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Lymphoid Chemokines in the CNS

Stephen J. Lalor and **Benjamin M. Segal***
Holtom-Garrett Program in Neuroimmunology, Department of Neurology, University of Michigan, 4013 BSRB, 109 Zina Pitcher Place, Ann Arbor, MI 48109-2200, USA

Abstract

Lymphoid chemokines, including CCL19, CCL21 and CXCL13, are critical in the development and organization of secondary lymphoid tissues and in the generation of adaptive immune responses. These molecules have also been implicated in the development of ectopic lymphoid structures in the setting of chronic inflammation. Here we review current knowledge on the production of lymphoid chemokines in the central nervous system during both homeostatic conditions and in disease states. Accumulating evidence suggests that constitutive expression of CCL19 plays a critical immunosurveillance role in healthy individuals. In contrast, aberrant induction of CCL19, CCL21 and CXCL13 may support the establishment of chronic autoimmunity and hematopoetic tumors within the CNS.

Keywords

Chemokines; experimental autoimmune encephalomyelitis; multiple sclerosis; Primary CNS lymphoma; lymphoid neogenesis; neuroborreliosis

1. Introduction

The lymphoid chemokines, CCL19, CCL21 and CXCL13, constitute a subclass of chemoattractant molecules originally identified based on their roles in the development and maintenance of secondary lymphoid tissues. These chemokines are constitutively expressed in lymph nodes, spleen and Peyer's patches. They regulate the homing of lymphocytes and myeloid cells to spatially segregated compartments within secondary lymphoid tissues, thereby facilitating cognate antigen-specific interactions and the generation of adaptive immune responses (Yoshie *et al*., 1997; Zlotnik *et al*., 1999; Mantovani, 1999).

CCL19 and CCL21 are produced by stromal cells in the T cell zones of lymph nodes and spleen. CCL21 is also expressed in high endothelial venules (HEVs) and lymphatic vessels (Cyster *et al*., 1999). Both of these chemokines bind to CCR7, which is expressed on the surface of naive T cells and mature dendritic cells (DC). CCL21 also binds CXCR3 with lower affinity. CXCL13 is produced by follicular DC within B cell follicles and binds to the receptor, CXCR5. It drives the migration of B cells and $CD4^+$ helper T cells (follicular Th cells) to B cell rich areas (Förster *et al*., 1996; Legler *et al*., 1998; Ansel *et al*., 2000; Moser *et al*., 2002; Müller *et al*., 2003). Hence, CCL19 and CCL21 expedite antigen-specific $CD4^+$

* *Tel*.: +1 734 615 5635; *Fax*: +1 734 615 7300 bmsegal@med.umich.edu.

T cell immunity, while CXCL13 is critical for normal germinal center formation as well as for efficient immunoglobulin cross-switching and affinity maturation (Junt *et al.* 2005).

In addition to their role in the development and maintenance of secondary lymphoid tissues, CCL19, CCL21 and CXCL13 can be induced in non-lymphoid tissues during chronic inflammation. In this setting they have been implicated in the process of lymphoid neogenesis – the organization of infiltrating immune cells and stromal elements into structured aggregates that recapitulate many of the features of secondary lymphoid tissues (Hjelmstrom *et al*., 2001). When formed in response to persistent infection, these ectopic lymphoid structures have been shown to contain the spread of microbes (Halle *et al*., 2009; Moyron-Quiroz *et al*., 2004). However, lymphoid neogenesis also has the potential to aggravate autoimmune inflammation via the recruitment and activation of autoantigen-specific T and B lymphocytes within the target organ (Aloisi and Pujol-Borrell, 2006). Ectopic lymphoid follicles have been found in the salivary glands in Sjogren's syndrome (Salomonsson *et al*., 2002), the synovium in rheumatoid arthritis (Takemura *et al*., 2001) and the thyroid gland in Hashimoto's thyroiditis (Hjelmstrom *et al*., 2001).

Here, we will review the literature concerning the production of lymphoid chemokines in the central nervous system (CNS), both during homeostasis and in disease states. In certain circumstances, CNS lymphoid chemokines act to the benefit of the host. For example, there is growing evidence that constitutive expression of CCL19 and CCL21 in cerebrovascular endothelium and the choroid plexus plays a critical role in immune surveillance of the subarachnoid and perivascular spaces of healthy individuals. In addition, induction of lymphoid chemokines in CNS tissues might participate in the clearance of local infections, such as neuroborreliosis and Toxoplasmosis gondii. Conversely, aberrant production of CCL19, CCL21 and CXCL13 may support the establishment of hematopoetic tumors or chronic autoimmunity in the brain and spinal cord.

2. CCR7-CCL19 interactions in immune surveillance of the CNS

Low numbers of T cells are consistently found in normal human and rat brains and cerebrospinal fluid (CSF)(Booss *et al*., 1983; Wekerle *et al*, 1987), indicating that the CNS is continuously patrolled by activated T cells. The importance of such immune surveillance is suggested by the emergence of opportunistic infections and tumors in the brains and spinal cords of immunosuppressed individuals (Snider *et al*., 1983; Schneck and Penn, 1971). Disruption of leukocyte trafficking to the CNS by adhesion molecule blockade in patients with multiple sclerosis (MS) increases the risk of progressive multifocal leukoencephalopathy, a demyelinating disease mediated by the JC virus (Langer-Gould *et al*., 2005). Furthermore, primary CNS lymphoma occurs in the context of AIDS as well as global immunosuppressive treatments to prevent transplant rejection (Snider *et al*., 1983; Schneck and Penn, 1971). Still, relatively little is known about the chemokines that regulate leukocyte migration to the uninflamed CNS.

