B Cells Accumulate in the Cerebrospinal Fluid in Inflammatory Neurological Diseases

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Abstract

The contribution of B lymphocytes and their products to the pathogenesis of inflammatory diseases of the central nervous system is still not fully understood. Beside their role as precursors of antibody-secreting cells, B cells participate in the regulation of T cell activation through their antigen-presenting capacity, the production of cytokines and chemokines and the formation of ectopic germinal centers in intermeningeal spaces. This article reviews the current knowledge on B cells within the cerebrospinal fluid in inflammatory diseases affecting the central nervous system. Here, we will focus on two prototypical inflammatory diseases of the central nervous system: multiple sclerosis, an autoimmune-mediated inflammatory disease, and infection-triggered inflammation in Lyme neuroborreliosis.

Keywords: Cerebrospinal fluid; B cells; Inflammation; Multiple sclerosis; Neuroborreliosis

Abbreviations: CD: Cluster of Differentiation; CIS: Clinically Isolated Syndrome; CNS: Central Nervous System; CPMS: Chronic Progressive Multiple Sclerosis; CSF: Cerebrospinal Fluid; HIV: Human Immunodeficiency Virus; Ig: Immunoglobulin; MBP: Myelin Basic Protein; MOG: Myelin Oligodendrocyte Glycoprotein; MRI: Magnetic Resonance Imaging; MS: Multiple Sclerosis; OspA: Outer Surface Protein A; PLP: Proteo Lipid Protein; PPMs: Primary Progressive Multiple Sclerosis; RRMS: Relapsing Remitting Multiple Sclerosis; SPMS: Secondary Progressive Multiple Sclerosis; VCAM-1: Vascular Cell Adhesion Molecule 1; VLA-4: Very Late Activation Antigen 4

B cells in Central Nervous System Inflammation

After the differentiation from pro- and pre-B cell precursors in the bone marrow, naive immature B cells migrate to secondary lymphoid tissues, predominantly the spleen, in order to emerge as CD19++CD20+surfaceIgM-surfaceIgD+ mature naïve B cells [1]. Following an antigen-driven germinal center reaction in secondary lymphoid organs and stimulation by dendritic cells and T cells, B cells get activated and subsequently differentiate into CD19+CD20++CD27++CD38++CD138- memory B cells and antibody-secreting effector cells, consisting of CD19+CD20-CD27++CD38++CD138-HLA-DR++ plasma blasts and CD19-CD20-CD27++CD38++CD138-HLA-DR- plasma cells [2]. Whereas plasma blasts are short-lived and disappear quickly after removal of their challenging antigen, plasma cells persist for several months to years in their specific survival niches, such as inflamed tissue or the bone marrow [3]. Different functions and phenotypes of various B-cell populations are shown in Figure 1.

B cells are largely absent in the cerebrospinal fluid (CSF) in non-inflammatory conditions, but accumulate during central nervous system (CNS) inflammation [4]. In acute infectious diseases or chronic inflammatory disorders, B cells represent up to 30% of all cells in the CSF [5-8]. Oligoclonal bands and intrathecal immunoglobulin (Ig) synthesis are found in a variety of subacute and chronic infections of the CNS, like human T cell lymphotropic virus-associated myelopathy, subacute sclerosing panencephalitis, human immunodeficiency virus (HIV) infection of the CNS, neurosyphilis, cryptococcal and mumps meningitis and neuroborreliosis [8-10]. In these diseases, the intrathecal Ig response is specific to the underlying infectious agent [11-15], whereas the target of the intrathecal antibody response in multiple sclerosis (MS) is still unknown.

