The Usual Suspects: Chemokines and Microbial Infection of the Central Nervous System

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1 Introduction

For many years, the central nervous system (CNS) was considered an “immunologically privileged site” – a perspective based on limited immune surveillance when compared to peripheral tissue, muted expression of MHC molecules in the context of an apparent lack of professional antigen presenting cells, and the absence of a classical lymphatic drainage system. Together, these observations supported the notion that the CNS was unable to mount and/or support an immune response. However, over time this view evolved and it is now clear that CNS tissue is neither immunologically inert nor privileged, rather, its immune response is exquisitely sensitive to antigenic challenge. Indeed, overwhelming evidence now indicates that upon microbial infection of the CNS there is often a dynamic and orchestrated localized immune response that culminates with infiltration of antigen-specific lymphocytes, usually resulting in control and elimination of the invading pathogen. It is important to note that not all effective immune responses originating in the CNS are completely beneficial to the host; alternatively, there are instances where immune cell infiltration following infection is associated with severe neuropathology resulting in death or chronic neurodegenerative disease.

The signaling events governing leukocyte infiltration into the CNS in response to infection are complex and depend on many factors including type of pathogen, e.g. intracellular or extracellular, the route of infection, cellular tropism (neuron and/or glial cells), and genetic background of the host. Nevertheless, it is now apparent that leukocyte trafficking is partially dependent on a class of small (7–17 kDa) chemotactic cytokines known as chemokines (chemotatic cytokine). Chemokines represent a family of over 40 proteins, which for the most part are secreted into the environment and function by binding to chemokine receptors in the form of G protein-coupled receptors (GPCRs) that are expressed on numerous cell types. Four sub-families of chemokines have been identified and defined based on structural criteria relating to the location of conserved cysteine residues within the amino-terminus of the protein. Chemokines were initially discovered close to 30 years ago and their strong association with various human inflammatory diseases led some researches to theorize, and later confirm, that they influence leukocyte recruitment into inflamed tissue. In fact, it is now accepted that this
superfamily of proteins plays an important role in numerous biological processes ranging from maintaining the organizational integrity of secondary lymphoid tissue to participating in various aspects of both innate and adaptive immune responses following microbial infection. For excellent reviews on chemokine and chemokine receptor signaling as well as a comprehensive overview of identified chemokine receptors and their ligands, please see (Charo and Ransohoff 2006; Le et al., 2004; Luster 1998).

This chapter will focus on highlighting recent insights into chemokine and chemokine receptor expression in both host defense and disease following either viral or bacterial infections of the CNS. In all cases, we have tried to provide information pertaining to regulation of chemokine expression and functional roles within the context of animal models of disease as well as clinical disease. We certainly acknowledge that other microbial pathogens e.g. fungi and parasites are capable of infecting and replicating within the CNS and chemokines/chemokine receptors have been suggested to participate in host responses. However, our focus was weighted by the fact that the overwhelming majority of clinical infections involving the CNS relate to viral or bacterial infection.

2 Viral Infection of the CNS

Evaluation of the immune response following viral infection of the CNS is important as a number of viruses are capable of infecting the CNS (Table 1). Indeed, exposure to a neurotropic virus at some point during the course of a normal human lifespan is almost inevitable. While the majority of these encounters will result in a benign and clinically silent course of infection often associated with life-long

| Pathogen       | Primary host cell(s) | Disease                                      |
|----------------|----------------------|----------------------------------------------|
| Virus          |                      |                                              |
| RNA            |                      |                                              |
| LCMV           | Neurons, astrocytes, glia | Meningitis, meningoencephalitis             |
| Measles        | Neurons              | Meningitis, demyelination, encephalitis      |
| West Nile      | Neurons              | Meningitis, encephalitis                     |
| Coronavirus    | Glia; neurons        | Encephalomyelitis, demyelination             |
| TMEV           | Microglia, macrophages | Encephalomyelitis, demyelination             |
| DNA            |                      |                                              |
| HSV            | Neurons              | Blindness, corneal scarring                  |
| CMV            | Astrocytes           | Encephalitis                                 |
| Retrovirus     |                      |                                              |
| HIV            | Microglia            | HAD, HIV                                     |
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persistence of virus, others can be quite severe and potentially life-threatening. A common pathological feature of many neurotropic viruses is acute meningitis and/or encephalitis that occur as a result of neuroinflammation due to chemotactic signals derived within the CNS in response to infection. While immune cell infiltration may be beneficial by attracting antigen-specific T cells required for reducing viral load, in many of these cases, the amplified inflammatory responses can also be dangerous and lead to permanent, immune-mediated neurologic damage and/or death. It is now widely accepted that resident cells of the CNS are quite capable of secreting a number of different chemokines that undoubtedly contribute to directing distinct subsets of leukocytes into the CNS based upon surface expression of chemokine receptors (Lane et al., 2006). Therefore, defining chemokine/chemokine receptor expression profiles within the CNS of viral-infected hosts provides important information on potential targets for therapeutic intervention.

2.1 Lymphocytic Choriomeningitis Virus (LCMV)

LCMV is an ambisense RNA virus and a member of the Arenaviridae family (Emonet et al., 2006). It is a rodent-borne viral infectious disease in humans that presents as aseptic meningitis, encephalitis or meningoencephalitis (Jahrling and Peters 1992). Infection of the murine CNS with LCMV results in a well-established model of viral meningitis (Buchmeier et al., 1980; Buchmeier and Zajac 1999). LCMV infection of the CNS 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.) (Buchmeier et al., 1980). Specifically, infiltrating CD8+ T cells are essential in contributing to cell damage and death in LCMV-infected mice. Instillation of LCMV into the CNS of immunocompetent mice reveals that expression of chemokine RNA in the CNS precedes the infiltration of immune cells (Asensio and Campbell 1997). Transcripts for CXCL10, CCL2, CCL4, CCL5, and CCL7 are apparent in the CNS of LCMV infected mice 3 days p.i. and increase by day 6, correlating with accumulation of activated T cells (Asensio and Campbell 1997). Astrocytes are, in part, responsible for expression of certain chemokines including CXCL10 following LCMV infection (Asensio et al., 1999). These early results suggested that chemokine expression in the CNS represents an early host response to intracranial (i.c.) infection with LCMV and drives subsequent immunopathology.

Characterization of chemokine receptors has revealed that CCR5 and CXCR3 are readily detected on infiltrating T cells present within the CNS of LCMV-infected mice (Christensen et al., 2004; Nansen et al., 2000, 2002). Studies using CCR5-deficient mice (CCR5−/− mice) suggested that CCR5 signaling on T cells is dispensable with regards to regulating T cell migration into the CNS, as LCMV-infection of CCR5−/− mice results in a lethal T cell-mediated meningitis with no change in...
the composition of the cellular infiltrate within the CNS of CCR5−/− mice compared to controls (Nansen et al., 2002). In contrast, LCMV infection of the CNS in CXCR3−/− mice results in a dramatic increase in survival that correlated with paucity in CD8+ T cells migrating into the parenchyma from the meninges; however, no alterations in the distribution of viral antigen between infected CXCR3−/− mice and wildtype control mice were observed (Christensen et al., 2004). Importantly, reconstitution of LCMV-infected CXCR3−/− mice with CXCR3+/+ CD8+ T cells restored susceptibility to viral-induced meningitis (Christensen et al., 2004). These data highlight the importance of CXCR3 in allowing positional migration of effector T cells to sites of infection during on-going viral-induced neuroinflammation.

Ligands capable of binding to CXCR3 include the non-ELR CXC chemokines CXCL9, -10, and -11. These chemokines lack the ELR motif that is a common feature in the amino terminus of the other members within the CXC subfamily. Subsequent studies indicated that CXCL10, but not CXCL9 or CXCL11, is the key signaling molecule responsible for recruiting effector T cells into the CNS of LCMV-infected mice (Christensen et al., 2006). However, given the overlapping chemokine expression profiles that exist within the CNS of LCMV-infected mice there is the possibility of functional redundancy as well as compensatory mechanisms that may be employed in the absence of specific chemokine signaling pathways. Indeed, LCMV-infection of mice deficient in both CXCR3 and CCR5 (CXCR3/CCR5-deficient mice) indicated that lack of both receptors does not impair generation of virus-specific T cells suggesting that signaling through these receptors is not critical in generating virus-specific T cells (de Lemos et al., 2005). Although T cell infiltration into the CNS of either CXCR3−/− or CXCR3/CCR5-deficient mice was reduced compared to wild-type mice, there were greater numbers of CD8+ T cells present in the neural parenchyma of double-deficient mice compared to CXCR3−/− mice, indicating that CCR5 may function to negatively regulate the antiviral CD8+ T cell response (de Lemos et al., 2005).

2.2 West Nile Virus (WNV)

West Nile Virus (WNV) is a flavivirus that cycles between primary and secondary hosts, including avian/mosquito vectors and humans/mammals, respectively (Sejvar and Marfin 2006). WNV was first isolated from an infected patient in Uganda in 1937 and has caused sporadic outbreaks in Africa and Asia. WNV represents a re-emerging viral pathogen as the virus was isolated from a flamingo in New York City in 1999. Subsequently, the virus spread west and, in the process, has had significant impact on specific populations of birds. In addition, humans are susceptible to infection, which can range from mild flu-like symptoms (West Nile Fever) to more serious neurological disease characterized by meningitis and encephalitis.

Peripheral infection of susceptible mice (C57BL/6) with neurotropic clinical isolates of WNV results in viral entry and disease that recapitulates many of the
viral and pathological parameters of the human disease. Neurons are the primary targets of viral infection resulting in multifocal encephalitis characterized by infiltrating T cells and macrophages. Upon WNV infection, neurons secrete CXCL10 that presumably serves to attract CXCR3 + T cells into the CNS. In support of this are studies indicating that either antibody neutralization of CXCL10 or infection of CXCL10−/− mice results in diminished infiltration of CXCR3 + CD8 + T cells into the brain accompanied by an increase in virus and disease severity (Klein et al., 2005). These data support a protective role for CXCL10 in host defense against WNV infection by attracting virus-specific effector T cells into the CNS.