Lymphocyte trafficking through the intact blood brain barrier (BBB) is tightly controlled by specialized cerebrovascular endothelial cells and astrocytic processes. The presence of the BBB and the absence of lymphatic vessels has led some to view the CNS as an immunologically privileged site. However, adoptive transfer studies have shown that activated T cells access the CNS as readily as other tissues (Hickey *et al*., 1991; Wekerle *et al*., 1987). In addition to traversing the cerebrovascular endothelium, leukocytes could bypass the BBB by entering the subarachnoid space via the meningeal veins or the choroid plexus (Krumbholz *et al*., 2007; Kivisakk *et al*., 2004). Accumulating evidence indicates that these are the primary routes by which lymphocytes survey the CNS during homeostasis

and that CCL19/ CCR7 interactions regulate this process (Axtell *et al*., 2009; Kivisaak *et al*., 2003).

More than 80% of cells in the CSF of healthy individuals are central memory T (T_{CM}) cells that express high levels of CCR7, CD27, CD45RO and L-selectin (Giunti *et al*., 2003; Kivisakk *et al*., 2003; Kivisakk *et al*., 2004). The presence of L-selectin and CCR7 suggests these cells are not terminally committed effector T cells (T_{EFF}) but instead retain the ability to recirculate to secondary lymphoid organs. CCL19 has been detected in the CSF of healthy humans (Krumbholz *et al*., 2007; Pashenkov *et al*., 2003). Furthermore, CCL21 is constitutively expressed in the choroid plexus (Kivisakk *et al*., 2004). Taken together, these data support the hypothesis that T_{CM} enter the subarachnoid space in response to CCL19/ CCL21 gradients to carry out routine surveillance. If a patrolling T_{CM} encounters its cognate antigen presented by a resident antigen presenting cell, it will downregulate CCR7 and CD27 and upregulate CCR5 and CXCR3, evolving to an effector phenotype (T_{EFF}) with the ability to traverse the glia limitans and infiltrate the parenchyma (Giunti *et al*., 2003; Kivisakk *et al*., 2003). In support of this model, T_{EFF} accumulate in active demyelinating lesions but are sparse in the CSF of healthy individuals and MS patients (Giunti *et al*., 2003; Kivisakk *et al*., 2004; Hintzen *et al*., 1995).

3. Lymphoid chemokines in non-inflammatory neurological disease

A. CCL21/CXCR3 interactions in cerebrovascular ischemia and glutamate mediated excitotoxicity

As will be discussed below, induction of lymphoid chemokines in the CNS is most prominently associated with inflammatory processes, such as autoimmune demyelinating disease and Lyme neuroborreliosis. However, non-inflammatory insults can also trigger their expression by CNS resident cells. Hence, CCL21 mRNA is rapidly and persistently upregulated in coritcal neurons in response to ischemic injury (Biber *et al*., 2001). Consistent with these findings, CCL21 is expressed in cultured neurons, but not in astrocytes or microglia, that have been subjected to various treatments known to induce cell death. Moreover, the isoform of CCL21 expressed by neurons in the ischemic CNS or in culture is identical to that expressed by stromal cells in the T cell rich areas of the spleen and lymph nodes (Vassileva *et al*., 1999; Rappert *et al*., 2002). The related chemokine, CCL19, which shows a similar expression pattern to CCL21 in peripheral lymphoid organs, is not induced in response to ischemic insult (Biber *et al*., 2001).

The main target of the neuronal derived CCL21 appears to be CXCR3 expressing glial cells. CCR7, the principal receptor for CCL19 and CCL21 (Yoshida *et al*., 1997; Yoshida *et al*., 1998), is not present on cultured glia or neurons, or in ischemic brain tissue (Biber *et al*., 2001). Given the lack of CCR7 on infiltrating leukocytes, it is unlikely that CCL21 upregulation mediates their recruitment from the circulation to the ischemic brain. In contrast, CXCR3, which also binds CCL21 (Soto *et al*., 1998), is expressed on both cultured microglia as well as in ischemic brain tissue (Biber *et al*., 2001). CCL21 exerts direct biological effects on microglia via a CXCR3 dependent pathway. Hence, Kettenmann and colleagues reported that CCL21 triggers a chloride current and chemotaxis in cultured murine microglia (Rappert *et al*., 2002). Microglia from CXCR3-deficient, but not CCR7-deficient, mice were unresponsive to CCL21. Furthermore, CCL19, which binds CCR7 but not CXCR3, had no effect on the microglia.

Glutamate mediated excitotoxicity is an important mechanism that underlies neuronal death in stroke (Castillo *et al*., 1996). It was recently demonstrated that cultured cortical neurons and hippocampal neurons in organotypic slices release CCL21 following exposure to high concentrations of glutamate (de Jong *et al*., 2005). These investigators found that CCL21 is

stored in secretory granules that are transported along neuronal processes to presynaptic structures. Furthermore, microglia migrate towards supernatants from glutamate-treated neuronal cultures. Interestingly, microglia cultured from CXCR3-deficient mice can still migrate towards these supernatants, albeit less effectively, indicating the presence of as yet unidentified factors released by the neurons that act as chemoattractants for microglia (de Jong *et al*., 2005). Collectively, these data indicate that production and release of CCL21 by neurons is a stereotypic response to excitotoxic insults and provides a bridge between neuronal stress and microglial activation and chemotaxis. Furthermore, axonal transport of CCL21 in secretory granules suggests a mechanism for the activation of microglia remote from the primary lesion, a phenomenon that has been observed in both stroke and traumatic brain and spinal cord injury (Williams, *et al.* 2007).

B. Lymphoid chemokines in hematopoetic tumors of the CNS

A growing body of data suggests that local production of lymphoid chemokines plays an important role in the establishment and/or maintenance of hematopoietic tumors within the CNS. The specific chemokine(s) involved depend on the cell of origin of the neoplasm. Hence, CCL19 was recently implicated in CNS infiltration by T cell acute lymphoblastic leukemia (T-ALL), while CXCL13 has been associated with the development of diffuse large B cell lymphomas in the CNS (Buonamici *et al*., 2009; Smith *et al*., 2003; Tun *et al*., 2008).

