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# B Cells in Multiple Sclerosis: Connecting the Dots

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## Abstract

Over the last two decades B cells have increasingly moved into the spotlight in multiple sclerosis (MS) research. This interest was fuelled by growing understanding and acceptance of pathological involvement of B cells and antibodies in MS. Data derived from animal models of MS, human histopathological studies, and analyses of B cells in the peripheral blood and cerebrospinal fluid (CSF) have permitted the integration of B cells in our overall picture of MS immunopathogenesis. The as yet strongest direct evidence for a central role of B cells in MS autoimmunity was the demonstration that peripheral B cell depletion leads to a rapid decline of disease-activity in MS. While lending formidable impact to peripheral blood B cells as mediators of disease activity, the effects of anti-CD20 treatment also seemingly challenged the paradigm of a role of antibodies in targeted central nervous system (CNS) myelin destruction. This review shall attempt to provide an overview of our current understanding of B cell and antibody mediated mechanisms relevant to MS. We will include findings from, both, human studies, and animal models to highlight the complexity of B cell function as it pertains to MS. B cells appear to be effective drivers of inflammatory activity in MS by way of a diverse toolset of cellular functions. These functions appear to be closely linked to B cells that can be found in the periphery. However, by serving as the source of antibodies, B cells offer a direct humoral response that may target the CNS and lead to tissue specific destruction. Therefore, B cells participate in MS pathogenesis on both sides of the blood-brain barrier.

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## Introduction

This review article will highlight B cell functions relevant to MS immunopathogenesis. Owing to the boost of knowledge gained from studies of B cell-depleting therapy in MS, we will discuss B cell functions also in view of potential rituximab effects.

The original impetus to test CD20-targeted B cell depletion was based upon the substantial, albeit indirect, body of evidence that autoantibodies - products of terminally differentiated B cells - must somehow be involved in MS immunopathogenesis. It was thought that anti-CD20 treatment should reduce autoreactive and demyelinating antibodies. Rituximab is a monoclonal antibody of the IgG1 isotype targeting CD20, capable of triggering rapid complement and natural killer (NK) cell-mediated depletion of CD20-expressing B cells [1]. Importantly, CD20-targeted B cell depletion does not affect the CD19+CD20- pro-B cell and CD20-CD138+ plasma cell populations, both predominantly residing in the bone marrow. Within about 6 to 8 months following a standard course of rituximab treatment the CD20+ B cell compartment will begin to replenish [2]. Rituximab was found to significantly reduce MS disease activity within a few weeks of administration, which corresponded to the rapid near complete peripheral B cells depletion [3]. Following anti-CD20 treatment, CSF B cells were also partially depleted; however, serum and CSF immunoglobulin titers and oligoclonal bands remain mainly unchanged [4,5], likely reflecting the long half life of immunoglobulins and/or persistence of CD20-negative plasma cells. While it is to be expected that in patients undergoing long-term therapy no new plasma cells will develop, survival of long-lived plasma cells may provide a stable antibody repertoire even during long-term-therapy. The emerging view based on current knowledge of B cell involvement in MS is that both antibody-independent and antibody-dependent mechanisms of B cells are now thought to contribute to MS the MS disease process.

## Evidence for antibody involvement in MS

### What is known about antibody specificity in MS?

In 1947 Kabat et al. hypothesized that antibodies against myelin constituents may be involved in human CNS demyelinating disease based on immunization experiments in macaques [6]. In MS, a number of key discoveries have later on supported Kabat's hypothesis and have greatly enhanced the general acceptance of an immunopathological role of antibodies. Nonetheless, many questions remain to be answered.

