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The role of B cells in multiple sclerosis: current and future therapies

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Abstract

While it was long held that T cells were the primary mediators of multiple sclerosis (MS) pathogenesis, the beneficial effects observed in response to treatment with Rituximab, a monoclonal antibody (mAb) targeting CD20, shed light on a key contributor to MS that had been previously underappreciated: B cells. This has been reaffirmed by results from clinical trials testing the efficacy of subsequently developed B cell-depleting mAbs targeting CD20 as well as studies revisiting the effects of previous disease-modifying therapies (DMTs) on B cell subsets thought to modulate disease severity. In this review, we summarize current knowledge regarding the complex roles of B cells in MS pathogenesis and current and potential future B cell-directed therapies.

Introduction

The contribution of B cells to autoimmune disease pathogenesis has historically been viewed primarily via the production of pathogenic autoantibodies, as exemplified in diseases such as myasthenia gravis (MG) and neuromyelitis optica (NMO). However, seminal observations in recent years have challenged this simplistic view[1]. It is now well-established that, in addition to the production of autoantibodies, B cells are able to drive autoimmunity through the presentation of autoantigen to autoreactive T cells, the secretion of proinflammatory cytokines, and the establishment of tertiary lymphoid organs (TLOs) in chronically inflamed tissues[2]. Paradoxically, B cells have also been shown to exert several regulatory functions critical for the prevention or resolution of inflammation accompanying several autoimmune diseases[3–7]. Collectively, these findings demonstrate a complex role for B cells as regulators of autoimmunity. B cells have become a focal point in recent years regarding their degree of involvement in the pathogenesis of multiple sclerosis (MS), which has canonically been

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considered a predominantly T cell-mediated autoimmune disease[1,8–10]. Corroborated by the success of clinical trials of B cell depleting therapies, this newfound role for B cells has warranted a shift in the approach to the treatment of MS.

B cells were first implicated in the pathogenesis of MS through the discovery of oligoclonal bands (OCBs) or abnormal production of clonally expanded IgG in the cerebral spinal fluid (CSF), but not plasma of patients with MS[11]. Since then, cells isolated from the CSF and peripheral blood of patients with MS have been found to produce these oligoclonal bands[12–14]. However, unlike what is observed in NMO and MG, there is significant heterogeneity in the antigen specificity of these oligoclonal antibodies, which may target pathogens as well as autoantigens[8]. Multiple studies have demonstrated the presence of clonally expanded B cells within lesions, as well as TLOs, and B cells can be found within the parenchyma, CSF, and meninges of patients with multiple sclerosis[15–24]. The clinical success of B cell depleting therapies such as anti-CD20 monoclonal antibodies (mAbs) corroborated these results, solidifying the contribution of B cells in the pathogenesis of multiple sclerosis[25–27].

B cell tolerance: an overview

The adaptive immune response requires not only the ability of B and T cells to detect and respond to any encountered foreign antigen, but to do so in a highly specific way[28,29]. In order to accomplish this, each cell type expresses an antigen receptor with a particular specificity, conducive to their respective roles in this process. However, the B and T cell receptor (BCR and TCR, respectively) differ in important aspects: Firstly, the affinity of the BCR for antigen is several orders of magnitude higher than that of the TCR, allowing the BCR to recognize soluble antigens whereas antigen presentation to the TCR is determined by binding of peptides to major histocompatibility complex (MHC) molecules[30]. Secondly, the specificity and binding affinity of the BCR is not static, in contrast to the TCR, but can be edited through participation in a germinal center (GC) reaction[31,32], the process responsible for the T cell-dependent generation of high affinity memory B cell and plasma cells.

Given that the specificities of the primary BCR repertoire are generated by random recombination of genes encoding the antigen binding region of the BCR, the generation of B cells possessing an autoreactive BCR seems inevitable[31]. Indeed, it has been established that a considerable majority of the initial BCR repertoire exhibits significant self-reactivity[32]. To prevent, or at least limit the emergence of autoreactive B cell responses, several mechanisms exist which effectively restrict the persistence of autoreactive B cells and thereby mitigate the risk of developing autoimmunity. The establishment of B cell tolerance can conceptually be divided into two separate “checkpoints”: Central tolerance, which occurs during the early stages of B cell development within the bone marrow, and peripheral tolerance, which occurs upon T cell-dependent activation and subsequent entry into the GC reaction[33,34].

Central tolerance

In the bone marrow, the process of B cell development has two critical goals: The first is to generate a primary repertoire of BCRs that is maximally diverse, so as to facilitate

recognition of foreign antigen. The second is to prevent the emigration of B cells expressing self-reactive BCRs into the periphery[29,32,35].

The diversification of the primary BCR repertoire is accomplished through the rearrangement of the variable (V), diversity (D), and joining (J) gene segments encoding the variable regions of the BCR, in a process termed V(D)J recombination[36]. However, as a consequence of the stochastic and error-prone nature of this process, a majority of B cells (50–80%) initially express a self-reactive BCR[33–35,37,38], at which point central tolerance mechanisms go into effect to eliminate these cells. The fate, i.e. death, survival, or anergy of these self-reactive B cells is determined by the strength of the signals derived from the bone marrow microenvironment and transduced through the BCR and its coreceptors[32]. However, B cells that strongly bind self-antigen can be rescued by reactivating V(D)J recombination in an attempt to generate a BCR that is either non-reactive or only slightly reactive to self-antigen, a process also termed receptor editing. B cells that still do not produce a BCR with lessened autoreactivity undergo apoptosis (clonal deletion)[37]. Lastly, any remaining B cells with BCRs that still weakly bind self-antigen are thought to become anergic. B cells that have undergone anergy are desensitized to BCR signaling and as a result typically will not undergo T-dependent activation and maturation into plasma cells or memory B cells in the periphery. Nevertheless, with sufficient stimulation, anergic B cells are still able to participate in GC responses[39].

Last, it must be noted that some self-reactive B cell clones may not encounter their respective antigen in the bone marrow, and thereby evade central tolerance mechanisms by “clonal ignorance”. The control of these autoreactive B cells therefore relies on peripheral tolerance mechanisms, as discussed below. It may be these clones that fail both central and peripheral tolerance that are key in promoting autoimmune pathology in many conditions.

Peripheral tolerance

While central tolerance mechanisms are estimated to eliminate 90% of the autoreactive B cell clones produced, there are still some cells that escape into the immune periphery and which need to be controlled to prevent undue development of autoimmune pathology[40]. Interestingly, in contrast to a number of autoimmune diseases that are thought to be the result of defects in both central and peripheral tolerance mechanisms, the development of MS is thought to result from a deficiency primarily in peripheral tolerance mechanisms[41,42], which will be discussed next. Peripheral B cell tolerance mechanisms rely on many of the signaling requirements for the activation of mature B cells prior to or upon participating in the GC reaction. GCs are specialized microenvironments that form in secondary lymphoid organs to facilitate the T cell-dependent activation and affinity maturation of mature B cells and subsequent generation of high affinity memory B cells and plasma cells. Briefly, B cells that have encountered their cognate antigen migrate from B cell-rich follicles to adjacent T cell-rich T cell zones[43]. These B cells internalize, process, and present a fragment of that antigen to $CD4^+$ T cells in the form of a linear peptide complexed with MHC class II (MHC II)[43]. Upon receiving T cell help in the form of cytokines and stimulation of costimulatory receptors (described in more detail later), the presenting B cells begin proliferating, thus initiating the GC reaction.