Studies by Murphy and colleagues suggest an alternative chemokine signaling pathway is important in host defense (Glass et al., 2005, 2006; Lim et al., 2006). WNV infection of CCR5−/− mice resulted in a rapid and uniformly fatal infection characterized by an impaired ability to clear virus from the brain and reduced numbers of T cells compared to infected CCR5 +/+ mice (Glass et al., 2005). Adoptive transfer of WNV-immune splenocytes into infected CCR5−/− mice restored protection that correlated with increased T cell infiltration into the CNS. Supporting the importance of CCR5 signaling in host defense against WNV-induced neurologic disease are findings that indicate humans harboring the CCRDelta32 deletion have increased susceptibility to WNV-induced disease (Glass 2006). Indeed, CCR5Delta32 homozygosity was significantly associated with fatal outcome after WNV infection in one patient cohort examined highlighting the importance of this receptor in defense following viral infection of the CNS.

In addition, another mosquito-borne flavivirus, Japanese encephalitis virus (JEV), induces an acute encephalitis, in which the role of the host immune response with regards to either host defense and/or disease has not been well-characterized (Solomon and Winter 2004). However, animal models of JEV-induced encephalitis suggest that disease severity correlates with increased expression of proinflammatory genes (Chen et al., 2004, 2000; Suzuki et al., 2000; Winter et al., 2004). Chemokines, including CCL5 (a ligand for CCR5) are expressed within the CNS of JEV-infected humans suggesting a potential role in controlling leukocyte infiltration into the CNS (Chen et al., 2000; Suzuki et al., 2000). While levels of IgM and IgG were higher within the cerebral spinal fluid (CSF) of survivors of JEV infection, expression of chemokines CCL8 and CCL5 were elevated in nonsurvivors, implying a potential role in disease pathogenesis (Winter et al., 2004).

2.3  Herpes Simplex Viruses (HSV)

Herpes simplex viruses (HSV) are extremely prevalent human pathogens with seroconversion rates approaching 60% worldwide (Carr and Tomanek 2006; Looker and Garnett 2005). Infection by HSV type 1 (HSV-1) is the leading cause of blindness in the industrialized world due to corneal infection that leads to the disease herpetic corneal keratitis. The inflammatory response associated with these viruses strongly correlates with morbidity as a result of the development of
lesions and subsequent scarring. Therefore, understanding the signals regulating inflammation in response to HSV-1 infection of the cornea may provide insight into relevant targets for immunotherapy.

The first thorough study to characterize the chemokine gene expression profile within the cornea of HSV-1-infected mice was performed in 1996 using PCR (Su et al., 1996). Chemokines detected included CXCL1, CXCL2, CXCL9, CXCL10 and CCL5 (Su et al., 1996). With the exception of CXCL10, expression of chemokines as well as proinflammatory cytokines is transient and occurs during the innate immune response. Recent investigations into molecular signaling pathways involved in initiating chemokine gene expression in response to HSV-1 infection of the CNS have revealed differential requirements for members of the TLR family with regards to chemokine gene expression. TLR2 is required for production of various proinflammatory cytokines as well as chemokines, including CXCL1, CXCL2, CXCL4, and CXCL5 (Aravalli et al., 2005). In contrast, increased production of CXCL9 and CXCL10 required both TLR9 as well as type I interferon signaling pathways (Wuest et al., 2006). HSV-1 infection of either TLR2−/− or TLR9−/− mice resulted in an attenuated inflammatory response highlighting a previously unappreciated role for TLR signaling pathways in regulating viral neuropathogenesis (Aravalli et al., 2005; Wuest et al., 2006). Additional studies by various groups employing either neutralizing antibodies or chemokine/chemokine receptor knock-out mice have also aided in defining the functional contributions of these molecules to disease. CXCL2 promotes neutrophil trafficking by signaling through the receptor CXCR2 expressed on the cell surface. Administration of anti-CXCL2 antibody to HSV-1-infected mice or infection of CXCR2−/− mice revealed limited neutrophil accumulation within the cornea (Maertzdorf et al., 2002; Yan et al., 1998). Blocking CXCL10 during the acute phase of HSV-1 ocular infection resulted in increased viral titers within the stroma and trigeminal ganglion, but limited corneal pathology, although spread of virus from the cornea stroma into the retina was markedly restricted in anti-CXCL10 treated mice (Carr et al., 2003). In addition, viral antigen was co-localized with infiltrating CD11b + cells suggesting that viral infection of inflammatory cells facilitates spread of the virus to other restricted anatomical regions of the eye (Carr et al., 2003). These findings led investigators to hypothesize that upon HSV-1 infection of the cornea, CXCL10 serves to orchestrate inflammatory responses that function to control viral replication, but also may serve to disseminate virus to other regions of the eye by enabling infection of inflammatory cells (Carr et al., 2003). HSV-1 infection of CXCR3−/− mice revealed somewhat surprising results as mice deficient in CXCR3 signaling exhibited an overall increase in survival rate compared to infected wildtype mice, which was associated with a 2-fold increase in the frequency of infiltrating T cells within the trigeminal ganglion (Wickham et al., 2005). Similarly, HSV-1 infection of mice lacking CCR5 allowed for characterization of the contributions of CCR5 ligands CCL3 and CCL5 to defense and/or disease. HSV-1 infection of CCR5−/− mice resulted in no difference in mortality despite deficiencies in controlling viral replication in the eye (Carr et al., 2006). This approach highlights the potential overlapping compensatory mechanisms that are inherent within the chemokine family.
2.4 **Measles Virus (MV)**

Measles virus is an enveloped negative-strand RNA virus, and a member of the paramyxovirus family (Griffin and Bellini 1996). Infection with MV typically results in an acute febrile illness and rash due to viral replication first in mono- and lymphocytic cells, and then in a variety of tissues (Katz 1995). MV infection is typically self-limiting due to the generation of the virus-specific immune response (Griffin and Bellini 1996). In the rare case, however, MV re-emerges in fully immunocompetent individuals in the CNS months to years following the resolution of the acute infection resulting in a fatal disease known as subacute sclerosing panencephalitis (SSPE) (ter Meulen et al., 1983). The pathological hallmarks of SSPE include demyelination, gliosis, and T lymphocyte infiltration into the CNS accompanied by expression of chemokines and cytokines (Anlar et al., 2001; Hofman et al., 1991; Nagano et al., 1994; Schnorr et al., 1997). As infiltration of activated immune cells into the CNS is important in disease pathogenesis in SSPE, understanding the signals regulating leukocyte invasion is critical to potential disease intervention. Infection of human astrocyte cultures with MV (either live virus or ultraviolet-inactivated) results in production of mRNA transcripts for CCL2, CCL3, CCL4, and CCL5 (Noe et al., 1999; Xiao et al., 1998). Addition of cytokines IFN-\(\gamma\) and TNF-\(\alpha\) to viral-infected cultures did not result in a synergistic effect with regards to chemokine transcript expression, but rather selectively inhibited CCL2 and CCL4 transcript levels (Xiao et al., 1998). These intriguing results suggest localized expression of cytokines may regulate chemokine gene expression and modulate disease by altering the composition of the cellular infiltrate. Analysis of the cerebral spinal fluid (CSF) of SSPE patients contained elevated levels of chemokines, in which CXCL10 was predominantly expressed (Saruhan-Direskeneli et al., 2005).

The receptor for MV, CD46, is expressed at low levels on neurons, oligodendrocytes, and astrocytes in normal brains (McQuaid and Cosby 2002). Mouse models for MV pathogenesis have been hampered by the fact that mice do not express CD46 and are normally not infected by MV. However, a transgenic mouse expressing human CD46 on neurons under the control of the neuron-specific enolase promoter (NSE) is susceptible to infection and replication of human MV strains (Rall et al., 1997). Intracranial infection of transgenic NSE-CD46 neonate (day 1) mice with MV results in widespread viral replication in neurons that leads to acute encephalitis, seizures, weight loss and ataxia, with death occurring by day 15–20 p.i. (Rall et al., 1997). T and B lymphocytes enter the CNS and are found in association with MV-antigen expressing neurons following MV infection in NSE-CD46 mice (Manchester et al., 1999). The contribution of these cells to host defense was determined using adoptive transfer of immune cell subsets into transgenic CD46-expressing mice. These studies revealed that CD4 + T lymphocytes in combination with CD8 + T lymphocytes or B cells are necessary for protection during MV infection (Tishon et al., 2006). Additionally, neurons contributed to chemokine expression in the CNS following MV infection of NSE-CD46 mice (Patterson et al.,
2003). In these experiments chemokine transcripts for CXCL10 and CCL5 were elevated in CNS tissues isolated from MV infected immunodeficient NSE-CD46/Rag-2 knock-out (ko) mice, as well as from MV-infected primary hippocampal neuron cultures established from embryonic NSE-CD46 mice (Patterson et al., 2003). Neutralization of CXCL10 and CCL5 with polyclonal neutralizing antisera resulted in significant reductions in CD4+ and CD8+ T cell infiltration into the CNS following MV infection of NSE-CD46 mice (Patterson et al., 2003). Therefore, these findings highlight the functional relevance of chemokines in the neuropathogenesis of MV infection and reveal potential targets for therapy in SSPE patients.

2.5 Cytomegalovirus

Cytomegalovirus (CMV) infection of humans can result in pathological manifestations within the CNS that include microglial nodule formation and ventriculoencephalitis (Arribas et al., 1995). Glial cells are sensitive to CMV infection and are capable of secreting chemokines. For example, CMV infection of astrocytes did not result in the production of antiviral cytokines but does generate chemokines CCL8 and CCL2 (Cheeran et al., 2001). However, CMV infection of microglial cells promoted production of anti-viral cytokines TNF-α and IL-6, in addition to chemokines, such as CXCL10 (Cheeran et al., 2003). It is possible that CMV-infected astrocytes attract microglial cells to sites of infection to aid in host defense via secretion of anti-viral cytokines. In support of this possibility are data demonstrating an ~60% reduction in viral gene expression in CMV-infected astrocytes co-cultured with microglia (Cheeran et al., 2001). CXCL10 expressed from CMV-infected microglia may aid in host defense by attracting T cells into the CNS (Cheeran et al., 2004). Infection of immunodeficient mice with murine CMV (MCMV) resulted in unrestricted viral replication within the brain and ultimately death (Cheeran et al., 2004). In addition, there were elevated levels of CCL2 and CXCL10 within the brains of MCMV-infected immunodeficient animals, which likely reflect the increase in viral burden. Transfer of splenocytes obtained from MCMV-primed animals into infected immunodeficient mice resulted in protection from lethal disease. These findings indicate that localized expression of chemokines is not sufficient to control viral replication in the absence of an adaptive immune response (Cheeran et al., 2004). Further, these findings suggest that expression of T cell chemoattractant chemokines, such as CXCL10, may exert a protective effect by attracting T cells to sites of viral replication (Cheeran et al., 2004).

