



REVIEW

**REVISED** Role of CXCL13 in the formation of the meningeal tertiary lymphoid organ in multiple sclerosis [version 2; referees: 2 approved]

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**Abstract**

Immunomodulatory therapies available for the treatment of patients with multiple sclerosis (MS) accomplish control and neutralization of peripheral immune cells involved in the activity of the disease cascade but their spectrum of action in the intrathecal space and brain tissue is limited, taking into consideration the persistence of oligoclonal bands and the variation of clones of lymphoid cells throughout the disease span. In animal models of experimental autoimmune encephalomyelitis (EAE), the presence of CXCL13 has been associated with disease activity and the blockade of this chemokine could work as a potential complementary therapeutic strategy in patients with MS in order to postpone disease progression. The development of therapeutic alternatives with ability to modify the intrathecal inflammatory activity of the meningeal tertiary lymphoid organ to ameliorate neurodegeneration is mandatory.

**Keywords**

multiple sclerosis, chemokines, CXCL13, B cells, tertiary lymphoid organ, meninges

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**REVISED Amendments from Version 1**

Our main goal during the writing of the revised version of this article (Version 2) entailed the clarification of concepts that should be considered as 'hypothetical', opposite to concepts that should be considered as facts in relation to the possible role that CXCL13 plays in the pathogenesis of MS and the possible therapeutic intervention for this disease by blocking the formation, or the effect, of this chemokine in the CNS. For this purpose, we did intensify the search for evidence of the role CXCL13 could play in the pathogenesis of EAE in the animal model of MS and the possible cellular sources of CXCL13 in the CNS, with special emphasis on the supporting findings involving the meningeal tissue. We included two new sections based on the current knowledge on the role of CXCL13 in other autoimmune diseases and the documented cellular sources of CXCL13 in neuroinflammation caused by diseases other than MS. We also included citations on the adverse effects of chemokine manipulation in the CNS of patients with MS, stressing the fact that the immune response in MS is significantly complex and not completely understood, raising flags of concern when considering extrapolation of therapeutic applications from bench to bedside. Overall, nearly 25% of the citations are new in this updated version of the manuscript.

See referee reports

## Introduction

Although disease modifying therapy (DMT) agents in multiple sclerosis (MS) have contributed to reduction of neuroinflammation, they have not succeeded in the prevention of progression of disease. Inflammation is the appropriate tissue response to infection, autoimmunity, cancer, injury and allograft transplantation<sup>1</sup>. When inflammation does not resolve appropriately, a prolonged immune response persists leading to tissue destruction and loss of function<sup>1</sup>. Chronic infiltration by immune cells in the meninges is believed to form transitory lymphoid cell aggregates which simulate secondary lymphoid organs (SLO), and are known as meningeal tertiary lymphoid organs (mTLO) which play an important role in the pathogenesis of autoimmunity<sup>1,2</sup>. The mTLO seem to play a role in the intrathecal activity of immune system cells in MS<sup>3</sup>. The SLO, such as lymph nodes, show a cellular organization that includes germinal centers (GC) containing antibody secreting and proliferating B-cells with follicular dendritic cells (FDC), a T-cell zone that incorporates naïve cells from the blood stream, high endothelial venules for extravasation of lymphocytes, and a stromal cell network that provides chemokines and extracellular matrix for cell migration and structural integrity<sup>1</sup>. Chemokines are a family of proteins with the specific property of regulating leukocytes in the immune system and they may play a role in neurotransmission and neuromodulation<sup>4</sup>. Leukocyte trafficking is mediated by inflammatory chemokines in inflamed tissues and by homeostatic chemokines in lymphoid sites<sup>5</sup> (Figure 1). In this review, we focus on the role that CXCL13 (also known as B cell attracting chemokine [BCA-1], C-X-C motif ligand 13, or B lymphocyte chemoattractant [BLC]) plays in the organization of the mTLO in MS.

### In normal conditions, the SLO acquire information and prepare for immune defense

The SLO have a genetically determined pattern of development and programming that allows trapping and concentration of

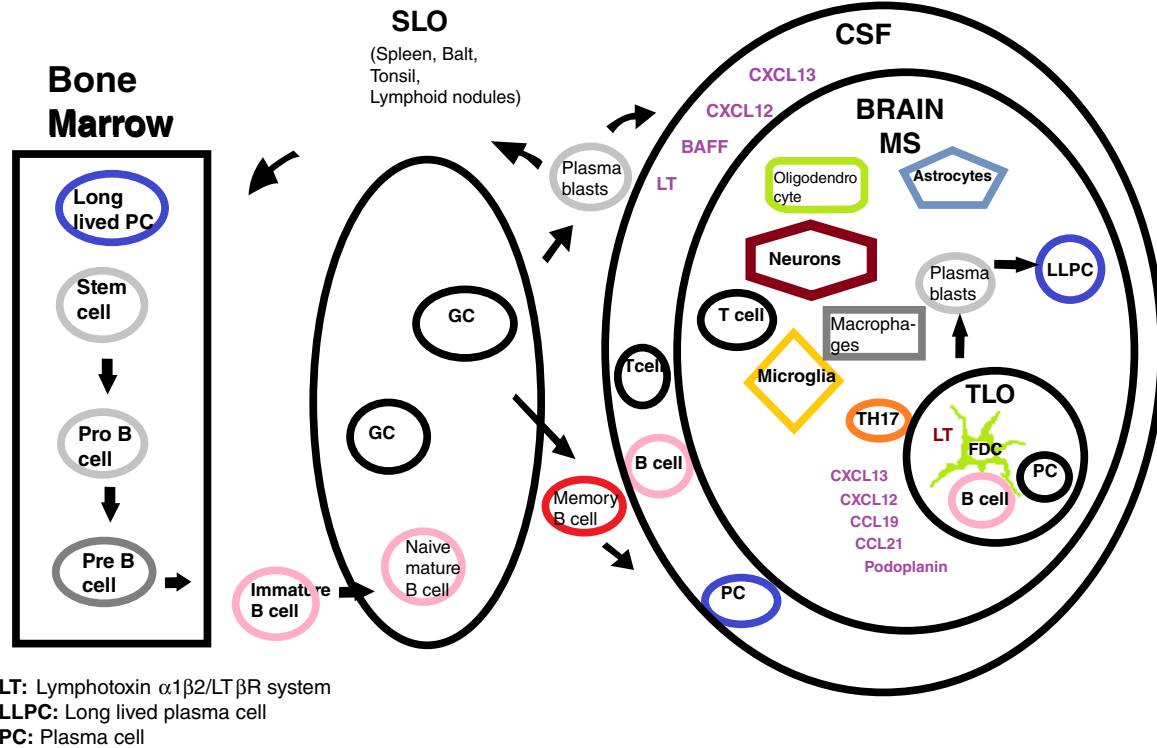
foreign antigens to initiate an adaptive immune response<sup>1</sup>. Mucosal associated and non-encapsulated lymphoid tissue (including the Peyer's patches, adenoid tissue of the nasopharynx, tonsils, and the bronchial associated lymphoid tissue), together with lymphoid nodes and spleen, constitute the SLO<sup>6,7</sup>. The lymph node cortex contains clusters or primary follicles that include packaged B cells and FDC, whereas the node para-cortex has a lesser number of dendritic cells (DC) and T cells<sup>6</sup>. Generation of B cells with ability to produce auto antibodies usually occur in physiological conditions<sup>8</sup>. These auto antibodies are low affinity IgM, which exhibit a wide spectrum of reactivity and strong preference for soluble self-antigens on the cell surface<sup>8</sup>. Auto reactive low affinity B cells suffer apoptosis being unlikely they represent danger in normal conditions<sup>8</sup>.

