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Targeting CXCL13 During Neuroinflammation

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Abstract

The chemokine, C-X-C motif ligand 13 (CXCL13), is constitutively expressed in lymphoid organs and controls the recruitment and compartmentalization of lymphocytes and antigen presenting cells within these specialized structures. Recent data, however, also find induction of this molecule during central nervous system (CNS) inflammation under a variety of circumstances. While its role(s) in the pathogenesis of neoplastic, infectious and autoimmune disorders of the CNS remain incompletely understood, several lines of evidence suggest that CXCL13 could become a relevant therapeutic target in at least some of these diseases. This review focuses on how CXCL13 contributes to the pathogenesis of selected CNS disorders involving both experimental animals and humans, paying particular attention to the issue of whether (and if so, how) blockade of this ligand or its receptor might benefit the host. Current blocking strategies largely involve the use of monoclonal antibodies, but an improved understanding of downstream signaling pathways makes small molecule inhibition a future possibility.

Keywords

CXCL13; lymphoid chemokines; CXCR5; neuroborreliosis; primary central nervous system lymphoma; multiple sclerosis; post-stroke dementia

INTRODUCTION

Lymphoid chemokines (also referred to as homeostatic chemokines) are constitutively expressed in lymphoid organs and control the recruitment and compartmentalization of both lymphocytes and antigen presenting cells (APC) within these specialized structures [1–12]. C-C motif ligand (CCL) 19 and CCL21 bind to C-C motif receptor (CCR) 7 and recruit T cells and dendritic cells (DC) to T cell areas of secondary lymphoid tissues [2, 3]. C-X-C motif ligand (CXCL) 12 binds to C-X-C motif receptor (CXCR) 4 and attracts multiple immune cell types to lymph nodes and spleen, and along with CXCL13, drives the formation of germinal centers (GC) [11, 12]. CXCL13 is made primarily by stromal cells and follicular DC (FDC) in B cell follicles, and recruits both B cells and CD4+ T follicular helper (Tfh) cells to these compartments via its cognate receptor, CXCR5 [1, 3, 8–12].

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CONFLICT OF INTEREST

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Induction of all lymphoid chemokines in both T and B cell areas of lymphoid organs depends on lymphotoxin (LT)- β and tumor necrosis factor (TNF)- α signaling in stromal cells and FDC that are the main physiologic sources of these chemoattractant molecules in these tissues [4–6].

Beyond their role in the development and maintenance of lymphoid tissues, the lymphoid chemokines also are implicated in propagating non-lymphoid tissue inflammation. Ectopic structures resembling the GC of lymphoid organs are found in the synovia of patients with rheumatoid arthritis and in the salivary glands of patients with Sjögren's disease [13]. Experimental evidence supporting a role for lymphoid chemokines in driving the process of "lymphoid neogenesis" comes from studies in transgenic mice where forced overexpression of CCL21 or CXCL13 in organs such as the pancreas or the thyroid gland is sufficient to cause the local formation of lymphoid structures resulting in diabetes and thyroiditis, respectively [14–17]. CXCL13 is particularly notorious in this regard; it is found in gastric mucosa-associated lymphoid structures that develop in response to *Helicobacter pylori* infections [18], and CXCL13-CXCR5 interactions likely contribute to the development of both *H. pylori*-associated gastritis and gastric lymphomas [18–21]. Importantly, CXCL13 can also be found in B cell aggregates that develop in the inflamed meninges of humans with progressive multiple sclerosis (MS) and in mice with experimental autoimmune encephalomyelitis (EAE), an important animal model of MS [22–25]. Thus, ectopic lymphoid follicles that form due to CXCL13 overexpression could be a natural feature of chronic organ-specific autoimmune inflammation [13, 23, 24, 26]. How CXCL13 gets induced in the central nervous system (CNS) in the setting of EAE and MS remains unknown. Here, after briefly considering the known consequences of systemic deletion or neutralization of CXCL13, we focus on its role in driving inflammation in the CNS and on the potential to target the CXCL13-CXCR5 axis as a therapeutic strategy in selected neuroinflammatory disorders.