The GC is comprised of the dark zone (DZ) and the light zone (LZ)[44]. In the DZ, activated B cells proliferate and undergo affinity maturation through somatic hypermutation (SHM) of the genes comprising the BCR. B cells that have completed this process enter the LZ where they reencounter antigen presented on the surface of follicular dendritic cells (FDCs) and compete for survival signals derived from follicular T helper (Tfh) cells. B cell clones are then selected based on affinity; those expressing higher affinity BCRs either reenter the DZ or differentiate directly into memory B cells or plasma cells, while lower affinity clones that do not receive Tfh cell help undergo apoptosis[37].

Mature B cells, upon receiving the first activating signal through stimulation of the BCR, require costimulatory signals typically derived from helper T cells sharing the same antigenic specificity to become fully activated, and subsequently either directly differentiate into plasmablasts or memory B cells, or to be recruited into the GC reaction. Cells that receive one signal without the other undergo apoptosis or become anergic[45]. The principal B cell surface receptors involved in this process are the BCR, the CD40 receptor, and the Fas receptor (CD95). The ultimate fate of a given B cell is the sum of the relative activity of these signaling pathways, briefly summarized here.

BCR stimulation initiates signaling cascades that result in survival and proliferation as well as apoptosis. Apoptosis, mediated by the BH3-only protein Bim, functions as a default program. Therefore, survival and proliferation require sufficient “positive” costimulatory signals, such as stimulation of CD40 by T cell-derived CD40 ligand (CD40L), to avoid apoptosis. In addition to apoptosis, BCR signaling in the absence of costimulation can also result in anergy. BCR binding occupancy is the deciding variable in this circumstance, with high occupancy favoring apoptosis and intermediate (5–45%) occupancy favoring anergy[46,47]. Goodnow and colleagues elegantly demonstrated this concept using transgenic mice expressing a BCR with high affinity for hen egg lysozyme (HEL) as well as soluble HEL. As anergic B cells exhibit attenuated BCR signaling, this mechanism primarily serves to prevent the aberrant activation of ignorant B cells (autoreactive B cells that have not encountered their antigen yet). Ignorant B cells are able to undergo the initial steps of activation with proficiency comparable to non-autoreactive naïve mature B cells. However, since autoreactive $CD4^+$ T cells are similarly regulated by self-tolerance mechanisms, the likelihood that corresponding autoreactive $CD4^+$ T cells exist in the periphery is low; therefore, without T cell help, these previously ignorant B cells will be activated in the absence of costimulation, and either undergo apoptosis[48] or become anergic[45]. CD40 stimulation is able to deter apoptosis temporarily, however it must be compounded with BCR signaling to fully activate B cells. Importantly, signaling via the CD40 molecule by CD40L expressed on T cells also induces the expression of CD95 on B cells, which is essential to the maintenance of peripheral tolerance[49]. CD40L, in turn, is upregulated upon cognate binding of a $CD4^+$ TCR to the peptide:MHC complex on antigen-presenting cells (APCs). Upon proper activation, T cells will also upregulate Fas ligand (FasL), which will then bind to CD95 on activated B cells, inducing caspase-mediated apoptosis. This mechanism is relevant specifically for the control of anergic B cell responses.

Anergic B cells have a shortened lifespan (2–5 days)[45] and exhibit impaired signaling through the BCR and repressed expression of the costimulatory molecule CD86[49]. Although

these cells are capable of overcoming anergy in the absence of costimulation given sufficient BCR or toll-like receptor (TLR) stimulation[49–51], anergic B cells are eliminated typically by follicular exclusion, in which these cells are outcompeted by non-autoreactive B cells for entry into the follicle[46] as well as for the critical survival cytokine, B cell-activating factor (BAFF)[45].

In the rare event that an anergic B cell enters the GC reaction and presents self-antigen to a T cell specific for the same autoantigen, it is signaled to undergo apoptosis via CD95[37], a consequence of the attenuated BCR signaling characteristic of anergic B cells. Without sufficient BCR or toll-like receptor (TLR) signaling to counter signaling through CD95, these B cells will undergo apoptosis[52]. Taken together, the control of aberrant ignorant and anergic B cell activation and proliferation, and therefore the maintenance of tolerance, relies on both activating and death-inducing stimuli.

It is important to note that SHM is able to result in the generation of an autoreactive BCR, but in addition to being controlled by the tolerance mechanisms summarized here, these B cells are additionally controlled by follicular regulatory T cells (Tfrs), although the mechanism by which they exert these suppressive effects is poorly understood[53]. Indeed, MS patients exhibit a decrease in circulating Tfrs as well as reduced suppressive function of the Tfrs present compared to healthy control subjects[54].

The role of B cells in the pathogenesis of MS

Initial evidence

Experimental autoimmune encephalomyelitis (EAE) induced in rodents or non-human primates has been widely studied as an animal model of human MS[55]. A number of rodent EAE models have been studied over the past decades, which differ in the nature of the neuroantigen used for disease induction, the use of different susceptible mouse strains, and whether disease is induced by immunization (“active EAE”), adoptive transfer of pathogenic lymphocytes (“passive EAE), or develops spontaneously in mouse lines transgenically expressing pathogenic T cell or B cell receptors. Many different neuroantigen preparations have been used for the induction of EAE, including whole spinal cord homogenate, purified or recombinant neuroantigen proteins, or immunodominant peptides identified from encephalitogenic neuroantigens. The choice of neuroantigen and mouse strain has important implications for the cellular autoimmune mechanisms driving EAE and the pathological phenotype. For example, induction of EAE in C57BL/6 mice with myelin oligodendrocyte (MOG) protein induces autoantibodies that are important for the effector mechanism driving disease in this model (i.e. B cells), whereas induction of disease with MOG peptide does not depend on autoantibody and B cells. Thus, the choice of EAE model may inform on the mechanism of B cell-depleting strategies and the role of autoantibodies in MS, which do not deplete plasma cells.

To assess the role of B cells in MS, and its animal model experimental autoimmune encephalomyelitis (EAE), B cell depletion has been studied using a number of different experimental approaches. One of the most utilized models for studying the contribution of B cells is μMT mice in which the IgM heavy chain is mutated, and where the animals therefore