Antibodies were found to be present in CSF and brain tissue [7] of MS patients and clonal IgG (OCB, oligoclonal bands) in the CSF were later found to be a relatively sensitive though not very specific marker for MS [8]. Importantly, positive OCB in the CSF of patients with CIS are associated with a greater risk to develop a second clinical relapse and thus to establish the diagnosis of clinically definite MS [9]. Antibody depositions within areas of demyelination and local complement activation have been described [10] by way of immunohistochemistry and have been associated with a specific lesion pattern (Type II) suggestive of antibody mediated demyelination [11]. However, others have argued that these findings may not necessarily be indicative of a myelin-destructive antibody response as similar immunoreactivity could also be detected in other neurological disease (OND)

including stroke [12]. Nonetheless, myelin oligodendrocyte glycoprotein (MOG)-specific antibodies have been detected in direct association with areas of active myelin-breakdown [13,14] and have been eluted from post-mortem MS lesion tissue [15]. Earlier studies have also demonstrated the presence of myelin-basic protein (MBP) and proteolipid protein (PLP)-specific antibodies in MS lesions and CSF [16,17]. An ongoing topic of discussion is the presence or absence and disease-relevance of myelin-reactive MS serum antibodies. Perhaps the most convincing evidence regarding this issue stems from recent studies showing high titers of anti-MOG antibodies in very early (i.e. pediatric) MS [18–20] and from a study demonstrating demyelination-enhancing potential of human anti-MOG antibodies in an animal model of MS [21].

Defining the antigenic specificity of antibodies present in the MS CNS has been a challenge and the subject of multiple studies over the years. In earlier work, oligoclonal bands were shown to bind to myelin proteins [22] but also Epstein-Barr virus antigens [23]. More recently, molecular technology has permitted the cloning of antibodies expressed by CSF B cells and plasma cells. In particular, single cell PCR technology has permitted the amplification of paired immunoglobulin heavy and light chain genes for the recombinant expression of monoclonal antibodies for target identification. A significant number of Fab fragments expressed in *E. coli* using variable region Ig genes expressed by single CSF CD19+ B cells displayed MBP-reactivity [24]. Two other studies examined the reactivity patterns of recombinant monoclonal IgG as expressed by clonally expanded CSF plasma cells from MS patients [25,26]. Neither study found MOG reactivity nor was able to confirm MBP-reactivity amongst CSF plasma cell expressed IgG. However, using frozen MS lesion, one study demonstrated specific reactivity of a number of monoclonal antibodies with myelin at the rim of demyelinating lesions [26] while the other study using paraffin embedded and fixed MS lesion tissue was unable to demonstrate myelin reactivity [25]. In addition to myelin-reactivity, the first study also described one antibody that reacted with astroglia in normal and MS white matter [26]. To complicate matters further, a study using a phage-displayed combinatorial antibody library approach demonstrated strong DNA-reactivity amongst CSF expressed IgG [27] and yet another study found potentially pathogenic antibodies against neurofascin 186 (NF186), a neuronal protein [28]. Interestingly, passive transfer of anti-NF186 antibodies and myelin reactive T cells in an animal model induced axonal injury and disease exacerbation [28]. Taken together, the identity of relevant target antigens of pathologenic antibodies in MS remains uncertain.

## What are the requirements for antibodies to be pathogenic?

The fact that plasma cells do not become depleted and CSF oligoclonal bands remain largely unchanged during rituximab treatment suggests that MS disease-activity can be diminished through mechanisms not involving antibodies, and that antibodies alone are not sufficient to trigger CNS tissue damage and MS relapses. However, in context of an appropriate inflammatory response antibodies may still be considered relevant players, and could be contribute to relapse severity and be involved in non-relapsing immunobiology as seen in progressive MS. Extensive data supporting a pathogenic role of antibodies in CNS autoimmune demyelination derives from models of experimental autoimmune encephalomyelitis (EAE). For example, in primate EAE, antibodies against MOG have

clearly been shown to possess myelin destructive potential [29,30]. In murine EAE, anti-MOG antibodies also appear to possess some disease enhancing potential but less so when compared to primate EAE. Importantly, antibodies against conformational epitopes of MOG appear to be the truly pathogenic ones in this model, while antibodies against MOG peptides are not clearly linked to myelin destruction; this hypothesis is true in both primate and rodent EAE [30,31]. Interestingly, this dichotomy appears to not be limited to antibodies but to also extend to the antigen-presenting (APC) function of B cells [32]. Thus, the capacity of antibodies and B cells to recognize conformational epitopes appears to be an important requirement for demyelinating pathogenicity.