KNOWN EFFECTS OF CXCL13 DELETION OR NEUTRALIZATION *IN VIVO*

Mice genetically deficient in CXCL13 were found to lack most of their lymph nodes (inguinal, iliac, sacral, brachial and axillary, but not mesenteric) and Peyer's patches from early development [1, 8]. Splenic architecture was also disrupted [1], and while circulating B cell numbers were slightly elevated, B cell homing to the peritoneal cavity was reduced in these hosts [1, 27]. When normal adult animals were given neutralizing anti-CXCL13 antibodies and immunized with a foreign antigen 24 hours later, treatment caused splenic follicles to rapidly disappear, B cells could no longer surround splenic T cell zones, and FDC became difficult to find [28]. Nonetheless, GC B cell numbers were preserved, and animals mounted an identical antigen-specific antibody response compared to mice treated with isotype control antibodies [28]. This indicates that organized splenic GC are not absolutely required for normal humoral immunity, and/or that other lymphoid organs can act to maintain antibody responses. Indeed, CXCL13 neutralization had no effect on FDC networks and GC in Peyer's patches [28]. Finally, in animal models of both Sjögren's syndrome and autoimmune arthritis, anti-CXCL13 antibodies given to mice with established symptoms could ameliorate disease but had much more limited effects on the size or appearance of the lymphoid-like structures found in diseased tissues [28]. These data

suggest that CXCL13 plays a more limited role to actively maintain these ectopic follicles, or that redundancy exists with other chemokines (CCL21, LT β) to exert these maintenance effects.

CXCL13 IN THE PATHOGENESIS OF PRIMARY CNS LYMPHOMA (PCNSL)

Primary CNS lymphoma (PCNSL) is a rare but often fatal form of non-Hodgkin B cell lymphoma arising within the CNS. These tumors have a low propensity to metastasize systemically, but inside the CNS they originate in and spread to involve the brain, leptomeninges, spinal cord, and intraocular compartments. Death typically occurs via direct compression of vital brain structures or from a global rise in intracranial pressure. Immune system compromise, such as in patients with the acquired immune deficiency syndrome (AIDS), is the strongest risk factor for the development of PCNSL [29]. The heightened susceptibility of immunocompromised hosts highlights the importance of normal CNS immune surveillance in preventing the formation of these tumors. When PCNSL arise in immunocompetent individuals, pathological specimens show extensive tumor infiltrating lymphocytes (TIL) indicative of an active anti-tumor host response [30]. Among these TIL, CD8⁺ T cells expressing both granzyme B and markers of proliferation are most common, and their localization and density in perivascular areas suggests that recruitment may be orchestrated by chemokines arising from perivascular macrophages [30]. Tumor cells expressing inhibitory ligands such as programmed death-ligand 1 (PD-L1) can suppress the local cytotoxic T lymphocyte (CTL) response and grow aggressively [31]. Thus, local CNS immunity is important for both the prevention and control of PCNSL.

Given the importance of CXCL13 in the homing and compartmentalization of B cells in lymphoid organs, its role in the pathogenesis of B cell lymphomas has now been carefully explored. Smith et al. were the first to report CXCL13 expression by malignant B cells in PCNSL [32], a finding since reproduced by multiple other groups [33–35]. Its cognate receptor, CXCR5, is also consistently present on tumor cells, although exactly what proportion of TIL are CXCR5 positive remains a matter of debate [32, 33]. Still, at least one paper describes that the highest density of TIL within PCNSL tissues is found in those areas where CXCL13 expression is most robust [35]. Fischer et al. compared serum and cerebrospinal fluid (CSF) concentrations of both CXCL12 and CXCL13 in a cohort of 30 patients with CNS lymphoma (23 individuals with PCNSL and 7 with systemic lymphoma that spread to the CNS) to samples from 40 non-lymphoma controls (10 with and 30 without other CNS malignancies). While neither serum nor CSF CXCL12 levels differed between CNS lymphoma patients and controls, CSF CXCL13 concentrations were significantly higher in the CNS lymphoma group, even as serum CXCL13 levels were consistently low across both cohorts [36]. Furthermore, when CSF CXCL13 concentrations were followed longitudinally in 7 of their patients with PCNSL, levels declined in all 5 individuals responding to chemotherapy and increased in both cases where lymphoma progression occurred [34]. In a separate and larger cohort of patients, Rubenstein et al. also found that mean CSF CXCL13 levels were significantly higher in patients with CNS lymphoma compared to a range of controls, and that progression-free survival with standard treatment was significantly longer for those patients with lower (<200 pg/mL) compared to higher (>200 pg/mL) CSF CXCL13 levels [37]. For the 5 PCNSL patients in this cohort with

undetectable CSF CXCL13 levels at the time of diagnosis, none progressed after treatment over a median follow up of 46 months [37]. Thus, both studies strongly suggest that CXCL13 plays an important role in PCNSL pathogenesis and that higher CNS levels signify a more aggressive form of disease.