2.6 Human Immunodeficiency Virus (HIV)

Human immunodeficiency virus-1 (HIV-1) induces severe damage to the immune system, as it is tropic for both CD4+ T cells and for cells of the monocyte/
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Macrophage lineage. Ultimately, significant depletion of effector memory CD4+ T cells leads to acquired immunodeficiency syndrome (AIDS) in which susceptibility to opportunistic infection is increased due to a limited adaptive immune system (Brenchley et al., 2006; Grossman et al., 2006). Infection with HIV can also result in damage to the CNS resulting in dementia. HIV-associated dementia (HAD) affects approximately 20% of individuals with advanced HIV disease, and is characterized clinically by numerous neurological and psychiatric symptoms with cognitive and motor impairments being the most prevalent (Albright et al., 2003; Janssen et al., 1991; Marder et al., 1996; Navia et al., 1986). HIV-1 encephalitis (HIVE), the histological correlate of HAD, occurs in the majority of HAD cases and the pathological hallmarks include blood–brain barrier (BBB) damage, productive viral infection, inflammatory infiltrates consisting of lymphocytes and macrophages, astrogliosis, microgliosis and neuronal loss (Budka 1991; Cartier et al., 2005).

Chemokines and chemokine receptors are implicated in both disease pathogenesis and protection during HAD, however, the precise mechanisms governing the damage are presently under investigation. Several chemokine receptors are directly involved in the infection, serving as CD4 coreceptors for viral entry into target cells (Cartier et al., 2005). CCR5 and CXCR4 are predominantly utilized, but additional studies support CCR2a (splice variant), CCR3, CCR8, CXCR6 and CX3CR1 as minor coreceptors for HIV-1 (Cartier et al., 2005).

The initial entry of HIV into the brain is thought to occur via migration of infected monocytes and CD4+ T lymphocytes across the BBB (Cartier et al., 2005; Speth et al., 2005). Although the molecular mechanisms are currently unknown, chemokines are thought to be central regulators of this process (Cartier et al., 2005; Speth et al., 2005). For example, recent studies demonstrate that the presence of CCL2 within the brain promotes migration of HIV-infected leukocytes into the CNS (Eugenin et al., 2006). CXC3CL1 and CXCL12 have also been implicated in the transmigration of lymphocytes and monocytes across the BBB (Nottet 1999). Once HIV enters the brain, virus-host interactions and viral protein release lead to altered chemokine expression that can result in activation or proliferation of microglia and astrocytes or activation of neuronal chemokine receptors resulting in neuronal dysfunction or death (Speth et al., 2005). High CCL2 concentrations in the CSF of SIV-infected macaques correlate with a significantly higher expression of macrophage/microglia and astrocyte activation markers suggesting involvement of CCL2 in microgliosis and astrogliosis (Zink et al., 2001). As an inducer of astrocyte proliferation, enhanced expression of CXCL12 during HIV encephalitis likely contributes to astrogliosis as well (Bonavia et al., 2003). Factors released by activated microglia and astrocytes may indirectly or directly contribute to neuronal damage. Additionally viral proteins such as gp120 can act as chemokine agonists and activate signaling pathways in astrocytes and neurons resulting in neurodegeneration (Kaul et al., 2005). Taken together, these studies support the notion that chemokines and chemokine receptors are serving, in part, as mediators of disease leading to the pathogenesis associated with HAD.
The duplicitous nature of chemokines and chemokine receptors is highlighted by studies demonstrating the protective abilities of these molecules during HAD. Among the protective functions that chemokines and chemokine receptors play are competition for HIV binding, modulation of astrocyte and microglia activation and protection from HIV-induced neurotoxicity (Speth et al., 2005). Corasaniti et al., (2001a, b) showed that CXCL12 elicits protection against gp120-induced neuronal apoptosis in rats and suggests that this protection is conferred through a shift in the competition of gp120 and CXCL12 binding to CXCR4 in favor of CXCL12. Regarding protection from neurotoxicity, studies support a role for CCL2 and CX3CL1 in protecting neurons from Tat-induced apoptosis (Eugenin et al., 2003; Tong et al., 2000). Diminished microglial production of proinflammatory cytokines and reactive oxygen species following CX3CL1 treatment has been observed, yet the mechanism is currently under investigation (Mizuno et al., 2003).

2.7 Coronavirus

Coronaviruses are enveloped positive-strand RNA viruses with a genome ranging from 30–34kb in size (Masters 2006). 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 (Holmes and Lai 1996; McIntosh 1996; Perlman et al., 1999). 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 (Holmes and Lai 1996; McIntosh 1996; Perlman et al., 1999). More recently, a human coronavirus (CoV) 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 (Holmes 2003). Although normally considered an upper respiratory tract pathogen, SARS CoV was recently isolated from brain tissue of a SARS patient with significant neurologic symptoms and neuropathology associated with neuronal necrosis and glial hyperplasia (Xu et al., 2005). Analysis of blood samples revealed a dramatic increase in CXCL9 and CXCL10 protein levels (Xu et al., 2005). Moreover, immunostaining showed discrete patterns of CXCL9 expression by glial cells that was associated with infiltrating T cells and macrophages. These findings demonstrate that SARS CoV is capable of infecting the CNS and is associated with neurologic disease characterized by immune cell infiltration in which chemokine signaling may be important.

Supporting this possibility are studies utilizing a murine coronavirus, mouse hepatitis virus (MHV), to characterize the functional contributions of chemokine signaling in leukocyte trafficking and accumulation within the CNS (reviewed in Glass et al., 2002; Lane et al., 2006). Instillation of MHV into the
CNS of susceptible mice results in an acute encephalomyelitis characterized by viral infection and replication in either neurons and/or glial cells (Lane et al., 2006). Mice that survive the acute disease often develop a chronic demyelinating disease with virus persisting in white matter tracts and infiltrating T cells and macrophages contribute to myelin damage. MHV infection results in an orchestrated expression of chemokines including CCL2, CCL3, CCL4, CCL5, CXCL9, and CXCL10 (Lane et al., 1998). Analysis of the temporal expression profiles of chemokine transcripts revealed that at all stages of disease examined e.g. acute and chronic, CXCL10 was the prominent chemokine detected.

Inhibition of chemokine signaling through use of genetic knock-out mice or neutralizing antibodies has provided a powerful approach to designate both redundant and non-redundant roles for chemokines in MHV-induced CNS disease. Indeed, MHV infection of CCL3−/− mice results in deficient activation and accumulation of myeloid dendritic cells into draining cervical lymph nodes that results in muted activation of virus-specific T cells (Trifilo et al., 2003; Trifilo and Lane 2004). For example, virus-specific CD8 + T cells are unable to undergo egress from lymphatic tissue and migrate into the CNS as a result of impaired expression of tissue-specific homing receptors e.g. chemokine receptors CXCR3 and CCR5 (Trifilo et al., 2003). In addition, virus-specific CD4 + T cells derived from MHV-infected CCL3−/− mice produce increased levels of IL-10 and diminished IFN-γ when stimulated with viral antigen (Trifilo and Lane 2004). Therefore, these data indicate that early expression of chemokines such as CCL3 serve an important role in linking innate and adaptive immune responses following MHV infection of the CNS.

Blocking either CXCL9 or CXCL10 during acute disease resulted in increased mortality that correlated with reduced infiltration of CXCR3 + T cells into the CNS and increased viral titers, demonstrating an important role for these chemokines in host defense by attracting T cells into the CNS (Dufour et al., 2002; Liu et al., 2000, 2001a). However, blocking CXCL10 during chronic disease was beneficial as animals recovered locomotor activity that was associated with reduced T cell infiltration into the CNS and extensive remyelination (Liu et al., 2001b). Therefore, CXCL10 is either protective or contributes to disease depending on the stage of disease. Further support for the importance of CXCL10 in modulating disease is derived from studies demonstrating that antibody-targeting of CXCR3 improves clinical outcome during chronic disease by reducing T cell accumulation within the CNS (Stiles et al., 2006b). Importantly, the influence of blocking CXCL10 signaling appears to be at the level of trafficking rather than muting specific T cell effector functions e.g. cytokine secretion or proliferation (Stiles et al., 2006a). In addition to CXCL10, the chemokine CCL5 also promotes protection during acute disease as well as amplifies disease severity during chronic disease (Glass et al., 2004, 2001). CCL5 is capable of recognizing the receptors CCR1 and CCR5, which are surface receptors present on activated macrophages and T cells. MHV infection of CCR5−/− did not significantly impact host defense, but demyelination was reduced and this correlated with paucity in macrophage accumulation within the
CNS (Glass et al., 2001). These findings suggest that CCR5 signaling promotes macrophage trafficking but does not significantly impact T cell migration. However, MHV infection of mice deficient in CCR1 signaling (CCR1−/− mice) did not affect viral clearance from the brain, although there was a reduction in CD8+ T cell accumulation (Hickey et al., 2007). Further, MHV-infected CCR1−/− mice ultimately succumbed to fatal disease during the chronic stage of infection in the absence of any significant change in the composition of the cellular infiltrate (Hickey et al., 2007). Therefore, these data demonstrate that neither CCR1 nor CCR5 signaling alone is necessary for optimal host defense during acute disease, but both receptors influence the disease pathogenesis during chronic disease.