Importantly, it has long been assumed that CNS-reactive antibodies may not be pathogenic by themselves, i.e. in the absence of a localized inflammatory response and/or destructive effectors. Recent studies in animal models of neuromyelitis optica (NMO), a CNS demyelinating disease with an aquaporin-4-directed pathogenic antibody response, have provided strong evidence for this hypothesis to be true. Direct injections of IgG from NMO patients into rat brain were only able to mediate local tissue damage when co-injected with complement, but not when injected alone [33]. This knowledge may support the hypothesis that in MS a pathogenic antibody response alone, be it localized or systemic, would be unable to lead to CNS demyelination.

## Disease-relevant B cells in MS

### Origin of Disease-Relevant B cells

One of the key questions in MS B cell immunology is at which stage during B cell development do potentially disease-relevant B cells arise and which developmental stages of B cells (i.e. naïve vs. memory) provide ongoing immunopathogenic support.

B cells derive from multipotent, hematopoietic precursors in the bone marrow. Prior to exiting the bone marrow, B cells will begin to express mature B cell receptors (BCR). B cell receptors are surface-expressed immunoglobulin (Ig) molecules resulting from somatic recombination of various germline segments. Somatic recombination follows a tightly regulated program and early developmental stages of B cells can be discerned based on the degree of recombination and maturity of the BCR. CD19+CD20- pro-B cells have yet to rearrange immunoglobulin genes. Therefore, their persistence during CD20-targeted therapy will provide the opportunity of a virtual reset of the B cell repertoire. Accordingly, following anti-CD20 B cell depletion essentially all re-emerging B cells are of the naïve, non-antigen experienced (CD27-) phenotype [34,35]. Indeed, some collateral experimental data obtained during clinical rituximab trials suggests sustained suppression of inflammatory activity even after replenishment of the B cell repertoire [34,36]. At present it is unknown how long this effect will persist after cessation of B cell depleting therapy. It is important to keep in mind that BCRs expressed on mature naïve B cells emigrating from the bone marrow are frequently poly-or autoreactive [37]. Defective mechanisms of B cell tolerance, as described to possibly be involved in other autoimmune disorders like systemic lupus erythematosus (SLE)[38,39] or rheumatoid arthritis (RA)[40], could also permit the survival and further evolution of autoreactive B cells in MS. Nonetheless, continued suppression of new relapsing MS disease-activity following B cell replenishment – if truly sustained –

could suggest that it is likely not an *a priori* autoreactive load of early B cells providing the basis for the development of pathogenic B cell repertoires in MS.

## Functional aspects of B cells in MS immunopathology

B cells emerging from germinal centers (GC) generally express the memory B cell marker CD27. These cells have frequently undergone extensive somatic hypermutation (SHM) suggesting T cell-dependent affinity-maturation for specific target antigens. Quite likely, CD27+ memory B cells are the B cell subtype of the greatest disease-driving relevance in MS. Importantly, memory B cells and other subsets can perform a number of diverse functions:

1. Memory B cells can rapidly become activated to differentiate into antibody secreting plasma cells and/or plasma blasts. In the CSF antibodies are produced by terminally differentiated plasma blasts and plasma cells [41], including the antibodies that make up oligoclonal bands [42].

2. B cells act as sophisticated and highly selective antigen presenting cells (APC) [32]. Studies in humans have identified antigen-presenting CD80 positive memory B cells [43]. In MS, memory B cells from some patients have recently been described as potent APC for myelin antigens including myelin-basic protein (MBP) and myelin-oligodendrocyte glycoprotein (MOG)[44]. Particularly with respect to the APC function of B cells, studies in experimental autoimmune encepcd2halomyelitis (EAE) have greatly enhanced our understanding. In 1999, Lyons et al demonstrated, that B cells were a required element for induction of EAE following immunization with MOG protein (recombinant MOG, rMOG)[45]. On the contrary, disease induction by immunizations with an encephalitogenic peptide (MOG35-55) appeared to be B cell-independent [45]. This difference is likely based on the fact, that B cells are able to recognize antigens of complex structure via the BCR, a function that is not required for processing and presentation of short linear peptides to CNS-reactive T cells.