lack mature B cells[56]. Another approach consists of using monoclonal antibodies, for example anti-CD19 or anti-CD20 mAbs, to deplete subsets of B cells[57]. CD19 is expressed on B cells from the pro-B cell stage on, but to a lower extent in plasma cells[57]. CD20 is expressed on pre-B cells and continues to be expressed on B cells until the plasmablast stage[57]. A subset of $CD3^+$ T cells also expresses CD20 and are depleted with anti-CD20 mAb treatment; however the effect that this has on therapeutic efficacy is not known[57]. Studies indicate that the pathogenic nature of B cells depends greatly on autoantigen, subset of B cells depleted, and activation of the B cells. B cell-deficient μMT mice on the C57BL/6 background immunized with truncated recombinant human myelin oligodendrocyte glycoprotein1–120 (rhMOG1–120) protein do not develop EAE, but μMT mice immunized with MOG35–55 peptide are still susceptible to EAE[58]. Similarly, μMT mice on the B10.PL background and immunized with $MBP_{1–11}$ peptide are still susceptible to EAE[6]. Interestingly, the μMT mice in this latter study showed impaired recovery from EAE, which provided seminal evidence for a regulatory function of B cells in neuroinflammatory disease. Regulatory B cells will be discussed later. Irrespective, mice immunized with recombinant mouse $MOG_{1–117}$ protein ($rmMOG_{1–117}$) and treated with anti-CD20 mAb weekly 21 days prior to immunization or after EAE was fully developed also showed less severe disease, while $MOG_{35–55}$ peptide-immunized mice treated with anti-CD20 mAb 21 days prior to immunization or after EAE was fully developed showed exacerbated disease[59]. Similarly, human CD20 transgenic mice backcrossed on the C57BL/6 background that were immunized with $rhMOG_{1–120}$ protein and treated with Rituximab for three days prior to immunization showed less severe disease than untreated mice[60]. μMT mice that were reconstituted with activated $rhMOG_{1–120}$ protein-primed B cells restored the susceptibility to EAE induced with protein myelin antigen[58]. A caveat to these studies is that μMT mice still produce IgA^+ B cells[61] and have also been shown to produce IgG1, IgG2a, and IgE under certain conditions[62]. In another B cell depletion model, Weber, et al. demonstrated that, when mice are immunized with $rmMOG_{1–117}$ protein, anti-CD20 mAb depletion reduced disease severity by decreasing the differentiation of $rmMOG_{1–117}$ protein-specific Th1 and Th17 cells, but not in the $MOG_{35–55}$ peptide-induced EAE model. They hypothesized that this was due to activation of B cells and subsequent antigen processing and presentation by B cells in the $rmMOG_{1–117}$ protein model, but not the $MOG_{35–55}$ peptide model[59] In contrast, in a relapsing-remitting EAE model where Biozzi mice, which produce high titers of antibodies, were immunized with spinal cord homogenate, anti-CD20 mAb treatment did not rescue the mice from disease[63]. This could be due to the type of antigen utilized, depletion of regulatory B cells, or intrinsic differences of the Biozzi mouse EAE model compared with the recombinant MOG protein and $MOG_{35–55}$ peptide models. When anti-CD20 mAb was administered 21 days post-sensitization in the marmoset model of active EAE using $MOG_{34–56}$ peptide or $rhMOG_{1–125}$ protein, clinical disease was lower or absent in the anti-CD20 mAb treated group[64,65]. Thus, the results in models inducing EAE with myelin protein antigen are consistent with the clinical data in MS patients showing reduced relapse rates after anti-CD20 mAb treatment in relapsing-remitting MS (RRMS) patients. However, anti-CD20 mAb treatment may not significantly modulate disease overall in patients with progressive MS[25,26,66,67]. Anti-CD19 mAb treatment for B cell depletion is also explored in EAE and MS. Treatment of active EAE induced with $rhMOG_{1–125}$ protein with a single dose anti-CD19 mAb before disease induction as well as

7 days post-immunization ameliorated disease severity and has been reported to inhibit disease to a greater extent than anti-CD20 mAb treatment[68,69]. In MS, anti-CD19 mAb treatment reduced lesion size in newly forming lesions[70]. Taken together, current data support that B cells play a primarily pathogenic role in MS and EAE, and it seems that disease outcome depends greatly upon the autoantigen, the cooperation between T and B cells, the stage at which the depletion occurs, and the subset of B cells that are targeted. However, it is important to note that there is evidence for specific B cell effector functions that are protective in MS and EAE, which will be discussed. In the following sections we will provide an overview of the current understanding of pathogenic and protective mechanisms of B cell effector functions in MS and its murine model EAE.

Antibody-dependent mechanisms

I. Production of autoantibodies—As mentioned above, circulating B cells possessing a self-reactive BCR exist in the immune periphery of most healthy individuals, suggesting that they have escaped central tolerance mechanisms[34,37,71,72]. Most autoreactive B cells remain ignorant or anergic and are subsequently eliminated or are controlled through the peripheral tolerance mechanisms described earlier. While this should prevent the production of autoantibodies, it is important to note that autoantibodies, such as B1 cell-derived natural autoantibodies, typically of the IgM isotype, also exist in the serum of healthy individuals and are believed to carry out beneficial, rather than pathogenic functions, such as the clearance of apoptotic cells[73,74].

Autoantibody-driven pathogenesis was first implicated by the detection of OCBs and the presence of class-switched autoantibodies in lesions and CSF of MS patients[11,20,22,75,76]. In contrast to autoimmune diseases classically considered to be autoantibody-mediated, such as MG or Grave's disease[77], where the nature of the autoantigens targeted by autoantibodies is often known, the molecular targets and pathogenic role of autoantibodies in MS patients are still not fully resolved, with some notable exceptions such as in neuromyelitis optica[1] (NMO). Nevertheless, studies from CSF and blood of patients with MS and from EAE have provided some insights into ways that antibodies may contribute to MS.

In a small clinical study of 14 MS patients, IgG was isolated from lesions of post-mortem patients and was found to bind to rhMOG$_{1–121}$ protein[78]. In corroboration of this observation, higher titers of autoantibodies specific for full-length human MOG protein were found in MS patients compared with controls and antibodies from these patients enhanced disease severity when transferred to mice with EAE when compared with diseased mice that were not treated with these antibodies[79]. Similarly, Lyons and colleagues reconstituted μMT mice with serum from rhMOG$_{1–120}$ protein-immunized mice and found that this serum was capable of restoring EAE induced with MOG protein in μMT mice[58]. In further support of this notion, mice with guinea pig myelin basic protein (MBP)-induced active and passive EAE that have been treated with an anti-MOG mAb exhibit more demyelination than controls[80], and when EAE is induced with MOG$_{35–55}$ peptide, depletion of IgG antibodies leads to significantly reduced disease scores[81]. Another study assessed the proteolytic

[1]NMO was considered a subtype of MS until the discovery of aquaporin-4 (AQP4) autoantibodies.

activity of MBP-specific antibodies from MS patients and found increased cleavage of MBP protein correlate with worse scores on the MS expanded disability status scale (EDSS)[82].

Thus, some evidence suggests a pathogenic role for autoantibodies in MS. However, autoantibodies detected in the serum of patients with clinically isolated syndrome (CIS) do not correlate with progression to definite MS[83], and myelin-binding autoantibodies can also be found in the serum of healthy controls[84]. This indicates that specific conditions may be necessary for myelin-binding autoantibodies to mediate pathogenic function in MS, and leaves room for investigation of specific effector mechanisms such as antibody-induced demyelination[79,82,85–87], complement activation[88–91], and opsonization and phagocytosis[76].

Antibodies have been associated with demyelinating lesions in both MS and EAE[86,92,93]. As mentioned before, IgG isolated from lesions of MS patients can proteolytically cleave MBP protein, and this correlated with EDSS scores[82]. This indicates that antibodies may cause damage to the myelin sheath. Along these lines, anti-MOG protein-specific antibodies have been associated with CNS demyelination[86,92,94], which may be due to the ability of these antibodies to destabilize and alter the structure of the myelin sheaths[95,96] (Fig. 1a).

Autoantibodies have also been associated with pathogenesis of EAE by complement activation as indicated by the presence of active complement in MS lesions[88]. Complement activation was characteristic in recombinant rat $MOG_{1–125}$ ($rrMOG_{1–125}$) protein-induced EAE[87], and mice that have been decomplemented show a delay of disease onset[91]. Studies have demonstrated autoantibody-driven, complement-dependent demyelination during MBP–PLP fusion protein-induced EAE and in brains of patients with MS[90,97].

Autoantibodies can also act to increase opsonization and phagocytosis of autoantigens by macrophages and dendritic cells to present to autoreactive T cells[98]. Macrophages that bound myelin antigen as well as immunoglobulin and activated complement have been previously identified in a patient with MS[88], and incubation with IgG antibodies isolated from the CSF of EAE-induced mice increases uptake of myelin by macrophages[99]. Kinzel, et al. demonstrated that when MOG protein-specific antibodies are deposited, APCs take up the antigen via the Fc receptor and proceed to process and present the antigen and activate T cells[98,100]. Autoantibodies specific for MOG protein opsonize to the greatest extent when compared with antibodies against other myelin antigens, and the anti-MOG mAb with the highest myelin uptake was shown to enhance EAE and demyelination[101]. MBP protein-specific antibodies were also able to increase myelin uptake, and increase inflammation during EAE[101].