It has long been observed that T-ALL tends to relapse in the CNS. However, the factors controlling T-ALL cell migration across the BBB remain poorly understood. Recently, leptomeningeal invasion by leukemic cells was shown to be CCR7-dependent in two animal models of T-ALL (Buonamici *et al*., 2009). Here, CCR7 expression is driven by the T-ALL oncogene, Notch-1. Similarly, CCR7 is expressed in the 80% or more of the human T-ALL tumors that carry Notch 1 activating mutations. CCL19 was detected in endothelial cells of brain venules in the vicinity of the infiltrating cancer cells. Deficiency of either CCR7 or CCL19 prevented the recruitment of cancer cells to the CNS. Notably, CCR7 expression did not correlate with neoplastic infiltration of non-CNS tissues, and CCR7 expression was not required for CNS involvement in mouse models of B cell ALL (Buonamici *et al*., 2009).

Primary CNS lymphoma (PCNSL) is a diffuse large B cell lymphoma that is confined to the CNS. In several studies, development of this rare tumor has been associated with ectopic expression of CXCL13 in the brain. Smith and colleagues found that CXCL13 was expressed in all 24 PCNSL specimens, but none of the healthy brain biopsy specimens they examined (Smith *et al*., 2003). Immunohistochemical staining localized the chemokine to the leukemic cells themselves as well as to the cerebrovascular endothelium. However, *in situ* hybridization pinpointed the leukemic cells as the primary source. Tumor cells stained positively for CXCR5, the primary receptor for CXCL13. In a complementary study, CXCL13 was found to be elevated in the CSF of patients with PCNSL compared to patients with other CNS malignancies or with systemic lymphomas without CNS involvement (Fischer *et al*., 2009). CSF CXCL13 levels fell in a small number of patients who responded to chemotherapy. Finally, microarray analyses identified CXCL13 and regulator of G-protein signaling 13 (RGS13), a modulator of CXCR5 signaling, as molecules upregulated in PCNSL in comparison to non-CNS diffuse B cell lymphoma (Tun *et al*., 2008). The question of whether CXCL13 alters the survival or spread of the neoplastic B cells, or represents an epiphenomenon, remains unanswered.

4. Lymphoid chemokines in infections of the CNS

Lyme neuroborreliosis (NB) is a CNS infection caused by the spirochete *Borrelia burgdoferi* (*B. burgdoferi*). It can manifest as a basilar meningitis with cranial neuropathies,

radiculitis, transverse myelitis, or encephalitis causing focal white matter lesions (Ruppercht *et al.*, 2008). Myeloid cells are a major source of CNS CXCL13 in animal models of NB. CXCL13 has been localized to microglia and infiltrating macrophages/ DC in brain and spinal cord sections of rhesus macaques with acute NB (Ramesh *et al.*, 2009; Narayan *et al.*, 2005). Interestingly, sonicates of *B. burgdoferi* stimulate human myeloid and plasmacytoid DC to produce CXCL13 *in vitro* (Narayan *et al.*, 2005).

Multiple laboratories have found that CXCL13 is significantly elevated in the CSF of patients with NB compared to other neurological diseases, including Guillane Barre syndrome and Bell's palsy (Ruppercht *et al.*, 2009; Senel *et al.*, 2009). There is no correlation between CSF CXCL13 and serum CXCL13 or measures of BBB disruption, indicating that the chemokine is produced intrathecally. In a recent study, CSF CXCL13 revealed a higher combined sensitivity and specificity for the diagnosis of NB than any other parameter investigated (Senel *et al.*, 2009). Its level fell in response to antibiotic treatment faster than the other parameters. In a separate study, B cell chemotaxis towards CSF from patients with acute NB was reduced over 50% when the samples were preincubated with an anti-CXCL13 neutralizing antibody (Ruppercht *et al.*, 2009). This suggests that CXCL13 may be involved in the recruitment of B cells to the subarachnoid space of *B. burgdoferi* infected patients.

CCL19 and CCL21 have clearly been demonstrated to play a protective role in an animal model of another CNS infection. Hence, transcripts encoding both chemokines rise significantly in the CNS following infection of mice with the protozoan parasite, Toxoplasmosis gondii (Noor *et al*., 2010). CCR7-deficient mice succumb to disease early in association with increased parasite burden in the brain. Their increased susceptibility may be due to impaired priming of effector T cells in the periphery as well as to a reduction in leukocyte trafficking to the brain, since serum IFNγ levels are reduced in the infected knock-out mice.

5. CCL19/CCL21 expression in neuroinflammation associated with autoimmune demyelination

CCL19 and CCL21 have been detected in inflamed CNS venules and in perivascular cuffs in EAE lesions, both by *in situ* hybridization and immunohistochemistry (Alt *et al*., 2002; Columba-Cabezas *et al.*, 2003). Several groups have reported that CCL19, CCL21 and CCR7 levels rise in the spinal cord in association with clinical relapses and chronic progression of EAE (Bagaeva *et al*., 2006; Columba-Cabezas *et al*., 2003; Dijkstra *et al*., 2006). In parallel, $CCR7^+$ cells accumulate within inflammatory cuffs and meningeal infiltrates (Alt *et al*., 2002; Columba-Cabezas *et al.*, 2003). Encephalitogenic lymphoblasts express CCR7 and CXCR3 proteins on the cell surface, migrate towards CCL19 and CCL21 *in vitro*, and specifically bind to exposed cerebral vessels in frozen sections of EAE brains in a CCR7/ CXCR3 dependent manner. CCR7 is also upregulated on activated microglia in white matter adjacent to active EAE lesions (Dijkstra *et al*., 2006), and cultured microglia upregulate CCR7 and migrate towards CCL21 following treatment with the TLR4 agonist, LPS (Dijkstra *et al*., 2004). By extension, proinflammatory factors might induce CCR7 expression by microglia *in vivo*.