2.8 Theiler’s Murine Encephalomyelitis Virus (TMEV)

Theiler’s Murine Encephalomyelitis Virus (TMEV) is a positive-strand RNA picornavirus that does not infect humans, but does induce an acute encephalomyelitis and chronic demyelinating disease following instillation into the CNS of mice (Oleszak et al., 2004). Importantly, TMEV infection serves as an excellent model for the human demyelinating disease MS due to similarities in neuropathology. TMEV infection results in activation of various chemokine genes within the CNS including CCL2, CCL3, CCL4, CCL5, CXCL9, and CXCL0 that precedes and accompanies leukocyte infiltration into the CNS and is associated with onset of clinical disease (Hoffman et al., 1999; Ransohoff 2002). In vitro studies have revealed that TMEV infection results in a dramatic increase in the synthesis of chemokine mRNA transcripts (Palma and Kim 2001; Palma and Kim 2004; So et al., 2006). Chemokine gene activation was largely independent of type I IFN signaling but completely dependent upon NFκB and IRF/ISRE pathways (Palma and Kim 2004; So et al., 2006). More recently, induction of chemokine gene expression was found to occur in a TLR3-dependent manner (Palma and Kim 2004; So et al., 2006). Functional studies have ruled out CXCL10 as important in either host defense or disease following TMEV infection, indicating that alternative signaling pathways promote these separate events (Tsunoda et al., 2004). Moreover, antibody targeting of either CXCL9 or CCL5 results in increased viral antigen expression within the CNS and increased spinal cord pathology, suggesting that these chemokines serve to restrict viral gene expression (Ure et al., 2005). Therefore, the TMEV model of viral-induced neurologic disease is distinct from others with regards to chemokines participating in leukocyte infiltration into the CNS and/or disease progression. Indeed, CNS-derived chemokine gene expression in TMEV-infected mice during chronic disease appears to be dictated primarily by viral persistence and is independent of genetic factors related to susceptibility, severity of neuropathology, and the presence or absence of regulatory T cells (Ransohoff 2002).
3 Bacterial Infections of the CNS

Several different types of bacteria infect the CNS and cause severe and often fatal diseases, thus highlighting the importance of studying the host immune response following infection (Table 2). Unlike viruses, the majority of these bacteria do not require a host cell for survival and replication within the CNS. Rather, many bacterial infections of the CNS stem from the colonization of the CSF. In general, these pathogens gain access to the CNS following septicemia, which facilitates passage of the bacterium and immune cells across the BBB, culminating in diseases such as meningitis, encephalitis and brain abscess. Notably, bacterial meningitis accounts for one of the top ten infectious causes of death throughout the world (Nau and Bruck 2002; Scheld et al., 2002). The high mortality rate associated with these infections and the prevalent neurologic damage incurred by survivors is the result of pathological changes within the CNS due to (1) direct damage elicited by the pathogen itself and (2) the strong host inflammatory response within the CNS during the bacterial infection. For instance, gram-positive bacteria induce a robust neuroinflammatory response via immunoreaction to the peptidoglycan-techoic acid that constitutes the bacterial cell wall. Similarly, the lipopolysaccharide (LPS) contained within the outer membrane of gram-negative bacteria is highly antigenic and contributes to bacterial-mediated CNS pathogenesis. The resulting host inflammatory response contributes to CNS damage at the site of infection due to the toxic effects of immune mediators including cytokines, chemokines, oxidative agents and proteolytic enzymes (Koedel et al., 2002; Pfister and Scheld 1997). Similar to the viral infections discussed in the first section of this chapter, recruitment and activation of leukocytes is a hallmark of acute inflammation in response to bacteria and it is evident that chemokines also play a vital role in this process. Therefore, understanding the role of chemokine/chemokine receptor expression within the CNS of bacteria-infected hosts may uncover potential targets for therapeutic intervention.

Despite the dramatic clinical impact of the neurotropic bacterial pathogens on morbidity and mortality, work dedicated to understanding the mechanisms governing immune cell infiltration following infection are limited compared to viral-based

| Pathogen                  | Disease                                      |
|---------------------------|----------------------------------------------|
| Bacteria                  |                                              |
| Gram-positive             |                                              |
| Streptococcus pneumoniae  | Meningitis, meningoencephalitis, brain abscess|
| Listeria monocytogenes    | Meningitis, meningoencephalitis, brain abscess|
| Staphylococcus aureus     | Brain abscess                                |
| Gram-negative             |                                              |
| Neisseria meningitidis    | Meningitis, meningoencephalitis              |
| Haemophilus influenza     | Meningitis                                   |
| Spirochetes               |                                              |
| Borrelia burgdorferi      | Meningitis, encephalitis                     |
studies. Nonetheless, several important studies are beginning to shed light on the subject. From this work it is apparent that bacterial infections within the CNS tend to result in similar chemokine profiles compared to virus (Kielian 2004b; Lahrtz et al., 1998). Indeed, in many instances the level and duration of chemokine expression in response to bacterial infection surpasses what is often observed following viral infections. Presumably, the presence of higher levels of antigens, including cell wall products and/or various secreted virulence factors likely contribute to this feature of the bacteria-induced inflammation. Targeted studies have revealed the presence of elevated levels of CXCL8, CXCL1, CCL2, CCL3, CCL4 and CCL5 in the cerebrospinal fluid (CSF) of patients with bacterial meningitis compared to controls (Lahrtz et al., 1998; Spanaus et al., 1997; Sprenger et al., 1996). More recently, global screening for a broad range of chemokines in the CSF of similar patients has also demonstrated significantly enhanced levels of CXCL5, CXCL7, CXCL10, CCL8 and CCL20 (Kastenbauer et al., 2005). Importantly, CXCL8 expression correlates well with neutrophil infiltration, which are the predominant leukocytes present during bacteria-mediated CNS infections (Halstensen et al., 1993; Lahrtz et al., 1998; Mastroianni et al., 1998; Spanaus et al., 1997; Sprenger et al., 1996). However, the frequent presence of chemokines, such as, CCL2, CCL3 and CCL4, in the context of comparatively low monocyte infiltration during CNS infections caused by bacteria suggests that these chemokines play a broader role in the host anti-bacterial response beyond leukocyte trafficking, and emphasizes the importance of future research dedicated to this field (Inaba et al., 1997; Lahrtz et al., 1998; Mastroianni et al., 1998; Spanaus et al., 1997; Sprenger et al., 1996). The following section of this chapter is intended to highlight the common bacteria associated with CNS disease that have been studied in the context of chemokine regulation and signaling.

### 3.1 Streptococcus pneumoniae

*Streptococcus pneumoniae* (*S. pneumoniae*) is a gram-positive pathogenic bacterium that is the primary causative agent of otitis media and bacterial pneumoniae (Bridy-Pappas et al., 2005). Importantly, it is also the primary cause of bacterial meningitis in adults, accounting for nearly 50% of all reported cases (Bridy-Pappas et al., 2005; Robinson et al., 2001). *S. pneumoniae* commonly resides within the nasopharynx of healthy individuals, but in many cases, colonization in other areas of the host results in septicemia, subsequent breaching of the BBB and infection of the meninges. Pneumococcal meningitis has a nearly 30% mortality rate and in the majority of cases a high frequency of neurologic defects in survivors (Bohr et al., 1984; Bridy-Pappas et al., 2005; Edwards et al., 1985; Pfister et al., 1993). The high rate of fatal cases is speculated to be a result of an initial suboptimal host immune response against *S. pneumoniae*. The poor immune response can be attributed in part to the polysaccharide capsule surrounding *S. pneumoniae* that has been shown to facilitate immune evasion, thus giving it the advantage of reaching high bacterial
titers, which ultimately contributes to an unfavorable inflammatory environment (Musher 1992).

Indeed, chemokine expression is a hallmark of *S. pneumoniae* infection and several chemokines, such as CXCL1, CXCL8, CCL2, CCL3, CCL4 and CCL5 are expressed in the CSF of patients with meningitis (Lahrtz et al., 1998; Spanaus et al., 1997; Sprenger et al., 1996). A similar chemokine protein profile was seen in resident mouse microglia stimulated with the highly immunogenic cell wall components from *S. pneumoniae* (Hausler et al., 2002). In a mouse model of pneumococcal meningitis that mimics the natural route of infection in humans, CXCL2 was highly expressed following intranasal infection (Zwijnenburg et al., 2001). These and other studies have focused on determining the function and regulation of chemokines in the context of pneumococcal meningitis. For instance, CSF from pneumococcal meningitis patients was chemotactic for neutrophils and mononuclear leukocytes (Lahrtz et al., 1998; Spanaus et al., 1997; Sprenger et al., 1996). Given that neutrophils are the predominant immune cell infiltrate during acute disease, other groups have taken a closer look at the function of CXCL8, and its mouse homolog, CXCL2. In a rabbit model of LPS induced meningitis, CXCL8 blockade resulted in reduced leukocyte recruitment (Dumont et al., 2000). Likewise, intravenous treatment with anti-CXCL8 antibody in a rabbit model of *S. pneumoniae*-induced meningitis impaired leukocyte accumulation in the CSF (Ostergaard et al., 2000). CXCL2 expression in mice appears to be dependent on toll-like receptor (TLR) signaling. Mice deficient for MyD88, an integral signal transduction molecule involved in TLR signaling, exhibited a reduced inflammatory host response, including significant reduction in CXCL2 expression, which resulted in higher bacterial titers in the CNS and increased aggravation of disease compared to wild-type mice (Koedel et al., 2004). While these results are interesting with regards to CXCL8, it is apparent that more work needs to be focused on understanding the role of the other chemokines expressed during pneumococcal meningitis. Recent work has demonstrated an even broader array of chemokine protein expression including CXCL5, CXCL7, CXCL10, CCL8 and CCL20 in CSF of meningitis patients (Kastenbauer et al., 2005); and at least one study implicates the cytokine IFN-γ in differentially modulating chemokine production in resident CNS macrophages in response to components of the *S. pneumoniae* cell wall (Hausler et al., 2002). In summary, these studies suggest that inflammation in the CNS following *S. pneumoniae* infection is dynamically regulated by a distinct chemokine profile and highlights the importance of microglia as key mediators of this active process.