### Lymphoid cells are able to learn and exchange information at the GC

The GC present remarkable lymphocytic mitosis within SLO follicles<sup>9</sup>. Weyand *et al.* stated the GC are critical in the development of the B-cell normal immune response by driving cell division and maturation, B-cell selection with high affinity for immunoglobulin receptors and differentiation of B-cells and plasma cells (PC)<sup>3</sup>. Real time imaging technology has allowed visualization of the transit of the B cells from the dark zone to the light zone, and vice versa, during the maturation of the GC<sup>9-11</sup>. The GC light zone displays a predominance of FDC and follicular T-helper (Tfh) cells, whereas the dark zone contains closely packed lymphocytes and stromal cells<sup>9,12</sup>. The chemokine receptor CXCR4 is required for the positioning of the B cells in the dark zone where its ligand, CXCL12, is more abundant and is produced by stromal cells<sup>13</sup>. At the light zone, CXCL13 chemokine is concentrated in the FDC processes and, in conjunction with CXCR5, they contribute to the accumulation of B cells in this zone<sup>12,13</sup>. T-cells in the GC are essential to maintain signaling and represent approximately 5–20% of cell population<sup>9</sup>. Tfh cells are characterized by the expression of CXCR5 and ICOS, which has an inducible T cell co-stimulatory effect<sup>9,14</sup>. B cell growth and differentiation at the germinal center are supported by IL10 and ICOS<sup>14</sup>. Within the light zone, the three possible different outcomes for the centrocytes include death due to apoptosis; differentiation into memory B-cell or long lived plasma cells (LLPC); and re-entrance to the dark zone for a further round of cell mutation and selection<sup>15</sup>. The relevant function of the GC is, most likely, the primary production of memory B-cells and LLPC<sup>15,16</sup> (Figure 2). Recent studies analyzing IgG heavy chain variable region genes in B cells from MS patients revealed that B cells are able to enter and exit the blood brain barrier (BBB) in order get exposed to somatic hypermutation (SMH) at the GC<sup>17-21</sup>.

### Chemokines direct traffic of lymphocytes during the cell search for specific information

The induction of lymphoid chemokines, depends on lymphotoxin  $\beta$  (LT- $\beta$ ) and the tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) signaling on stromal cells and FDC<sup>22</sup>. Lymphotoxin  $\alpha 1\beta 2$  (LT $\alpha 1\beta 2$ ) is expressed in the surface of B and T cells in the adult immune system and ligates to the lymphotoxin  $\beta$  receptor (LT $\beta$ R) in reticular stromal cells thus inducing expression of lymphoid chemokines, such as CCL19, CCL21 and CXCL13<sup>23</sup>. These chemokines regulate the homeostatic traffic of lymphocytes in



**Figure 1. B cells lineage from bone marrow to CNS.** B-cells originating in the bone marrow exit toward the blood stream as immature B-cells; they enter the SLO and specialize in the germinal centers producing memory B cells and plasmablasts, which in pathologic conditions, are able to gain access to the CNS. The TLO is formed in the meninges during chronic inflammation in the deep brain cortical sulci and share organogenesis with the SLO<sup>24</sup>. Podoplanin and the Th17 signature cytokine IL-17 have been associated with ectopic lymphoneogenesis in human diseases whereas BAFF is a key factor for mutation and survival of B cells which is produced by astrocytes in the CNS<sup>3,25</sup>. **BAFF:** B-cell activating factor of the tumor necrosis factor family; **BALT:** bronchial associated lymphoid tissue; **CCL19:** chemokine (c-c motif) ligand 19; **CSF:** cerebrospinal fluid; **CXCL13:** chemokine (C-X-C motif) ligand 13; **FDC:** follicular dendritic cells; **GC:** germinal center; **LT:** lymphotoxin  $\alpha 1\beta 2$ /LT $\beta$ R system; **LLPC:** long lived plasma cells; **MS:** multiple sclerosis; **PC:** plasma cell; **SLO:** secondary lymphoid organ; **TLO:** tertiary lymphoid organ.

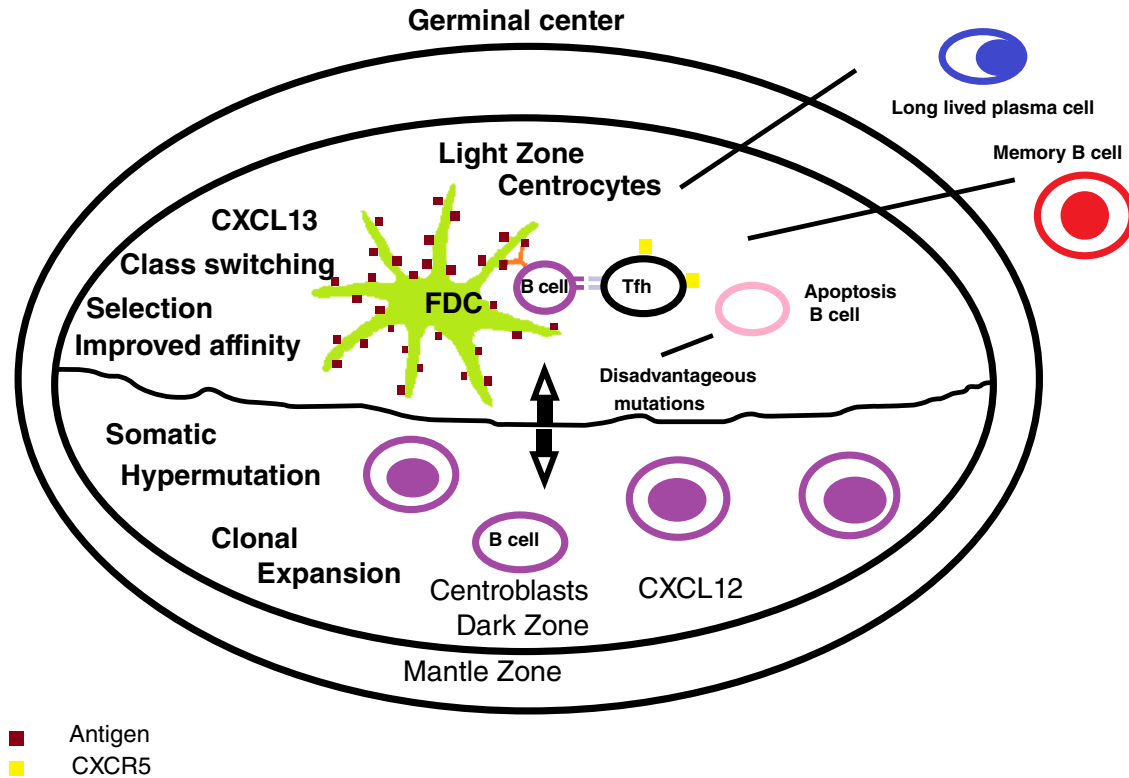
lymphoid organs and their distribution in the GC<sup>23</sup>. Homeostatic chemokines promote secretion of LT $\alpha 1\beta 2$  by T and B cells, establishing a feedback loop that perpetuates the recruitment of lymphocytes and positional organization in the GC<sup>1</sup>. The chemokine CXCL13 has the following relevant properties:

1. CXCL13 increases its own production by stimulating the growth of FDC after regulating LT $\alpha 1\beta 2$  on the membrane of B cells<sup>5,26</sup>.
2. CXCL13 is produced in the SLO by FDC and macrophages and is an important chemoattractant to the CNS<sup>27,28</sup>.
3. Follicular stromal cells express CXCL13, which is needed for nesting CXCR5<sup>+</sup>B cells and a subset of T cells in the follicular compartment<sup>6</sup>.
4. CXCL13 primarily works through CXCR5 expressed in mature B lymphocytes<sup>8</sup>, CD4<sup>+</sup> Tfh<sup>29</sup>, CD4<sup>+</sup> Th17 cells<sup>30</sup>, minor subset of CD8<sup>+</sup> T cells and activated tonsil Treg cells<sup>8,30</sup>.
5. CXCL13 has no relation with CD138<sup>+</sup> and CD38<sup>+</sup> plasmablasts, and PC<sup>17</sup>.