Similar observations have been made in MS specimens. Hence, CCL19 transcripts are elevated in both active and inactive lesions (Krumbholz *et al.* 2007), and CCL19 concentrations are elevated in the CSF from MS patients compared to controls (Pashenkov *et al*., 2003 Krumbholz *et al*., 2007; Giunti *et al*., 2003). CCL21 expression has also been described in the choroid plexus of MS specimens (Kivisakk *et al*., 2004; Krumbholz *et al*., 2007). CSF CCL19 levels correlate with intrathecal IgG production, suggesting that CCL19

may play a role in the maintenance or expansion of B cells in the MS brain (Krumbholz *et al*., 2007; Pashenkov *et al*., 2003). Interestingly, lymphocytes infiltrating MS lesions do not express CCR7, although microglia and infiltrating DC do (Kivisakk *et al*., 2004; Serafini *et al*., 2006). This may be secondary to downregulation of the receptor following the migration of lymphocytes across the BBB. In support of this theory, CCR7 is consistently found on $CD4^+$ memory T cells in the CSF of healthy individuals and of patients with MS, prior to their conversion to effector cells. CCR7 is also expressed by a subset of DC in the CSF of MS patients (Krumbholz *et al*., 2007; Kivisakk *et al*., 2004).

While the above findings support a role for CCL19 and/ or CCL21 in lymphocyte recruitment and microglial activation/ chemotaxis during autoimmune demyelinating disease, a cause-and-effect relationship has not been established. In fact, transgenic expression of CCL21 or CCL19 in oligodendrocytes did not result in lymphocyte recruitment or lymphoid neogenesis in white matter (Chen *et al*., 2002). However, transgenic expression of CCL21 did cause a severe neuropathological condition, characterized by neutrophil and eosinophil infiltration, hypomyelination, spongiform myelinopathy, myelin breakdown and reactive astrogliosis. These mice died within the first four weeks of life (Chen *et al*., 2002). One explanation for this unexpected phenotype is that CCL21 constitutively produced by oligodendrocytes does not simulate the biological functions of the chemokine induced in endothelial cells during neuroinflammation. Pathological changes were not observed in the CNS of CCL19 transgenic mice, suggesting a role for CXCR3, rather than CCR7, in mediating the effects of oligodendrocyte derived CCL21.

6. CXCL13 in autoimmune demyelination

CXCL13 is not expressed in the healthy CNS. However, we and others have found that it is upregulated in the spinal cord of mice with EAE (Bagaeva *et al*., 2006; Magliozzi *et al*., 2004). This observation was consistent across several different mouse strains, autoantigens and methods of disease induction. CXCL13 is first detectable in the CNS at the onset of the initial clinical episode. Its levels rise during subsequent relapses and during chronic progression. We showed that both CXCL13 deficiency and CXCL13 neutralization reduce the severity of the early clinical course (Bagaeva *et al*., 2006). The role of CXCL13 at this stage of disease does not seem to be recruitment of leukocytes to the CNS, since the frequency of infiltrating $CXCR5^+$ leukocytes did not differ between myelin immunized CXCL13 deficient and wildtype mice. Our studies indicated that infiltrating myeloid DC are at least one source of CXCL13 in the inflamed CNS. This observation lead us to postulate that CXCL13 induces the migration of encephalitogenic T cells towards DC in the CNS, thereby increasing the frequency of their cognate interaction within the target organ. Myeloid DC have been described as highly efficient antigen presenting cells in the CNS during EAE (McMahon *et al*., 2005).

Later in the disease course, CXCL13 might assume the alternative role of initiating lymphoid neogenesis. CXCL13-expressing lymphoid follices were detected in the meninges of Biozzi mice during the chronic progressive stage of EAE (Magliozzi *et al*., 2004). These follicles contained B cells and a network of $CXCL13^+$ and $FDC\text{-}M1^+$ follicular DC. Blockade of $LT\alpha1\beta2$ (LTβ, which is expressed on B cells and subsets of encephalitogenic T cells) suppressed clinical EAE and prevented the induction of CXCL13 as well as the formation of organized ectopic lymphoid follicles in the CNS of myelin-immunized mice (Columba-Cabezas *et al*., 2006). These observations suggest that, analogous to the regulation of CXCL13 in secondary lymphoid tissues, engagement of LTβ receptor on stromal cells by LTβ on lymphocytes, induces CXCL13 expression in the CNS.

Similar observations have been made in MS. Hence, LT and CXCL13 are upregulated in the CSF of MS patients compared to healthy controls or patients with non-inflammatory neurological diseases (Corcione *et al*., 2004; Krumbholz *et al*., 2006; Sellebjerg *et al*., 2009). CSF CXCL13 expression correlated with other indices of inflammation, including the presence of B cells, plasmablasts, and T cells and intrathecal immunoglobulin synthesis (Krumbholz *et al*., 2006; Sellebjerg *et al*., 2009). The CXCL13 receptor, CXCR5, was expressed on all CSF B cells and approximately 20% of CSF T cells (Krumbholz *et al*., 2006). Furthermore, serum CXCL13 fluctuated in association with radiological disease activity (Festa *et al*., 2009). Conversely, CXCL13 levels and CSF B cell counts fell after treatment of MS patients with natalizumab or methylprednislone (Sellebjerg *et al*., 2009; Stüve *et al*., 2006; Krumbholz *et al*., 2008). If the above studies are reproduced in additional cohorts of patients, CSF and/ or serum CXCL13 might become useful as a biomarker of MS disease activity.

In complementary studies, CXCL13 was detected in active, but not inactive, MS lesions (Corcione *et al*., 2004; Krumbholz *et al*., 2006; Sellebjerg *et al*., 2009). CXCL13 expression localized to infiltrating immune cells, particularly macrophages, both in the perivascular cuffs and within the parenchyma. Similar to the findings in Biozzi mice with chronic EAE, lymphoid follicles were discovered in the meninges of 40% of brain autopsy specimens from individuals with secondary progressive MS (Serafini *et al*., 2004; Magliozzi *et al*., 2007). These follicles contained a well developed network of proliferating B cells, plasma cells, T cells and CXCL13-expressing follicular DC in close association with inflamed meningeal blood vessels. Future studies will be required to determine whether the immunoglobulins produced in the meningeal follicles (or less structured B cell aggregates during earlier phases of MS) are the main source of oligoclonal bands in the CSF, a frequent finding in MS.