II. Autoantigen presentation to T cells—Dendritic cells (DCs) are considered the archetypal professional APCs. However, studies using transgenic mice lacking MHC II expression selectively on B cells[59,102] have underscored the importance of B cell APC function in the pathogenesis of several autoimmune diseases[1].

Specifically, memory B cells are particularly efficient APCs and myelin-specific memory B cells have been found in the peripheral blood of MS patients[103,104]. This is primarily attributed to the unique ability of B cells to present antigens that are present in very low

concentrations[105–107] through BCR-mediated uptake, which is of vastly superior efficiency compared with pinocytosis, the primary mechanism of antigen uptake employed by other APCs[108–111].

B cells can activate T cells through MHC II-dependent antigen presentation and CD80 co-stimulation. Evidence from MS patients to support this notion include that there is an increase in $CD80^+$ B cells in patients with active MS[112], and that B cell-depleting therapies also cause a decrease in T cells in the CSF of patients with MS[25]. In fact, in certain EAE models it has been shown that mice that have MHC II-deficient B cells are resistant to EAE regardless of the presence of myelin-specific antibodies[102]. Moreover, T cells from B cell-deficient mice could infiltrate into the CNS when EAE was induced with $rrMOG_{1–125}$ protein, but T cell reactivation did not occur[113], and it has been shown that when B cells have the same antigen specificity as T cells there is more differentiation towards proinflammatory Th1 and Th17 cells[59]. Of particular interest, disease severity is increased when T and B cells share antigen specificity[58–60,87,98,114,115]. In summary, antigen presentation by B cells, particularly when T and B cells overlap in their specificity for myelin antigen, and B cell-derived costimulation are important pathogenic processes during EAE and MS (Fig. 1b).

Antibody-independent mechanisms

I. Secretion of proinflammatory cytokines—Another form of stimulation B cells provide is via proinflammatory cytokine and chemokine secretion. B cells can secrete lymphotoxin (LT), tumor necrosis factor (TNF)-α, interleukin (IL)-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), BAFF, a proliferation-inducing ligand (APRIL), and IL-15 (Fig. 1c). Patients with MS show an increased LT to IL-10 ratio, and increased levels of LT and TNF-α upon reactivation with TLR-9 stimulation or interferon (IFN)-γ stimulation[116]. Culture of oligodendrocytes with LT and TNF-α demonstrates the ability of these two molecules to induce oligodendrocyte apoptosis[117]. Somewhat in disagreement with this observation was a study investigating the effect of co-culturing B cells and B cell secretory products from healthy controls and patients with MS with oligodendrocytes, which found no correlation between the levels of LT or TNF-α and oligodendrocyte death. However, secretory products from MS patient B cells induced more oligodendrocyte death than those from healthy controls[118]. In a $MOG_{35–55}$ peptide-induced EAE model, LT-deficient mice show lower EAE disease severity than WT controls[119]. Moreover, it has been demonstrated that LT-β receptor is important for the establishment of TLOs (which will be discussed later) during EAE[120]. However, LT-deficient mice lack lymph nodes, which impairs T and B cell priming, and therefore could account for the observation that these animals do not develop EAE[121,122]; thus, the contribution of B cell-derived LT to EAE may not have been conclusively addressed yet. TNF-α confers both protective and pathogenic effects. TNF-α is important for induction of EAE[123], but also for mediating CNS remyelination *in vivo*[124] and regulating the induction of regulatory T cells[125]. These effects are likely due to different downstream effects of signals through TNF receptor 1 vs TNF receptor 2. However, B cell-specific TNF-signaling deficiency has not been studied. B cells from patients with MS produce more IL-6 than B cells from healthy

controls, and mice had reduced EAE scores when B cells were deficient for IL-6[126]. This indicates a pathogenic role for B cell derived IL-6.

Recently, evidence from EAE models has suggested an important role of GM-CSF in MS. GM-CSF deficient C57BL/6 mice are resistant to development of EAE[127]. Moreover, GM-CSF promotes chronic EAE, where mice do not remit from disease[128]. Furthermore, lack of the GM-CSF receptor on T cells altered the extent of chronic EAE disease severity[128], mice with GM-CSF retrovirally transduced antigen-specific T cells show severe and chronic disease compared with controls[129], and patients with MS show significantly more GM-CSF + T cells than controls[130]. Additionally, T cell derived GM-CSF is important for the effector phase of EAE[131], and is required to activate microglia during EAE[132]. Interestingly, GM-CSF-producing B cells have been identified in patients with MS and these cells could activate myeloid cells *in vitro*[133]. However, the effect of B cell-specific GM-CSF (e.g. in mice with B cell-specific knockout of GM-CSF or GM-CSF receptor) has so far not been directly tested in EAE models.

BAFF and APRIL are regulators of B cell survival, maturation, and activation that are dysregulated in patients with MS[134–138]. APRIL has a soluble or membrane bound form and binds the transmembrane activator and cyclophilin ligand interactor (TACI), and the B cell maturation antigen (BCMA) receptors. Marmosets induced with EAE and treated with anti-APRIL antibodies showed decreased disease severity[139], but the exact mechanism by which this cytokine acts in this context is unknown. Like APRIL, BAFF can be membrane-bound or soluble and can signal through the receptors BCMA, TACI, and, unlike APRIL, BAFF receptor (BAFF-R)[137]. BAFF-overexpressing mice show increased disease severity, ligand KO mice show decreased disease severity when compared with WT mice[140], and marmosets induced with EAE and treated with anti-BAFF antibodies show a decrease in disease severity[139]. BAFF has also been implicated in the induction of TLOs[134]. Overall, current evidence indicates that BAFF signaling is pathogenic in EAE. Studies on the receptors of BAFF reveal a more pleotropic role for BAFF signaling. BAFF-R-deficient mice show exacerbated EAE[141], but treatment of mice with a ligand antagonist that binds to BCMA suppresses disease[142], and anti-TACI mAb treatment has been shown to reduce the number of pathogenic Th1 and Th17 T cell subsets, but not memory T cell subsets, but disease severity was not reported in this study[143]. Taken together, this indicates that there are either specific roles unique to each BAFF receptor, or specific roles for subsets of B cells that express these receptors. Specifically, while BAFF-R stimulation seems to be protective, BCMA and TACI signaling seems to be pathogenic during EAE. In addition to potentially signaling to induce pathogenic T cells[140,143], BAFF is known to inhibit the production of a B cell-produced cytokine, IL-15[144], potentially through the TACI receptor[143]. While the main function of IL-15 is to drive proliferation of $CD8^+$ T cells and NK cells[145], IL-15 KO mice exhibit exacerbated disease[146]. This could be because IL-15 is known to induce proliferation of a subset of $CD8^+$ $CD122^+$ T cells that have the capacity to suppress EAE by modulating Th17 cells[147]. While inhibiting BAFF-R signaling is protective in mice, the translational potential of targeting this pathway in MS patients was cast into question by adverse outcomes observed in a clinical trial with the BAFF- and APRIL-depleting TACI-Fc fusion protein, atacicept[148], which will be elaborated on later.

