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Chapter 3.5

Chemokine Responses in Virus-Induced Neurologic Disease: Balancing Host Defense and Neuropathology

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1. INTRODUCTION

Viral infection of the central nervous system (CNS) presents unique challenges to the immune system’s ability to recognize, and eliminate foreign antigen. Factors that increase the complexity of CNS immune responses include (1) the presence of the blood–brain barrier (BBB) which provides a physical and physiological obstruction that is difficult for cells and macromolecules to cross, (2) the relative absence of MHC class I and II expression on crucial CNS cells like astrocytes and neurons, and (3) lack of abundant antigen presenting cells (APC) which are required for the generation of an adaptive immune response. However, in spite of these obstacles, activated leukocytes do enter the CNS and participate in elimination of virus through a variety of highly efficient mechanisms. In addition, the presence of activated immune cells, e.g. T lymphocytes and macrophages, has been proven to contribute to the development of neuropathology in several viral model systems.

The underlying molecular and cellular mechanisms governing leukocyte migration and entry into the CNS following viral infection are just now being understood. Over the past several years, there has been a surge of interest reflected in the literature demonstrating the importance of chemokines in orchestrating events surrounding inflammation following microbial infection or injury. The chemokines represent an ever-growing family of small, molecular weight secreted proteins that have been shown to target the migration of specific populations of leukocytes during periods of inflammation. 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, macrophage, granulocytes, neutrophil, and T lymphocytes (functional criteria). Chemokines bind to specific receptors expressed on the surface of a wide variety of cells. The majority of chemokine receptors identified to date belong to the seven-transmembrane G protein-coupled receptor superfamily [1]. Following chemokine binding to 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 [2]. In addition, there is growing evidence that chemokine receptors influence the polarization of T cell immune response (i.e. Th1 vs. Th2) in tissue [3].

Viral infection of the CNS can result in a temporal expression of both chemokines and chemokine receptors by resident cells of the CNS as well as by inflammatory cells. Numerous studies have demonstrated a relationship between viral infection of the CNS and the expression of chemokine and/or chemokine receptors with leukocyte entry and disease development. There is an increasing amount of evidence indicating that chemokine expression represents a double-edged sword in the sense that expression is beneficial to the host by attracting T
lymphocytes and macrophages which participate in host defense through elimination of foreign antigen, e.g. virus. Conversely, chemokine expression in response to CNS viral infection also clearly contributes to neuropathology by attracting activated T lymphocytes and macrophages into the CNS which then release cytotoxic factors. This chapter will focus on the chemokine response to viral infection of the CNS with an emphasis on the functional significance of chemokine expression as it relates to both host defense and neuropathology.

2. VIRAL INFECTION OF GLIAL CELLS AND CHEMOKINE EXPRESSION

At first glance the CNS does not appear equipped to effectively cope with viral infection due to the aforementioned barriers. However, experimental evidence indicates otherwise. Activated glial cells can express MHC class I and/or class II molecules suggesting that these cells may function in an APC capacity and contribute to the development of an adaptive response and viral clearance [4]. Moreover, both activated microglia and astrocytes release reactive oxygen and nitrogen intermediates that may interfere with viral replication [5,6].

