen to T cells as well as secrete chemokines such as CCL3 and CXCL10 that enhance polarization to a T_H1 response. In turn, MHV-specific T cells express chemokine receptors including CXCR3 and CCR5 that enable them to traffic into the CNS as a result of localized expression of ligands CXCL9 and CXCL10 (ligands for CXCR3) as well as CCL5 (ligand for CCR5). In addition, our contention is that expression of CCR2 by endothelial cells of the BBB is also important in increasing the permeability of this structure.

With regards to chronic disease, MHV persistence within the CNS results in chronic expression of CXCL10 and CCL5 which together contribute to the maintenance of a chronic inflammatory disease by attracting both T cells and macrophages (Fig. 2). Local secretion of CXCL10 and CCL5 may also contribute to demyelination by enhancing specific T cell effector functions including (1) secretion of IFN-γ that activates local inflammatory macrophage and resident microglia, as well as directly damaging oligodendrocytes and (2) increasing CTL activity by CD8$^+$ T cells.
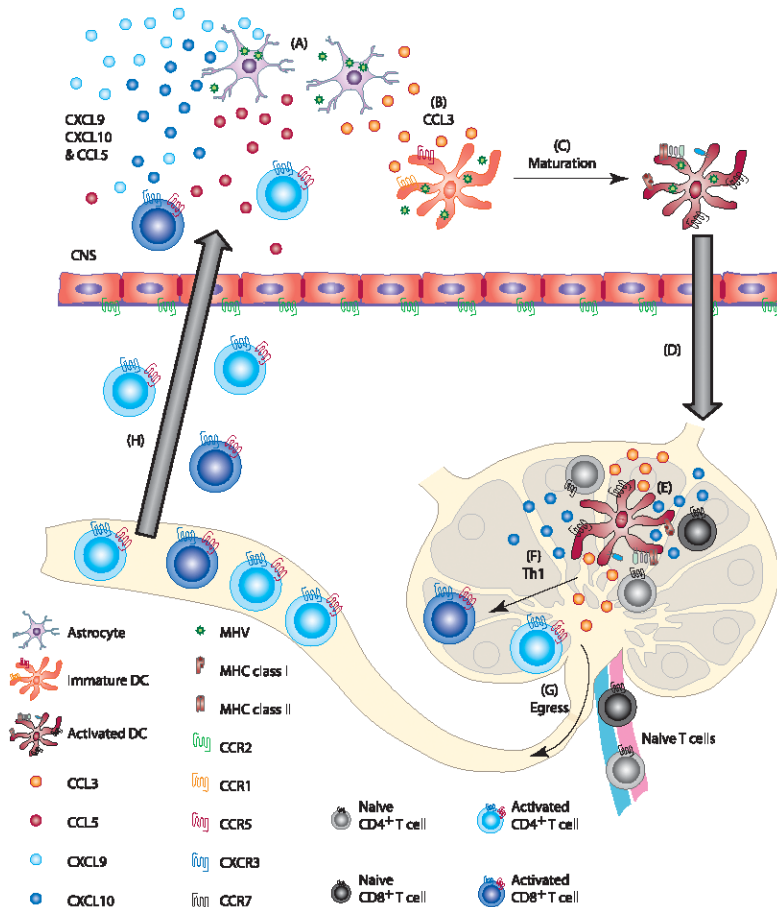
Fig. 1A–H Chemokines and innate/adaptive immune response following MHV infection of the CNS. Instillation of MHV into the CNS of susceptible mice results in infection of astrocytes that are an important source of chemokines including CXCL10, CCL5, and CCL3 (**A**). In addition, immature DC-like cells may also be susceptible to infection and secrete CCL3 (**B**) that functions in a paracrine and autocrine manner to bind to CCR1 expressed on immature DC-like cells. As a result of CCL3 signaling and MHV infection, the DC-like cells undergo maturation and activation (**C**) resulting in a remodulation of the plasma membrane characterized by decreased expression of CCR1 accompanied by increased expression of CCR7 as well as major histocompatibility complex (MHC) class I and