Before we highlight the role of CSF B cells in neurological diseases, it is necessary to introduce the reader to the current understanding of immune cell trafficking into the brain. Cells of the immune system have access to the three distinct anatomical compartments (CSF, meninges and brain parenchyma), which are all relevant for CNS inflammation [16,17]. Whereas the migration of leukocytes into the brain parenchyma occurs at the blood-brain barrier (BBB), a highly specialized membrane, the migration of leukocytes into the CSF occurs through the choroid plexus into the subarachnoid space which contains the CSF, as illustrated in Figure 2. The choroid plexus is a specific anatomical structure located in brain ventricles. The CSF is secreted by modified epithelial cells of the choroid plexus, which are also known as Kolmer cells. In contrast to the BBB, the choroid plexus allows immune cells, particularly lymphocytes, an easier passage into the CSF [17]. Thus, the CSF contains less innate immune cells and more lymphocytes than peripheral blood [18]. Trafficking of immune cells into the CSF is increased in CNS inflammation and according to the hallmark publication of Reiber and Peter in 2001, the total cell count in CSF represents the most sensitive parameter for characterization of an acute inflammatory disease of the CNS. Whereas in normal CSF 0–4 leukocytes/μl are observed, CSF leukocyte counts are moderately increased in MS and significantly higher in acute infectious brain inflammation such as meningitis or meningoencephalitis [19]. In summary, inflammatory CNS disorders are characterized by the occurrence of intrathecal Ig synthesis and pleocytosis in the CSF.

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Multiple Sclerosis

MS is the most frequent, chronic inflammatory demyelinating disease of the CNS, mainly affecting young adults and characterized by a heterogeneous clinical presentation [20,21]. Although, the etiology of MS is still unknown, it is widely considered as autoimmune disease triggered by environmental factors in genetically susceptible individuals [22]. Worldwide, approximately 2.5 million people suffer from MS. 85-90% of MS patients initially present with relapsing-remitting MS (RRMS) characterized by acute relapses followed by complete or incomplete remission [21]. The majority of these patients later convert to a secondary progressive disease course (SPMS). 10-15% of patients suffer from primary progressive MS (PPMS), a malignant disease with steady progression from the onset. Current treatments for MS attempt to reduce the inflammatory activity by immunomodulation, prevent the entry of immune cells into the CNS, deplete specific subsets of immune cells, or suppress immune responses in an unspecific manner [21]. The discovery of intermeningeal ectopic germinal centers, associated with B cells and high concentrations of germinal center promoting cytokines and chemokines like CxCL-13, suggest the de novo formation of ectopic lymphoid structures (neolymphogenesis) within the CNS [23-26]. These observations foster the crucial contribution of B cells in MS. Although, intensive and controversial research on the role of B cells in MS is ongoing for several years [27].

Intrathecal Ig Synthesis in MS

More than 90% of MS patients show a persistent increased intrathecal production of Ig, mainly consisting of oligoclonal IgG [28]. Oligoclonal IgG bands are restricted to the CSF [29] and their presence is an important marker for the diagnosis of MS [10]. Additionally, oligoclonal IgM synthesis in the CSF occurs in about 40% of MS patients [30]. Although many efforts have been made to identify the targets of the intrathecally produced antibodies, they are still largely unknown [31]. Recently, Owens et al. described that CSF IgG antibodies do not target myelin oligodendrocyte protein (MOG), myelin basic protein (MBP) and proteo lipid protein (PLP), three of the major myelin proteins, and do only weakly react with MS brain tissue [32]. In contrast, myelin lipids were identified as main targets for oligoclonal IgM bands in more than 70% of cases [33,34] and a higher IgM index at clinical onset of MS correlates with faster progression of the disease, thereby predicting an aggressive MS disease course [33,35-37]. Furthermore, a follow-up study showed that the more aggressive MS disease course in the presence of lipid-specific IgM bands is characterized by the occurrence of more relapses and higher disability [34]. Villar et al. [34] found an increased number of persisting IgM secreting CD19+CD5+ B cells in the CSF of MS patients with anti-lipid IgM oligoclonal bands compared to patients with IgM bands lacking myelin lipospecificity. By longitudinal analysis of CSF and serum samples from oligoclonal IgM band positive patients with and without lipid-specificity, it was shown that lipid-specific oligoclonal IgM bands persist for approximately two years, thus indicating a persistent immune response. In contrast, oligoclonal IgM bands without lipid-specificity at disease onset disappeared 18 months later, thus suggesting a transient immune response [34]. In summary, intrathecal synthesis of immunoglobulins, especially IgG is a useful marker for the diagnosis of MS, although the targets of these antibodies are not fully resolved yet. Moreover, B cells, plasma cells and excess immunoglobulins are found in MS lesions and B cell follicle-like structures in the meninges of MS patients have been discovered [23,38,39]. Recent data of successful clinical trials using rituximab, a monoclonal chimeric antibody targeting CD20, expressed on B cells, support the crucial role of B cells in MS pathology, thus indicating B cells as key players in MS disease activity [40].