In every instance, ectopic follicles were located deep within the sulci, adjacent to underlying cortical plaques. Extensive subpial demyelination, pronounced microglial activation and neurite loss and an increased number of active lesions were present within the cortical areas adjacent to ectopic B-cell follicles (Magliozzi *et al*., 2007). This finding supports the view that ectopic follicles have a direct role in cortical injury, possibly as a source of diffusible factors such as autoantibodies, proinflammatory cytokines and/or proteolytic enzymes that cause neuronal injury either directly or via microglial activation (Magliozzi *et al*., 2007).

8. Conclusions

Evidence is accumulating that lymphoid chemokines play pleiotrophic roles when expressed in the CNS. During homeostasis, CCL19 and CCL21 may facilitate immune surveillance of the subarachnoid space via recruitment of central memory T cells. Conversely, ectopic expression of these chemokines by CNS resident cells may trigger microglial activation and/ or the recruitment and organization of infiltrating leukocytes in response to specific danger signals. These chemokines are induced *de novo* (CXCL13) or above baseline levels (CCL19 and CCL21) in response to certain CNS infections and neoplasms, as well as in the setting of autoimmune inflammation. In addition to regulating leukocyte entry across the BBB, they have been associated with the development of ectopic lymphoid structures in the meninges during chronic EAE and secondary progressive MS. Data from animal models suggest that CNS lymphoid chemokines play a beneficial role in homeostatic immune surveillance and clearance of immune infections. Conversely, aberrant expression of these molecules appears to be detrimental with regard to the development of hematopoetic tumors and autoimmune demyelination within the CNS. A causal relationship between CNS lymphoid chemokines and pathological and clinical outcomes remains to be established in human disease.

Nonetheless, the current data suggests that CXCL13, CCL19 and CCL21 will ultimately be useful as biomarkers and/ or therapeutic targets.

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References

Aloisi F, Pujol-Borrell R. Lymphoid neogenesis in chronic inflammatory diseases. Nat Rev Immunol. 2006; 6:205–217. [PubMed: 16498451]

Alt C, Laschinger M, Engelhardt B. Functional expression of the lymphoid chemokines CCL19 (ELC) and CCL 21 (SLC) at the blood-brain barrier suggests their involvement in G-protein-dependent lymphocyte recruitment into the central nervous system during experimental autoimmune encephalomyelitis. Eur J Immunol. 2002; 32:2133–2144. [PubMed: 12209625]

Ansel K, McHeyzer-Williams L, Ngo V, McHeyzer-Williams M, Cyster J. In vivo-activated CD4 T cells upregulate CXC chemokine receptor 5 and reprogram their response to lymphoid chemokines. J Exp Med. 1999; 190:1123–1134. [PubMed: 10523610]

Axtell R, Steinman L. Gaining entry to an uninflamed brain. Nat Immunol. 2009; 10:453–455. [PubMed: 19381137]

Bagaeva L, Rao P, Powers J, Segal B. CXC chemokine ligand 13 plays a role in experimental autoimmune encephalomyelitis. J Immunol. 2006; 176:7676–7685. [PubMed: 16751415]

Biber K, Sauter A, Brouwer N, Copray S, Boddeke H. Ischemia-induced neuronal expression of the microglia attracting chemokine Secondary Lymphoid-tissue Chemokine (SLC). Glia. 2001; 34:121–133. [PubMed: 11307161]

Booss J, Esiri MM, Tourtellotte WW, Mason DY. Immunohistological analysis of T lymphocyte subsets in the central nervous system in chronic progressive multiple sclerosis. Journal of the Neurological Sciences. 1983; 62:219–232. [PubMed: 6607973]

Buonamici S, Trimarchi T, Ruocco M, Reavie L, Cathelin S, Mar B, Klinakis A, Lukyanov Y, Tseng J, Sen F, Gehrie E, Li M, Newcomb E, Zavadil J, Meruelo D, Lipp M, Ibrahim S, Efstratiadis A, Zagzag D, Bromberg J, Dustin M, Aifantis I. CCR7 signalling as an essential regulator of CNS infiltration in T-cell leukaemia. Nature. 2009; 459:1000–1004. [PubMed: 19536265]

Castillo J, Dávalos A, Naveiro J, Noya M. Neuroexcitatory amino acids and their relation to infarct size and neurological deficit in ischemic stroke. Stroke. 1996; 27:1060–1065. [PubMed: 8650715]

Chen S, Leach M, Chen Y, Cai X, Sullivan L, Wiekowski M, Dovey-Hartman B, Zlotnik A, Lira S. Central nervous system inflammation and neurological disease in transgenic mice expressing the CC chemokine CCL21 in oligodendrocytes. J Immunol. 2002; 168:1009–1017. [PubMed: 11801633]

Columba-Cabezas S, Griguoli M, Rosicarelli B, Magliozzi R, Ria F, Serafini B, Aloisi F. Suppression of established experimental autoimmune encephalomyelitis and formation of meningeal lymphoid follicles by lymphotoxin beta receptor-Ig fusion protein. J Neuroimmunol. 2006; 179:76–86. [PubMed: 16870269]

Columba-Cabezas S, Serafini B, Ambrosini E, Aloisi F. Lymphoid chemokines CCL19 and CCL21 are expressed in the central nervous system during experimental autoimmune encephalomyelitis: implications for the maintenance of chronic neuroinflammation. Brain Pathol. 2003; 13:38–51. [PubMed: 12580544]

Corcione A, Casazza S, Ferretti E, Giunti D, Zappia E, Pistorio A, Gambini C, Mancardi G, Uccelli A, Pistoia V. Recapitulation of B cell differentiation in the central nervous system of patients with multiple sclerosis. Proc Natl Acad Sci U S A. 2004; 101:11064–11069. [PubMed: 15263096]