In addition, cultured glial cells release cytokines in response to viral infection that may work in both an autocrine and/or paracrine manner in the regulation of the local immune response as well as enhance recruitment of protective T lymphocytes required for efficient clearance of viral antigen or conversely, disease development. Viral infection of glial cells results in robust expression of numerous chemokines. In vitro studies have demonstrated that human microglia and astrocytes are able to express chemokine genes such as MCP-1/CCL2, RANTES/CCL5, and MIP-1α/CCL3 and β/CCL4 [7] following infection with various viral pathogens including measles virus [8] and human coronavirus [9]. Infection of rat astrocytes and microglia with the Paramyxovirus, Newcastle disease virus (NDV), results in rapid expression of mRNA transcripts for CCL5 and non-ELR (glutamic acid–leucine–arginine) CXC chemokine IP-10/CXCL10 [10,11]. Unlike TNF-α, which requires live virus to trigger expression, UV-inactivated NDV was sufficient to trigger robust chemokine gene expression. Interestingly, chemokine transcripts are readily detectable even following infection with a low dose of virus suggesting that a soluble factor(s), e.g. cytokines, may be released which triggers chemokine expression by neighboring cells that are uninfected. In support of this are studies demonstrating that interferon treatment of glial cells can result in robust chemokine gene expression [13,14]. Moreover, a recent report has indicated that treatment of primary cultures of mouse astrocytes with nanomolar concentrations of KC/CXCL2 and CXCL1 results in chemokine gene expression [15]. Therefore, cytokines and chemokines produced by virally infected cells are sufficient to function in a paracrine manner to induce expression of these soluble molecules in uninfected cells. In addition, it is important not to underestimate the impact of glial cell production of chemokines as it relates to acute and/or chronic disease development following viral infection of the CNS. Normally, chemokine expression is associated with inflammation suggesting that stimulatory factors provided by leukocytes are required for expression. However, it is clear that cultured glial cells may also exhibit prolonged expression of chemokine genes in the apparent absence of exogenous stimuli such as cytokines suggesting that these molecules can either function in promoting tissue damage or possibly in a repair/protective mechanism within the CNS.
3. IN VIVO MODELS OF VIRAL-INDUCED NEURODEGENERATIVE DISEASE: CORRELATION OF DISEASE SEVERITY WITH CHEMOKINE GENE EXPRESSION

From the studies discussed above, it is apparent that glial cells respond to viral infection by expressing numerous chemokine genes. Expression may be in response to a primary event or represent a secondary response to cytokine(s) released from infected cells as discussed above. Numerous laboratories have extended in vitro investigations involving viral trig- gerring of chemokine expression in glial cells and have turned their attention to determining whether viral infection of the CNS of humans and/or animals results in chemokine gene expression.

Chemokines are expressed within the CNS of virus-infected humans and current evidence suggests that these molecules contribute to the pathogene- sis of disease. For example, during herpes simplex type 1 (HSV-1) encephalitis, increased levels of CCL2 detected within the cerebral spinal fluid (CSF) of patients correlated with clinical disease severity [16]. Similarly, CSF levels of IL-8/CXCL8 correlated with increased neutrophil accumulation and increased disease severity in patients infected with Japanese encephalitis virus [17].

The demonstration of chemokine gene expression within the CNS of humans infected with neurotropic viruses supports the hypothesis that these molecules are important during the disease process. This is further evidenced by recent studies using various animal models of viral pathogenesis. CNS infection with different classes of viruses results in robust inflam- matory responses characterized by recruitment of large numbers of leukocytes into the CNS suggesting that chemokines may contribute to this process. In support of this are numerous studies demonstrating expression of multiple chemokines within the CNS following infection with a wide variety of viruses (see Table 1). The following offers brief descriptions of recent in vivo studies analyzing the chemokine re- sponse to CNS infection with several different types of viruses.

3.1. Lymphocytic Choriomeningitis Virus (LCMV)

LCMV is an ambisense RNA virus and a member of the Arenaviridae. Infection of the murine CNS with LCMV results in a well-established model of viral meningitis [18,19]. LCMV infection of adult, immunocompetent mice results in a monophasic disease characterized by leukocyte infiltration into distinct anatomic regions of the brain and ultimately death between 6–8 days post-infection (p.i.) [18]. Others have determined that multiple chemokine genes are expressed following LCMV infection of the CNS during the acute stage of disease which cor- relates with onset of choriomeningitis. Chemokines expressed include CCL2, CCL4, CCL5, C10/CCL6, CCL7, and CXCL10 [20,21]. Expression of the CXC chemokine CXCL10 was analyzed in more detail as this chemokine was prominently expressed through- out the infection. In situ hybridization for CXCL10 mRNA and viral RNA revealed overlap in expres- sion suggesting that CXCL10 expression is sensitive to areas of viral infection and replication. Interest- ingly, CNS infection of athymic nude mice (lack- ing conventionally educated lymphocytes) resulted in an identical chemokine profile when compared to LCMV-infected euthymic mice indicating that resi- dent glial cells are responsible for local production of chemokines and do not require secondary signals provided by inflammatory leukocytes. This is sup- ported by dual-labeling analysis which revealed that astrocytes were the major cellular source of CXCL10 following LCMV infection [20].