Stromal cells from the T cell zone express the chemokines CCL19 and CCL21, which share the receptor CCR7 that directs naïve, central memory T cells and DC to the T cell compartment<sup>6,31</sup>. CXCR5 is expressed in 20 to 30% of CD4<sup>+</sup>T cells in blood and CSF, and virtually in all B cells in blood and the majority of B cells in the CSF compartment<sup>32</sup>. Mice lacking CXCL13, or its receptor CXCR5, fail to develop peripheral lymph nodes<sup>1</sup>. Khademi *et al.* determined the concentration of CXCL13 in CSF of individuals with MS, other neurological diseases including viral and bacterial infection, and healthy controls finding higher levels of the chemokine in subjects with infections followed to a lesser extent by the patients with MS<sup>33</sup>. The levels of CXCL13 correlated negatively with disease span, concluding that early determination of CXCL13 might predict prognosis of disease<sup>33</sup>.

#### Could the TLO become an operation center with ability to magnify an autoimmune response?

By maintaining antibody diversity, B cell differentiation, isotype switching, oligoclonal expansion, and local production of autoreactive PCs, the TLO perpetuate disease in response to environmental inputs<sup>34</sup>. Lymphoid organogenesis and formation



**Figure 2. The germinal center.** B-cells enter the dark zone of the germinal center (centroblasts), a step which depends on the expression of CXCR4 in the surface, where the cells go through proliferation and somatic hypermutation (SHM). Subsequently, the cells migrate to the light zone (centrocytes) where they capture antigens through the mutated B cell receptors and are internalized for presentation to the T cells. The centrocytes differentiate from the centroblasts by the level of expression of surface proteins. Centrocytes are CXCR4<sup>low</sup>, CD83<sup>high</sup>, CD86<sup>high</sup> and the centroblasts are CXCR4<sup>high</sup>, CD83<sup>low</sup> y CD86<sup>low</sup>. The fluctuation between centroblasts and centrocytes is part of a synchronized cellular program which permits a temporal separation of the processes of mitosis and SHM from selection. The functional output of the TLO, in comparison to the SLO, could result from the dysregulated nature of their GC response supporting a breakout of autoimmune variants and the development of long lasting humoral autoimmunity characterized by presence of B cells with minimal memory and LLPC<sup>15,16</sup>. **FDC**: follicular dendritic cells; **LLPC**: long lasting plasma cells; **Tfh**: T follicular helper cells.

of mTLO may be facilitated by expression of lymphotoxin  $\alpha$  (LT- $\alpha$ ) at the external layer of meningeal inflamed vessels leading to the compartmentalization of the immune response in MS<sup>17,35</sup>. It has been postulated that the perpetuation of neuroinflammation and disease progression results from mTLO induced differentiation and maturation of antigen specific effector lymphocytes<sup>28</sup>. The identification of independent centroblasts in the CSF of MS patients has suggested that there is an intrathecal B cell differentiation which is not dependent on the immune activity in the blood compartment<sup>17</sup>. The TLO, besides SLO, provide a thriving environment where PC differentiate from plasmablasts<sup>6,28</sup>. In the absence of recirculating immune cells from the periphery, the TLO exerts its remarkable ability to remain active for several weeks<sup>36</sup>. Therefore, the neutralization of TLO could play a significant role by blocking the re-emergency of auto reactive clones that could be able to drive relapses or resistance to therapy<sup>36</sup>. Th17 cells, Tfh and a subtype of activated B cells, which are critical in systemic inflammation related with presence of TLO, are strongly associated with MS progression<sup>37</sup>. In an animal model of experimental autoimmune encephalitis (EAE), Peters *et al.* found the following: 1) Expression of CXCL13

by Th17 cells in the CNS; 2) evidence that IL17 and the surface molecule podoplanin contribute to the development of ectopic lymphoid follicle in target organs; and 3) evidence of GC-like reactions in some of the mTLO as suggested by the presence of CXCL13, PNA- and GL7- positive T and B cells and plasma cells<sup>25</sup>. By using next-generation RepSeq analysis, Lehmann-Horn *et al.* demonstrated the presence of SHM and class switch recombination (CSR) in the mTLO B-cell aggregates which had been initiated by activation of the induced cytidine deaminase enzyme (AICDA) in the Thx2D2 mice animal model of chronic EAE<sup>38</sup>. The B-cell repertoires found at the mTLO were different than those found at the SLO suggesting that the mTLO represent autonomous sites of immune activity and that GC activity in the mTLO was antigen driven<sup>38</sup>.

#### In absence of CXCL13, a reduced inflammatory response emerges from studies on animal models and human pathology

Disorganized B cell follicles in SLO have shown reduced capacity to originate natural antibody responses in CXCL13-/- mice<sup>26,39</sup>. Deficiency of CXCL13 results in a moderate course

of disease characterized by a better recovery with attenuation of white matter inflammation and gliosis during the acute and chronic stage of EAE<sup>40</sup>. Krumbholz *et al.* showed there was a direct correlation between CXCL13 levels and the number of B cells, T cells and plasmablasts in the CSF of MS patients<sup>5</sup>. Clonal expansion and SMH of B cells have been observed in the CSF of patients with MS<sup>41</sup>. CXCL13 was upregulated in active MS lesions but not in chronic inactive lesions and, in a similar range, in the serum of patients with relapsing remitting MS (RRMS) and control subjects indicating the intrathecal production of this chemokine<sup>5</sup>. CXCL13 was identified by immunohistochemistry in intrameningeal B-cell follicles, but not in the cerebral parenchyma, of chronic active or inactive MS lesions<sup>42</sup>. Patients with clinically isolated syndrome, who had shown conversion to clinically definitive MS within 2 years, had high levels of CXCL13 in the CSF<sup>33,43,44</sup>. Elevated levels of CXCL13 in CSF have also been reported in patients with RRMS compared to controls and the CSF levels have been significantly increased during relapses but declining after initiation of B cell depleting therapy<sup>22,33,45</sup>.

### Facts learned from the role of CXCL13 in other autoimmune diseases

Chronic and active inflammation in target organs such as thymus, thyroid, synovial tissue and salivary gland can be driven by formation of TLO in the corresponding target organs<sup>2</sup>. Expression of CXCL13 in pancreatic tissue has been associated with formation of ectopic lymphoid follicles and the induction of cascade events leading to diabetes in a transgenic mice model<sup>46</sup>. In patients with *Helicobacter pylori* the blockade of CXCL13 has prevented the development of mucosa-associated lymphoid tissue (MALT) lymphoma and the propagation of inflammation by CXCL13 have been documented in non-lymphoid tissue<sup>47</sup>. Although formation of pulmonary lymphoid follicles has been seen in patients with complicated rheumatoid arthritis, idiopathic pulmonary hypertension and Sjogren's syndrome, the presence of lymphoid follicles has correlated with positive outcome in patients with lung cancer and infections of the respiratory tract<sup>48</sup>. Lung B cells are a major source of CXCL13 and it has a positive role in the lymphoid neogenesis in chronic obstructive pulmonary disease through a LT receptor and toll-like receptor signaling<sup>48</sup>.