II. CCR7-expressing, activated DCs home to the draining cervical lymph node (**D**). Upon entry, activated DCs express a variety of soluble factors including CCL3 and CXCL10 (**E**) that activate and enhance polarization of virus-specific T cells to a T_H1 phenotype (**F**). Activated T cells exit the lymph node via the efferent lymph (**G**), enter the blood stream, and migrate to the CNS via expression of the chemokine receptors CXCR3 and CCR5 (**H**)
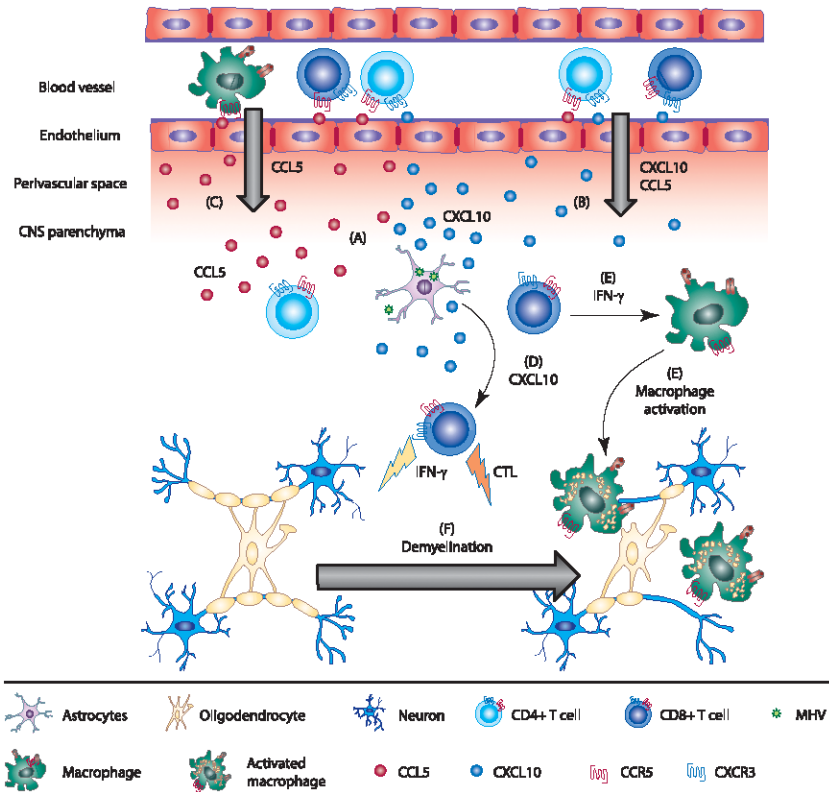
Fig. 2A–F Chemokines and MHV-induced demyelination. Persistent MHV infection within astrocytes leads to chronic CXCL10 and CCL5 expression (**A**) that serves to recruit CXCR3^{+} and CCR5^{+} T cells into the CNS (**B**). In addition, activated CD4^{+} T cells secrete CCL5 that enhances macrophage migration into the CNS (**C**). We believe that CXCL10 may also influence T cell effector functions within the CNS, including CTL activity (**D**) and IFN-γ secretion (**E**), leading to macrophage activation. Both IFN-γ production and CTL activity may enhance tissue destruction as well as macrophage activation that amplifies myelin destruction (**F**)

Clearly, these observations indicate that chemokine signaling is an integral component involved in eliciting protective immunity in response to viral infection of the CNS. Conversely, our studies also indicate that chronic localized secretion of select chemokines ultimately amplifies disease severity through maintaining inflammation within the CNS. Importantly, studies derived from the MHV system demonstrate that antibody targeting of select chemokines offers a powerful approach towards delineating the functional contributions

of these molecules in a model of immune-mediated demyelination. Further, these studies highlight the relevancy of such an approach in treating human neuroinflammatory and demyelinating diseases such as MS.