3. Regulatory functions of B cells. In mice, IL-10 expressing (B10) B cells have been demonstrated to regulate autoimmunity [46], which is why they are also referred to as Breg (B regulatory) cells. In humans the situation regarding Breg is less clear. One study showed that in normal human B cells IL-10 is mainly produced by naïve B cells and found decreased average production of IL-10 in MS B cells [35]. Another study confirmed the predominant role of naïve B cells as IL-10 producers [44]. In contrast, another study showed that in some MS patients IL-10 was mainly produced by CD27+ memory B cell [47], however, the majority of MS patient in this study were undergoing treatment with either immunosuppressive or immunomodulatory substances. Further studies are required to clearly define the characteristics and functions of IL-10 expressing B cells in humans. In addition to down-regulating autoimmunity, B cells have also been shown to be potent polarizers of encephalitogenic T cells. For example, in rMOG induced murine EAE, B cells functioning as APC were able to polarize a TH1 and TH17 response, which was reversible by B cell deletion [32]. In contrast, in MOG35-55 induced

EAE B cells were not required as APC and did not polarize TH responses; interestingly, in MOG35-55 EAE B cell depletion led to a disease exacerbation. Irrespective of the antigen used for EAE induction, B cell depletion reduced the number of CD4+CD25+Treg. These findings may suggest a capacity of B cells to regulate T cell autoreactivity mainly when not involved in a conformation (i.e. rMOG) directed immune response.

4. Generation of ectopic GC-like structures. In MS, like in other tissue specific autoimmune diseases, structures reminiscent of ectopic GC with the potential capacity to modify immunoglobulin genes expressed by B cells have been described. Historically, in 1979 Prineas was the first to describe peri-vascular lymphoid tissue which resembled the medullary region of lymph nodes and suggested an ongoing immune response against disease-relevant antigens in situ [48]. Even earlier studies described the presence of plasma cells in CSF [49]. These observations were expanded by the description of meningeal ectopic B cell follicle-like structures with germinal center characteristics including CD35+ follicular dendritic cells and lymphoid chemokines CCL21 and CXCL 13[50]. Furthermore, very recently overlapping B cell repertoires were described defining a B cell network connecting MS brain parenchyma, CSF and meningeal follicular structures [51,52]. B cells present within the borders of the blood brain barrier carry features that clearly suggest them to be derived as part of antigen-driven responses that to some degree occur within the CNS compartment: B cells in the CSF were repeatedly demonstrated to have undergone Ig class-switching to express IgG [53,54]; clonal expansion is a prominent feature of CSF and CNS B cell repertoires [55,56]; significant SHM as common to B cells undergoing affinity maturation during target-specific immune responses was shown to shape CNS and CSF B cell repertoires [57].

5. Bystander activation. Very recently, Bar-Or et al. demonstrated that in MS patients B cells respond to non-CNS specific activating stimuli (i.e. CpG DNA or IFN-γ) by expressing an exaggerated pro-inflammatory cytokine response profile [34]. Depletion of these B cells by way of rituximab treatment resulted in diminished TH1 and TH17 pro-inflammatory T cell activity. Importantly, these findings provided a possible reason for why episodic, non-CNS specific triggering of B cells in MS could potentially result in relapsing disease activity.

## Challenges

Important and as yet unanswered questions include whether antigen-experienced B cells that reside behind the blood-brain-barrier result from a local immune response undergoing a local GC reaction, or whether B cells undergo affinity-maturation in the periphery prior to entering the CNS compartment. With respect to the first scenario, pre- and post-germinal center B cells have been found to be present in the CSF of patients with early MS (CIS, clinically isolated syndrome)[58] with the majority belonging to the CD19+CD27+IgG+ memory B cell compartment. However, the clonal relationship between naïve-mature and memory B cell subtypes has not been further studied. With respect to the second scenario, unequivocal demonstration of the presence or absence of clonally related B cells on both

sides of the blood-brain-barrier (i.e. in the periphery and the CNS) would greatly enhance our understanding of the dynamics of disease-relevant B cell responses.