Cyster J. Chemokines and the homing of dendritic cells to the T cell areas of lymphoid organs. J Exp Med. 1999; 189:447–450. [PubMed: 9927506]

de Jong E, Dijkstra I, Hensens M, Brouwer N, van Amerongen M, Liem R, Boddeke H, Biber K. Vesicle-mediated transport and release of CCL21 in endangered neurons: a possible explanation

for microglia activation remote from a primary lesion. J Neurosci. 2005; 25:7548–7557. [PubMed: 16107642]

Dijkstra I, de Haas A, Brouwer N, Boddeke H, Biber K. Challenge with innate and protein antigens induces CCR7 expression by microglia in vitro and in vivo. Glia. 2006; 54:861–872. [PubMed: 16977602]

Dijkstra I, Hulshof S, van der Valk P, Boddeke H, Biber K. Cutting edge: activity of human adult microglia in response to CC chemokine ligand 21. J Immunol. 2004; 172:2744–2747. [PubMed: 14978072]

Festa E, Hankiewicz K, Kim S, Skurnick J, Wolansky L, Cook S, Cadavid D. Serum levels of CXCL13 are elevated in active multiple sclerosis. Mult Scler. 2009; 15:1271–1279. [PubMed: 19805441]

Fischer L, Korfel A, Pfeiffer S, Kiewe P, Volk H, Cakiroglu H, Widmann T, Thiel E. CXCL13 and CXCL12 in central nervous system lymphoma patients. Clin Cancer Res. 2009; 15:5968–5973. [PubMed: 19773382]

Förster R, Mattis A, Kremmer E, Wolf E, Brem G, Lipp M. A putative chemokine receptor, BLR1, directs B cell migration to defined lymphoid organs and specific anatomic compartments of the spleen. Cell. 1996; 87:1037–1047. [PubMed: 8978608]

Giunti D, Borsellino G, Benelli R, Marchese M, Capello E, Valle M, Pedemonte E, Noonan D, Albini A, Bernardi G, Mancardi G, Battistini L, Uccelli A. Phenotypic and functional analysis of T cells homing into the CSF of subjects with inflammatory diseases of the CNS. J Leukoc Biol. 2003; 73:584–590. [PubMed: 12714572]

Halle S, Dujardin H, Bakocevic N, Fleige H, Danzer H, Willenzon S, Suezer Y, Hämmerling G, Garbi N, Sutter G, Worbs T, Förster R. Induced bronchus-associated lymphoid tissue serves as a general priming site for T cells and is maintained by dendritic cells. J Exp Med. 2009; 206:2593–2601. [PubMed: 19917776]

Hickey W, Hsu B, Kimura H. T-lymphocyte entry into the central nervous system. J Neurosci Res. 1991; 28:254–260. [PubMed: 2033653]

Hintzen R, Fiszer U, Fredrikson S, Rep M, Polman C, van Lier R, Link H. Analysis of CD27 surface expression on T cell subsets in MS patients and control individuals. J Neuroimmunol. 1995; 56:99–105. [PubMed: 7822487]

Hjelmström P. Lymphoid neogenesis: de novo formation of lymphoid tissue in chronic inflammation through expression of homing chemokines. J Leukoc Biol. 2001; 69:331–339. [PubMed: 11261778]

Junt T, Fink K, Förster R, Senn B, Lipp M, Muramatsu M, Zinkernagel R, Ludewig B, Hengartner H. CXCR5-dependent seeding of follicular niches by B and Th cells augments antiviral B cell responses. J Immunol. 2005; 175:7109–7116. [PubMed: 16301613]

Kivisäkk P, Mahad D, Callahan M, Sikora K, Trebst C, Tucky B, Wujek J, Ravid R, Staugaitis S, Lassmann H, Ransohoff R. Expression of CCR7 in multiple sclerosis: implications for CNS immunity. Ann Neurol. 2004; 55:627–638. [PubMed: 15122702]

Kivisäkk P, Mahad D, Callahan M, Trebst C, Tucky B, Wei T, Wu L, Baekkevold E, Lassmann H, Staugaitis S, Campbell J, Ransohoff R. Human cerebrospinal fluid central memory CD4+ T cells: evidence for trafficking through choroid plexus and meninges via P-selectin. Proc Natl Acad Sci U S A. 2003; 100:8389–8394. [PubMed: 12829791]

Krumbholz M, Meinl I, Kümpfel T, Hohlfeld R, Meinl E. Natalizumab disproportionately increases circulating pre-B and B cells in multiple sclerosis. Neurology. 2008; 71:1350–1354. [PubMed: 18936427]

Krumbholz M, Theil D, Cepok S, Hemmer B, Kivisäkk P, Ransohoff R, Hofbauer M, Farina C, Derfuss T, Hartle C, Newcombe J, Hohlfeld R, Meinl E. Chemokines in multiple sclerosis: CXCL12 and CXCL13 up-regulation is differentially linked to CNS immune cell recruitment. Brain. 2006; 129:200–211. [PubMed: 16280350]

Krumbholz M, Theil D, Steinmeyer F, Cepok S, Hemmer B, Hofbauer M, Farina C, Derfuss T, Junker A, Arzberger T, Sinicina I, Hartle C, Newcombe J, Hohlfeld R, Meinl E. CCL19 is constitutively expressed in the CNS, up-regulated in neuroinflammation, active and also inactive multiple sclerosis lesions. J Neuroimmunol. 2007; 190:72–79. [PubMed: 17825430]

Langer-Gould A, Atlas S, Green A, Bollen A, Pelletier D. Progressive multifocal leukoencephalopathy in a patient treated with natalizumab. N Engl J Med. 2005; 353:375–381. [PubMed: 15947078]