### Possible cellular sources of CXCL13 in the CNS

CXCL13 plays an important role in the formation of the GC in ectopic lymphoid follicles of several organs affected by inflammatory or autoimmune disease, or by infection<sup>1</sup>. Important sources of CXCL13 are follicular stromal and FDC<sup>26,49,50</sup>. In human lung tissue of patients with COPD, Litsious *et al.* found that stromal cells and DC produce CXCL13 upon stimulation by lymphocytes –mainly B cells– that express LT thus acquiring lymphoid tissue inducer (LTi) cells function in the TLO<sup>48</sup>. The LT stimulates the expression of CXCL13 by stromal cells mainly through the LT $\beta$  receptor in the SLO<sup>13,26,48,51</sup>. Pikor *et al.* reported the production of CXCL13 by meningeal stromal cells when they are in the presence of LT $\beta$ R in the relapsing-remitting model of EAE in SJL/J mice<sup>52</sup>. The source

of intrathecal production of CXCL13 during neuroinflammation has not been determined with certainty. However, stromal cells within the B cell follicles have been considered to be responsible for the chemokine production and the notion of simple passive transfer from the blood stream to the intrathecal compartment due to dysfunction of the BBB has been detracted<sup>13,26,51,53</sup>. According to Essen and collaborators, stromal cells in the meninges could produce CXCL13 in special circumstances and drive the focal accumulation and organization of lymphoid cells in specific sites<sup>51</sup>. In their study, they were able to demonstrate that microglia cells, *in vivo* and *in vitro*, are the main producers of CXCL13 in acute neuroinflammation induced by the Sindbis virus, which is not associated with demyelination<sup>51</sup>. They also found that type-1 interferon could suppress the production of CXCL13 by microglial cells<sup>51</sup>. In the rhesus macaque model of neuroborreliosis, Ramesh *et al.* and Narayan *et al.* found that infiltrating microglia and macrophage/DC myeloid cells could be one source of CXCL13 in the CNS during inflammation<sup>54,55</sup>. In biopsy specimens from patients with primary CNS lymphoma, Smith and collaborators encountered the following: 1) FDC were not present in the analyzed specimens; 2) there was expression of CXCR5 and CXCL13 in malignant B cells with positive production of BCA-1 (CXCL13) mRNA; and 3) CXCL13 was present in endothelial vascular cells which had a negative production of BCA-1 (CXCL13) mRNA by *in situ* hybridization, a finding that could be attributed to transcytosis<sup>56</sup>. In different types of EAE, CXCL13 and BAFF mRNA transcripts were found to be significantly upregulated in the CNS of mice which developed the relapsing-remitting and the chronic-relapsing courses of disease opposite to those which developed a chronic progressive course. Besides, cells expressing CXCL13 were exclusively found in the brain stem meninges where infiltrating leukocyte proliferation was intense and vascular endothelial cells did not express CXCL13<sup>57</sup>. In specimens from patients with giant cell arteritis, arterial TLO with FDC precursors and lymphoid ducts were detected in the medial layer of the temporal arteries expressing CXCL13, BAFF, APRIL, IL 7, IL 17 and LT $\beta$ <sup>58</sup>.

### A forthcoming research task: How early are the mTLO formed in the lifespan of MS?

Pikor *et al.* conducted studies in the animal model of relapsing-remitting EAE (SJL/J mice) and observed that at the onset of disease the TLO predominantly have T lymphocytes whereas in the acute relapsing phase, the meningeal aggregates exhibited both T and B cells<sup>52</sup>. Meningeal infiltrates can be dispersed or well organized encompassing mTLO, whose lifespan is unknown<sup>28,42</sup>. The presence of follicles containing proliferating B cells, T cells, PC and FDC that express CXCL13 in the proximity of inflamed blood vessels in the meninges of patients with secondary progressive MS (SPMS) has been documented<sup>42</sup>. The mTLO correlated with neuronal loss, adjacent cortical demyelination and a more rapid progression of disease<sup>22</sup>. Patients with SPMS with positive mTLO have shown wide gray matter demyelination associated with loss of neurons, oligodendrocytes, and astrocytes; cortical atrophy, and microglial activation in the outer layer of the cortex<sup>59,60</sup>. It remains to be determined

whether the formation of mTLO depends on the subtype of disease or it is the result of inflammation or consequence of chronicity<sup>36</sup>.

### Could CXCL13 be neutralized by direct action on itself, its receptor (CXCR5) or the lymphotoxin $\beta$ (LT- $\beta$ )?

A novel therapeutic monoclonal antibody against CXCL13 (Mab 5261 and Mab 5261-muIg) has been shown to induce functional *in vitro* inhibition of the chemokine in humans and mice<sup>8</sup>. An LT $\beta$  receptor blocking immunoglobulin inhibits CXCL13 interactions, suppresses the formation of mTLO in the CNS and ameliorates the symptoms of EAE in rodents<sup>23</sup>. In EAE induced by the transfer of myelin-specific Th17 cells (Th17 EAE), Quinn *et al.* confirmed a role of Tfh cells by blocking Tfh trafficking using antibody against CXCL13 and found that this treatment significantly reduced expression of disease<sup>61</sup>. Some DMT available for the treatment for MS ameliorate levels of CXCL13, but the mechanisms by which it occurs are not completely understood. In patients with RRMS treated with natalizumab, a significant reduction in CXCL13 in CSF was observed in comparison to  $\beta$ -interferon<sup>62</sup>. In another study, Novakova *et al.* evaluated the effect of treatment with fingolimod in CSF biomarkers, including CXCL13, of MS patients who had previously been on  $\beta$ -interferon, glatiramer acetate, teriflunomide (and had to switch therapy because of breakthrough disease activity) or natalizumab (who had to switch due to risk of PML) observing significant reduction of CXCL13 in the CSF of patients in both groups<sup>63</sup>. Also, Alvarez *et al.* found that in patients with active RRMS, in spite of treatment with  $\beta$ -interferon or glatiramer acetate, the administration of rituximab led to a normalization of the CSF level of CXCL13 in the majority of patients, thus suggesting that high levels of CXCL13 in CSF at baseline could predict a forthcoming therapeutic response to B cell depletion<sup>64</sup>. Piccio *et al.* found that in patients with RRMS treated with IV rituximab, concomitant with either  $\beta$ -interferon or glatiramer acetate, there was a reduction of CXCL13 and CCL19 in CSF, which correlated with significant reduction of B cells (95%) and T cells (50%) in CSF<sup>32</sup>. Perry *et al.* found intrathecal reduction of CXCL13 (50.4%) and IgG index (13.5%) resulting from inhibition of development of LT $\beta$  cells in patients with MS treated with daclizumab<sup>65</sup>. Braendstrup *et al.* reported the case of a patient with MS who had undergone allogeneic hematopoietic stem cells transplant for treatment of follicular lymphoma and who after two years presented negative determination of oligoclonal bands and detectable CXCL13 in CSF<sup>66</sup>. Esen *et al.* suggested that blockade of CXCL13 could be a possible therapeutic target in EAE with advanced state of inflammation<sup>51</sup>. However, past experience manipulating some of the chemokines in the treatment of MS has been unfavorable<sup>67,68</sup>. Atacept is a recombinant fusion protein that ligands to the cytokines BLys/BAFF and APRIL which are involved in the differentiation, maturation and survival of B cells<sup>67,68</sup>. Although atacept does not accomplish depletion of progenitor or memory B cells<sup>68</sup>, it has the ability to disrupt B cell pathways, thus possibly stimulating a T cell response that leads to the creation of a pro-inflammatory environment<sup>67</sup>. In a clinical trial in patients with MS, atacept was able to

reduce the concentration of immunoglobulins and the number of circulating mature B cells correlating with an increment in relapses without changes in the CNS lesion load by MRI<sup>68</sup>. Upon discontinuation of atacept and a 60-week follow-up, laboratory findings and activity of disease normalized<sup>68</sup>. In fact, atacept and infliximab (a TNF blocking drug) have the ability to induce increment of memory B cells in the blood, enhance their ability to enter the CNS, and increase disease relapse rate and lesion load by MRI<sup>69</sup>. Also, Badr *et al.* have reported evidence of synergy between BAFF and CXCL13 which could have important implications for homeostasis of B cells<sup>70</sup>. Altogether these findings have led to conclude that the immune response in MS is unpredictable and complex and that additional studies must be conducted with significant focus on patient safety<sup>67,68</sup>.