II. Induction/establishment of tertiary lymphoid organs—Finally, B cells can contribute to the establishment of structures known as TLOs, a feature of diseases characterized by chronic inflammation, and as such a feature of disease such as rheumatoid arthritis (RA), Sjögren's disease, and MS[149]. At the induction of TLO architecture, B cells along with T cells express $LT\alpha_1/\beta_2$, promoting the differentiation of resident stromal cells into FDCs[150–152]. Additionally, B cells will express CXCL13[103] and BAFF[134], ultimately facilitating the formation of an architecture similar to GCs in secondary lymphoid organs[1,149] (Fig. 1d). As these GC-like structures persist, they can potentially become a continual reservoir of autoreactive plasmablasts, plasma cells, and memory B cells[1,153–155], perpetuating or even exacerbating autoimmune disease[149]. These structures have been identified in patients with MS and are thought to be important in relapses of MS, and in the trafficking and re-priming of other leukocytes relevant to the pathogenesis of MS[23,24,153,154,156–160].

Protective B cell functions

The protective functions of B cells in MS are well-documented and include the production of anti-inflammatory cytokines and the use of inhibitory surface receptors to regulate other immune cells. Some studies have also shown that antibodies can exert beneficial functions in MS, such as inducing remyelination[161,162] or clearing apoptotic cells[73,74].

Regulatory B cells (Bregs), particularly the B10 subset in humans, have the capacity to secrete anti-inflammatory cytokines such as IL-10, TGF-β, and IL-35[163–166]. B cell-derived TGF-β has protective effects during EAE by inhibiting APC function[167]. IL-10 production is a common feature of several Breg subsets that are found in both humans and mice[165,168] and have been shown to be necessary for recovery in EAE[7]. Interestingly, plasma cells can also secrete IL-10 in humans[7,9]. Finally, EAE studies showed that Breg binding of glucocorticoid-induced tumor necrosis factor-related receptor (GITR) by its ligand, (GITR-L), which is expressed on B cells, resulted in the expansion of regulatory T cell (Treg) populations independent of IL-10[1,169]. Moreover, B cells can protect against EAE by suppressing T cell responses through the inhibitory receptor PD-1[170]. Collectively, these protective functions are thought to be a major contributor to the beneficial effects that accompany existing therapies, which we describe later in this review.

B cell-depleting therapies

Several B cell-targeted therapies have been developed based on the growing body of evidence, from both mouse and human studies, of significant B cell involvement in the pathogenesis of MS. The most effective therapies employ mAbs to deplete B cells through NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and antibody-triggered apoptosis[1,3,171] (Fig. 2a). Of note, these therapies do not typically reduce the levels of serum IgG, calling into question the role of autoantibodies in MS[25,66,172]. In clinical trials, B cell depletion therapies using mAbs directed against CD20 or CD19 have yielded promising results for the treatment of MS[111].

Anti-CD20 mAbs

CD20 is a surface receptor present primarily on B cells, functioning as an ion channel which serves to amplify calcium signals transduced by the BCR[57,173]. Importantly, CD20 is not expressed on pre-B cells or plasma cells, enabling reconstitution of the B cell compartment following depletion as well as preservation of pre-existing humoral immunity[57,174]. Following depletion, reconstitution primarily consists of naïve and immature B cells, while the memory B cells population remains low[10,173,175]. Considering that memory B cells exhibit an enhanced ability to stimulate myelin-reactive T cells, the sustained reduction in memory B cells suggests that targeting the APC function of B cells in MS might significantly contribute to the efficacy of anti-CD20 antibody therapies[173].

Additionally, the reconstituted B cells exhibit reduced proinflammatory (GM-CSF, LT-α, TNF-α) and increased anti-inflammatory (IL-10) cytokine production[173]. Of note, a decrease in $CD3^+$ T cells in the CSF of treated MS patients is also observed[57]. This could be a result of the depletion of $CD20^+$ T cells, which are known to produce more inflammatory cytokines[176,177] compared with $CD20^-$ T cells, although this may be somewhat speculative; more likely this decrease may be the result of the increased regulatory B cell activity[173].

Taken together, these observations suggest that the depletion and subsequent reconstitution of the peripheral B cell compartment effectively shifts the B cell response from proinflammatory to anti-inflammatory[111].

Presently there are 4 different anti-CD20 mAbs that have moved to phase III clinical trials for the treatment of RRMS: rituximab (RTX), ocrelizumab (OCR), ofatumumab (OFT), and ublituximab (UTX). In clinical trials, all four mAbs displayed similar efficacy and had favorable safety profiles, differing slightly in structure, target epitope, and predominant mechanism of B cell depletion[3,111,178] (Fig. 2b).

RTX—Rituximab is a mouse-human chimeric IgG1 mAb to CD20, depleting B cells primarily through CDC[3,178]. RTX was the first anti-CD20 mAb tested as a treatment for MS and, after yielding surprisingly promising results in its initial phase II clinical trial, provided the rationale for investigating anti-CD20 as an effective treatment for MS, RRMS specifically[111,173,178]. In spite of the striking reduction in the number of new contrast-enhancing lesions as well as relapse rates, RTX has not entered subsequent phase III clinical trials for various reasons and therefore is used as an off-label therapy for MS patients[173].

Ocrelizumab—The next mAb developed was ocrelizumab. Importantly, OCR differed from RTX in that it was a humanized IgG1 mAb. The reasoning for this was two-fold: firstly, since the chimeric nature of RTX resulted in 24.6% of patients developing anti-idiotypic antibodies in response to RTX treatment, a humanized mAb would be less immunogenic[111,178]; secondly, the humanized Fc region displayed a higher affinity for the FcγRIIIa receptors present on NK cells[173], thereby enhancing the ADCC activity of OCR[171,173,179,180]. The efficacy of OCR was tested in three phase III trials, the results of which established OCR as the first anti-CD20 mAb to be approved by the FDA to treat both RRMS as well as primary progressive MS (PPMS)[57].

Ofatumumab—Ofatumumab is a fully human mAb and, consequently, is less immunogenic than OCR[181]. A second unique feature is that OFT not only binds an epitope that is completely distinct from that of RTX or OCR[178], but it does so with slower rate of dissociation from CD20 compared with RTX, resulting in enhanced CDC activity[57,182–186]. A phase III clinical trial (ASCLEPIOS I) testing the efficacy and safety of OFT compared to teriflunomide in the treatment of RRMS is currently underway (Clinicaltrials.gov number: NCT02792218).

Ublituximab—Ublituximab is, like OFT, a fully human mAb and binds a unique epitope on CD20. However, the Fc region has been glycoengineered to increase the affinity for FcγRIIIa, resulting in increased ADCC activity[187]. The results from a recently concluded phase II clinical trial are promising, and recruiting is underway for a phase III trial (ULTIMATE 1) comparing the efficacy of UTX to that of teriflunomide (Clinicaltrials.gov number: NCT03277261).

Anti-CD19 mAb

While both CD19 and CD20 are involved in B cell activation and differentiation, specifically through the modulation of BCR signaling[57], they differ in the timing of their expression during the course of B cell development and maturation. Importantly, CD19 is expressed on plasmablasts and some plasma cells after CD20 expression is lost[57]. This differential expression allows for anti-CD19 mAb therapies to have a longer-lasting depletion effect[188,189] and to eliminate pathogenic short-lived antibody-secreting cells, while preserving long-lived plasma cell populations.

MEDI-551 (inebilizumab), in addition to being humanized, which confers reduced immunogenicity, also lacks a fucose residue in the Fc region, which enables tighter binding to FcγRIIIa. MEDI-551, therefore, exhibits superior NK cell- and macrophage-mediated ADCC[57,189].

In the EAE model, MEDI-551 has been shown to reduce leukocyte infiltration into the spinal cord and reduce the numbers of short- and long-lived autoreactive plasma cells in the spleen and bone marrow, while maintaining and expanding protective Breg and myelin-specific Treg populations, respectively[57].

In human clinical trials, MEDI-551 has also been shown to reduce the mean number of cumulative new or enlarging MRI lesions over 24 weeks[57].