High-dose chemotherapy regimens, especially those incorporating methotrexate, are the current standard of care for patients with PCNSL [29, 38]. Immunotherapy for this disorder remains in its infancy; many treatment regimens utilize rituximab (anti-CD20, a reagent that binds and deletes both normal and malignant B cells) based on its pivotal role in systemic B cell lymphomas, but compelling efficacy for rituximab in PCNSL remains the subject of some debate [39]. A highly potent oral Bruton tyrosine kinase inhibitor, ibrutinib, has been shown to block CXCL13-driven migration of malignant B cells *in vitro* and to lower plasma concentrations of CXCL13 in patients with mantle cell lymphoma, a rare form of systemic non-Hodgkin lymphoma, *in vivo* [40, 41]. This compound is currently in development for the treatment of B cell lymphoproliferative disorders. Other tyrosine kinases downstream of CXCR5 are amenable to small molecule inhibition and can block the CXCL13-driven migration of B cells [42]. However, no study has yet attempted to directly target the CXCL13-CXCR5 axis in PCNSL, although the rationale to do so appears reasonable once the appropriate therapeutic reagents capable of accessing the CNS become available.

CXCL13 EXPRESSION DURING LYME NEUROBORRELIOSIS (LNB)

Lyme disease is caused by infection with the tick-borne spirochete, *Borrelia burgdorferi*. Early signs include a characteristic skin rash, erythema chronicum migrans, indicative of local pathogen replication at the inoculation site and marking its dissemination in the blood. Later disease manifestations occur with involvement of the heart, joints and nervous system. An array of neurological complications, collectively referred to as Lyme neuroborreliosis (LNB), can include meningitis, encephalopathy, cranial nerve palsies, myelitis, polyradiculitis, and peripheral neuropathy [43, 44]. Non-human primates have been used to model LNB; neurological involvement in rhesus macaques consistently follows intradermal challenge with a neurotropic *B. burgdorferi* isolate [45, 46]. In this experimental setting, signs of LNB can be caused by pathogen-induced inflammation, rather than by widespread neural injury or overwhelming pathogen replication in the target tissue. The humoral immune response is important for clearing spirochetes from the CNS; infection of both humans and experimental animals is characterized by B cell hyperactivity [47], and accumulation of plasma cells in both neural tissues and CSF [48–50]. Some investigators have even suggested that the CNS resembles an ectopic germinal center during LNB based on neuropathological features observed in the primate model [51]. These data have naturally led to questions regarding CXCL13 and other lymphoid chemokines in the pathogenesis of this disorder.

In cultured brain slices from nonhuman primates exposed to *B. burgdorferi* *in vitro*, glial cells rapidly produce a range of immune mediators, including CXCL13 [52]. Spirochetal lipoproteins activate Toll-like receptor (TLR) 2 on human monocytes *in vitro* also triggering CXCL13 release [53]. Direct inoculation of *B. burgdorferi* into the cisterna magna of rhesus macaques elicits a rapid lymphocytic and monocytic pleocytosis in the CSF, accompanied

by rapid rises of interleukin (IL)-6, IL-8, CCL2 and CXCL13 [54]. B cells making pathogen-specific antibodies first arise in the periphery after several weeks and then traffic into the CNS [54]. In humans with LNB, CSF levels of CXCL13 can be extremely high [55–61], and multiple studies claim that such a finding in the appropriate clinical context is both a sensitive and specific diagnostic marker for this disorder [57–59, 61]. When the composition of CSF inflammatory cell infiltrates from LNB patients are analyzed by flow cytometry, CXCL13 is identified as a key regulator of B cell recruitment to this compartment [55, 60]. CXCL13 levels in the CSF typically fall with antimicrobial treatment [56, 61]; persistent elevations suggest that the pathogen has evaded clearance and remains infective. Again, while local humoral immunity supports pathogen clearance from the CNS [47–50, 62], self-reactive antibodies can also emerge with notable frequency in the setting of chronic infection [63–65]. Some of these antibodies are directed at epitopes such as gangliosides that may be shared between the pathogen and neural tissues [66], but others are directed at myelin or neuronal proteins whose emergence cannot be readily explained by molecular mimicry [64, 65]. If B cells making these anti-myelin antibodies clonally expand within the CNS as one study suggests [65], then how and where they are generated, how they are recruited, and how they persist within the brain requires further study. Chronic LNB remains poorly understood and may in fact represent multiple disorders that in some cases reflect pathogen persistence in the CNS and in others a more *bona fide* autoimmune process [66].