### Would a complementary intrathecal therapy for deactivation of the mTLO be necessary to arrest disease progression?

A self-sustained intrathecal inflammation fostered by CSF chemokines involved in the traffic and survival of inflammatory cells occurs early in disease and is orchestrated by mTLO<sup>3</sup>. Studies have shown that lineage of B cells can travel through peripheral blood, cervical lymphoid nodes, and the intrathecal compartment where they can be exposed to SMH in the mTLO and return to peripheral blood<sup>17</sup>. As mentioned above, Piccio *et al.* found that CSF CXCL13 and CCL19 were decreased at week 24 after IV rituximab<sup>32</sup>. However, Topping *et al.* found that therapy with intrathecal rituximab in patients with RRMS and SPMS resulted in no variation of CXCL13 levels in serum and CSF during the period of evaluation<sup>71</sup>. Bonnan has hypothesized that, in order to prevent an unwanted generalized immune suppression resulting from systemic targeting of resident TLO, intrathecal immune reset should be attempted with a combination of monoclonal antibodies targeting each cell sub-type and aimed at eliminating simultaneously B cells, T cells, PC and FDC, via the intrathecal route. Excepting rituximab, candidate drugs still require preclinical studies for validation<sup>3</sup>. Komori *et al.* reported that in patients with progressive MS who received therapy with intrathecal rituximab the depletion of B cells in the CSF compartment was transient and incomplete and could be facilitated by complement dependent cytotoxicity and to a lesser degree by antibody dependent cellular cytotoxicity<sup>72</sup>.

### Conclusion

An early neutralization of CXCL13 could interfere with the organization and function of the mTLO thus modifying and reducing inflammation in the CNS of patients with MS. Studies in animal models where CXCL13 has been neutralized, or is not expressed (such as the CXCL13<sup>-/-</sup> mice), confirm its crucial role maintaining, rather than initiating, inflammation and its manipulation could lead to modification of disease in these models<sup>39</sup>. However, any therapeutic strategy unable to neutralize LLPCs or antibody secreting cells will not be successful in an attempt to impede the chronic progression of disease<sup>73</sup>. Neutralization of the CXCL13 should be carefully sought as complementary therapy to the DMT in MS.

## Data availability

No data is associated with this article.

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## References

- Jones GW, Jones SA: **Ectopic lymphoid follicles: inducible centres for generating antigen-specific immune responses within tissues.** *Immunology*. 2016; **147**(2): 141–151.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Weyand CM, Kurtin PJ, Goronzy JJ: **Ectopic lymphoid organogenesis: a fast track for autoimmunity.** *Am J Pathol*. 2001; **159**(3): 787–93.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bonnan M: **Intrathecal immune reset in multiple sclerosis: exploring a new concept.** *Med Hypotheses*. 2014; **82**(3): 300–309.  
[PubMed Abstract](#) | [Publisher Full Text](#)
- Mélik-Parsadaniantz S, Rostène W: **Chemokines and neuromodulation.** *J Neuroimmunol*. 2008; **198**(1–2): 62–68.  
[PubMed Abstract](#) | [Publisher Full Text](#)
- Krumbholz M, Theil D, Cepok S, et al.: **Chemokines in multiple sclerosis: CXCL12 and CXCL13 up-regulation is differentially linked to CNS immune cell recruitment.** *Brain*. 2006; **129**(Pt 1): 200–211.  
[PubMed Abstract](#) | [Publisher Full Text](#)
- Drayton DL, Liao S, Mounzer RH, et al.: **Lymphoid organ development: from ontogeny to neogenesis.** *Nat Immunol*. 2006; **7**(4): 344–53.  
[PubMed Abstract](#) | [Publisher Full Text](#)
- Ruddle NH: **Lymphoid neo-organogenesis: lymphotoxin's role in inflammation and development.** *Immunol Res*. 1999; **19**(2–3): 119–125.  
[PubMed Abstract](#) | [Publisher Full Text](#)
- Klitmatcheva E, Pandina T, Reilly C, et al.: **CXCL13 antibody for the treatment of autoimmune disorders.** *BMC Immunol*. 2015; **16**(1): 6.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Allen CD, Okada T, Cyster JG: **Germinal-center organization and cellular dynamics.** *Immunity*. 2007; **27**(2): 190–202.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Hauser AE, Junt T, Mempel TR, et al.: **Definition of germinal-center B cell migration *in vivo* reveals predominant intrazonal circulation patterns.** *Immunity*. 2007; **26**(5): 655–667.  
[PubMed Abstract](#) | [Publisher Full Text](#)
- Schwickert TA, Lindquist RL, Shakhar G, et al.: ***In vivo* imaging of germinal centres reveals a dynamic open structure.** *Nature*. 2007; **446**(7131): 83–87.  
[PubMed Abstract](#) | [Publisher Full Text](#)
- Cyster JG, Ansel KM, Reif K, et al.: **Follicular stromal cells and lymphocyte homing to follicles.** *Immunol Rev*. 2000; **176**(1): 181–193.  
[PubMed Abstract](#) | [Publisher Full Text](#)
- Allen CD, Ansel KM, Low C, et al.: **Germinal center dark and light zone organization is mediated by CXCR4 and CXCR5.** *Nat Immunol*. 2004; **5**(9): 943–952.  
[PubMed Abstract](#) | [Publisher Full Text](#)
- Vinuesa CG, Tangye SG, Moser B, et al.: **Follicular B helper T cells in antibody responses and autoimmunity.** *Nat Rev Immunol*. 2005; **5**(11): 853–865.  
[PubMed Abstract](#) | [Publisher Full Text](#)
- Alsughayyir J, Pettigrew GJ, Motallebzadeh R: **Spoiling for a Fight: B Lymphocytes as Initiator and Effector Populations within Tertiary Lymphoid Organs in Autoimmunity and Transplantation.** *Front Immunol*. 2017; **8**: 1639.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bannard O, Horton RM, Allen CD, et al.: **Germinal center centroblasts transition to a centrocyte phenotype according to a timed program and depend on the dark zone for effective selection.** *Immunity*. 2013; **39**(5): 912–924.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Blauth K, Owens GP, Bennett JL: **The Ins and Outs of B Cells in Multiple Sclerosis.** *Front Immunol*. 2015; **6**: 565.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bittner S, Ruck T, Wiendl H, et al.: **Targeting B cells in relapsing-remitting multiple sclerosis: from pathophysiology to optimal clinical management.** *Ther Adv Neurol Disord*. 2017; **10**(1): 51–66.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Von Büdingen HC, Kuo TC, Sirota M, et al.: **B cell exchange across the blood-brain-barrier in multiple sclerosis.** *J Clin Invest*. 2012; **122**(12): 4533–43.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Palanichamy A, Apeltsin L, Kuo TC, et al.: **Immunoglobulin class-switched B cells form an active immune axis between CNS and periphery in multiple sclerosis.** *Sci Transl Med*. 2014; **6**(248): 248ra106.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Stern JN, Yaari G, Vander Heiden JA, et al.: **B cells populating the multiple sclerosis brain mature in the draining cervical lymph nodes.** *Sci Transl Med*. 2014; **6**(248): 248ra107.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Irani DN: **Regulated Production of CXCL13 within the Central Nervous System.** *J Clin Cell Immunol*. 2016; **7**(5): pii: 460.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Columba-Cabezas S, Griguoli M, Rosicarelli B, et al.: **Suppression of established experimental autoimmune encephalomyelitis and formation of meningeal lymphoid follicles by lymphotoxin beta receptor-Ig fusion protein.** *J Neuroimmunol*. 2006; **179**(1–2): 76–86.  
[PubMed Abstract](#) | [Publisher Full Text](#)
- Meinl E, Krumbholz M, Hohlfeld R: **B lineage cells in the inflammatory central nervous system environment: migration, maintenance, local antibody production, and therapeutic modulation.** *Ann Neurol*. 2006; **59**(6): 880–892.  
[PubMed Abstract](#) | [Publisher Full Text](#)
- Peters A, Pitcher LA, Sullivan JM, et al.: **Th17 cells induce ectopic lymphoid follicles in central nervous system tissue inflammation.** *Immunity*. 2011; **35**(6): 986–996.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Ansel KM, Ngo VN, Hyman PL, et al.: **A chemokine-driven positive feedback loop organizes lymphoid follicles.** *Nature*. 2000; **406**(6793): 309–314.  
[PubMed Abstract](#) | [Publisher Full Text](#)
- Carlsen HS, Baekkevold ES, Morton HC, et al.: **Monocyte-like and mature macrophages produce CXCL13 (B cell-attracting chemokine 1) in inflammatory lesions with lymphoid neogenesis.** *Blood*. 2004; **104**(10): 3021–7.  
[PubMed Abstract](#) | [Publisher Full Text](#)
- Mitsdoerffer M, Peters A: **Tertiary Lymphoid Organs in Central Nervous System Autoimmunity.** *Front Immunol*. 2016; **7**: 451.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Fazilleau N, Mark L, McHeyzer-Williams LJ, et al.: **Follicular helper T cells: lineage and location.** *Immunity*. 2009; **30**(3): 324–335.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Lim HW, Hillsamer P, Kim CH: **Regulatory T cells can migrate to follicles upon T cell activation and suppress GC-Th cells and GC-Th cell-driven B cell responses.** *J Clin Invest*. 2004; **114**(11): 1640–9.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Luther SA, Tang HL, Hyman PL, et al.: **Coexpression of the chemokines ELC and SLC by T zone stromal cells and deletion of the ELC gene in the *plt/plt* mouse.** *Proc Natl Acad Sci U S A*. 2000; **97**(23): 12694–12699.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Piccio L, Naismith RT, Trinkaus K, et al.: **Changes in B- and T-lymphocyte and chemokine levels with rituximab treatment in multiple sclerosis.** *Arch Neurol*. 2010; **67**(6): 707–14.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Khademi M, Kockum I, Andersson ML, et al.: **Cerebrospinal fluid CXCL13 in multiple sclerosis: a suggestive prognostic marker for the disease course.** *Mult Scler*. 2011; **17**(3): 335–343.  
[PubMed Abstract](#) | [Publisher Full Text](#)
- Corsiero E, Nerviani A, Bombardieri M, et al.: **Ectopic Lymphoid Structures: Powerhouse of Autoimmunity.** *Front Immunol*. 2016; **7**: 430.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Corcione A, Casazza S, Ferretti E, et al.: **Recapitulation of B cell differentiation in the central nervous system of patients with multiple sclerosis.** *Proc Natl*