3.2. Adenovirus

Adenoviruses are icosahedral, double-stranded DNA viruses that are capable of infecting a wide variety of
vertebrate hosts. Infection of certain strains of adult mice with mouse adenovirus (MAV) can result in fatal encephalitis within 4–6 days p.i. CNS disease is characterized by viral infection of cerebral vascular endothelial cells and resulting vascular pathology. C57BL/6 (B6) mice are susceptible to MAV-induced CNS disease whereas BALB/c mice are resistant [22,23]. Experiments designed to determine the underlying difference in disease susceptibility between B6 and BALB/c mice found no alterations in innate and/or adaptive immune responses or differences in cytokine expression profiles following viral infection [24]. MAV infection of B6 mice results in increased expression of the chemokines CXCL10 and CCL3–5 within the CNS [24]. CXCL10 was the prominent chemokine expressed within the CNS of infected B6 mice. In situ hybridization analysis determined that expression was associated with meningeal vessels as well as perivascular glial cells. MAV infection of resistant BALB/c mice resulted in restricted chemokine gene expression with only CXCL1 being detected. Interestingly, both strains of mice exhibited equivalent levels of expression of chemokine receptors CCR1–5. The authors conclude that productive infection in the CNS with MAV in mice susceptible to disease, i.e. B6 mice, results in a regulated pattern of chemokine gene expression and may point to a contribution by these molecules in disease pathogenesis.

3.3. Borna Disease Virus (BDV)

BDV is a negative-strand RNA virus that can cause a lethal meningoencephalitis in susceptible hosts [25]. Experimental infection of mice with BDV results in mice and rats results in a CD8+ T lymphocyte-mediated neurologic disease [26]. The underlying mechanisms contributing to leukocyte entry into the CNS of BDV-infected rodents are just now being understood. Numerous studies have demonstrated expression of proinflammatory cytokines within the CNS of BDV-infected mice and rats. In addition, these investigators have identified a dramatic increase in chemokine gene expression following BDV infection. CXCL10 and CCL5 transcripts are strongly expressed within the CNS of infected mice and rats. Similar to LCMV infection of the CNS, BDV infection of immunodeficient mice, e.g. lacking T lymphocytes or receptors for types I and II interferons, resulted in CXCL10 and CCL5 expression suggesting that resident glial cells are capable of expressing these chemokines in the absence of stimulation by factors released by inflammatory lymphocytes. Astrocytes were determined to be the primary source of CXCL10 within the brains of infected rodents. The authors speculate that localized expression of chemokine genes by resident glial cells is important in the early immune response to viral infection and is required for the initiation of virus-induced neuroinflammation. Furthermore, the authors consider the possibility that sustained production of chemokines may contribute to behavioral and anatomic changes in BDV-infected rodents. For example, BDV infection of newborn rats ultimately results in the selective loss of cerebellar Purkinje cells and chronic expression of chemokines may contribute to this loss. Additional studies are required to further sort out the functions/roles chemokines may have in the complex model of BDV infection of the CNS.