Revisiting the effects of earlier DMTs on B cells

As mentioned previously, B cells can contribute by different effector mechanisms to the pathogenesis of MS, namely via autoantibody production, antigen presentation to and stimulation of autoreactive T cells, establishment of ectopic lymphoid structures, and secretion of proinflammatory cytokines such as IL-6 and LT-α[9]. While several therapies have been developed which directly target B cells, it has also been shown that earlier disease-modifying therapies (DMTs) have appreciable effects on the B cell contribution to MS[9,112,173,190–199] (Fig. 2c), although the exact mechanism responsible for the beneficial

effects observed remain incompletely understood[3]. These DMTs are thought to exert their effects on multiple cell types, further underscoring the complex inter-cellular dynamics that collectively result in the pathogenesis of MS. Here we will summarize the effects of earlier DMTs on the B cell component of MS pathogenesis.

Interferon-β

The mechanism underlying the therapeutic efficacy of IFN-β is poorly understood, but is generally attributed to the combination of various anti-inflammatory effects on several immune cell types. While IFN-β treatment results in a shift from Th1 to Th2 responses, it reduces the percentages of naïve B cells expressing the costimulatory molecule CD86[196] and the chemokine receptor CCR5[200], as well as reducing the memory B cell population[201]. Further adding to its cumulative anti-inflammatory effects, IFN-β also increases the frequency of IL-10-producing regulatory transitional B cells[202,203]. This was coupled with an increase in BAFF levels in the serum and plasma, the source of which was suggested to be monocytes and granulocytes[8].

Glatiramer acetate

Glatiramer acetate (GA) is a synthetic amino acid copolymer comprised of L-alanine, L-lysine, L-glutamic acid, and L-tyrosine. GA, along with IFN-β, is a first-line medication used to treat MS. Although GA is known to act on B and T cells, the exact protective mechanisms remain incompletely understood[204]. Similar to IFN-β, GA treatment results in an increase in B cell-derived IL-10[205] with a concomitant decrease in the production of IL-6, LT-α,[3] and TNF-α[173] in humans, corroborated by results from EAE studies showing a shift towards a regulatory B cell phenotype[3]. In RRMS patients, GA treatment causes a decrease in the expression of CXCR5 and intracellular adhesion molecule (ICAM)-3 in B cells, reducing their migratory potential[182,206], as well as a decrease in the total circulating memory B cells and plasmablasts[8], likely due to the reduced levels of BAFF in the CNS[173].

Daclizumab

Daclizumab is a humanized IgG1 mAb to CD25, the alpha subunit of the IL-2 receptor. This treatment is primarily utilized to deplete the autoreactive CD4 T cell pool, however, it also reduces the memory B cell population, of which 60% express CD25[171,207], and in addition a specific Breg subset[208]. However, daclizumab has been withdrawn from the market in light of 12 recent reports of serious inflammatory brain disorders, three of them being fatal[209].

Fingolimod

Fingolimod, an antagonist to the sphingosine-1-phosphate (S1P) receptor, results in a reduction of circulating lymphocytes as well as decreasing the infiltration of potentially pathogenic or autoreactive lymphocytes into CNS[8]. In the periphery, the relative frequency of naïve and memory B cells is reduced[198,210–213]. Although the total number of B cells within the CNS is not significantly changed, there is an increase in the frequency of IL-10-producing Breg subsets in CSF[3,208,213–220] as well as the peripheral blood[173]. Overall, treatment with fingolimod favors an increase in the frequency of regulatory B cells[210,212,213,221].

Dimethyl fumarate

Although the exact mechanism of action is not well understood, the overall effect on B cells is similar to fingolimod. DMF treatment results in a decrease in total circulating B cells, a decrease in the frequency of naïve and memory B cells[199,222–225] with a concurrent increase in IL-10-producing B cells in peripheral blood[226], and a shift from a proinflammatory to an anti-inflammatory cytokine profile.

Natalizumab

Natalizumab prevents leukocyte migration into the CNS through the blood brain barrier (BBB)[227] by targeting VLA-4, an adhesion molecule expressed on T and B cells, as well as on monocytes, basophils, neutrophils, and eosinophils[228]. The beneficial effects were initially attributed to the effects on T cell migration, however it was later shown *in vitro* that human B cell migration across endothelial cells was reduced to a greater extent than T cells[229]. Treatment causes an increase in memory B cells and a decrease in naïve B cells in the periphery[230], but leads to B cell reduction in the CSF[231,232]. Natalizumab has also been reported to reduce the amount of IgG, IgM, and OCBs in the CSF[3,173,231,233–236]. An increase in peripheral memory B cells is also seen following treatment due to their high level of VLA-4 expression[229,237,238], which could be potentially problematic upon discontinuation[207]. Natalizumab treatment is also associated with an increase in regulatory B cells[171].

Alemtuzumab

Alemtuzumab is a humanized mAb to CD52, which is expressed on over 95% of B and T cells, but is also expressed on multiple other leukocyte populations[239]. Though its definitive function is unknown, evidence suggests that it serves as a costimulatory molecule for T cell activation and contributes to T cell migration[240]. Treatment with anti-CD52 mAb results in the ADCC- and CDC-dependent depletion of B and T cells[173,182]. Notably, immature transitional B cells and naïve mature B cells are increased upon reconstitution of the B cell compartment, while memory B cells remain reduced[3].

Positive clinical outcomes from phase III clinical trials showing reduced relapse rates compared with IFN-β1α, are tempered by concerns over increasing occurrence of secondary autoimmune disease[241], specifically antibody-mediated autoimmunity such as Graves' disease and hypothyroidism among others[182]. It is thought that this effect could possibly be due to accelerated repopulation of immature B cells[3] in the absence of regulation by T cells[242], as B cells repopulate much faster than T cells[3,173]. Increases in serum IL-21, a cytokine implicated in the induction of Th17 cells, B cell differentiation and antibody production, and inhibition of Treg cells, correlates with the incidence of secondary autoimmunity following alemtuzumab treatment[243] and is therefore a second contributor to this adverse effect.

Cladribine

Cladribine (NCT03364036/Mavenclad) is a chlorinated analogue of deoxyadenosine that eliminates B and T cells by manipulating the purine salvage pathway to ultimately impair DNA synthesis and repair[244]. The phosphorylation of cladribine is catalyzed by DCK but

can be abrogated by the enzyme 5'-neucleotidase (5'-NTase)[245]. B and T cells express low levels of 5'-NTase relative to DCK[245]. Previous reports emphasized the primary target population as T cells, however the depletion of B cells is comparatively greater[245,246]. This selectivity is likely due to the finding that most B cell subsets express higher concentrations of DCK compared with T cells[245]. Importantly, it was recently shown that cladribine exerts its protective effects through the long-term depletion of memory B cells[245] and that it has the ability to penetrate the BBB, a shortcoming of B cell-targeting mAbs[246–248]. Cladribine was shown to be efficacious in the treatment of RRMS in a phase III clinical trial. A 2-year phase IV clinical trial is currently recruiting to determine the onset of action in subjects with highly active RRMS.

Plasmapheresis

One of the earliest clinical observations pointing to the involvement of B cells in MS was the detection of OCBs in the CSF of MS patients[57]. In response to this, plasmapheresis, which is the removal of soluble products from the plasma, namely proinflammatory Ig and cytokines[178,249], was applied to the treatment for MS. Although intrathecal OCBs are one of the hallmarks of MS, detected in the CSF of >95% of patients[250–253], only patients exhibiting the most common demyelination pattern, pattern II, respond favorably to plasmapheresis[3,254], suggesting that the role of B cells in MS is not primarily antibody-mediated. This is further supported by the maintenance of CSF immunoglobulin levels in patients treated with RTX or OCR[3,178]. However, plasmapheresis, plasma absorption, and therapeutic plasma exchange (TPE) are still used as suitable treatments for patients with severe and refractory MS relapses, particularly those unresponsive to steroids[8], though whether the beneficial outcome is due to the removal of autoreactive immunoglobulin remains unknown[3].