### 3.2 Neisseria meningitidis

*Neisseria meningitidis* (*N. meningitidis*) is known best as a primary pathogen of meningitis that has the potential to cause epidemic outbreaks (Manchanda et al., 2006). *N. meningitidis* is gram-negative bacterium that elicits a robust host immune
response through its LPS-rich cell wall. In fact, the severity of clinical presentation appears to correlate with the level of LPS within the CSF (Moller et al., 2005). Like *S. pneumoniae*, *N. meningitidis* resides within the human nasopharynx and is transmitted by respiratory spread. Its antiphagocytic capsule also gives *N. meningitidis* the advantage of evading immune clearance, which can result in high titers of the pathogen in the blood that ultimately facilitates breaching of the BBB. Upon infection of the CNS, meningeal cells appear to be a prominent source of chemokine production during meningococcal infection (Fowler et al., 2006). Binding of *N. meningitidis* to these cells results in high levels of CCL2, CCL5 and CXCL8 expression (Fowler et al., 2006), BBB damage and access to the CNS. Targeted studies, have demonstrated that the chemokines, CCL2, CCL3, CCL4, CCL5, CXCL1 and CXCL8 are expressed in the CSF from patients with meningococcal meningitis (Moller et al., 2005; Spanaus et al., 1997). Importantly, CSF from these patients is also chemotactic for inflammatory immune cells (Spanaus et al., 1997), supporting the functional role of chemokines for positional migration of immune cells in the context of bacterial meningitis. Additional evidence lies in the observation that the CSF from similar patients infected with *N. meningitidis* also contained significant levels of CCL2, CCL3, CCL5 and CXCL8, which had an inverse correlation with the relative bacterial load (Moller et al., 2005), and in vivo and ex vivo studies demonstrated that LPS was the major mediator of chemokine secretion (Moller et al., 2005). Enhanced CCL2, CCL3 and CXCL8 protein production was associated with increasing concentrations of LPS; however, the plasma CCL5 levels were inversely related (Moller et al., 2005), suggesting that CCL5 does not play a major role in leukocyte recruitment to the site of CNS infection. Meningeal cells appear to be a prominent source of chemokine production during a meningococcal infection, as CCL2, CCL5 and CXCL8 are each highly expressed following in vitro binding studies with *N. meningitidis* (Fowler et al., 2006). This robust host neuroinflammatory response elicited at the site of CNS infection may indeed have consequences beyond direct damage to CNS tissue. Leukopenia is a common feature in patients with systemic meningococcal infections often resulting in fatal septic shock (Flaegstad et al., 1995; Kornelisse et al., 1997). It is interesting to speculate that strong expression of chemokines at the site of infection may effectively deplete the levels of circulating leukocytes, ultimately contributing to high bacterial titers and the resulting fatal disease outcome.

### 3.3 Haemophilus influenza

Pathogenic strains of the gram-negative bacterium *Haemophilus influenza* (*H. influenza*) are defined by the presence of a capsule and are grouped into six types (A-F) that cause a variety of diseases affecting the ear, upper respiratory tract and CNS (Murray et al., 2005). *H. influenza* type B (HiB) was the number one cause of acute meningitis in infant and young children in the United States until widespread use of the HiB vaccine reduced reported cases to less than 10% since 1990.
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(Schuchat et al., 1997). Similar to *S. pneumoniae* and *N. meningitidis*, the CSF from patients with Hib contains significant amounts of CCL2, CCL3, CCL4, CCL5, CXCL1 and CXCL8 (Spanaus et al., 1997). In addition, similar findings were observed in a rat model of Hib, as significant levels of CCL2, CCL3, CCL5 and CXCL2 were expressed within 24–48 h post infection (Diab et al., 1999). Importantly, expression of these chemokines correlated well with the recruitment of infiltrating neutrophils and macrophages into the meninges, and also with disease severity (Diab et al., 1999). CXCL2 and CCL3 expression was detected in neutrophils and macrophages in the subarachnoid space that separates the meningeal cells from the lateral ventricles. CCL2 expression localized to infiltrating neutrophils and macrophages, and to some extent, astrocytes, whereas CCL5 expression occurred predominantly in astrocytes and resident microglia. The functional roles of CXCL2, CCL2 and CCL3 in immune cell trafficking during HiB infection were also determined by using neutralizing antibody studies (Diab et al., 1999). Treatment with anti-CCL2 significantly reduced macrophage infiltration into the subarachnoid space, while anti-CXCL2 treatment impaired neutrophil trafficking. It is important to note that this study was the first to show that anti-CCL3 also abrogated neutrophil recruitment, despite the observation that this chemokine is not capable of neutrophil recruitment in vitro. However, other studies are starting to show a role of CCL3 in neutrophil trafficking in vivo (Ajuebor et al. 2004; Ramos et al. 2005; Standiford et al. 1995). Interestingly, selective chemokine blockade also modulated the expression of the other chemokines; e.g. anti-CCL3 administration resulted in downregulation of CCL5, but simultaneous upregulation of CCL2 and CXCL2 (Diab et al. 1999). Therefore, it is possible that blocking CCL3 expression may have an indirect effect on neutrophil recruitment by modulating the expression of other chemokines, again highlighting the complex interplay between the regulatory events controlling chemokine regulation and immune infiltration during infection.

3.4 *Listeria monocytogenes*

*Listeria monocytogenes* (*L. monocytogenes*) is a gram-positive bacterium that is an important human pathogen, causing diseases ranging from mild gastroenteritis to fatal septicemia, encephalitis and up to 15% of the reported cases of meningitis in adults (Calder 1997). Transmission of *L. monocytogenes* and the pathogenesis of listeriosis are facilitated by several key features (Murray et al. 2005). For instance, it is a hardy bacterium that survives in soil, high heat and is able to replicate at low temperatures, thus facilitating transmission as a food-borne pathogen. Furthermore, it is a facultative intracellular bacterium that evades immune surveillance by surviving within macrophages and endothelial cells and can move from cell-to-cell via actin-based motility without exposure to other immune factors, such as antibodies and complement. In fact, *Listeria* can infect a variety of cell types, including neurons, astrocytes and meningeal cells. Chemokine
signaling is considered important for controlling intracellular replication of *Listeria* by recruiting activated monocytes across the BBB and into the subarachnoid space (Frei et al. 1993). In an experimental mouse model, Seebach, et al. (1995) showed that the immune cell infiltration to the meninges is temporally regulated following intracerebral infection with *L. monocytogenes*. Within 24 h p.i., roughly 80% of the infiltrating cells were neutrophils, with monocytes making up approximately 50% of the inflammatory cells after 72 h p.i. (Seebach et al., 1995). The immune cell influx correlated well with time-dependent chemokine expression, as CCL3 and CCL4 were expressed predominantly by neutrophils within the meninges early in the infection (12 h p.i.), and CXCL2, CCL3 and CCL4 were expressed by both neutrophils and monocytes by 24–48 h p.i. Furthermore, CCL3, CCL4 and CXCL2 were present in CSF from infected mice and in vitro antibody treatment with anti-CXCL2 or anti-CCL3 showed that these chemokines were partly responsible for neutrophil and monocyte recruitment, respectively. In light of the in vitro results it is interesting that the temporal expression of CCL3/CCL4, followed by CXCL2/CCL3/CCL4 is associated with a switch from a neutrophil-rich to a monocyte dominant immune cell environment. As we have seen, the in vivo inflammatory environment and in vitro model are often not correlated and can represent two widely distinct situations. The in vivo findings suggest that CCL3 is capable of either directly or indirectly recruiting neutrophils to areas inflammation, including the sites of bacterial-induced meningitis, once again emphasizing the importance of the dynamic regulatory networks that likely modulate chemokine and chemokine receptor function and signaling.

### 3.5 Borrelia burgdorferi

*Borrelia burgdorferi* (*B. burgdoferi*) is an obligate intracellular, spirochete bacterium, known best as the causative agent of Lyme’s disease (Murray et al., 2005). It is most commonly transmitted to humans by an infected tick. Lyme borreliosis can also affect the CNS, generally manifesting as meningitis. In fact, other spirochetes have long been associated with CNS infection, including the causative agents of syphilis (*Treponema pallidum*) and leptospirosis (*Leptospira*) (Pachner 1986). In the case of borreliosis, host immune evasion is mediated through the ability of *B. burgdorferi* to constantly modify its surface structure by modulating lipoprotein expression. However, the surface structure of borrelia can also induce a strong and often damaging inflammatory response (Morrison et al., 1997), which is mediated in part by chemokine expression. As we have seen, inflammatory changes in the CSF mediate breaching of the BBB and infiltration of immune cells within the CNS, causing subsequent pathogenesis. Indeed, the CSF of patients with neuroborreliosis contained significant amounts of CCL3, CCL4 and CCL8 compared to control patients (Grygorczuk et al., 2003). Furthermore, human monocytes infected with *Borrelia* strongly expressed and secreted CCL2, CCL5, CCL8, CXCL1 (Sprenger et al., 1997). It appears that activated CD8 + T cells expressing the chemokine receptor,
CCR5, may be directed to the CNS via these or other unidentified chemokines during the early phase of neuroborreliosis (Jacobsen et al., 2003). Interestingly, CCR5 signaling on CD8 + T cells during Borrelia infection is also speculated to contribute to chronic autoimmune driven disease (Hemmer et al., 1999).

### 3.6 Staphylococcus aureus

*Staphylococcus aureus* is a gram-positive bacterium that is well known for causing food poisoning, skin infections and acute endocarditis (Murray et al., 2005). It resides within the human nasal mucosa and/or the skin and is transmitted by skin-to-skin contact and by sneezing. *S. aureus* is a common hospital-acquired infection and methicillin-resistant strains of *S. aureus* (MRSA) complicate effective drug treatment strategies. For example, *S. aureus* represents a significant nosocomial infection and is a prominent cause of brain abscesses, a disease that affects roughly 1 out of every 10,000 hospital-admitted patients in the U.S. (Kielian 2004a; Townsend and Scheld 1998). In addition, *S. aureus* is able to survive intracellularly within neutrophils and endothelial cells further interfering with the success of host-immune clearance (Gresham et al., 2000). Chemokine signaling in response to CNS infection with *S. aureus* has been studied in a murine experimental brain abscess model. Using this model, Keilan, et al. (2001) demonstrated that CCL1, CCL2, CCL3, CCL4, CXCL2 were expressed within 6h post bacterial exposure. Microglia and astrocytes appear to play a prominent role in immune cell recruitment in this model system as the primary reservoirs for chemokine production and secretion. CCL1, CCL2, CCL3, CCL4, CXCL2 were expressed on primary cultures of murine microglia and astrocytes infected with *S. aureus* (Kielian et al., 2001), and microglia or astrocytes stimulated with heat inactivated *S. aureus* or gram-positive peptidoglycan expressed CCL1, CCL2, CCL3, CCL4, CCL5, CXCL10 (Kielian et al., 2002), and CCL2, CCL4 and CXCL2 (Esen et al., 2004). The functional role of chemokine signaling was further examined using CXCR2 ko mice, which had impaired neutrophil trafficking and increased bacterial burden within the CNS (Kielian et al., 2001), indicating that CXCR2 ligands provide the main chemotactic signal driving neutrophil influx into the CNS. In addition, CXCL2 is chronically expressed in the experimental brain abscess model and correlated with continued infiltration of neutrophils in the presence of low bacterial titers within the CNS (Baldwin and Kielian 2004). It appears that CXCL2 expression in microglia is dependent on MyD88 signaling, suggesting that *S. aureus*-mediated microglia activation and chemokine production are dependent on TLR recognition, in part by TLR2 (Esen and Kielian 2006). These studies indicate that CXCL2:CXCR2 signaling is the dominant cue for neutrophil recruitment to sites of *S. aureus* infection, which in themselves may be the primary mediators of CNS damage. To gain a better understanding of the mechanisms that induce tissue injury within the CNS it is of interest in the future to determine the functional importance of the other chemokines expressed during the course of *S. aureus* infection.
4 Perspectives

Microbial infection of the CNS often results in a tightly regulated inflammatory immune response in which chemokine signaling helps to control leukocyte entry and positional migration to sites of infection. Although functional redundancy is associated with many chemokine ligands as a result of receptor promiscuity, chemokine expression profiles often significantly shape the immune response to infection of the CNS based on various criteria including pathogen, route of infection, and cellular tropism. Careful consideration of chemokine and chemokine receptor expression during the course of disease could help in the derivation of treatments for patients infected with CNS invading pathogens with the ultimate goal of limiting inflammation/pathology without muting specific anti-microbial effector responses. Indeed, numerous treatments have been developed to control inflammation within the context of infection and autoimmune diseases by targeting specific proinflammatory molecules. With this in mind, various approaches are currently being developed and/or are in various stages of clinical trials to disrupt chemokine ligand interactions with specific signaling receptors with the hopes of improving disease outcome (Charo and Ransohoff 2006). Given the relatively rapid pace at which our understanding of the biology of chemokines and chemokine receptors has progressed over the past decade, it is likely that successful clinical approaches will be developed to mute specific chemokine signaling pathways and improve clinical outcome in response to microbial infection of the CNS.