3.4. Theiler’s Murine Encephalomyelitis Virus (TMEV)

Intracranial infection of mice with TMEV, a member of the Picornaviridae, results in an acute polioencephalitis followed by a chronic demyelinating disease in susceptible strains of mice [27]. TMEV infection of the CNS results in dramatic increase in cytokine expression accompanied by lymphocyte infiltration [28]. Recent studies have indicated that chemokines are expressed within the CNS of TMEV-infected mice during both acute and chronic stages of disease [29,30]. Chemokines expressed during acute disease included CXCL2, CXCL10, and CCL2-5 and it is thought that these molecules cooperate in the initiation of events involved in leukocyte recruitment into the CNS. Following the acute stage of disease, the level of inflammation is reduced which coincides with a reduction in chemokine gene expression [30]. However, the onset of chronic demyelination within these mice coincides with at resurgence in CXCL10, CCL2, and CCL5 expression suggesting these molecules may contribute to the maintenance of chronic inflammation and demyelination accompanied by persistent viral replication. At this point, no functional studies have indicated whether expression of CXCL10, CCL2, or CCL5 is required for
driving demyelination through exerting a chemotactic effect upon leukocytes. Collectively, these studies imply that chemokine expression is strictly regulated and expression is dictated by the phase of disease (e.g. acute versus chronic).

3.5. Mouse Hepatitis Virus (MHV)

Infection of susceptible mice with neurotropic strains of MHV results in an acute encephalomyelitis characterized by widespread viral infection of neurons and glial cells. Depending upon the viral strain, cellular infiltration usually peaks around 5–7 days and consists primarily of T lymphocytes and macrophages. We have recently determined that a complex, well-orchestrated expression of chemokine genes occurs following MHV infection of the CNS which appears to be dictated, in part, by viral burden [12]. As with the LCMV model, CXCL10 mRNA transcripts are detected soon after CNS infection and co-localize with areas of MHV replication (Fig. 1). 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 CXCL10, CCL2–5, and CCL7. Double-labeling revealed that astrocytes and microglia expressed mRNA transcripts for CXCL10 during the acute stage of disease. Furthermore, analysis of the chemokine receptor mRNA profile indicates increased expression of CCR1, CCR5, and CXCR3 (Fig. 2). Confocal microscopy revealed that macrophages (determined by F4/80 antigen expression) express CCR5 (Fig. 3). In addition, studies have shown that both CD4+ and CD8+ T cells infiltrating into the brain express CXCR3 [31].

By day 12 p.i., MHV-infected mice that have survived the acute stage of disease develop an immune-mediated demyelinating disease with clinical and histologic features similar to the human demyelinating disease multiple sclerosis (MS). A recent study demonstrating that infiltration of CD4+ T lymphocytes and macrophages perhaps driven by CCL5 expression is a key event linked to demyelination in mice persistently infected with MHV [32]. Mice have cleared infectious virus (as determined by plaque assay) by 12 days, yet viral RNA persists within white matter tracts for months after infection. Similar to the scenario with TMEV, the level of CNS infiltration subsides and this correlates with reduced expression of chemokine transcripts. Analysis of chemokine message expression within the brains and spinal cords of MHV-infected mice during the chronic demyelinating phase of disease (day 35) indicated that CXCL10 and CCL5 are the two prominent chemokines expressed [12]. In situ hybridization for chemokine transcripts indicated that expression was limited primarily to areas of viral persistence undergoing demyelination (Fig. 4). Astrocytes were determined to be the cellular source

Figure 1. Co-localization of MHV RNA and CXCL10 mRNA transcripts. In situ hybridization was performed on sequential brain sections representing day 2 p.i. with MHV. Sections were probed with 35S-labeled antisense riboprobes specific for MHV (top panel), CXCL10 (middle panel), or a labeled CXCL10 sense probe (bottom panel). Film autoradiography was performed to localize signal within the brain sections. Note the co-localization of MHV signal with CXCL10 specifically around the lateral ventricle and meninges.
Figure 2. Chemokine receptor mRNA expression within the CNS of MHV-infected mice. Mice were infected intracranially with MHV and the chemokine receptor mRNA profile within the brain was analyzed by ribonuclease protection assay at days 2, 7, and 35 p.i. Levels of receptor mRNAs are presented as normalized units determined by performing densitometric analysis of receptor signal intensity. Prominent chemokine receptors expressed following viral infection included CXCR3, CCR5, and CCR1. Data presented represent average signal intensities determined from a minimum of three mice per time point.