B cell-directed treatment strategies currently unexplored in MS

Anti-CD22 mAb (epratuzumab)

Epratuzumab is a humanized mAb targeting CD22, which is thought to be a negative regulator of BCR-derived activation signals and primarily expressed on mature B cells[178]. Epratuzumab induces only partial B cell depletion and was well tolerated in a phase III clinical trial for systemic lupus erythematosus (SLE), but has not yet been explored as a treatment for MS[255].

Targeting plasmablasts and plasma cells

Treatments are also being explored which would target plasmablasts and plasma cells specifically. Daratumumab, a mAb to CD38, which is thought to selectively deplete plasmablasts and some plasma cells, is currently used to treat multiple myeloma and plasma cell malignancies, but is also being considered for MS treatment[111,173]. Anti-CD38 mAb treatment also depletes memory B cells, the reduction of which is thought to significantly contribute to the efficacy of several of the aforementioned DMTs[256]. However, the potential effectiveness of this treatment is called into question by studies showing that plasmablasts in humans are a source of IL-10, along with immature/transitional B cells, which also highly express CD38[10,214,215]. Regulatory myeloid-derived suppressor cells (MDSCs) and a novel

subpopulation of Tregs also highly express CD38 and are therefore susceptible to depletion by daratumumab, although the degree to which this affects clinical outcome requires further investigation[256].

Bortezomib, a small molecule proteasome inhibitor, has been approved for treatment of multiple myeloma. Additional studies using animal models of SLE, RA, autoimmune hemolytic anemia, and MG have shown promise in effectively depleting plasma cells[10,178], though it is noteworthy that there is a lack of definitive evidence implicating plasmablasts and plasma cells as significant contributors to MS pathology.

Targeting B cell survival factors

BAFF and APRIL are soluble factors essential for the maturation, activation, and survival of B cells[8,178,257]. There are three separate receptors capable of binding both molecules, albeit with different affinities: BAFF-R, TACI, and BCMA. Adding to the complexity of this system is the observation that expression of these receptors varies greatly depending on the context. Evidence of increased serum levels of BAFF in MS patients suggested it as a potential target. However, in a phase II clinical trial for the TACI-Fc fusion protein atacicept, which depletes soluble BAFF and APRIL, treatment unexpectedly worsened MS in the participants and the trial was halted[8,148,258]. Additionally, in a phase II clinical trial to control optic neuritis, atacicept precipitated CNS inflammation and augmented the conversion of people with optic neuritis to clinically definite MS. Proposed reasons for its failure include the possible depletion of Breg populations, the evidence that APRIL has been suggested as a negative regulator of autoimmunity, and the relative increase in memory B cells[173], although the exact reason is unknown[8].

Since the atacicept trial, four additional antibodies targeting components of this system have been developed: belimumab and tabalumab, which are humanized mAbs to BAFF; VAY736, a fully human IgG1 anti-BAFF-R mAb; and human BCMA fused to IgG1 Fc (hBCMA-Fc)[259]. However, none of these have progressed past phase II clinical trials[8,178].

Targeting B cell-produced GM-CSF

GM-CSF is thought to be involved in multiple aspects of MS pathogenesis and it is secreted by multiple cell types[260]. The frequency of GM-CSF-producing B cells is increased in MS patients, which is thought to enhance the proinflammatory myeloid cell responses[133,261]. Results from EAE studies showed that using antibodies against GM-CSF or its receptor ameliorates disease or prevented its onset[260]. Otilimab (MOR103/GSK3196165), a human recombinant antibody with high specificity for GM-CSF, has been developed, initially to treat RA. It has been tested on RRMS and secondary progressive MS (SPMS) patients in a small phase Ib clinical trial, the results showing that it was well-tolerated. Thus, selective suppression of GM-CSF production by pathogenic B cells may be beneficial because these cells are significant producers of this cytokine in MS[260].

Targeting B cell signaling pathways

Signals received within inflamed tissues are known to skew B cells towards more pro-inflammatory and pathogenic subsets[9]. Therefore, interfering with intracellular signaling

cascades in B cells using small molecule inhibitors has generated interest as a potential treatment for MS[173]. Particularly attractive is the potential of small molecular compounds to cross the BBB, a current deficiency of mAb therapies. One example is tofacitinib, which interferes with the JAK/STAT signaling pathway through the selective inhibition of Jak1 and Jak3. Tofacitinib treatment was shown to impair plasmablast development and thereby reduce Ig secretion in mouse and human B cells[262]. Memory B cell formation was also impaired, however, class switching was unaffected[262]. Ibrutinib, an inhibitor of Bruton's tyrosine kinase, has been studied in the treatment of SLE, providing the basis for exploring its efficacy in the treatment of MS[9].

Inducing IL-10-producing regulatory B cells

Given that the enhanced frequency and activity of IL-10-producing regulatory B cells is a key feature of several approved therapies, as detailed above, induction of regulatory B cell subsets would represent a promising treatment of MS[3,10,263]. Unfortunately, numerous obstacles have hindered the development of this therapeutic approach, namely the disparities between animal and human models regarding the phenotype of regulatory B cells[5,168] and lack of suitable methods for inducing them[10]. One proposed method to facilitate the generation of regulatory B cells involves the expansion of IL-10$^+$ B cells from patient PBMCs *in vivo* and subsequent transfer back into the patient[10]. However, further advances in characterizing the function and phenotype of B cell subsets in humans will be essential before this particular treatment becomes clinically feasible.

Future challenges/Concluding remarks

The benefit of targeting the B cell component in MS pathogenesis has been firmly established in light of the striking efficacy seen in clinical trials for B cell-depleting mAbs. However, variations in disease outcome represent a significant shortcoming of this type of therapy[10]. Several possible explanations for this have been postulated, including the inability to reach pathogenic B cell populations within the CNS, the depletion of beneficial regulatory B cell populations, and genetic polymorphisms affecting ADCC. Last, one might have to consider the likelihood that MS is the common clinical outcome of several differing pathogenic mechanisms, some which may not depend on B cells.

As depletion occurs only in the peripheral blood, perivascular space, and, less effectively, within CSF[259], B cell-depleting mAbs may not sufficiently reach areas within the CNS in which pathogenic B cell populations are thought to reside, such as meningeal TLOs[247,248,264]. This is further supported by evidence showing that current anti-CD20 mAbs do not efficiently cross the BBB, primarily as a result of their size[29,173]. However, advances in antibody engineering are seeking to overcome this by exploiting receptor-mediated transcytosis (RMT) as a way for mAbs to cross the BBB without its disruption[265]. For example, engineered bispecific mAbs (bsAbs) where the antigen-binding fragment (Fab) of one antibody arm is directed against a physiological receptor on the BBB (e.g. transferrin receptor) and the other Fab is comprised of a "therapeutic arm" directed against a therapeutic target, show enhanced passage across the BBB[265,266]. Results using bsAbs in

mouse models of Alzheimer's showed that they could be a promising approach for improving B cell-targeted mAb treatments[265].