References

Ajuebor MN, Kunkel SL, Hogaboam CM. 2004. The role of CCL3/macrophage inflammatory protein-1alpha in experimental colitis. *Eur J Pharmacol* 497(3):343–349.
Albright AV, Soldan SS, Gonzalez-Scarano F. 2003. Pathogenesis of human immunodeficiency virus-induced neurological disease. *J Neurovirol* 9(2):222–227.
Anlar B, Soylemazoglu F, Aysun S, Kose G, Belen D, Yalaz K. 2001. Tissue inflammatory response in subacute sclerosing panencephalitis (SSPE). *J Child Neurol* 16(12):895–900.
Aravalli RN, Hu S, Rowen TN, Palmquist JM, Lokensgard JR. 2005. Cutting edge: TLR2-mediated proinflammatory cytokine and chemokine production by microglial cells in response to herpes simplex virus. *J Immunol* 175(7):4189–4193.
Arribas JR, Clifford DB, Fichtenbaum CJ, Commins DL, Powderly WG, Storch GA. 1995. Level of cytomegalovirus (CMV) DNA in cerebrospinal fluid of subjects with AIDS and CMV infection of the central nervous system. *J Infect Dis* 172(2):527–531.
Asensio VC, Campbell IL. 1997. Chemokine gene expression in the brains of mice with lymphocytic choriomeningitis. *J Virol* 71(10):7832–7840.
Asensio VC, Kincaid C, Campbell IL. 1999. Chemokines and the inflammatory response to viral infection in the central nervous system with a focus on lymphocytic choriomeningitis virus. *J Neurovirol* 5(1):65–75.
Baldwin AC, Kielian T. 2004. Persistent immune activation associated with a mouse model of *Staphylococcus aureus*-induced experimental brain abscess. *J Neuroimmunol* 151(1–2):24–32.
Bohr V, Paulson OB, Rasmussen N. 1984. Pneumococcal meningitis. Late neurologic sequelae and features of prognostic impact. *Arch Neurol* 41(10):1045–1049.
Bonavia R, Bajetto A, Barbero S, Pirani P, Florio T, Schettini G. 2003. Chemokines and their receptors in the CNS: expression of CXCL12/SDF-1 and CXCR4 and their role in astrocyte proliferation. Toxicol Lett 139(2–3):181–189.

Brenchley JM, Price DA, Douek DC. 2006. HIV disease: fallout from a mucosal catastrophe? Nat Immunol 7(3):235–239.

Bridy-Pappas AE, Margolis MB, Center KJ, Isaacman DJ. 2005. Streptococcus pneumoniae: description of the pathogen, disease epidemiology, treatment, and prevention. Pharmacotherapy 25(9):1193–1212.

Buchmeier MJ, Zajac AJ. 1999. Lymphocytic choriomeningitis virus. In Persistent Viral Infections, eds. R Ahmed, J Chen, pp. 575–605. London: John Wiley and Sons, Ltd.

Buchmeier MJ, Welsh RM, Dutko FJ, Oldstone MB. 1980. The virology and immunobiology of lymphocytic choriomeningitis virus infection. Adv Immunol 30:275–331.

Budka H. 1991. Neuropathology of human immunodeficiency virus infection. Brain Pathol 1(3):163–175.

Calder JA. 1997. Listeria meningitis in adults. Lancet 350(9074):307–308.

Carr DJ, Tomanek L. 2006. Herpes simplex virus and the chemokines that mediate the inflammation. Curr Top Microbiol Immunol 303:47–65.

Carr DJ, Chodosh J, Ash J, Lane TE. 2003. Effect of anti-CXCL10 monoclonal antibody on herpes simplex virus type 1 keratitis and retinal infection. J Virol 77(18):10037–10046.

Carr DJ, Ash J, Lane TE, Kuziel WA. 2006. Abnormal immune response of CCR5-deficient mice to ocular infection with herpes simplex virus type 1. J Gen Virol 87(3):489–499.

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(1):16–42.

Charo IF, Ransohoff RM. 2006. The many roles of chemokines and chemokine receptors in inflammation. N Engl J Med 354(6):610–621.

Cheeran MC, Hu S, Yager SL, Gekker G, Peterson PK, Lokensgard JR. 2001. Cytomegalovirus induces cytokine and chemokine production differentially in microglia and astrocytes: antiviral implications. J Neurovirol 7(2):135–147.

Cheeran MC, Hu S, Sheng WS, Peterson PK, Lokensgard JR. 2003. CXCL10 production from cytomegalovirus-stimulated microglia is regulated by both human and viral interleukin-10. J Virol 77(8):4502–4515.

Cheeran MC, Gekker G, Hu S, Min X, Cox D, Lokensgard JR. 2004. Intracerebral infection with murine cytomegalovirus induces CXCL10 and is restricted by adoptive transfer of splenocytes. J Neurovirol 10(3):152–162.

Chen CJ, Liao SL, Kuo MD, Wang YM. 2000. Astrocytic alteration induced by Japanese encephalitis virus infection. Neuroreport 11(9):1933–1937.

Chen CJ, Chen JH, Chen SY, Liao SL, Raung SL. 2004. Upregulation of RANTES gene expression in neuroglia by Japanese encephalitis virus infection. J Virol 78(22):12107–12119.

Christensen JE, Nansen A, Moos T, Lu B, Gerard C, Christensen JP, Thomsen AR. 2004. Efficient T-cell surveillance of the CNS requires expression of the CXC chemokine receptor 3. J Neurosci 24(20):4849–4858.

Christensen JE, de Lemos C, Moos T, Christensen JP, Thomsen AR. 2006. CXCL10 is the key ligand for CXCR3 on CD8 + effector T cells involved in immune surveillance of the lymphocytic choriomeningitis virus-infected central nervous system. J Immunol 176(7):4235–4243.

Corasaniti MT, Bilotta A, Strongoli MC, Navarra M, Bagetta G, Di Renzo G. 2001a. HIV-1 coat protein gp120 stimulates interleukin-1beta secretion from human neuroblastoma cells: evidence for a role in the mechanism of cell death. Br J Pharmacol 134(6):1344–1350.

Corasaniti MT, Piccirilli S, Paolotti A, Nisticò R, Stringaro A, Malorni W, Finazzi-Agro A, Bagetta G. 2001b. Evidence that the HIV-1 coat protein gp120 causes neuronal apoptosis in the neocortex of rat via a mechanism involving CXCR4 chemokine receptor. Neurosci Lett 312(2):67–70.

de Lemos C, Christensen JE, Nansen A, Moos T, Lu B, Gerard C, Christensen JP, Thomsen AR. 2005. Opposing effects of CXCR3 and CCR5 deficiency on CD8 + T cell-mediated inflammation in the central nervous system of virus-infected mice. J Immunol 175(3):1767–1775.
Diab A, Abdalla H, Li HL, Shi FD, Zhu J, Hojberg B, Lindquist L, Wretlind B, Bakhiet M, Link H. 1999. Neutralization of macrophage inflammatory protein 2 (MIP-2) and MIP-1alpha attenuates neutrophil recruitment in the central nervous system during experimental bacterial meningitis. Infect Immun 67(5):2590–2601.

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(7):3195–3204.

Dumont RA, Car BD, Voitenok NN, Junker U, Moser B, Zak O, O’Reilly T. 2000. Systemic neutralization of interleukin-8 markedly reduces neutrophilic pleocytosis during experimental lipopolysaccharide-induced meningitis in rabbits. Infect Immun 68(10):5756–5763.

Edwards MS, Rench MA, Haffar AA, Murphy MA, Desmond MM, Baker CJ. 1985. Long-term sequelae of group B streptococcal meningitis in infants. J Pediatr 106(5):717–722.

Emonet S, Lemasson JJ, Gonzalez JP, de Lamballerie X, Charrel RN. 2006. Phylogeny and evolution of old world arenaviruses. Virology 350(2):251–257.

Esen N, Kielian T. 2006. Central role for MyD88 in the responses of microglia to pathogen-associated molecular patterns. J Immunol 176(11):6802–6811.

Esen N, Tanga FY, DeLeo JA, Kielian T. 2004. Toll-like receptor 2 (TLR2) mediates astrocyte activation in response to the Gram-positive bacterium Staphylococcus aureus. J Neurochem 88(3):746–758.

Eugenin EA, D’Aversa TG, Lopez L, Calderon TM, Berman JW. 2003. MCP-1 (CCL2) protects human neurons and astrocytes from NMDA or HIV-tat-induced apoptosis. J Neurochem 85(5):1299–1311.

Eugenin EA, Osiecki K, Lopez L, Goldstein H, Calderon TM, Berman JW. 2006. CCL2/monocyte chemoattractant protein-1 mediates enhanced transmigration of human immunodeficiency virus (HIV)-infected leukocytes across the blood–brain barrier: a potential mechanism of HIV-CNS invasion and NeuroAIDS. J Neurosci 26(4):1098–1106.