Figure 3. Macrophage/microglia express CCR5 within the CNS of MHV-infected mice. Confocal microscopy was performed to co-localize CCR5 expression with macrophage/microglial cells (determined by F4/80 antigen) expression at day 6 p.i. with MHV. Representative staining showed individual cells staining for CCR5 (green) and F4/80 (red) while dual-labeling indicated double-positive cells (yellow).

4. FUNCTIONAL SIGNIFICANCE OF CHEMOKINE EXPRESSION DURING ACUTE VIRAL ENCEPHALOMYELITIS

The demonstration of chemokine and chemokine receptor expression within the CNS of virally infected mice is provocative and suggests that these molecules are important in contributing to events culminating in leukocyte entry into the CNS. However, despite these compelling observations, experimental evidence supporting a functional role for chemokines in events surrounding neuroinflammation following viral infection of the CNS is lacking. Studies by Karpus and colleagues have convincingly demonstrated that antibody-mediated neutralization of CCL2 or CCL3 is effective in reducing disease severity in mice with experimental autoimmune encephalomyelitis (EAE) by blocking monocyte entry into the CNS [3,34]. Therefore, it is not unreasonable to suggest that targeted inhibition by antibody of selected chemokines expressed within the CNS of virally infected mice may modulate the severity of inflammation. By extension, these experiments may illuminate specific targets or pathways for chemotherapeutic targeting in chronic neuroinflammatory disease.

In support of this hypothesis are a series of recent studies examining the contributions of Mig/CXCL9, CXCL-10 exerts a potent chemotactic effect on T lymphocytes through binding to CXCR3 [33].
CXCL10, and CCL5 to neuroinflammation, host defense, and neurologic disease following MHV infection of mice. Both CD4$^+$ and CD8$^+$ T lymphocytes are required for efficient clearance of MHV from the CNS and one mechanism by which activated T lymphocytes eliminate MHV is through the release of IFN-$\gamma$ [31,32,35]. CXCL9 and CXCL10 are non-ELR CXC chemokines that attract activated T lymphocytes following binding to CXCR3. CXCL10 expression is inducible by both IFN-$\alpha$/$\beta$ and IFN-$\gamma$ while CXCL9 expression is strictly dependent upon IFN-$\gamma$ [36–38]. Analysis of CXCL9 and CXCL10 mRNA expression within the CNS of MHV-infected mice revealed that CXCL10 was clearly detectable by day 2 p.i. and was prominently expressed at days 7, 12, and 35 p.i. (Fig. 5). In contrast, CXCL9 transcripts were only detected at days 7 and 12 p.i. and this correlated with the presence of IFN-$\gamma$ mRNA transcripts (not shown). Based upon these data, it is interesting to speculate that early expression of CXCL10 may reflect local production of IFN-$\alpha$/$\beta$ in response to MHV infection which, in turn, activates both infected and non-infected cells to produce CXCL10. CXCL9 expression is delayed until T lymphocytes enter the CNS and produce IFN-$\gamma$. Furthermore, these data suggested that both CXCL9 and CXCL10 may be important in host defense by attracting anti-viral T lymphocytes into the CNS. In support of this is the observation that administration of rabbit antisera specific for either CXCL9 or CXCL10 to MHV-infected mice during the acute stage of disease results in a dramatic increase in mortality. Treatment of MHV-infected mice with either anti-CXCL9 or anti-CXCL10 resulted in a significant decrease in numbers of CD4$^+$ and CD8$^+$ T lym-