B cells in the CSF of MS Patients

Whereas there are no differences in the distribution of leukocyte subsets in the peripheral blood, impressing variations in leukocyte populations in the CSF of MS patients compared to non-inflammatory neurological diseases can be monitored [4]. CD19+ B cells, which are nearly absent in the CSF of healthy individuals and patients with non-inflammatory neurological diseases, were shown to accumulate in the CSF during CNS inflammation [4,7]. In addition, a more progressive...
In CNS inflammatory conditions, most B cells in the CSF are CD27 expressing antigen-experienced memory B cells and short-lived CD19+CD27++CD138+ plasma blasts [6,7,24,42,43]. Cepok and colleagues showed that almost 50% of B lymphocytes in the CSF are plasma blasts, a phenotype which is only infrequently found in the peripheral blood of MS patients [6], but which is abundant in the peripheral blood of patients with systemic lupus erythematosus [44,45]. Plasma blasts in the CSF were found to be discriminated from naïve as well as memory B cells by their increased size and density, representing a higher activation state [6]. Moreover, it was shown that the quantity of CD19+CD138+ plasma blasts in the CSF correlates with intrathecal IgG synthesis and inflammatory parenchymal disease activity, as monitored by the number and volume of gadolinium-enhancing lesions in T1-weighted magnetic resonance images (MRI) [6,7]. By analyzing CD19+CD27+ memory B cells in more detail, two distinct subsets depending on the strength of CD27 expression have been identified. One subset is characterized by a high expression (CD19+CD27++) while the other exhibits only an intermediate expression of CD27 (CD19+CD27+) [6]. By further analyzing the two distinct subsets of CD19+CD27+ memory B cells for the expression of CD138, high expression of CD138 was mainly found on CD19+CD27++ B cells. Furthermore, the CD19+CD27++ B cell subset was shown to express lower levels of CD19 than naïve CD19+CD27- and memory CD19+CD27+ B cells [6]. In addition, CD19+CD27++ B cells express high levels of CD38, thereby representing a phenotype comparable with plasma blasts [6]. Recently, our group could demonstrate that the number of CSF B cells correlates with intrathecal production of matrix metalloproteinase-9 (MMP-9) and CxCL-13, two mediators promoting B cell migration through the BBB and maintenance of immune responses within the CNS [7]. MMP-9, which may be secreted by leukocytes and CNS-resident cells under inflammatory conditions, is involved in the degradation of the extracellular matrix, thereby promoting BBB leakage and subsequent transmigration of leukocytes into the brain [7,46-48]. CxCL-13 is a key regulator for B cells within lymphoid tissues and follicles, produced by stromal cells of germinal centers but also by monocytes, macrophages and dendritic cells [49-53]. It was shown several times that both mediators, MMP-9 and CxCL-13 are increased in the CSF of patients affected with MS as well as neuroborreliosis thereby explicating the accumulation of B cells in the CSF [50,54-60]. In addition, an obvious accumulation of CD19+CD138- mature B cells and CD19+CD138+ plasma blasts was observed in the CSF of patients affected with CIS and RRMS, but not in Chronic Progressive MS (CPMS), suggesting active inflammation [7]. The quantity of mature B cells and plasma blasts was associated with higher disease activity, as measured by the number of T2 lesions as well as the presence of gadolinium-enhancing lesions, referring to acute brain inflammation and BBB dysfunction [7]. Cepok and colleagues investigated the expression of CD19 and CD27 on CD138+ B cells in more detail [6]. They found that the majority of CD138+ B cells expresses high levels of CD27, HLA-DR as well as intermediate to high levels of CD19, a phenotype comparable to short-lived plasma blasts [6]. By longitudinally investigating the num-

**Figure 2: Transmigration of immune cells across the choroid plexus into the CSF.**

Under the influence of chemokines immune cells in the small choroidal capillaries undergo adhesion, rolling and finally diapedesis into the stroma. There, the cells might be attracted by additional chemokines, produced by the CSF-secreting epithelial cells of the choroid plexus (Kolmer cells), thus resulting in migration through the epithelial cell layer into the ventricle.