Flaegstad T, Kaaresen PI, Stokland T, Gutteberg T. 1995. Factors associated with fatal outcome in childhood meningococcal disease. Acta Paediatr 84(10):1137–1142.

Fowler MI, Ho Wang Yin KY, Humphries HE, Heckels JE, Christodoulides M. 2006. The inflammatory response of human meningeal cells following challenge with Neisseria lactamica: comparison with Neisseria lactamica. Infect Immun 74(11):6467–6478.

Frei K, Nadal D, Pfister HW, Fontana A. 1993. Listeria meningitis: identification of a cerebrospinal fluid inhibitor of macrophage listericidal function as interleukin 10. J Exp Med 178(4):1255–1261.

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(1):8–17.

Glass WG, Chen BP, Liu MT, Lane TE. 2002. Mouse hepatitis virus infection of the central nervous system: chemokine-mediated regulation of host defense and disease. Viral Immunol 15(2):261–272.

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(7):4018–4025.

Glass WG, Lim JK, Cholera R, Pletnev AG, Gao JL, Murphy PM. 2005. Chemokine receptor CCR5 promotes leukocyte trafficking to the brain and survival in West Nile virus infection. J Exp Med 202(8):1087–1098.

Glass WG, McDermott DH, Lim JK, Lekhong S, Yu SF, Frank WA, Pape J, Cheshier RC, Murphy PM. 2006. CCR5 deficiency increases risk of symptomatic West Nile virus infection. J Exp Med 203(1):35–40.

Gresham HD, Lowrance JH, Caver TE, Wilson BS, Cheung AL, Lindberg FP. 2000. Survival of Staphylococcus aureus inside neutrophils contributes to infection. J Immunol 164(7):3713–3722.

Griffin DD, Bellini WJ. 1996. Measles virus. In Fields Virology. 3rd edition. Vol. 1, eds. BN Fields, DM Knipe, PM Howley, pp. 1267–1312. Philadelphia, PA: Lippincott-Raven Publishers.
Grossman Z, Meier-Schellersheim M, Paul WE, Picker LJ. 2006. Pathogenesis of HIV infection: what the virus spares is as important as what it destroys. Nat Med 12(3):289–295.

Grygorczuk S, Pancwicz S, Kondrusik M, Swierzbinska R, Zajkowska J, Hermanowska-Szpakowicz T. 2003. [Serum and cerebrospinal fluid concentration of inflammatory proteins MIP-1-alpha and MIP-1-beta and of interleukin 8 in the course of borreliosis]. Neurol Neurochir Pol 37(1):73–87.

Halstensen A, Ceska M, Brandtzæg P, Redl H, Naess A, Waage A. 1993. Interleukin-8 in serum and cerebrospinal fluid from patients with meningococcal disease. J Infect Dis 167(2):471–475.

Hausler KG, Prinz M, Nolte C, Weber JR, Schumann RR, Kettenmann H, Hansch UK. 2002. Interferon-gamma differentially modulates the release of cytokines and chemokines in lipopolysaccharide- and pneumococcal cell wall-stimulated mouse microglia and macrophages. Eur J Neurosci 16(11):2113–2122.

Hemmer B, Gran B, Zhao Y, Marques A, Paschal T, Tzou A, Kondo T, Cortese I, Bielekova B, Straus SE and others. 1999. Identification of candidate T-cell epitopes and molecular mimics in chronic Lyme disease. Nat Med 5(12):1375–1382.

Hickey MJ, Held K.S., Baum E., Gao J.L., Murphy P.M., Lau T.E. 2007. CCR5 deficiency increases susceptibility to fatal corona virus infection of the central nervous system. Viral Immunology. In press.

Hoffman LM, Fife BT, Begolka WS, Miller SD, Karpus WJ. 1999. Central nervous system chemokine expression during Theiler’s virus-induced demyelinating disease. J Neurovirol 5(6):435–42.

Holmes KV. 2003. SARS-associated coronavirus. N Engl J Med 348(20):1948–1951.

Inaba Y, Ishiguro A, Shimbo T. 1997. The production of macrophage inflammatory protein-1alpha in the cerebrospinal fluid at the initial stage of meninitis in children. Pediatr Res 42(6):788–793.

Janssen R, Cornblath D, Epstein L, Foa R, McArthur J, Price R, Asbury A, Beckett A, Benson D, Bridge T. 1991. Nomenclature and research case definitions for neurologic manifestations of human immunodeficiency virus-type 1 (HIV-1) infection. Report of a Working Group of the American Academy of Neurology AIDS Task Force. Neurology 41(6):778–785.

Kastenbauer S, Angele B, Sporer B, Pfister HW, Koedel U. 2005. Patterns of protein expression in infectious meningitis: a cerebrospinal fluid protein array analysis. J Neuroimmunol 164(1–2):134–139.

Katz M. 1995. Clinical spectrum of measles. Curr Top Microbiol Immunol 191:1–12.

Kaul M, Zheng J, Okamoto S, Gendelman HE, Lipton SA. 2005. HIV-1 infection and AIDS: consequences for the central nervous system. Cell Death Differ 12 Suppl 1:878–892.

Kielian T. 2004a. Immunopathogenesis of brain abscess. J Neuroinflammation 1(1):16.

Kielian T. 2004b. Microglia and chemokines in infectious diseases of the nervous system: views and reviews. Front Biosci 9:732–750.

Kielian T, Barry B, Hickey WF. 2001. CXC chemokine receptor-2 ligands are required for neutrophil-mediated host defense in experimental brain abscesses. J Immunol 166(7):4634–4643.

Klein RS, Lin E, Zhang B, Luster AD, Tollett J, Samuel MA, Engle M, Diamond MS. 2005. Neuronal CXCL10 directs CD8+ T-cell recruitment and control of West Nile virus encephalitis. J Virol 79(17):11457–11466.

Koedel U, Rupprecht T, Angele B, Heesemann J, Wagner H, Pfister HW, Kirschning CJ. 2004. MyD88 is required for mounting a robust host immune response to Streptococcus pneumoniae in the CNS. Brain 127(Pt 6):1437–1445.
Koedel U, Scheld WM, Pfister HW. 2002. Pathogenesis and pathophysiology of pneumococcal meningitis. *Lancet Infect Dis* 2(12):721–736.

Kornelisse RF, Hazenletz JA, Hop WC, Spanjaard L, Suur MH, van der Voort E, de Groot R. 1997. Meningococcal septic shock in children: clinical and laboratory features, outcome, and development of a prognostic score. *Clin Infect Dis* 25(3):640–646.

Lahartz F, Piali L, Spanaus KS, Seebach J, Fontana A. 1998. Chemokines and chemotaxis of leukocytes in infectious meningitis. *J Neuroimmunol* 85(1):33–43.

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(2):970–978.

Lane TE, Hardison JL, Walsh KB. 2006. Functional diversity of chemokines and chemokine receptors in response to viral infection of the central nervous system. *Curr Top Microbiol Immunol* 303:1–27.

Le Y, Zhou Y, Iribarren P, Wang J. 2004. Chemokines and chemokine receptors: their manifold roles in homeostasis and disease. *Cell Mol Immunol* 1(2):95–104.

Lim JK, Glass WG, McDermott DH, Murphy PM. 2006. CCR5: no longer a “good for nothing” gene–chemokine control of West Nile virus infection. *Trends Immunol* 27(7):308–312.

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(5):2327–2330.

Liu MT, Chen BP, Oertel P, Buchmeier MJ, Hamilton TA, Armstrong DA, Lane TE. 2001a. The CXC chemokines IP-10 and Mig are essential in host defense following infection with a neurotropic coronavirus. *Adv Exp Med Biol* 494:323–327.

Liu MT, Keirstead HS, Lane TE. 2001b. 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(7):4091–4097.

Looker KJ, Garnett GP. 2005. A systematic review of the epidemiology and interaction of herpes simplex virus types 1 and 2. *Sex Transm Infect* 81(2):103–107.

Luster AD. 1998. Chemokines – chemotactic cytokines that mediate inflammation. *N Engl J Med* 338(7):436–445.

Maertzdorf J, Osterhaus AD, Verjans GM. 2002. IL-17 expression in human herpetic stromal keratitis: modulatory effects on chemokine production by corneal fibroblasts. *J Immunol* 169(10):5897–903.

Manchanda V, Gupta S, Bhalla P. 2006. Meningococcal disease: history, epidemiology, pathogenesis, clinical manifestations, diagnosis, antimicrobial susceptibility and prevention. *Indian J Med Microbiol* 24(1):7–19.

Manchester M, Eto DS, Oldstone MB. 1999. Characterization of the inflammatory response during acute measles encephalitis in NSE-CD46 transgenic mice. *J Neuroimmunol* 96(2):207–217.

Marder K, Albert S, Dooneief G, Stern Y, Ramachandran G, Epstein L. 1996. Clinical confirmation of the American Academy of Neurology algorithm for HIV-1-associated cognitive/motor disorder. The Dana Consortium on Therapy for HIV Dementia and Related Cognitive Disorders. *Neurology* 47(5):1247–1253.

Masters PS. 2006. The molecular biology of coronaviruses. *Adv Virus Res* 66:193–292.

Mastroianni CM, Lancell L, Mengoni F, Lichtner M, Santopadre P, D’Agostino C, Ticca F, Vullo V. 1998. Chemokine profiles in the cerebrospinal fluid (CSF) during the course of pyogenic and tuberculous meningitis. *Clin Exp Immunol* 114(2):210–214.

McIntosh K. 1996. Coronaviruses. In *Fields Virology*. 3rd edition. Vol. 1, eds. BN Fields, DM Knipe, PM Howley, pp. 401–430. Philadelphia, PA: Lippincott-Raven Publishers.

McQuaid S, Cosby SL. 2002. An immunohistochemical study of the distribution of the measles virus receptors, CD46 and SLAM, in normal human tissues and subacute sclerosing panencephalitis. *Lab Invest* 82(4):403–9.