- Acad Sci U S A*. 2004; **101**(30): 11064–9.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
36. Pitzalis C, Jones GW, Bombardieri M, *et al.*: **Ectopic lymphoid-like structures in infection, cancer and autoimmunity.** *Nat Rev Immunol*. 2014; **14**(7): 447–462.  
[PubMed Abstract](#) | [Publisher Full Text](#)
37. Romme Christensen J, Börnsen L, Ratzler R, *et al.*: **Systemic inflammation in progressive multiple sclerosis involves follicular T-helper, Th17- and activated B-cells and correlates with progression.** *PLoS One*. 2013; **8**(3): e57820.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
38. Lehmann-Horn K, Wang SZ, Sagan SA, *et al.*: **B cell repertoire expansion occurs in meningeal ectopic lymphoid tissue.** *JCI Insight*. 2016; **1**(20): e87234.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
39. Rainey-Barger EK, Rumble JM, Lalor SJ, *et al.*: **The lymphoid chemokine, CXCL13, is dispensable for the initial recruitment of B cells to the acutely inflamed central nervous system.** *Brain Behav Immun*. 2011; **25**(5): 922–931.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
40. Bagaeva LV, Rao P, Powers JM, *et al.*: **CXC chemokine ligand 13 plays a role in experimental autoimmune encephalomyelitis.** *J Immunol*. 2006; **176**(12): 7676–7685.  
[PubMed Abstract](#) | [Publisher Full Text](#)
41. Colombo M, Dono M, Gazzola P, *et al.*: **Accumulation of clonally related B lymphocytes in the cerebrospinal fluid of multiple sclerosis patients.** *J Immunol*. 2000; **164**(5): 2782–9.  
[PubMed Abstract](#) | [Publisher Full Text](#)
42. Serafini B, Rosicarelli B, Magliozzi R, *et al.*: **Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis.** *Brain Pathol*. 2004; **14**(2): 164–74.  
[PubMed Abstract](#) | [Publisher Full Text](#)
43. Ferraro D, Galli V, Vitetta F, *et al.*: **Cerebrospinal fluid CXCL13 in clinically isolated syndrome patients: Association with oligoclonal IgM bands and prediction of Multiple Sclerosis diagnosis.** *J Neuroimmunol*. 2015; **283**: 64–69.  
[PubMed Abstract](#) | [Publisher Full Text](#)
44. Bretschneider J, Czerwonik A, Senel M, *et al.*: **The chemokine CXCL13 is a prognostic marker in clinically isolated syndrome (CIS).** *PLoS One*. 2010; **5**(8): e11986.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
45. Sellebjerg F, Börnsen L, Khademi M, *et al.*: **Increased cerebrospinal fluid concentrations of the chemokine CXCL13 in active MS.** *Neurology*. 2009; **73**(23): 2003–2010.  
[PubMed Abstract](#) | [Publisher Full Text](#)
46. Luther SA, Lopez T, Bai W, *et al.*: **BLC expression in pancreatic islets causes B cell recruitment and lymphotoxin-dependent lymphoid neogenesis.** *Immunity*. 2000; **12**(5): 471–481.  
[PubMed Abstract](#) | [Publisher Full Text](#)
47. Yamamoto K, Nishiumi S, Yang L, *et al.*: **Anti-CXCL13 antibody can inhibit the formation of gastric lymphoid follicles induced by *Helicobacter* infection.** *Mucosal Immunol*. 2014; **7**(5): 1244–54.  
[PubMed Abstract](#) | [Publisher Full Text](#)
48. Litsious E, Semitekolou M, Galani IE, *et al.*: **CXCL13 production in B cells via Toll-like receptor/lymphotoxin receptor signaling is involved in lymphoid neogenesis in chronic obstructive pulmonary disease.** *Am J Respir Crit Care Med*. 2013; **187**(11): 1194–1202.  
[PubMed Abstract](#) | [Publisher Full Text](#)
49. Sáez de Guinoa J, Barrio L, Mellado M, *et al.*: **CXCL13/CXCR5 signaling enhances BCR-triggered B-cell activation by shaping cell dynamics.** *Blood*. 2011; **118**(6): 1560–9.  
[PubMed Abstract](#) | [Publisher Full Text](#)
50. Bajénoff M: **Stromal cells control soluble material and cellular transport in lymph nodes.** *Front Immunol*. 2012; **3**: 304.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
51. Esen N, Rainey-Barger EK, Huber AK, *et al.*: **Type-I interferons suppress microglial production of the lymphoid chemokine, CXCL13.** *Glia*. 2014; **62**(9): 1452–1462.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
52. Pikor NB, Astarita JL, Summer-Deluca L, *et al.*: **Integration of Th17- and Lymphotoxin-Derived Signals Initiates Meningeal-Resident Stromal Cell Remodeling to Propagate Neuroinflammation.** *Immunity*. 2015; **43**(6): 1160–1173.  
[PubMed Abstract](#) | [Publisher Full Text](#)
53. Lalor SJ, Segal BM: **Lymphoid chemokines in the CNS.** *J Neuroimmunol*. 2010; **224**(1–2): 56–61.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
54. Ramesh G, Borda JT, Gill A, *et al.*: **Possible role of glial cells in the onset and progression of Lyme neuroborreliosis.** *J Neuroinflammation*. 2009; **6**: 23.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
55. Narayan K, Dail D, Li L, *et al.*: **The nervous system as ectopic germinal center: CXCL13 and IgG in lyme neuroborreliosis.** *Ann Neurol*. 2005; **57**(6): 813–823.  
[PubMed Abstract](#) | [Publisher Full Text](#)
56. Smith JR, Brazier RM, Paoletti S, *et al.*: **Expression of B-cell-attracting chemokine 1 (CXCL13) by malignant lymphocytes and vascular endothelium in primary central nervous system lymphoma.** *Blood*. 2003; **101**(3): 815–821.  
[PubMed Abstract](#) | [Publisher Full Text](#)
57. Magliozzi R, Columba-Cabezas S, Serafini B, *et al.*: **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**(1–2): 11–23.  
[PubMed Abstract](#) | [Publisher Full Text](#)
58. Ciccía F, Rizzo A, Maugeri R, *et al.*: **Ectopic expression of CXCL13, BAFF, APRIL and LT- $\beta$  is associated with artery tertiary lymphoid organs in giant cell arteritis.** *Ann Rheum Dis*. 2017; **76**(1): 235–243.  
[PubMed Abstract](#) | [Publisher Full Text](#)
59. Magliozzi R, Howell OW, Reeves C, *et al.*: **A Gradient of neuronal loss and meningeal inflammation in multiple sclerosis.** *Ann Neurol*. 2010; **68**(4): 477–493.  
[PubMed Abstract](#) | [Publisher Full Text](#)
60. Howell OW, Reeves CA, Nicholas R, *et al.*: **Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis.** *Brain*. 2011; **134**(Pt 9): 2755–2771.  
[PubMed Abstract](#) | [Publisher Full Text](#)
61. Quinn JL, Kumar G, Agasing A, *et al.*: **Role of TFH cells in Promoting T Helper 17-Induced Neuroinflammation.** *Front Immunol*. 2018; **9**: 382.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
62. Novakova L, Axelsson M, Khademi M, *et al.*: **Cerebrospinal fluid biomarkers as a measure of disease activity and treatment efficacy in relapsing-remitting multiple sclerosis.** *J Neurochem*. 2017; **141**(2): 296–304.  
[PubMed Abstract](#) | [Publisher Full Text](#)
63. Novakova L, Axelsson M, Khademi M, *et al.*: **Cerebrospinal fluid biomarkers of inflammation and degeneration as measures of fingolimod efficacy in multiple sclerosis.** *Mult Scler*. 2017; **23**(1): 62–71.  
[PubMed Abstract](#) | [Publisher Full Text](#)
64. Alvarez E, Piccio L, Mikesell RJ, *et al.*: **Predicting optimal response to B-cell depletion with rituximab in multiple sclerosis using CXCL13 index, magnetic resonance imaging and clinical measures.** *Mult Scler J Exp Transl clin*. 2015; **1**: 2055217315623800.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
65. Perry JS, Han S, Xu Q, *et al.*: **Inhibition of LT1 cell development by CD25 blockade is associated with decreased intrathecal inflammation in multiple sclerosis.** *Sci Transl Med*. 2012; **4**(145): 145ra106.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
66. Braendstrup P, Langkilde AR, Schreiber K, *et al.*: **Progression and CSF Inflammation after Eradication of Oligoclonal Bands in an MS Patient Treated with Allogeneic Hematopoietic Cell Transplantation for Follicular Lymphoma.** *Case Rep Neurol*. 2012; **4**(2): 101–106.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
67. Bible E: **Multiple sclerosis: Atacept increases relapse rates in multiple sclerosis.** *Nat Rev Neurol*. 2014; **10**(4): 182.  
[PubMed Abstract](#) | [Publisher Full Text](#)
68. Kappos L, Hartung HP, Freedman MS, *et al.*: **Atacept in multiple sclerosis (ATAMS): a randomised, placebo-controlled, double-blind, phase 2 trial.** *Lancet Neurol*. 2014; **13**(4): 353–63.  
[PubMed Abstract](#) | [Publisher Full Text](#)
69. Baker D, Marta M, Pryce G, *et al.*: **Memory B Cells are Major Targets for Effective Immunotherapy in Relapsing Multiple Sclerosis.** *EBioMedicine*. 2017; **16**: 41–50.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
70. Badr G, Borhis G, Lefevre EA, *et al.*: **BAFF enhances chemotaxis of primary human B cells: a particular synergy between BAFF and CXCL13 on memory B cells.** *Blood*. 2008; **111**(5): 2744–54.  
[PubMed Abstract](#) | [Publisher Full Text](#)
71. Topping J, Dobson R, Lapin S, *et al.*: **The effects of intrathecal rituximab on biomarkers in multiple sclerosis.** *Mult Scler Relat Disord*. 2016; **6**: 49–53.  
[PubMed Abstract](#) | [Publisher Full Text](#)
72. Komori M, Lin YC, Cortese I, *et al.*: **Insufficient disease inhibition by intrathecal rituximab in progressive multiple sclerosis.** *Ann Clin Transl Neurol*. 2016; **3**(3): 166–179.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
73. Eggers EL, Michel BA, Wu H, *et al.*: **Clonal relationships of CSF B cells in treatment-naive multiple sclerosis patients.** *JCI Insight*. 2017; **2**(22): pii: 92724.  
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