**Abbreviation:** CSF: Cerebro Spinal Fluid.

Illustration modified from Wilson et al. [17] and Ransohoff et al. [16].
Numer of CD19+CD138+ plasma blasts in the CSF of 61 MS patients as well as 10 patients affected with other inflammatory neurological diseases, Cepok and colleagues showed that plasma blasts persist throughout the disease course of MS. However, they disappear from the CSF of patients affected with infectious inflammatory CNS disorders like neuroborreliosis or viral meningitis after the clearance of the pathogen [6]. In contrast to plasma blasts in the CSF, CD19+CD138- mature B cells were shown to persist for years in various inflammatory CNS diseases (MS, neuroborreliosis and viral meningitis) [6]. Another study aimed to analyze the heterogeneous group of CD19+CD27+ memory B cells in the CSF of various inflammatory diseases like MS, CIS, viral meningitis or meningencephalitis in more detail and found a selective enrichment of class-switched IgM-IgD-CD27+ memory B cells [42]. In contrast, the transmigration of CD19+CD27- naïve B cells from the periphery into the CNS was found to be largely prohibited [42]. Since the majority of patients suffer from RRMS, analysis of the immune cell subpopulations in the CSF during remission and clinical relapses were performed. Investigations in five RRMS patients revealed variations in the absolute white cell count whereas the distribution of different immune cell subpopulations (monocytes and B cells) remained stable [4]. The authors speculate that the patterns of CSF cytology in MS patients do not correspond to the various disease phases; however they suggest that the variations may reflect individual differences in immune reactivity with a predominance of B cells in some patients and monocytes in others. Furthermore, higher numbers of B cells relative to monocytes correlate with faster disease progression [4]. In addition, in a subgroup of patients suffering from RRMS and SPMS they found that high numbers of B cells and low numbers of monocytes are associated with a higher progression rate [4]. There are inconsistent reports on the frequency of plasma cells in the CSF of MS patients. While some data suggest increased numbers of CD19-CD138+ long-living plasma cells in the CSF from MS patients [24], others report only low counts of plasma cells with unchanged frequencies, regardless of the disease duration and the MS disease course [6,7]. Moreover, Corcione et al. [24] reported CD19+CD38+CD77+ centroblasts in the CSF of MS patients. In addition to analysis of the CSF B cell composition in untreated MS patients, several approaches aimed to evaluate the effect of various MS treatments on CSF B cells. Thereby, the effects of natalizumab on CSFB cells have been investigated. Natalizumab is a humanized monoclonal antibody, specific for very late activation antigen 4 (VLA-4), an adhesion molecule which is expressed by all white blood cells except neutrophils. Binding of natalizumab to VLA-4 inhibits the interaction of VLA-4 with its ligand vascular cell adhesion molecule 1 (VCAM-1), thus preventing leukocyte transmigration into the CNS [61]. By analyzing the number of white blood cells in the CSF, it was shown that natalizumab results in a decline in all major leukocyte subsets [61]. This effect was sustained even six months after termination of therapy, where among others, the numbers of CD19+B cells and CD138+ plasma cells remained lower in treated MS patients, compared to untreated MS patients [61]. However, the quantity of lymphocytes reverted to normal 14 months after cessation of natalizumab treatment [62]. Since beneficial effects of therapies targeting CD20 in MS have been reported, the effects of B cell depletion using rituximab were investigated in the peripheral blood and in the CSF [40,63]. Rituximab was found to decrease the quantity of B cells in the peripheral blood as well as in the CSF [64-67]. By analyzing B cells for the expression of co-stimulatory molecules pre- and post rituximab, Piccio et al. found that the total number of B cells in the CSF significantly decreased post-treatment. However, the proportion of B lymphocytes expressing co-stimulatory molecules CD80 and CD86 was significantly increased post-treatment [64]. Moreover, it was shown that rituximab effectively depletes B cells in the cerebral perivascular spaces, the main CNS compartment in which antigen presentation and T cell reactivation occurs [67]. Fingolimod (FTY720), an oral immunomodulatory drug, was recently approved for the treatment of RRMS [68-70]. One study showed that although fingolimod significantly decreases B cells in the periphery, it only had little impact on CSF B cells [71]. In conclusion, the accumulation of B cells, particularly of CD19+CD27+ and CD138+ plasma blasts, in the CSF establishes them as one of the central players in active MS. Furthermore, their numbers were found to correlate with intrathecal immunoglobulin synthesis, intrathecal production of CXCL-13 and MMP-9 as well as acute brain inflammation. Moreover, recent promising results of novel therapeutic approaches, either targeting the entry of leukocytes into the brain or depleting B cells, underpin their importance in chronic inflammatory demyelinating diseases, like MS.