It is quite likely that B cell and antibody responses occurring during CNS tissue damage may further diversify to include targets other than those exclusively contained in myelin. Therefore, a diverse population of antibody reactivities may be no major surprise. The challenge that remains will be to unequivocally determine which antigen or antigens are relevant targets of damaging autoantibodies in MS. From a B cell/antibody point-of-view the most promising tools available to tackle this challenge are monoclonal antibodies representing the antigenic specificity of disease-relevant B cells and plasma cells. Nonetheless, this process is greatly complicated by the diversity described above.

Aside from defining antigens that may be B cell targets, a challenge of at least equal importance will be to clearly identify disease-relevant B cells and their defining properties. Importantly, such properties can be entirely detached from the antigen-specific function mediated by the BCR but may, for example, extend to non-antigen specific activation via toll-like receptors as was demonstrated very recently [34].

Constant progress is made towards a better understanding of B cell mechanisms involved in disease-initiation and disease-perpetuation. Such knowledge along with future discovery of therapeutics targeting detrimental B cell functions, will possibly allow for much more precise approaches than pan-CD20+ B cell depletion.

## Summary

Owing to the convincing effect of peripheral B cell depletion, it appears safe to assume that B cells in the periphery are important for initiation and perpetuation of CNS directed inflammatory activity. B cells that reside behind the blood-brain-barrier may be important in their function to establish ectopic GC and to develop into antibody producing cells. We know that peripheral B cell depletion will also partially deplete CSF B cells but will not affect existing antibody producing cells, neither in the bone marrow nor in the CNS. Based on our current understanding, antibodies unfold their pathological capacities at the site of tissue injury (i.e. CNS myelin). However, antibodies alone cannot initiate tissue damage but require appropriate effector mechanisms or an actively inflammatory milieu. This inflammatory milieu likely becomes inactivated following peripheral B cell depletion. The ability of B cells to target conformational features of as yet unknown antigens may be a pre-requisite for B cell-dependent APC function and for the production of potentially destructive autoantibodies. Last but no least, non-antigen specific stimuli of B cells must be considered important component in the equation.

Taken together, B cells are multi-potent players in CNS inflammation and demyelination. Our knowledge of B cell mechanisms involved in MS has grown tremendously, particularly over the last decade. This knowledge is likely to increase as more sophisticated technologies become available to solve the B cell puzzle piece-by-piece.