References

1. Baggiolini M (2001) Chemokines in pathology and medicine. J Intern Med 250:91–104
2. Balashov KE, Rottman JB, Weiner HL, Hancock WW (1999) CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1alpha and IP-10 are expressed in demyelinating brain lesions. Proc Natl Acad Sci U S A 96:6873–6878
3. Banisor I, Leist TP, Kalman B (2005) Involvement of beta-chemokines in the development of inflammatory demyelination. J Neuroinflammation 2:7
4. Bartosik-Psujek H, Stelmasiak Z (2005) The levels of chemokines CXCL8, CCL2 and CCL5 in multiple sclerosis patients are linked to the activity of the disease. Eur J Neurol 12:49–54
5. Bazan JF, Bacon KB, Hardiman G, Wang W, Soo K, Rossi D, Greaves DR, Zlotnik A, Schall TJ (1997) A new class of membrane-bound chemokine with a CX3C motif. Nature 385:640–644
6. Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP (1999) Natural killer cells in antiviral defense: function and regulation by innate cytokines. Annu Rev Immunol 17:189–220
7. Boring L, Gosling J, Monteclaro FS, Lusis AJ, Tsou CL, Charo IF (1996) Molecular cloning and functional expression of murine JE (monocyte chemoattractant protein 1) and murine macrophage inflammatory protein 1alpha receptors: evidence for two closely linked C-C chemokine receptors on chromosome 9. J Biol Chem 271:7551–7558
8. Boring L, Gosling J, Cleary M, Charo IF (1998) Decreased lesion formation in $^{-/-}$CCR2−/− mice reveals a role for chemokines in the initiation of atherosclerosis. Nature 394:894–897
9. Buchmeier MJ, Lewicki HA, Talbot PJ, Knobler RL (1984) Murine hepatitis virus-4 (strain JHM)-induced neurologic disease is modulated in vivo by monoclonal antibody. Virology 132:261–270
10. Cartier L, Hartley O, Dubois-Dauphin M, Krause KH (2005) Chemokine receptors in the central nervous system: role in brain inflammation and neurodegenerative diseases. Brain Res Brain Res Rev 48:16–42
11. Castro RF, Perlman S (1995) CD8+ T-cell epitopes within the surface glycoprotein of a neurotropic coronavirus and correlation with pathogenicity. J Virol 69:8127–8131

12. Cheever FS, Daniels JB, Pappenheimer AM, Bailey OT (1949) A murine virus (JHM) causing disseminated encephalomyelitis with extensive destruction of myelin. J Exp Med 90:181–194

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

14. Clark-Lewis I, Kim KS, Rajarathnam K, Gong JH, Dewald B, Moser B, Baggiolini M, Sykes BD (1995) Structure-activity relationships of chemokines. J Leukoc Biol 57:703–711

15. Cook DN, Beck MA, Coffman TM, Kirby SL, Sheridan JF, Pragnell IB, Smithies O (1995) Requirement of MIP-1 alpha for an inflammatory response to viral infection. Science 269:1583–1585

16. Dandekar AA, Perlman S (2002) Virus-induced demyelination in nude mice is mediated by gamma delta T cells. Am J Pathol 161:1255–1263

17. Domachowske JB, Bonville CA, Gao JL, Murphy PM, Easton AJ, Rosenberg HF (2000) The chemokine macrophage-inflammatory protein-1 alpha and its receptor CCR1 control pulmonary inflammation and antiviral host defense in paramyxovirus infection. J Immunol 165:2677–2682

18. Dufour JH, Dziejman M, Liu MT, Leung JH, Lane TE, Luster AD (2002) IFN-gamma-inducible protein 10 (IP-10; CXCL10)-deficient mice reveal a role for IP-10 in effector T cell generation and trafficking. J Immunol 168:3195–3204