Treatment of LNB mainly involves the use of antibiotics that are capable of penetrating the blood-brain barrier and are active against *B. burgdorferi* [65]. Most regimens continue for up to 4 weeks; there is no compelling clinical trial-based evidence that prolonged treatment beyond this interval has any added benefit, even for patients with ongoing CNS symptoms [67]. The role of corticosteroids as an adjunctive immunotherapy in managing LNB also remains unclear. While early anecdotal reports suggested that the concomitant use of steroids augments the effects of antibiotics, no prospective trial has addressed this issue and larger retrospective studies have failed to show any convincing benefit compared to antibiotics alone [67]. In the rhesus macaque model of LNB, corticosteroids prevented normal isotype class switching of the anti-borrelial immunoglobulin (Ig) M to IgG response and led to higher pathogen loads in the CNS of treated animals [68]. Thus, early disruption of CXCL13-CXCR5 signaling that would presumably modulate or dampen CNS humoral response could delay or inhibit the recovery of humans from LNB. On the other hand, blockade at a late disease stage could turn out to benefit previously infected hosts in whom self-reactive antibodies emerge. If nothing else, CSF CXCL13 levels in LNB patients may become a reliable marker of the intrathecal humoral immune response against infection.

CXCL13 AND THE IMMUNOPATHOGENESIS OF MULTIPLE SCLEROSIS (MS)

Multiple sclerosis (MS) is the most common autoimmune disorder of the CNS, and one of the most common causes of non-traumatic disability in younger adults. For patients with the relapsing-remitting form of MS (RRMS), a number of therapies targeting the immune system have been shown in randomized clinical trials to slow relapse rate, disability

progression, and the formation of new brain and spinal cord lesions on magnetic resonance imaging (MRI) scans. The pathogenesis of MS remains poorly understood, but genetic and environmental factors both contribute to its onset and progression. Host immunity targeted against myelin proteins, triggered by unknown mechanisms, is presumed to initiate the formation of demyelinating lesions within the CNS. Experimental autoimmune encephalomyelitis (EAE) can be provoked in both rodents and non-human primates by either direct immunization with myelin proteins or via the adoptive transfer of myelin-specific CD4⁺ T cells, and these systems model certain features of human MS. An important role for CXCL13 and other lymphoid chemokines in the pathogenesis of MS and EAE has recently emerged, and these molecules continue to be an area of active investigation in this field.

CXCL13 has been found within B cell aggregates that develop in the inflamed meninges of both mice with EAE and a subset of humans with progressive MS [22–25]. In MS tissue specimens, these ectopic follicle-like structures contain proliferating B cells, plasma cells, T helper (Th) cells and FDC, and their presence correlates with adjacent cortical demyelination, neuronal loss and more rapid disease progression [69]. Quantitative PCR analyses of autopsy tissue demonstrate that *cxc13* mRNA is also induced directly in active demyelinating MS lesions, and immunostaining shows that both perivascular mononuclear cells and glial cells express CXCL13 protein in active MS plaques [70]. Microglia are an important source of this mediator *in vitro*, and both pro- and anti-inflammatory stimuli dynamically regulate CXCL13 production by these cells [71, 72]. More recent reports demonstrate that CXCL13 concentrations are increased in the CSF of patients with RRMS compared to controls, and that levels are higher during active disease relapses and decline following successful application of B cell-directed therapies [70, 73–77]. Even in cases of progressive MS where the long-term clinical benefits of such immunotherapies are unclear, CSF levels of CXCL13 still consistently fall after 6 months of systemic treatment with compounds that blocks lymphocyte entry into the CNS [78]. Together, these data suggest that changes in intrathecal CXCL13 concentrations may reflect lymphocyte traffic through the CNS more than local glial production, and that such changes also serve as a potential biomarker of treatment responsiveness across the spectrum of MS subtypes [79].