**B cells in Neuroborreliosis**

Lyme borreliosis is a multisystem disease caused by infections with the spirochete *Borrelia (B.burgdorferiensulato)* transmitted by ticks of the species *Ixodes* [72-75]. The prevalence of Lyme borreliosis ranges from 20-100 per 100,000 in the US to 100-130 per 100,000 in Europe [76,77]. So far, four human pathogenic species of *B.burgdorferiensulato* have been described [77]. In Europe the genospecies *B.burgdorferiensustricto, B.garini, B.afzelii* and *B.piemontii* exist. By contrast, in the US only one human pathogenic species, *B.burgdorferiensustricto* is present [72,77-81]. Whereas all genospecies may cause erythema migrans, *B.afzelii* has been mainly associated with *Acanthamoebatitis Chronic Atrophicans, B.burgdorferiensustricto* with arthritis and *B.garini* is predominantly associated with neurologistical manifestations of the disease [78]. Lyme neuroborreliosis is a severe inflammatory manifestation affecting the peripheral and central nervous system [77,82]. It may lead to multiple pathological and clinical symptoms like lymphocytic meningitis, meningencephalitis, cranial or peripheral neuritis and painful meningoradiculitis [74,77,83,84]. Acute painful meningoradiculitis is the most frequent CNS manifestation observed in Europe [5]. In meningoradiculitis it is assumed that the spirochete *B.burgdorferi* infects the brain and the meninges, although the patho mechanism is not fully understood so far [5]. In the majority of cases, the immune response in the CNS successfully clears the infection, resulting in remission of the symptoms [5]. Nevertheless, chronic neurologic diseases, like encephalomyelitis can occur in about 10-20% of untreated patients infected with *B.burgdorferi*, even after long periods of latent infection, thereby aggravating correct diagnosis [5,76,84]. Neuroborreliosis leads to local inflammation in the host and accumulation of leukocytes in the CSF [5,85]. MRI studies in late, chronic disease stages showed diffuse white matter lesions [86]. In addition to CSF-derived T cells, reactive with either *B.burgdorferi*-derived antigens or CNS-self antigens [87], T cells cross-reactive with both were detected [88,89]. Thus indicating that autoimmunity triggering the chronic disease is provoked by molecular mimicry [88,89]. The presence of antigen-triggered immunity is fostered by the presence of oligoclonal IgG and IgM bands in the CSF of affected individuals [5]. The presence of intrathecal *B.burgdorferi*-specific antibody production is partly considered to be essential for the diagnosis of neuroborreliosis [84]. The intrathecal synthesis of immunoglobulins may persist for several months or years after treatment [84]. Some approaches have analyzed the targets of immunoglobulins in the CSF of patients affected with neuroborreliosis.
These studies detected IgG specific for *B. burgdorferi* [90,91] but also CNS autoantigens [92]. Another study analyzed independently clonally expanded CD138+ plasma cells from the CSF and found distinct reactivities, for *B. burgdorferi*-derived antigens and CNS autoantigens, thus indicating that mechanisms involving molecular mimicry are absent in neuroborreliosis [83]. Cepok and colleagues reported high percentages of B cells and plasma cells in the CSF of patients affected with *B. burgdorferi* meningoencephalitis at disease onset, compared to non-infectious neurological diseases and viral meningitis [5]. During the first weeks of recovery, CD19+B cells were found to persist or even relatively increase in the CSF, and high B cell counts were found even after more than 100 days in the CSF [5]. In contrast to B cells, the number of CD19-CD138+ plasma cells in the CSF was found to decrease immediately to less than 0.5% one monthafter initiation of antibiotic treatment. Furthermore, lower numbers of natural killer T cells and monocytes were detected in the CSF of patients affected with neuroborreliosis is compared to non-infectious disorders [5]. In addition, it was shown that the percentage of plasma cells correlates with the absolute levels of intrathecally synthesized IgG and IgM, whereas it does not correlate with *B. burgdorferi*-specific IgG antibody production. However, *B. burgdorferi*-specific IgG antibody synthesis was found to correlate with the percentage of CSF B cells [5]. In contrast to variations in the cellular distribution in the CSF, no differences of immune cell subsets in the peripheral blood of patients affected with neuroborreliosis is compared to non-infectious controls have been detected [5]. Furthermore, it was shown that the lipid moiety of Borrelia outer surface protein A (OspA) provokes polyclonal B cell activation [93], which can subsequently lead to B cell maturation into plasma cells associated with the secretion of *B. burgdorferi*-specific antibodies [72]. The large numbers of infiltrating B cells in the CSF [5,6] are probably attracted by the relatively high intrathecal levels of CxCL-13, observed in neuroborreliosis [54,77,94]. Within the CSF, attracted B cells may mature into antibody-secreting plasma cells, thus producing *B. burgdorferi*-specific antibodies [72]. By longitudinally analyzing paired CSF and serum samples from one patient with definite neuroborreliosis, Tumani et al. [82] found a mononuclear cell pleocytosis, activated B cells and intrathecal humoral immune response with IgM predominance as well as blood-CSF barrier dysfunction which persisted for several weeks. Like in MS, differences in the distribution of various B cell subsets in neuroborreliosis are exclusively observed in the CSF, thus underpinning the role of CSF B cells in neuroinflammation. Moreover, in the CSF of patients affected with neuroborreliosis, intrathecally synthesized IgG as well as IgM, reactive against *B. burgdorferi*-specific antigens as well as CNS autoantigens were detected, thus fostering the impact of humoral immunity.