Legler D, Loetscher M, Roos R, Clark-Lewis I, Baggiolini M, Moser B. B cell-attracting chemokine 1, a human CXC chemokine expressed in lymphoid tissues, selectively attracts B lymphocytes via BLR1/CXCR5. J Exp Med. 1998; 187:655–660. [PubMed: 9463416]

Magliozzi R, Columba-Cabezas S, Serafini B, Aloisi F. Intracerebral expression of CXCL13 and BAFF is accompanied by formation of lymphoid follicle-like structures in the meninges of mice with relapsing experimental autoimmune encephalomyelitis. J Neuroimmunol. 2004; 148:11–23. [PubMed: 14975582]

Magliozzi R, Howell O, Vora A, Serafini B, Nicholas R, Puopolo M, Reynolds R, Aloisi F. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain. 2007; 130:1089–1104. [PubMed: 17438020]

Mantovani A. The chemokine system: redundancy for robust outputs. Immunol Today. 1999; 20:254–257. [PubMed: 10354549]

McMahon E, Bailey S, Castenada C, Waldner H, Miller S. Epitope spreading initiates in the CNS in two mouse models of multiple sclerosis. Nat Med. 2005; 11:335–339. [PubMed: 15735651]

Moser B, Schaerli P, Loetscher P. CXCR5(+) T cells: follicular homing takes center stage in T-helper-cell responses. Trends Immunol. 2002; 23:250–254. [PubMed: 12102746]

Moyron-Quiroz J, Rangel-Moreno J, Kusser K, Hartson L, Sprague F, Goodrich S, Woodland D, Lund F, Randall T. Role of inducible bronchus associated lymphoid tissue (iBALT) in respiratory immunity. Nat Med. 2004; 10:927–934. [PubMed: 15311275]

Müller G, Lipp M. Shaping up adaptive immunity: the impact of CCR7 and CXCR5 on lymphocyte trafficking. Microcirculation. 2003; 10:325–334. [PubMed: 12851649]

Narayan K, Dail D, Li L, Cadavid D, Amrute S, Fitzgerald-Bocarsly P, Pachner A. The nervous system as ectopic germinal center: CXCL13 and IgG in lyme neuroborreliosis. Ann Neurol. 2005; 57:813–823. [PubMed: 15929033]

Noor S, Habashy A, Nance J, Clark R, Nemati K, Carson M, Wilson E. CCR7 dependent immunity during acute Toxoplasma gondii infection. Infect Immun. 2010

Pashenkov M, Söderström M, Link H. Secondary lymphoid organ chemokines are elevated in the cerebrospinal fluid during central nervous system inflammation. J Neuroimmunol. 2003; 135:154–160. [PubMed: 12576236]

Ramesh G, Borda J, Gill A, Ribka E, Morici L, Mottram P, Martin D, Jacobs M, Didier P, Philipp M. Possible role of glial cells in the onset and progression of Lyme neuroborreliosis. J Neuroinflammation. 2009; 6:23. [PubMed: 19706181]

Rappert A, Biber K, Nolte C, Lipp M, Schubel A, Lu B, Gerard N, Gerard C, Boddeke H, Kettenmann H. Secondary lymphoid tissue chemokine (CCL21) activates CXCR3 to trigger a Cl- current and chemotaxis in murine microglia. J Immunol. 2002; 168:3221–3226. [PubMed: 11907075]

Rupprecht T, Elstner M, Weil S, Pfister H. Autoimmune-mediated polyneuropathy triggered by borrelial infection? Muscle Nerve. 2008; 37:781–785. [PubMed: 18288714]

Salomonsson S, Larsson P, Tengnér P, Mellquist E, Hjelmström P, Wahren-Herlenius M. Expression of the B cell-attracting chemokine CXCL13 in the target organ and autoantibody production in ectopic lymphoid tissue in the chronic inflammatory disease Sjögren's syndrome. Scand J Immunol. 2002; 55:336–342. [PubMed: 11967114]

Schneck S, Penn I. De-novo brain tumours in renal-transplant recipients. Lancet. 1971; 1:983–986. [PubMed: 4102451]

Sellebjerg F, Börnsen L, Khademi M, Krakauer M, Olsson T, Frederiksen J, Sørensen P. Increased cerebrospinal fluid concentrations of the chemokine CXCL13 in active MS. Neurology. 2009; 73:2003–2010. [PubMed: 19996075]

Senel M, Rupprecht T, Tumani H, Pfister H, Ludolph A, Brettschneider J. The chemokine CXCL13 in acute neuroborreliosis. J Neurol Neurosurg Psychiatry. 2009

Serafini B, Rosicarelli B, Magliozzi R, Stigliano E, Aloisi F. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. Brain Pathol. 2004; 14:164–174. [PubMed: 15193029]

Serafini B, Rosicarelli B, Magliozzi R, Stigliano E, Capello E, Mancardi G, Aloisi F. Dendritic cells in multiple sclerosis lesions: maturation stage, myelin uptake, and interaction with proliferating T cells. J Neuropathol Exp Neurol. 2006; 65:124–141. [PubMed: 16462204]

Smith J, Braziel R, Paoletti S, Lipp M, Uguccioni M, Rosenbaum J. Expression of B-cell-attracting chemokine 1 (CXCL13) by malignant lymphocytes and vascular endothelium in primary central nervous system lymphoma. Blood. 2003; 101:815–821. [PubMed: 12393412]

Snider W, Simpson D, Aronyk K, Nielsen S. Primary lymphoma of the nervous system associated with acquired immune-deficiency syndrome. N Engl J Med. 1983; 308:45. [PubMed: 6847922]

Soto H, Wang W, Strieter R, Copeland N, Gilbert D, Jenkins N, Hedrick J, Zlotnik A. The CC chemokine 6Ckine binds the CXC chemokine receptor CXCR3. Proc Natl Acad Sci U S A. 1998; 95:8205–8210. [PubMed: 9653165]

Stüve O, Marra C, Jerome K, Cook L, Cravens P, Cepok S, Frohman E, Phillips J, Arendt G, Hemmer B, Monson N, Racke M. Immune surveillance in multiple sclerosis patients treated with natalizumab. Ann Neurol. 2006; 59:743–747. [PubMed: 16634029]