19. Farber JM (1997) Mig and IP-10: CXC chemokines that target lymphocytes. J Leukoc Biol 61:246–257

20. Fife BT, Kennedy KJ, Paniagua MC, Lukacs NW, Kunkel SL, Luster AD, Karpus WJ (2001) CXCL10 (IFN-gamma-inducible protein-10) control of encephalitogenic CD4+ T cell accumulation in the central nervous system during experimental autoimmune encephalomyelitis. J Immunol 166:7617–7624

21. Fischer FR, Luo Y, Luo M, Santambrogio L, Dorf ME (2001) RANTES-induced chemokine cascade in dendritic cells. J Immunol 167:1637–1643

22. Fischer HG, Bonifas U, Reichmann G (2000) Phenotype and functions of brain dendritic cells emerging during chronic infection of mice with Toxoplasma gondii. J Immunol 164:4826–4834

23. Fischer HG, Reichmann G (2001) Brain dendritic cells and macrophages/microglia in central nervous system inflammation. J Immunol 166:2717–2726

24. Flesch IE, Stober D, Schirmbeck R, Reimann J (2000) Monocyte inflammatory protein-1 alpha facilitates priming of CD8(+) T cell responses to exogenous viral antigen. Int Immunol 12:1365–1370

25. Franciotta D, Martino G, Zardini E, Furlan R, Bergamaschi R, Andreoni L, Cosi V (2001) Serum and CSF levels of MCP-1 and IP-10 in multiple sclerosis patients with acute and stable disease and undergoing immunomodulatory therapies. J Neuroimmunol 115:192–198

26. Gade-Andavolu R, Comings DE, MacMurray J, Vuthoori RK, Tourtellotte WW, Nagra RM, Cone LA (2004) RANTES: a genetic risk marker for multiple sclerosis. Mult Scler 10:536–539

27. Galimberti D, Bresolin N, Scarpini E (2004) Chemokine network in multiple sclerosis: role in pathogenesis and targeting for future treatments. Expert Rev Neurother 4:439–453

28. Gerard C, Rollins BJ (2001) Chemokines and disease. Nat Immunol 2:108–115

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

30. Glass WG, Lane TE (2003) Functional analysis of the CC chemokine receptor 5 (CCR5) on virus-specific CD8+ T cells following coronavirus infection of the central nervous system. Virology 312:407–414

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

32. Glass WG, Hickey MJ, Hardison JL, Liu MT, Manning JE, Lane TE (2004) Antibody targeting of the CC chemokine ligand 5 results in diminished leukocyte infiltration into the central nervous system and reduced neurologic disease in a viral model of multiple sclerosis. J Immunol 172:4018–4025

33. Gosling J, Slaymaker S, Gu L, Tseng S, Zlot CH, Young SG, Rollins BJ, Charo IF (1999) MCP-1 deficiency reduces susceptibility to atherosclerosis in mice that overexpress human apolipoprotein B. J Clin Invest 103:773–778

34. Greter M, Heppner FL, Lemos MP, Odermatt BM, Goebels N, Laufer T, Noelle RJ, Becher B (2005) Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. Nat Med 11:328–334

35. Gu L, Tseng SC, Rollins BJ (1999) Monocyte chemoattractant protein-1. Chem Immunol 72:7–29

36. Gu L, Tseng S, Horner RM, Tam C, Loda M, Rollins BJ (2000) Control of TH2 polarization by the chemokine monocyte chemoattractant protein-1. Nature 404:407–411

37. Haring JS, Pewe LL, Perlman S (2001) High-magnitude, virus-specific CD4 T-cell response in the central nervous system of coronavirus-infected mice. J Virol 75:3043–3047

38. Haring JS, Perlman S (2003) Bystander CD4 T cells do not mediate demyelination in mice infected with a neurotropic coronavirus. J Neuroimmunol 137:42–50