**Figure 4.** CXCL10 and CCL5 are expressed within areas of demyelination. In situ hybridization was performed to localize CXCL10 and CCL5 mRNA transcripts within spinal cords of MHV-infected mice during chronic demyelinating disease. Spinal cords from mice at day 35 p.i. were probed with either $^{35}$S-labelled antisense riboprobes specific for CXCL10 or CCL5. The results indicate that these chemokines are prominently expressed within white matter tracts undergoing myelin stripping. Positive cells are indicated by overlaying silver grains (arrows).
phocyte infiltrating into the CNS which correlated with decreased expression of IFN-γ mRNA and protein and increased levels of virus [31,39]. Therefore, the collective evidence points to roles for both CCL9 and CXCL10 as important sentinel molecules in promoting a protective Th1 response against MHV infection of the CNS.

5. FUNCTIONAL SIGNIFICANCE OF CHEMOKINE EXPRESSION DURING CHRONIC VIRAL-INDUCED DISEASE

CXCL10 and CCL5 are the predominant chemokines expressed in the CNS of persistently infected mice undergoing demyelination [12]. Moreover, CXCR3 and CCR5 expression (receptors for CXCL10 and CCL5, respectively) is also detected at this time. These findings have parallels in human neurodegenerative diseases. Gliol cell expression of CCL5 and CXCL10 as well as CXCR3- and CCR5-positive mononuclear cells have been reported within demyelinating lesions present in MS patient [40]. 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 [40]. Collectively, these data illustrate the immunopathological consequences of expression of chemokines in human demyelinating diseases.

Experimental evidence supporting this hypothesis comes from studies using the MHV model of viral-induced demyelination. Intracranial infection of CD4⁻/⁻ and CD8⁻/⁻ mice with MHV resulted in increased mortality and delayed clearance of virus from the CNS which is consistent with previous studies demonstrating an important role for T lymphocytes in host defense against MHV-induced CNS disease [32]. Interestingly, infected CD4⁻/⁻ mice displayed significantly less severe inflammation and demyelination as compared to CD8⁻/⁻ and wild-type C57BL/6 (B6) 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 B6 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 microglia must be considered due to the fact that CCL5 mRNA transcripts and protein were 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 monocytes, 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 and the severity of disease evaluated. Treatment led to a disease in B6 mice similar to the phenotype observed in CD4⁻/⁻ mice with delayed viral clearance from the brain and decreased cellular infiltration as well as a significant reduction in the severity of demyelination. The decreased capacity to clear virus from the brains is explained by the limited infiltration of CD4⁺ and CD8⁺ T cells into the brain during the acute stage of disease. Furthermore, the decrease in macrophage infiltration correlated with the reduced severity of demyelination supporting the observations with MHV-infected CD4⁻/⁻ mice. These observations reinforce the functional significance of CCL5 expression during virus-induced CNS disease indicating that this chemokine has an important role in recruitment of both CD4⁺ and CD8⁺ T cells as well as macrophages into the CNS following MHV infection.

The functional significance of CXCL10 in contributing to chronic demyelinating disease in mice persistently infected with MHV has also been examined. Administration of neutralizing antibodies specific to CXCL10, but not CXCL9, to MHV-infected mice with established disease, e.g. hind-limb paralysis and demyelination, resulted in a dramatic reduction in clinical disease severity accompanied by an inhibition in the progression of demyelination that correlated with a significant decrease in CD4⁺ T cell and macrophage infiltration into the CNS [41]. Importantly, the reduction in neurologic and histologic disease severity in anti-CXCL10-treated mice...
correlated with a dramatic increase in the number of remyelinated axons. Further, CCL5 expression (mRNA and protein) was significantly reduced in mice treated with anti-CXCL10. Based on previous studies examining the contribution of CCL5 to MHV-induced CNS disease [32], these observations suggest that impaired CD4+ T cell infiltration into the CNS of anti-CXCL10-treated mice results in reduced expression of the macrophage chemoattractant CCL5 and this ultimately results in a dramatic reduction in demyelination. Molecular analysis of CXCR3 expression (RT-PCR) on infiltrating CD4+ and CD8+ T cells in persistently infected mice undergoing demyelination revealed that CD4+ T cells express markedly higher levels of CXCR3 mRNA when compared to CD8+ T cells. These data indicate that during chronic disease CD4+ T cells are the major responding T cell population to chronic CXCL10 expression. Collectively, these data indicate that elimination of effector cells, e.g. CD4+ T cells and macrophages, following establishment of disease allows the CNS time for repair of previously damaged axons. In addition, these studies support previous work in other models of neuroinflammatory disease that antibody-mediated targeting of chemokines may offer a viable treatment strategy for treatment of human neuroinflammatory diseases.