# Open Peer Review

Current Referee Status:  

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## Version 2

Referee Report 24 September 2018

doi:[10.5256/f1000research.17713.r38354](https://doi.org/10.5256/f1000research.17713.r38354)



### Anneli Peters

Institute of Clinical Neuroimmunology, Hospital and Biomedical Center of the Ludwig-Maximilians University, Martinsried-Planegg, Germany

The authors have addressed the concerns raised by the reviewers in the revised version.

In the new version there is one misleading statement in the paragraph about TLOs becoming operation centers (pg 5): "In ..EAE they found the following: 1) Expression of CXCL13 by Th17 cells in the CNS;". This statement is not correct - in this reference CXCL13 expression was detected in the CNS of Th17 recipient mice, but the source of CXCL13 are (most likely) not Th17 cells themselves but other cell types like stromal cells. This should be corrected.

**Competing Interests:** No competing interests were disclosed.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Referee Report 17 September 2018

doi:[10.5256/f1000research.17713.r38355](https://doi.org/10.5256/f1000research.17713.r38355)



### Hans Lassmann

Center for Brain Research, Medical University of Vienna, Vienna, Austria

The authors have revised the manuscript and have addressed all concerns raised by the reviewers.

**Competing Interests:** No competing interests were disclosed.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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## Version 1

Referee Report 16 July 2018

doi:10.5256/f1000research.15843.r35347



### Anneli Peters

Institute of Clinical Neuroimmunology, Hospital and Biomedical Center of the Ludwig-Maximilians University, Martinsried-Planegg, Germany

In this review article the authors describe the role of the chemokine CXCL13 in the formation of meningeal TLOs in MS and suggest it as a therapeutic target. The article is well written and the first half of the article provides a very detailed overview of the components and requirements for formation of secondary lymphoid organs. The authors then switch to tertiary lymphoid organs in the CNS assuming that all components and mechanisms of formation are identical to SLOs. While this may be the case for some of the most developed TLOs in some autoimmune diseases like Myasthenia gravis, it is not so clear which cell types and molecular players are required for formation of meningeal TLOs. In fact, to my knowledge it has not even been formerly proven that CXCL13 is required for formation of meningeal TLOs. Even though it is quite likely considering detection of CXCL13 in mTLOs and elevated CXCL13 levels in the CSF of MS patients, definitive proof even in the animal model is missing as also pointed out by reviewer 1, because a) CXCL13-deficient mice already have a defect in mounting proper immune responses in SLOs and b) active EAE induced by MOG-peptide/CFA immunization does not prominently feature mTLOs. The mouse models that do feature mTLOs such as the spontaneous 2D2xTh mouse have not been studied in the context of CXCL13 deficiency.

Furthermore, it would be very useful to discuss in this review cellular sources of CXCL13 in the CNS, as they may not be identical to SLOs. Thus, microglia (Ref 37) and meningeal stromal cells (Pikor et al., *Immunity*, 2015<sup>1</sup>) have been suggested as sources for CXCL13 and should be discussed.