Studies in the EAE model now have now demonstrated that CXCL13 and its related pathways actually contribute to disease pathogenesis and are not simply markers of fluctuating neuroinflammation. Columba-Cabezas et al. showed that treatment with an LTβ receptor-Ig fusion protein that blocks the interaction of LTβ with its receptor can: a) inhibit CXCL13 induction, b) suppress the formation of CNS lymphoid follicles, and c) reduce disease symptoms in mice with established EAE [80]. Around the same time, Bagaeva et al. showed that EAE onset occurs normally in CXCL13 knockout (KO) mice, but that disease severity wanes over time compared to wild-type mice because myelin-specific CD4⁺ T cell responses are not sustained [81]. These investigators also showed that administration of a neutralizing anti-CXCL13 antibody to mice at EAE onset causes a similar attenuation of disease [81]. Rainey-Barger et al. confirmed that CXCL13 deficiency leads to waning numbers of myelin-reactive Th1 and Th17 cells in the periphery of mice after peak EAE, and also showed that CXCL13 KO mice have no defects in the initial recruitment of B cells

to the inflamed CNS [82]. Since then, at least one pharmaceutical company has developed a human IgG1 anti-CXCL13 monoclonal antibody that specifically binds human, rodent and primate CXCL13 and neutralizes its actions in several *in vitro* functional assays [83]. Not only does this reagent ameliorate EAE induced by CXCR5⁺ Th17 cells *in vivo*, but it also reduces the number of ectopic follicles detected in several different autoimmune disease models, and it interferes with the trafficking of B cells to B cell areas of the spleen and lymph nodes in adoptive transfer studies [83]. Such findings reveal that pharmaceutical companies consider the CXCL13 pathway an important potential therapeutic target. Autoimmune diseases, perhaps more than any other condition considered here, may be the most likely first group of disorders where manipulation of the CXCL13-CXCR5 pathway is found to impact the course of a human disease.

DOES CXCL13 CONTRIBUTE TO THE PATHOGENESIS OF OTHER NEUROLOGICAL DISORDERS?

Despite experimental evidence casting some doubt on the direct role of CXCL13 in B cell recruitment to the acutely inflamed CNS [82], high CSF CXCL13 levels correlate closely with increased B cell recruitment to the intrathecal compartment during a range of human neurological disorders [36, 37, 55, 60, 73, 77]. It stands to reason that in other clinical settings where B cells or antibodies are involved in CNS disease pathogenesis, targeting the CXCL13 pathway may prove beneficial. During the autoimmune encephalitis of children and young adults caused by the emergence of anti-N-methyl-D-Aspartate (NMDA) receptor antibodies, more than 70% of patients have elevated CSF CXCL13 levels, and a prolonged or secondary rise in this mediator is associated with a poor response to treatment and a greater risk of clinical relapse [84]. A blocking study of some sort certainly could be considered in this setting. In a similar vein, both genetic deficiency and antibody-mediated ablation of B cells were shown to prevent the delayed appearance of cognitive deficits in mice following stroke [85]. This study also demonstrated abnormal numbers of B cells in the brains of human patients with post-stroke dementia [85]. While follow up investigations have not yet implicated CXCL13 in recruiting B cells to the brain in this setting, the potential benefit of rituximab or other B cell-directed therapies to prevent this common cerebrovascular disorder cannot be ignored. The availability of established models in animals will surely facilitate additional investigation of B cell trafficking into the CNS following stroke.

CONCLUSIONS

Humanized antibodies able to neutralize the biological activity of CXCL13 are now becoming available, and there are a number of well-defined neurological disorders where such reagents might be clinically effective. Anti-CD20-based treatments have now reached widespread use in many human neoplastic and autoimmune diseases, and while these drugs have proven reasonably safe, it is conceivable that therapies targeting B cell recruitment to the CNS will offer immunological advantages over wholesale B cell deletion. Of the clinical conditions described here, PCNSL and MS seem like viable candidates for manipulation of

the CXCL13-CXCR5 pathway to benefit patients with these devastating neurological disorders.

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