6. CHEMOKINE RECEPTORS AND VIRAL-INDUCED DISEASE

MHV infection of the CNS of susceptible mice results in a dramatic increase in both chemokines as well as chemokine receptors. Among the chemokine receptors that are prominently expressed during acute disease are CCR2 (major signaling receptors for CCL2) and CCR5 (receptor for various chemokine receptors). These experiments showed that the majority of cells expressing this receptor were macrophage/microglia [42]. 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. However, demyelination was significantly 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 a primary mechanism whereby macrophages are able to enter the CNS and contribute to demyelination.

Taken together, the studies on both CCR2 and CCR5 illustrated the complexity of chemokine receptor expression within the CNS as it relates to both host defense and disease development in a model of viral-induced CNS disease.

7. SUMMARY AND CONCLUSIONS

Available evidence demonstrates clearly that viral infection of the CNS results in a dramatic increase in chemokine gene expression. Moreover, production of chemokines in response to infection is highly focussed within areas of viral replication early in the disease process and areas of viral RNA persistence during the chronic stages of disease. Resident glial cells, e.g. microglia and astrocytes, are capable of generating a robust chemokine response following viral infection in the absence of inflammatory cells suggesting that this response may reflect an innate CNS response against viral infection analogous to the response of phagocytic cells in the periphery. Differences in virus and cellular tropism are likely explanations for the slight differences in chemokine profiles and duration of expression observed in the different models. However, what is striking in the studies discussed are the similarities with regards to the types of chemokines expressed. CC chemokines are the prominent chemokines expressed. In addition, the non-ELR CXC chemokine CXCL10 is often the predominant chemokine expressed early following viral infection suggesting an important role as a sentinel molecule in initiating neuroinflammation. Interestingly, the presence of T lymphocytes and macrophages within the CNS of virally infected mice is often associated with either host defense, aiding in clearance of virus, or the development of immunopathologic demyelinating disease. Studies on MHV-infected mice clearly illustrate the delicate balance that exists between the sentinel role of chemokine expression in host defense and the development of disease. Early production of chemokines such as CXCL9, CXCL10 and CCL5 are beneficial as they serve to attract T lymphocytes into the CNS which contribute to viral clearance [31,32,39]. However, chronic expression of CCL5 is detrimental as illustrated by experiments demonstrating a significant decrease in macrophage infiltration and demyelination following CCL5 depletion by antibody. The use of chemokine blocking agents, either receptor antagonists or antibodies, or chemokine/chemokine receptor knock-out mice will further our understanding of how these molecules drive defense and disease within the CNS following viral infection and may help to identify important targets for the development of selective new anti-inflammatory agents to attack MS. Based on studies presented in this brief overview, it is clear that targeting chemokines during either acute or chronic viral-induced CNS disease may offer exciting new insights into potentially novel interventional mechanisms in treating human neuroinflammatory diseases.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge support from the following sources: T. Lane is supported by grants NS37336 from the National Institutes of Health and RG30393A1/T from the National Multiple Sclerosis Society. M. Buchmeier is supported by grants AI43103 and AI 25913 from the National Institutes of Health.

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