39. Held KS, Chen BP, Kuziel WA, Rollins BJ, Lane TE (2004) Differential roles of CCL2 and CCR2 in host defense to coronavirus infection. Virology 329:251–260

40. Hildebrandt GC, Corrion LA, Olkiewicz KM, Lu B, Lowler K, Duffner UA, Moore BB, Kuziel WA, Liu C, Cooke KR (2004) Blockade of CXCR3 receptor:ligand interactions reduces leukocyte recruitment to the lung and the severity of experimental idiopathic pneumonia syndrome. J Immunol 173:2050–2059

41. Hogaboam CM, Lukacs NW, Chensue SW, Strieter RM, Kunkel SL (1998) Monocyte chemoattractant protein-1 synthesis by murine lung fibroblasts modulates CD4+ T cell activation. J Immunol 160:4606–4614

42. Holmes K, Lai M (1996) Coronaviridae: the viruses and their replication. In: Fields BN, Knipe DM, Howley PM (eds) Fields virology. Lippincott-Raven Publishers, New York, pp 1075–1094

43. Holmes KV (2003) SARS-associated coronavirus. N Engl J Med 348:1948–1951

44. Houck JC, Chang CM (1977) The purification and characterization of a lymphokine chemotactic for lymphocytes—lymphotactin. Inflammation 2:105–113

45. Huffnagle GB, McNeil LK, McDonald RA, Murphy JW, Toews GB, Maeda N, Kuziel WA (1999) Cutting edge: role of C-C chemokine receptor 5 in organ-specific and innate immunity to Cryptococcus neoformans. J Immunol 163:4642–4646

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

47. Kim TS, Perlman S (2005) Viral expression of CCL2 is sufficient to induce demyelination in $^{-/-}$RAG1−/− mice infected with a neurotropic coronavirus. J Virol 79:7113–7120

48. Kim TS, Perlman S (2005) Virus-specific antibody, in the absence of T cells, mediates demyelination in mice infected with a neurotropic coronavirus. Am J Pathol 166:801–809

49. Klein RS, Izikson L, Means T, Gibson HD, Lin E, Sobel RA, Weiner HL, Luster AD (2004) IFN-inducible protein 10/CXC chemokine ligand 10-independent induction of experimental autoimmune encephalomyelitis. J Immunol 172:550–559

50. Lai MM, Cavanagh D (1997) The molecular biology of coronaviruses. Adv Virus Res 48:1–100

51. Lane TE, Buchmeier MJ (1997) Murine coronavirus infection: a paradigm for virus-induced demyelinating disease. Trends Microbiol 5:9–14

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

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

54. Lazzeri E, Romagnani P (2005) CXCR3-binding chemokines: novel multifunctional therapeutic targets. Curr Drug Targets Immune Endocr Metabol Disord 5:109–118

55. Lin MT, Stohlman SA, Hinton DR (1997) Mouse hepatitis virus is cleared from the central nervous systems of mice lacking perforin-mediated cytolysis. J Virol 71:383–391