Takemura S, Braun A, Crowson C, Kurtin P, Cofield R, O'Fallon W, Goronzy J, Weyand C. Lymphoid neogenesis in rheumatoid synovitis. J Immunol. 2001; 167:1072–1080. [PubMed: 11441118]

Tun H, Personett D, Baskerville K, Menke D, Jaeckle K, Kreinest P, Edenfield B, Zubair A, O'Neill B, Lai W, Park P, McKinney M. Pathway analysis of primary central nervous system lymphoma. Blood. 2008; 111:3200–3210. [PubMed: 18184868]

Vassileva G, Soto H, Zlotnik A, Nakano H, Kakiuchi T, Hedrick J, Lira S. The reduced expression of 6Ckine in the plt mouse results from the deletion of one of two 6Ckine genes. J Exp Med. 1999; 190:1183–1188. [PubMed: 10523616]

Wekerle H, Sun D, Oropeza-Wekerle RL, Meyermann R. Immune reactivity in the nervous system: modulation of T-lymphocyte activation by glial cells. J Exp Biol. 1987; 132:43–57. [PubMed: 3323405]

Williams A, Wei H, Dave J, Tortella F. Acute and delayed neuroinflammatory response following experimental penetrating ballistic brain injury in the rat. J Neuroinflammation. 2007; 4:17. [PubMed: 17605820]

Yoshida R, Imai T, Hieshima K, Kusuda J, Baba M, Kitaura M, Nishimura M, Kakizaki M, Nomiyama H, Yoshie O. Molecular cloning of a novel human CC chemokine EBI1-ligand chemokine that is a specific functional ligand for EBI1, CCR7. J Biol Chem. 1997; 272:13803–13809. [PubMed: 9153236]

Yoshida R, Nagira M, Kitaura M, Imagawa N, Imai T, Yoshie O. Secondary lymphoid-tissue chemokine is a functional ligand for the CC chemokine receptor CCR7. J Biol Chem. 1998; 273:7118–7122. [PubMed: 9507024]

Yoshie O, Imai T, Nomiyama H. Novel lymphocyte-specific CC chemokines and their receptors. J Leukoc Biol. 1997; 62:634–644. [PubMed: 9365118]

Zlotnik A, Morales J, Hedrick J. Recent advances in chemokines and chemokine receptors. Crit Rev Immunol. 1999; 19:1–47. [PubMed: 9987599]

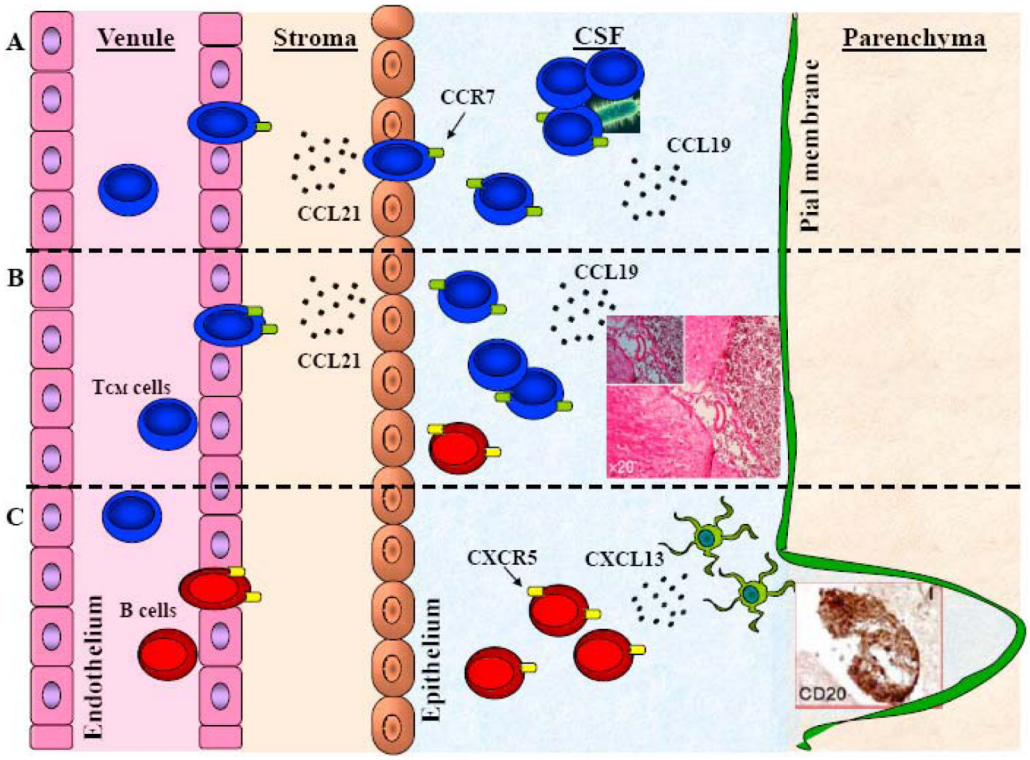

Figure 1. The role of lymphoid chemokines in CNS homeostatic and pathogenic states

CCL19 and CCL21 are constitutively expressed in cerebrovascular endothelium and the choroid plexus. The majority of cells in the CSF are T_{CM} cells that express CCR7 and may play a role in immunosurveillance of the subarachnoid and perivascular spaces, including in the clearance of local infections, A. Conversely, aberrant production of CCL19, CCL21 and CXCL13 may support CNS infiltration by leukemic T and B cells and the establishment of hematopoetic tumors (inset) in the brain and spinal cord, B. CXCL13 is not expressed in the healthy CNS but is upregulated during autoimmune inflammation where it may have a role in the cognate interaction of encephalitogenic T cells and DC, while later in disease CXCL13 has been associated with the development of ectopic lymphoid structures (inset) in the meninges, C.