Apart from bacterial infections, B cells are also involved in various viral infections of the CNS. B cell and plasma blast counts in the CSF were shown to be increased in patients infected with human immunodeficiency virus (HIV), compared to patients with non-infectious CNS disorders. It was shown that the quantity of B cells during early and late stages of HIV infections remain stable. This is in contrast to plasma blasts which were found in higher numbers during early stages of HIV infection. Moreover in HIV, the prevalence of CSF plasma blasts was shown to correlate with intrathecal IgG synthesis as well as with HIV RNA copy numbers in the CSF. In addition, initiation of antiviral treatment in HIV patients resulted in a decrease in the number of plasma blasts as well as reduced HIV RNA copy numbers within the CSF. The results indicate a HIV-triggered B cell response and furthermore, plasma blasts as the main virus-related B lymphocyte subset [8].

**Conclusion and Outlook**

Although MS is widely considered as T cell-mediated autoimmune disease of the CNS, there is accumulating evidence that B cells are key components in the pathogenesis of the disease. B cells may contribute at multiple sites to MS pathogenesis. As shown in Figure 1, B cells participate, next to their obvious role in antibody-secretion, in antigen-presentation as well as cytokine and chemokine production. Furthermore, the successful application of monoclonal antibodies targeting CD20 in MS patients confirmed, as stated by Barun and Bar-Or, “that it is no longer a question of whether B cells contribute, but how they contribute, to MS disease activity” [95].

Besides numerous other infectious diseases, B cells play an important role in Lyme neuroborreliosis. Via the secretion of *B. burgdorferi*-specific antibodies, B cells in the CNS contribute to the clearance of the underlying bacterial infection.

Although, several mechanisms of B cell-mediated immunity are well understood so far, their complex functions are not clarified in detail and require further studies.

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