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

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

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

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

60. Luster AD (1998) Chemokines—chemotactic cytokines that mediate inflammation. N Engl J Med 338:436–445
61. Mahad DJ, Ransohoff RM (2003) The role of MCP-1 (CCL2) and CCR2 in multiple sclerosis and experimental autoimmune encephalomyelitis (EAE). Semin Immunol 15:23–32
62. Marten NW, Stohlman SA, Bergmann CC (2000) Role of viral persistence in retaining CD8(+) T cells within the central nervous system. J Virol 74:7903–7910
63. Matsui M, Araya SI, Wang HY, Matsushima K, Saida T (2005) Differences in systemic and central nervous system cellular immunity relevant to relapsing-remitting multiple sclerosis. J Neurol 252:908–915
64. McIntosh K (1996) Diagnostic virology. In: Fields BN, Knipe DM, Howley PM (eds) Fields virology. Lippincott-Raven Publishers, New York, pp 401–430
65. McMahon EJ, Bailey SL, Castenada CV, Waldner H, Miller SD (2005) Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis. Nat Med 11:335–339
66. Megjugorac NJ, Young HA, Amrute SB, Olshalsky SL, Fitzgerald-Bocarsly P (2004) Virally stimulated plasmacytoid dendritic cells produce chemokines and induce migration of T and NK cells. J Leukoc Biol 75:504–514
67. Mehrad B, Moore TA, Standiford TJ (2000) Macrophage inflammatory protein-1 alpha is a critical mediator of host defense against invasive pulmonary aspergillosis in neutropenic hosts. J Immunol 165:962–968
68. Meyer A, Coyle AJ, Proudfoot AE, Wells TN, Power CA (1996) Cloning and characterization of a novel murine macrophage inflammatory protein-1 alpha receptor. J Biol Chem 271:14445–14451
69. Miller SD, Vanderlugt CL, Begolka WS, Pao W, Yauch RL, Neville KL, Katz-Levy Y, Carrizosa A, Kim BS (1997) Persistent infection with Theiler's virus leads to CNS autoimmunity via epitope spreading. Nat Med 3:1133–1136
70. Muller DM, Pender MP, Greer JM (2004) Chemokines and chemokine receptors: potential therapeutic targets in multiple sclerosis. Curr Drug Targets Inflamm Allergy 3:279–290
71. Narumi S, Kaburaki T, Yoneyama H, Iwamura H, Kobayashi Y, Matsushima K (2002) Neutralization of IFN-inducible protein 10/CXCL10 exacerbates experimental autoimmune encephalomyelitis. Eur J Immunol 32:1784–1791
72. Olszewski MA, Huffnagle GB, McDonald RA, Lindell DM, Moore BB, Cook DN, Toews GB (2000) The role of macrophage inflammatory protein-1 alpha/CCL3 in regulation of T cell-mediated immunity to Cryptococcus neoformans infection. J Immunol 165:6429–6436
73. Parra B, Hinton DR, Marten NW, Bergmann CC, Lin MT, Yang CS, Stohlman SA (1999) IFN-gamma is required for viral clearance from central nervous system oligodendroglia. J Immunol 162:1641–1647
74. Parra B, Lin MT, Stohlman SA, Bergmann CC, Atkinson R, Hinton DR (2000) Contributions of Fas-Fas ligand interactions to the pathogenesis of mouse hepatitis virus in the central nervous system. J Virol 74:2447–2450
75. Patterson CE, Daley JK, Echols LA, Lane TE, Rall GF (2003) Measles virus infection induces chemokine synthesis by neurons. J Immunol 171:3102–3109