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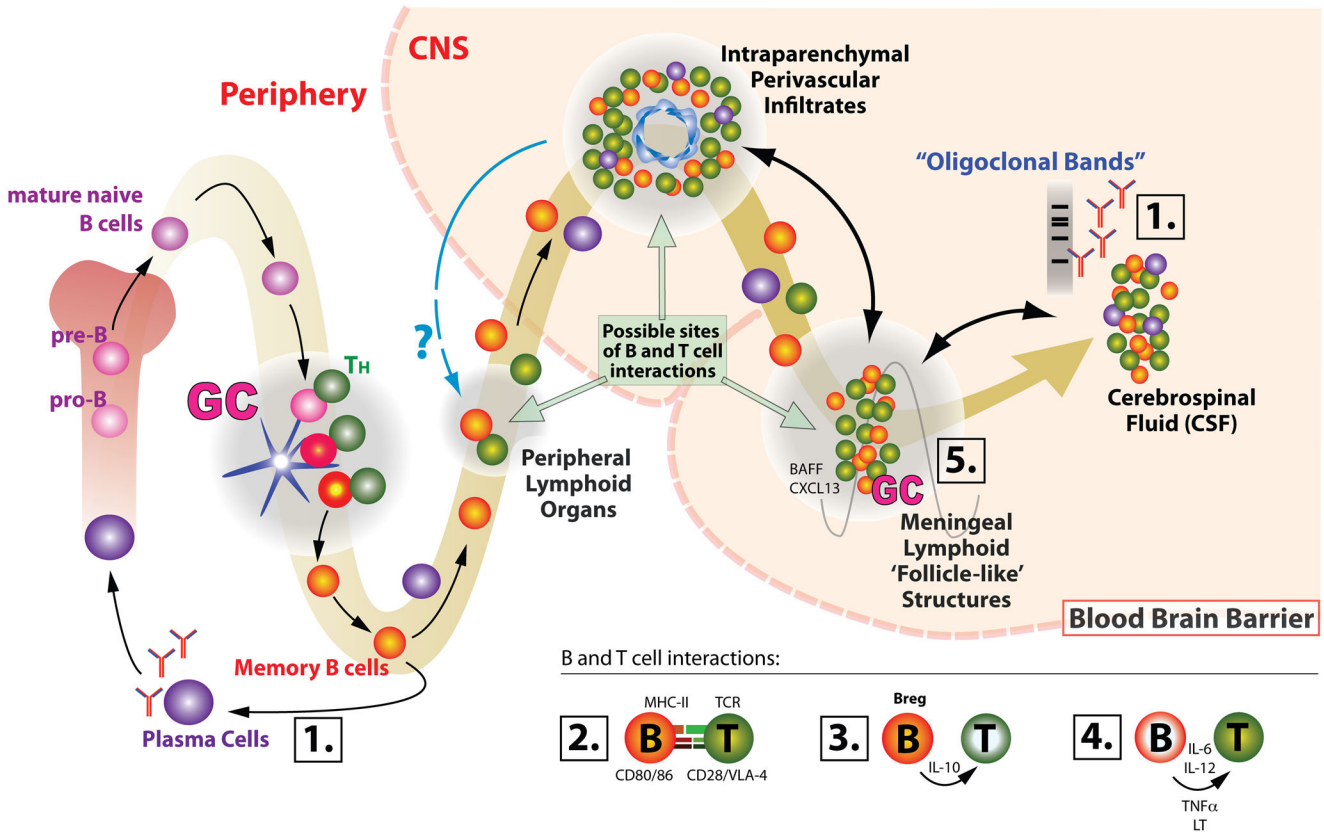


**Figure.**

This schematic summarizes current thinking about the development and migration of disease-relevant B cells and their functions in MS as described in this review article. The numbers in the figure correspond to the respective paragraphs in the *"Functional Aspects"* section. Upon leaving the bone marrow (BM) mature naïve B cells undergo antigen-training and affinity maturation in peripheral germinal centers (GC). During affinity maturation, immunoglobulin genes can become significantly mutated by somatic hypermutation (SHM). Which antigens drive the development of MS-relevant B cells remains controversial, but a number of possible candidates have been identified (see text). Following antigen training in the GC B cells undergo class-switch recombination and enter the CD27+ memory B cell compartment from which they can be rapidly activated to become antibody producing plasma cells or plasma blasts (1.). Memory B cells will persist and can act as efficient antigen-presenting cells (2.) to activate potentially CNS-reactive T cells. Activated CNS-reactive lymphocytes will cross the blood brain barrier (BBB) and establish perivascular infiltrates. Here, and at other sites, B and T cells reside in close proximity, which could allow for further *in situ* activation of inflammatory cells. The perivascular CNS parenchyma is likely the first site behind the BBB at which B and T cells will reside. Evidence exists for the formation of lymphoid follicle-like structures in the meninges (5.). In the cerebrospinal fluid, clonally expanded B cells and plasma cells can be identified, with the latter having been demonstrated to produce clonal IgG (oligoclonal bands, OCB). Overall, a vivid exchange of B cells appears to take place between the different compartments behind the BBB; it is likely that these different compartments (i.e. perivascular infiltrates, lymphoid

follicle-like structures, CSF) support different aspects of B cell involvement in MS immunopathogenesis. At present, it remains unknown whether there is a two-way communication of B cell clones between the periphery and the CNS, or whether migration of B cells into the CNS is a unidirectional process (see '?' in figure). In addition to the APC function, regulatory functions (3.) and T cell activation via non-antigen specific bystander B cell activation (4.) have been shown to be of relevance in MS.