Another important point is that the authors state that the "mTLO maintain differentiation and maturation of antigen-specific lymphocytes which perpetuate inflammation and disease progression". This is not a fact but a hypothesis and should be stated as such. While it is clearly an attractive hypothesis there is no proof neither in mouse models, nor in MS. We agree with the authors that in MS occurrence of TLOs has been *associated* with more severe disease course and cortical lesions, however, causality has not been demonstrated and even evidence for maturation of antigen-specific lymphocytes in mTLOs is very limited so far. Therefore, we believe that it is not justifiable to interfere with mTLO formation in MS patients, as long as their biological function and consequences are not much better understood.

As a side note, some sentences are a bit unclear, for example in the introduction "Inflammation is the appropriate immune response to...autoimmunity..." (pg 2) and "Tfh cells are characterized by expression of CXCR5 and ICOS, which is a subtype of Tfh cells" (pg 3).

Overall, the review has a very interesting and important topic, however, in my opinion as detailed above in several paragraphs the wording should be a bit more careful and precise in order not to be misleading.

### References

1. Pikor NB, Astarita JL, Summers-Deluca L, Galicia G, Qu J, Ward LA, Armstrong S, Dominguez CX, Malhotra D, Heiden B, Kay R, Castanov V, Touil H, Boon L, O'Connor P, Bar-Or A, Prat A, Ramaglia V, Ludwin S, Turley SJ, Gommerman JL: Integration of Th17- and Lymphotoxin-Derived Signals Initiates Meningeal-Resident Stromal Cell Remodeling to Propagate Neuroinflammation. *Immunity*. 2015; **43** (6): 1160-73 [PubMed Abstract](#) | [Publisher Full Text](#)

**Is the topic of the review discussed comprehensively in the context of the current literature?**

Yes

**Are all factual statements correct and adequately supported by citations?**

Partly

**Is the review written in accessible language?**

Yes

**Are the conclusions drawn appropriate in the context of the current research literature?**

Partly

**Competing Interests:** No competing interests were disclosed.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Author Response 10 Sep 2018

**Carlos Mora**, Georgetown University Hospital, Washington DC, USA

**Author response to Reviewer 2.**

We also thank Dr. Peters for her important observations to the content of the first version of the manuscript. We concur with the fact that the current knowledge on the formation of the secondary lymphoid organs (SLO) should not be unquestionably extrapolated and applied to the understanding of the genesis of the tertiary lymph nodes (TLO) especially in the context of neuroinflammation. We also understand that some of the existing concepts on this topic should still remain at a hypothetical, instead of conclusive, level of consideration. Yet, thanks to this reviewer comments and encouragement, especially in relation to discussion of possible cellular sources of CXCL13 in the CNS and to the search for further literature supporting a role for CXCL13 in the EAE animal models, we were able to expand in depth the content of the manuscript and bring more interesting material to the discussion giving further support to the role of this chemokine in the pathogenesis of MS. Certainly, patient safety is a demanding priority and any application of the knowledge acquired from *in vitro* or EAE animal model studies to the treatment of patients with MS should be considered with extreme caution.

**Competing Interests:** No competing interests were disclosed.

Referee Report 30 April 2018

doi:10.5256/f1000research.15843.r33520



**Hans Lassmann**

Center for Brain Research, Medical University of Vienna, Vienna, Austria

In the review article the authors highlight the potential importance of tertiary lymph follicle like structures in the meninges of MS patients as driving forces for tissue damage and in particular cortical demyelination. They provide a very good summary of immunological mechanisms, which are involved in the organization

and function of secondary lymph follicles in the peripheral lymphatic tissue and in particular highlight the importance of the interaction of CXCL13 with other cytokines and chemokines in these processes. They then describe in detail the evidence for the presence of structures with features of tertiary lymph follicles in the meninges of MS patients and their association with disease severity and cortical pathology. Finally they also review in detail the observations that CXCL13 is present in the CSF and may serve as a biomarker associated with poor prognosis of the disease. Based on the experimental observation that CXCL13 blockade or genetic ablation ameliorates EAE the authors propose that therapeutic blockade of CXCL13 in the CNS compartment of MS patients may be beneficial.

There is now good cumulative evidence that such follicle like inflammatory aggregates in the meninges are an important substrate of disease pathology in MS and that B-lymphocytes play an important, but so far not fully understood pathogenetic role in the disease. It is also clear that CXCL13 is an important chemokine, involved in B-cell recruitment into the central nervous system. However, it may be premature at the present time to propose intrathecal CXCR13 blockade as a therapy for MS patients. The EAE studies are only of limited value. It is not a surprise to ameliorate EAE with a therapy, which has major effects on the organization and function of peripheral lymphatic tissue. Although some EAE models show lymphocytic aggregates in the meninges, which share some features with those in MS, this is not the case in the majority of the models. Furthermore, in the respective mouse EAE models with lymph follicle like aggregates in the meninges there is no cortical demyelination. Thus lesion pathogenesis apparently is quite different between these models and MS. To what extent an intrathecal blockade of CXCL13 has an effect on CNS inflammation and what kind of effect will be achieved, is currently unknown. Whether this may induce dangerous side effects is also unclear. The suggestion to combine such a treatment with simultaneous intrathecal elimination of B-cells, T-cells and other immune cells is also far away from realization. Elimination with currently used antibodies requires complement of antibody dependent cellular cytotoxicity, and whether this is safe to induce within the CNS compartment in patients is also rather uncertain.

Thus this review addresses a topic, which is interesting in a disease such as multiple sclerosis, but the suggestions for therapeutic translation are currently premature and potentially dangerous.

**Is the topic of the review discussed comprehensively in the context of the current literature?**

Partly

**Are all factual statements correct and adequately supported by citations?**

Partly

**Is the review written in accessible language?**

Yes

**Are the conclusions drawn appropriate in the context of the current research literature?**

Partly

**Competing Interests:** No competing interests were disclosed.

**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.**

Author Response 30 May 2018

**Carlos Mora**, Georgetown University Hospital, Washington DC, USA

We thank Dr. Lassmann (Referee 1) for the valuable comments addressed upon the review of version No. 1 of our article. We agree on the inconclusive current state of knowledge on the possible effect of intrathecal blockade of CXCL13 during CNS inflammation. On the concept of efficacy of monoclonal antibodies as modulators of the immune response in the cerebrospinal fluid (CSF), special mention deserves the work reported by Komori et al. on the insufficient inhibition of activity of disease, upon the administration of intrathecal rituximab, in chronic progressive multiple sclerosis (MS). These investigators found out that the efficacy of a monoclonal antibody in CSF will not be substantial as long as the blood brain barrier remains closed. Following rituximab therapy, depletion of B-cells in the CSF was facilitated by complement dependent cytotoxicity (CDC) and, to a lesser degree, by antibody dependent cellular cytotoxicity. Although a decrement in the concentration of complement would reduce the efficacy of CDC, the addition of complement in the CSF could lead to adverse effects in the CNS tissue [reference: Komori M, Lin YC, Cortese I, Blake A, Ohayon J, Cherup J, et al. Insufficient disease inhibition by intrathecal rituximab in progressive multiple sclerosis. *Ann Clin Transl Neurol* 2016;3(3):166-179 doi: 10.1002/acn3.293]. In relation to the concept of 'therapeutic translation' mentioned in the review, specifically on the effect of an eventual combination of intrathecal blockade of CXCL13 with simultaneous intrathecal elimination of B-cells, T-cells and other immune cells, we do agree this therapeutic approach would be premature and could be potentially harmful for the recipients of such combined therapies clarifying that the hypothesis formulated by Bonnan [ref. 3 in the article] does not make any reference to the intrathecal blockade of CXCL13 in MS. We look forward to hearing further comments from reviewers prior to publication of version No. 2 of the article.

**Competing Interests:** No competing interests were disclosed.

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Referee Response ( *Member of the F1000 Faculty and F1000Research Advisory Board Member* ) 05 Jun 2018

**Hans Lassmann**, Department Neuroimmunology, Medical University of Vienna, Austria

I agree with the comment of the authors.

**Competing Interests:** No competing interests were disclosed.

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