76. Pearce BD, Hobbs MV, McGraw TS, Buchmeier MJ (1994) Cytokine induction during T-cell-mediated clearance of mouse hepatitis virus from neurons in vivo. J Virol 68:5483–5495
77. Penna G, Vulcano M, Roncari A, Facchetti F, Sozzani S, Adorini L (2002) Cutting edge: differential chemokine production by myeloid and plasmacytoid dendritic cells. J Immunol 169:6673–6676
78. Penna G, Vulcano M, Sozzani S, Adorini L (2002) Differential migration behavior and chemokine production by myeloid and plasmacytoid dendritic cells. Hum Immunol 63:1164–1171
79. Perlman SR, Lane TE, Buchmeier MJ (1999) Coronaviruses: hepatitis, peritonitis and central nervous system disease. In: Cunningham MW, Fujinami RS (eds) Effects of microbes on the immune system, vol. 1. Lippincott Williams and Wilkins, Philadelphia, pp 331–348
80. Pewe L, Haring J, Perlman S (2002) CD4 T-cell-mediated demyelination is increased in the absence of gamma interferon in mice infected with mouse hepatitis virus. J Virol 76:7329–7333
81. Pewe L, Perlman S (2002) Cutting edge: CD8 T cell-mediated demyelination is IFN-gamma dependent in mice infected with a neurotropic coronavirus. J Immunol 168:1547–1551
82. Phillips JJ, Chua M, Seo SH, Weiss SR (2001) Multiple regions of the murine coronavirus spike glycoprotein influence neurovirulence. J Neurovirol 7:421–431
83. Qin S, Rottman JB, Myers P, Kassam N, Weinblatt M, Loetscher M, Koch AE, Moser B, Mackay CR (1998) The chemokine receptors CXCR3 and CCR5 mark subsets of T cells associated with certain inflammatory reactions. J Clin Invest 101:746–754
84. Ramakrishna C, Stohlman SA, Atkinson RD, Shlomchik MJ, Bergmann CC (2002) Mechanisms of central nervous system viral persistence: the critical role of antibody and B cells. J Immunol 168:1204–1211
85. Ramakrishna C, Bergmann CC, Atkinson R, Stohlman SA (2003) Control of central nervous system viral persistence by neutralizing antibody. J Virol 77:4670–4678
86. Raport CJ, Gosling J, Schweickart VL, Gray PW, Charo IF (1996) Molecular cloning and functional characterization of a novel human CC chemokine receptor (CCR5) for RANTES, MIP-1beta, and MIP-1alpha. J Biol Chem 271:17161–17166
87. Salomon I, Netzer N, Wildbaum G, Schif-Zuck S, Maor G, Karin N (2002) Targeting the function of IFN-gamma-inducible protein 10 suppresses ongoing adjuvant arthritis. J Immunol 169:2685–2693
88. Sato N, Kuziel WA, Melby PC, Reddick RL, Kostecki V, Zhao W, Maeda N, Ahuja SK, Ahuja SS (1999) Defects in the generation of IFN-gamma are overcome to control infection with Leishmania donovani in CC chemokine receptor (CCR) 5-, macrophage inflammatory protein-1 alpha-, or CCR2-deficient mice. J Immunol 163:5519–5525
89. Sorensen TL, Tani M, Jensen J, Pierce V, Lucchinetti C, Folcik VA, Qin S, Rottman J, Sellebjerg F, Strieter RM, Frederiksen JL, Ransohoff RM (1999) Expression of specific chemokines and chemokine receptors in the central nervous system of multiple sclerosis patients. J Clin Invest 103:807–815

90. Sorensen TL, Trebst C, Kivisakk P, Klaege KL, Majmudar A, Ravid R, Lassmann H, Olsen DB, Strieter RM, Ransohoff RM, Sellebjerg F (2002) Multiple sclerosis: a study of CXCL10 and CXCR3 co-localization in the inflamed central nervous system. J Neuroimmunol 127:59–68

91. Stamatovic SM, Shakui P, Keep RF, Moore BB, Kunkel SL, Van Rooijen N, Andjelkovic AV (2005) Monocyte chemoattractant protein-1 regulation of blood-brain barrier permeability. J Cereb Blood Flow Metab 25:593–606

92. Stohlman SA, Bergmann CC, Lin MT, Cua DJ, Hinton DR (1998) CTL effector function within the central nervous system requires CD4+ T cells. J Immunol 160:2896–2904

93. Stohlman SA, Ramakrishna C, Tschen SI, Hinton DR, Bergmann CC (2002) The art of survival during viral persistence. J Neurovirol 8[Suppl 2]:53–58

94. Taub DD, Oppenheim JJ (1994) Chemokines, inflammation and the immune system. Ther Immunol 1:229–246

95. Trifilo MJ, Bergmann CC, Kuziel WA, Lane TE (2003) CC chemokine ligand 3 (CCL3) regulates CD8(+)-T-cell effector function and migration following viral infection. J Virol 77:4004–4014