The risk of eliminating beneficial anti-inflammatory regulatory B cells with global B cell-depleting therapies in addition to the intended pathogenic B cell populations may be ameliorated by developing novel therapies that more selectively target specific cell populations[3,9]. Additional hurdles stem from difficulties in definitively identifying certain B cell subsets in humans due to phenotypic differences between murine versus human B cells, namely memory B cell and regulatory B cell subsets[168,267]. Future advances in phenotypic and functional analysis of different B cell subsets will allow us to move toward developing therapies that are able to deplete only the pathogenic B cell populations while sparing those that are beneficial.

Another possible explanation for patients experiencing no significant improvement on B cell-depleting therapies is the existence of polymorphisms between patients in the Fc receptor region[149]. Since B cell-depleting mAbs utilize ADCC as a mechanism of action, these polymorphisms could potentially reduce the binding efficiency of the receptor to the depleting antibody, thereby limiting the effectiveness of treatment[149].

Last but not least, a possible reason for inconsistent treatment outcomes seen in B cell-directed therapies may rest with the fact that the exact role B cells in MS is still somewhat enigmatic. Great strides have been made towards a better understanding of which B cell subsets are pathogenic, where they reside, and how they function, but there is still much left to be discovered. Thus, development of more effective B cell-directed therapies will be contingent on an enhanced understanding of B cell biology in health and disease, as well as a better understanding of the pathogenesis of MS. The mounting body of work deconvoluting the involvement of B cells in MS pathogenesis provides key insights aiding in the development of more effective B cell-directed therapeutics going forward.

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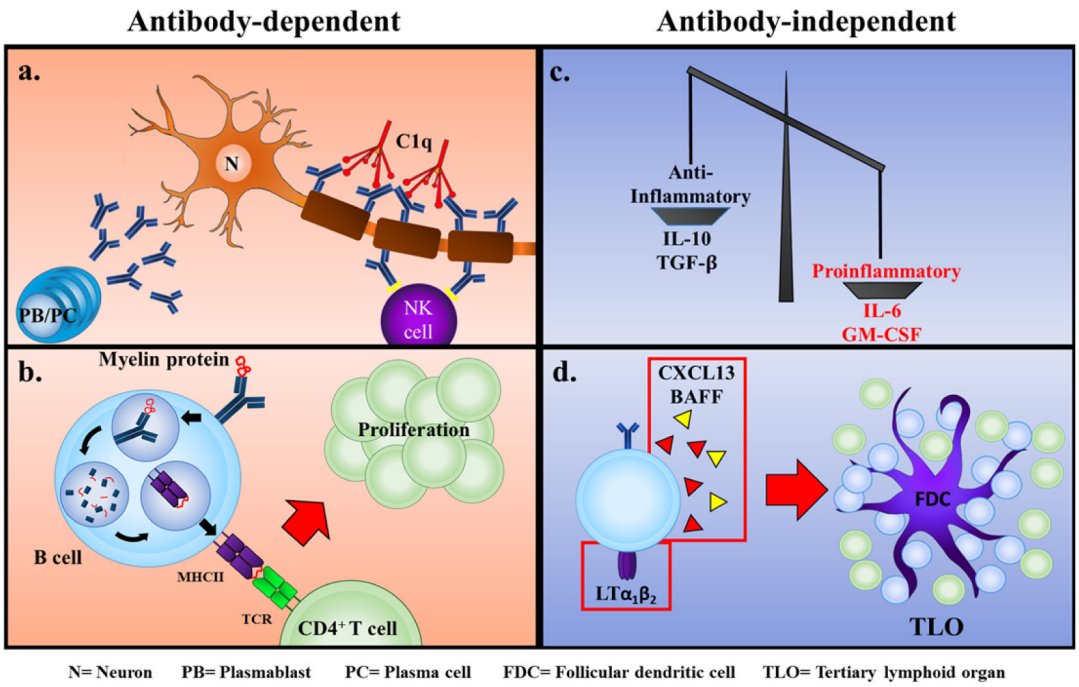

Figure 1. B cells mediate MS pathology through antibody-dependent and antibody-independent mechanisms.

(**a**) Myelin-reactive antibodies secreted by autoreactive plasmablasts and plasma cells mediate destruction of the myelin sheath by binding C1q and activating the classical complement pathway or initiating FcγRIIIa-mediated antibody-dependent cell-mediated cytotoxicity by NK cells. **b**) B cells expressing a myelin-reactive BCR are capable of processing myelin protein and presenting myelin peptide to autoreactive $CD4^+$ T cells, resulting in their activation and proliferation. (**c, d**) B cells are able to foster an environment conducive to autoimmunity by (**c**) secreting higher amounts of pro-inflammatory cytokines such as IL-6 and GM-CSF compared with regulatory cytokines such as IL-10 and TGF-b and (**d**) by secreting cytokines/chemokines including CXCL13 and BAFF which promote the differentiation of FDCs and establishment of TLOs, which facilitate the activation and expansion of autoreactive B and T cells. **N**= Neuron, **PB**= Plasmablast, **PC**= Plasma cell, **FDC**= Follicular dendritic cell, **TLO**= Tertiary lymphoid organ.

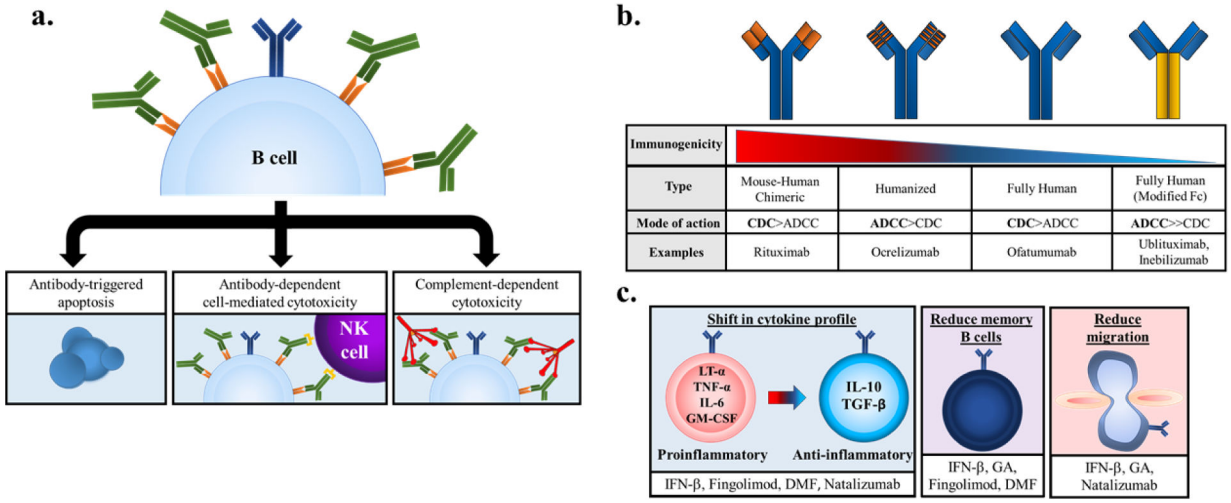

Figure 2. The mechanisms of action of B cell-depleting msAbs and earlier MS DMTs.

(**a**) Binding of anti-CD19 or anti-CD20 mAbs mediates B cell depletion via different mechanisms: directly catalyzing antibody-triggered apoptosis (*left*), initiating antibody-dependent cell-mediated cytotoxicity (ADCC) by binding of the mAb's Fc region by NK cell-expressed FcγRIIIa (*middle*), or initiating complement-dependent cytotoxicity (CDC) by binding C1q and activating the classical complement pathway (*right*). (**b**) B cell-depleting mAbs display differences in immunogenicity and preferential mechanism of action. (**c**) DMTs can shift B cell cytokine profiles from pro- to anti-inflammatory (*left*), reduce circulating memory B cells (*middle*), or impair migration through down-regulation or inhibition of surface receptors (*right*).