Mizuno T, Kawanokuchi J, Numata K, Suzumura A. 2003. Production and neuroprotective functions of fractalkine in the central nervous system. *Brain Res* 979(1–2):65–70.
Moller AS, Bjerre A, Brusletto B, Joo GB, Brandtzaeg P, Kierulf P. 2005. Chemokine patterns in meningococcal disease. J Infect Dis 191(5):768–775.
Morrison TB, Weis JH, Weis JJ. 1997. Borrelia burgdorferi outer surface protein A (OspA) activates and primes human neutrophils. J Immunol 158(10):4838–4845.
Murray PR, Pfaller MA, Rosenthal KS. 2005. Medical Microbiology. Elsevier Health Sciences, pp. 1–962. Amsterdam, The Netherlands.
Musher DM. 1992. Infections caused by Streptococcus pneumoniae: clinical spectrum, pathogenesis, immunity, and treatment. Clin Infect Dis 14(4):801–807.
Nagano I, Nakamura S, Yoshioka M, Onodera J, Kogure K, Itoyama Y. 1994. Expression of cytokines in brain lesions in subacute sclerosing panencephalitis. Neurology 44(4):710–715.
Nansen A, Marker O, Bartholdy C, Thomsen AR. 2000. CCR2 + and CCR5 + CD8 + T cells increase during viral infection and migrate to sites of infection. Eur J Immunol 30(7):1797–1806.
Nansen A, Christensen JP, Andreasen SO, Bartholdy C, Christensen JE, Thomsen AR. 2002. The role of CC chemokine receptor 5 in antiviral immunity. Blood 99(4):1237–1245.
Nau R, Bruck W. 2002. Neuronal injury in bacterial meningitis: mechanisms and implications for therapy. Trends Neurosci 25(1):38–45.
Navia BA, Cho ES, Petito CK, Price RW. 1986. The AIDS dementia complex: II. Neuropathology. Ann Neurol 19(6):525–535.
Noe KH, Cenciarelli C, Moyer SA, Rota PA, Shin ML. 1999. Requirements for measles virus induction of RANTES chemokine in human astrocytoma-derived U373 cells. J Virol 73(4):3117–3124.
Oleszak EL, Chang JR, Friedman H, Katsetos CD, Platsoucas CD. 2004. Theiler’s virus infection: a model for multiple sclerosis. Clin Microbiol Rev 17(1):174–207.
Ostergaard C, Yeng-Kow RV, Larsen CG, Mukaida N, Matsushima K, Benfield T, Frimodt-Moller N, Espersen F, Kharazmi A, Lundgren JD. 2000. Treatment with a monoclonal antibody to IL-8 attenuates the pleocytosis in experimental pneumococcal meningitis in rabbits when given intravenously, but not intracisternally. Clin Exp Immunol 122(2):207–211.
Pfister HW, Scheld WM. 1997. Brain injury in bacterial meningitis: therapeutic implications. Curr Opin Neurol 10(3):254–259.
Robinson KA, Baughman W, Rothrock G, Barrett NL, Pass M, Lexau C, Damaske B, Stefonek K, Barnes B, Patterson J and others. 2001. Epidemiology of invasive Streptococcus pneumoniae...
infections in the United States, 1995–1998: Opportunities for prevention in the conjugate vaccine era. JAMA 285(13):1729–1735.

Saruhan-Direskeneli G, Gurses C, Demirbilek V, Yentur SP, Yilmaz G, Onal E, Yapici Z, Yalcinkaya C, Cokar O, Akman-Demir G and others. 2005. Elevated interleukin-12 and CXCL10 in subacute subcutaneous panencephalitis. Cytokine 32(2):104–110.

Scheld WM, Koedel U, Nathan B, Pfister HW. 2002. Pathophysiology of bacterial meningitis: mechanism(s) of neuronal injury. J Infect Dis 186 Suppl 2:S225–S233.

Schnorr JJ, Xanthakos S, Keikavoussi P, Kampgen E, ter Meulen V, Schneider-Schaulies S. 1997. Induction of maturation of human blood dendritic cell precursors by measles virus is associated with immunosuppression. Proc Natl Acad Sci U S A 94(10):5326–5331.

Schuchat A, Robinson K, Wenger JD, Harrison LH, Farley M, Reingold AL, Lefkowitz L, Perkins BA. 1997. Bacterial meningitis in the United States in 1995. Active Surveillance Team. N Engl J Med 337(14):970–976.

Seebach J, Bartholdi D, Frei K, Spanaus KS, Ferrero E, Widmer U, Isenmann S, Strieter RM, Schwab M, Pfister H and others. 1997. Experimental Listeria meningoencephalitis. Macrophage inflammatory protein-1 alpha and -2 are produced intrathecally and mediate chemotactic activity in cerebrospinal fluid of infected mice. J Immunol 155(9):4367–4375.

Sejvar JJ, Marfin AA. 2006. Manifestations of West Nile neuroinvasive disease. Rev Med Virol 16(4):209–224.

So EY, Kang MH, Kim BS. 2006. Induction of chemokine and cytokine genes in astrocytes following infection with Theiler’s murine encephalomyelitis virus is mediated by the Toll-like receptor 3. Glia 53(8):858–867.

Solomon T, Winter PM. 2004. Neurovirulence and host factors in flavivirus encephalitis – evidence from clinical epidemiology. Arch Virol Suppl(18):161–170.

Spanaus KS, Nadal D, Pfister HW, Seebach J, Widmer U, Frei K, Glooor S, Fontana A. 1997. C-X-C and C-C chemokines are expressed in the cerebrospinal fluid in bacterial meningitis and mediate chemotactic activity on peripheral blood-derived polymorphonuclear and mononuclear cells in vitro. J Immunol 158(4):1956–1964.

Sprenger H, Rosler A, Tonn P, Braune HJ, Huffmann G, Gemsa D. 1996. Chemokines in the cerebrospinal fluid of patients with meningitis. Clin Immunol Immunopathol 80(2):155–161.

Sprenger H, Krause A, Kaufmann A, Priem S, Fabian D, Burmester GR, Gemsa D, Rittig MG. 1997. Borrelia burgdorferi induces chemokines in human monocytes. Infect Immun 65(11):4384–4388.

Standiford TJ, Kunkel SL, Lukacs NW, Greenberger MJ, Danforth JM, Kunkel RG, Strieter RM. 1995. Macrophage inflammatory protein-1 alpha mediates lung leukocyte recruitment, lung capillary leak, and early mortality in murine endotoxemia. J Immunol 155(3):1515–1524.

Stiles LN, Hardison JL, Schuamburg CS, Whitman LM, Lane TE. 2006a. T cell anti-viral effector function is not dependent on CXCL10 following murine coronavirus infection. J Immunol 177:8372–8380.

Stiles LN, Hosking MP, Edwards RA, Strieter RM, Lane TE. 2006b. Differential roles for CXCR3 in CD4+ and CD8+ T cell trafficking following viral infection of the CNS. Eur J Immunol 36(3):613–622.

Su YH, Yan XT, Oakes JE, Lausch RN. 1996. Protective antibody therapy is associated with reduced chemokine transcripts in herpes simplex virus type 1 corneal infection. J Virol 70(2):1277–1281.

Suzuki T, Ogata A, Tashiro K, Nagashima K, Tamura M, Yasui K, Nishihiira J. 2000. Japanese encephalitis virus up-regulates expression of macrophage migration inhibitory factor (MIF) mRNA in the mouse brain. Biochim Biophys Acta 1517(1):100–106.

ter Meulen V, Stephenson JR, Kreth HW. 1983. Comprehensive Virology. New York, NY: Plenum Press, pp. 105–159.
Tishon A, Lewicki H, Andaya A, McGavern D, Martin L, Oldstone MB. 2006. CD4 T cell control primary measles virus infection of the CNS: regulation is dependent on combined activity with either CD8 T cells or with B cells: CD4, CD8 or B cells alone are ineffective. *Virology* 347(1):234–245.

Tong N, Perry SW, Zhang Q, James HJ, Guo H, Brooks A, Bal H, Kinnear SA, Fine S, Epstein LG and others. 2000. Neuronal fractalkine expression in HIV-1 encephalitis: roles for macrophage recruitment and neuroprotection in the central nervous system. *J Immunol* 164(3):1333–1339.

Townsend GC, Scheld WM. 1998. Infections of the central nervous system. *Adv Intern Med* 43:403–447.

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(7):4004–4014.

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(1):8–15.

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(1):26–34.

Ure DR, Lane TE, Liu MT, Rodriguez M. 2005. Neutralization of chemokines RANTES and MIG increases virus antigen expression and spinal cord pathology during Thielers’s virus infection. *Int Immunol* 17(5):569–579.

Wickham S, Lu B, Ash J, Carr DJ. 2005. Chemokine receptor deficiency is associated with increased chemokine expression in the peripheral and central nervous systems and increased resistance to herpetic encephalitis. *J Neuroimmunol* 162(1–2):51–59.

Winter PM, Dung NM, Loan HT, Kneen R, Wills B, Thu le T, House D, White NJ, Farrar JJ, Hart CA and others. 2004. Proinflammatory cytokines and chemokines in humans with Japanese encephalitis. *J Infect Dis* 190(9):1618–1626.

Wuest T, Austin BA, Uematsu S, Thapa M, Akira S, Carr DJ. 2006. Intact TRL 9 and type I interferon signaling pathways are required to augment HSV-1 induced corneal CXCL9 and CXCL10. *J Neuroimmunol* 179(1–2):46–52.

Xiao BG, Mousa A, Kivisakk P, Seiger A, Bakhiet M, Link H. 1998. Induction of beta-family chemokines mRNA in human embryonic astrocytes by inflammatory cytokines and measles virus protein. *J Neurocytol* 27(8):575–580.

Xu J, Zhong S, Liu J, Li L, Li Y, Wu X, Li Z, Deng P, Zhang J, Zhong N and others. 2005. Detection of severe acute respiratory syndrome coronavirus in the brain: potential role of the chemokine mig in pathogenesis. *Clin Infect Dis* 41(8):1089–1096.

Yan XT, Tumpey TM, Kunkel SL, Oakes JE, Lausch RN. 1998. Role of MIP-2 in neutrophil migration and tissue injury in the herpes simplex virus-1-infected cornea. *Invest Ophthalmol Vis Sci* 39(10):1854–1862.

Zink MC, Coleman GD, Mankowski JL, Adams RJ, Tarwater PM, Fox K, Clements JE. 2001. Increased macrophage chemotactant protein-1 in cerebrospinal fluid precedes and predicts simian immunodeficiency virus encephalitis. *J Infect Dis* 184(8):1015–1021.

Zwijnenburg PJ, van der Poll T, Florquin S, van Deventer SJ, Roord JJ, van Furth AM. 2001. Experimental pneumococcal meningitis in mice: a model of intranasal infection. *J Infect Dis* 183(7):1143–1146.