96. Trifilo MJ, Lane TE (2004) The CC chemokine ligand 3 regulates CD11c+CD11b+ CD8alpha–dendritic cell maturation and activation following viral infection of the central nervous system: implications for a role in T cell activation. Virology 327:8–15

97. Trifilo MJ, Montalto-Morrison C, Stiles LN, Hurst KR, Hardison JL, Manning JE, Masters PS, Lane TE (2004) CXC chemokine ligand 10 controls viral infection in the central nervous system: evidence for a role in innate immune response through recruitment and activation of natural killer cells. J Virol 78:585–594

98. Tsunoda I, Lane TE, Blackett J, Fujinami RS (2004) Distinct roles for IP-10/CXCL10 in three animal models, Theiler's virus infection, EAE, and MHV infection, for multiple sclerosis: implication of differing roles for IP-10. Mult Scler 10:26–34

99. Warmington KS, Boring L, Ruth JH, Sonstein J, Hogaboam CM, Curtis JL, Kunkel SL, Charo IR, Chensue SW (1999) Effect of C-C chemokine receptor 2 (CCR2) knockout on type-2 (schistosomal antigen-elicited) pulmonary granuloma formation: analysis of cellular recruitment and cytokine responses. Am J Pathol 154:1407–1416

100. Watanabe R, Wege H, ter Meulen V (1983) Adoptive transfer of EAE-like lesions from rats with coronavirus-induced demyelinating encephalomyelitis. Nature 305:150–153

101. Whiting D, Hsieh G, Yun JJ, Banerji A, Yao W, Fishbein MC, Belperio J, Strieter RM, Bonavida B, Ardehali A (2004) Chemokine monokine induced by IFN-gamma/CXC chemokine ligand 9 stimulates T lymphocyte proliferation and effector cytokine production. J Immunol 172:7417–7424

102. Widney DP, Hu Y, Foreman-Wykert AK, Bui KC, Nguyen TT, Lu B, Gerard C, Miller JF, Smith JB (2005) CXCR3 and its ligands participate in the host response to Bordetella bronchiseptica infection of the mouse respiratory tract but are not required for clearance of bacteria from the lung. Infect Immun 73:485–493

103. Williamson JS, Stohlman SA (1990) Effective clearance of mouse hepatitis virus from the central nervous system requires both CD4+ and CD8+ T cells. J Virol 64:4589–4592

104. Williamson JS, Sykes KC, Stohlman SA (1991) Characterization of brain-infil-trating mononuclear cells during infection with mouse hepatitis virus strain JHM. J Neuroimmunol 32:199–207

105. Wu GF, Dandekar AA, Pewe L, Perlman S (2000) CD4 and CD8 T cells have redundant but not identical roles in virus-induced demyelination. J Immunol 165:2278–2286

106. Xie JH, Nomura N, Lu M, Chen SL, Koch GE, Weng Y, Rosa R, Di Salvo J, Mudgett J, Peterson LB, Wicker LS, DeMartino JA (2003) Antibody-mediated blockade of the CXCR3 chemokine receptor results in diminished recruitment of T helper 1 cells into sites of inflammation. J Leukoc Biol 73:771–780

107. Xue S, Jaszewski A, Perlman S (1995) Identification of a CD4+ T cell epitope within the M protein of a neurotropic coronavirus. Virology 208:173–179

108. Zhang Y, Yoneyama H, Wang Y, Ishikawa S, Hashimoto S, Gao JL, Murphy P, Matsushima K (2004) Mobilization of dendritic cell precursors into the circulation by administration of MIP-1alpha in mice. J Natl Cancer Inst 96:201–209

109. Zhou Y, Kurihara T, Ryseck RP, Yang Y, Ryan C, Loy J, Warr G, Bravo R (1998) Impaired macrophage function and enhanced T cell-dependent immune response in mice lacking CCR5, the mouse homologue of the major HIV-1 coreceptor. J Immunol 160:4018–4025