REVIEW ARTICLE | FOCUS
https://doi.org/10.1038/s41590-018-0135-x
nature immunology

Reassessing B cell contributions in multiple sclerosis

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There is growing recognition that B cell contributions to normal immune responses extend well beyond their potential to become antibody-producing cells, including roles at the innate–adaptive interface and their potential to modulate the responses of other immune cells such as T cells and myeloid cells. These B cell functions can have both pathogenic and protective effects in the context of central nervous system (CNS) inflammation. Here, we review recent advances in the field of multiple sclerosis (MS), which has traditionally been viewed as primarily a T cell–mediated disease, and we consider antibody-dependent and, particularly, emerging antibody-independent functions of B cells that may be relevant in both the peripheral and CNS disease compartments.

MS, one of the most common causes of neurological disability in young adults, is the prototypic acquired inflammatory demyelinating condition of the CNS, and it causes injury to both myelin and axons[1,2]. For most individuals with MS (approximately 85–90%), clinical onset is initially marked by relapsing and then remitting neurological deficits (which may appear to resolve fully or incompletely), a condition referred to as relapsing-remitting MS. The disease course often later exhibits ongoing worsening without obvious remission, a condition referred to as secondary progressive MS[3]. Approximately 10–15% of patients exhibit gradual worsening of neurological function from clinical onset (no remissions), a condition referred to as primary progressive MS (PPMS)[3]. The cause of MS, which remains unknown, is thought to reflect the consequences of several environmental exposures affecting a genetically susceptible host. Genome-wide association studies have implicated more than 200 genetic risk variants for MS[4], many of which encode molecules involved in immune-system responses, thus lending support to the prevailing concept that MS is primarily an immunologically mediated disease. Current studies aim to elucidate how the environmental risk factors implicated in MS (e.g., low levels of vitamin D, infection with Epstein–Barr virus, smoking and early-life obesity) interact with susceptibility genes and how they contribute to early disease mechanisms in the context of both the immune system and the CNS as the target organ[5]. Considerable evidence from studies of both patients with MS and the most commonly used MS animal model, experimental autoimmune encephalomyelitis (EAE)[6], has contributed to the common view of MS as a T cell–mediated disease[7], involving an abnormal balance between regulatory T cells (T_{reg} cells) and CNS-reactive effector T cells (Fig. 1a). Therapeutic efforts over the years have aimed to elucidate and correct or limit the presumed contributions of effector T cell responses and/or restore the balance between implicated effector and regulatory T cell responses[8,9].

Evolving concepts and distinct disease compartments in MS

An updated conceptual framework of MS immunopathophysiology has emerged on the basis of the striking ability of anti-CD20 antibodies to limit new MS relapses[10–15]. This finding has shifted attention to potential contributions of B cells to CNS inflammatory disease activity, particularly the antibody-independent functions of B cells that have been implicated in mediating new relapsing MS disease activity as part of cascades of cellular immunological interactions in the periphery. Another important conceptual advance in the field of MS has been the recognition that inflammatory CNS injury in MS involves both systemic and CNS-compartmentalized inflammatory responses[2,16–18]. Systemic responses, which contribute to the classical relapsing–remitting aspects of disease, involve cascades of aberrant activation of immune cells in the periphery and their subsequent trafficking across CNS endothelial barriers into the CNS, where they are thought to reactivate and contribute to injury in a perivascular distribution. This relapsing aspect of MS has been reasonably modeled in EAE[19]. In contrast, CNS-compartmentalized inflammation in MS, which is less well understood (and not as well modeled to date), might play a key role in propagating the steady, unrelenting CNS injury underlying progressive forms of the disease[16,18,20]. Chronically activated microglia and infiltrating macrophages within the CNS tissue[21–23], as well as B cell–rich aggregates of immune cells in the meninges surrounding the brain and spinal cord, are being actively studied as potential mediators of this CNS-compartmentalized inflammation[16,18,20]. Hence, with respect to the potential roles of B cell responses in MS, it is important to consider how B cells contribute to inflammatory disease processes both in the periphery and in the CNS in patients. Though we focus here on MS, the themes discussed have relevance across multiple immunologically mediated conditions including rheumatoid arthritis, type 1 diabetes and systemic lupus erythematosus (SLE), and researchers have also come to recognize the diversity of functional responses with which B cell populations might participate in disease modulation both outside and within the target organs involved. Further elucidation of the molecular mechanisms underlying the functional diversity of human B cells, their contribution to health and disease, and the potential to more selectively target them, are areas of active and shared interest across disease disciplines.

B cell tolerance and MS

Autoreactive B cells are present in the immunological repertoires of healthy individuals[24–26]. Their physiological roles as part of 'normal autoimmunity' remain incompletely understood, although they are normally maintained in a tolerant state. Two major checkpoints contribute to normal elimination and control of autoreactive B cells: central tolerance and peripheral tolerance[27]. Central B cell tolerance is established in the bone marrow and involves the elimination of approximately 75% of self-reactive B cells, whereas peripheral tolerance takes place in the secondary lymphoid organs, where most other self-reactive B cells are controlled. B cell receptor– and Toll-like receptor (TLR)-signaling pathways play important

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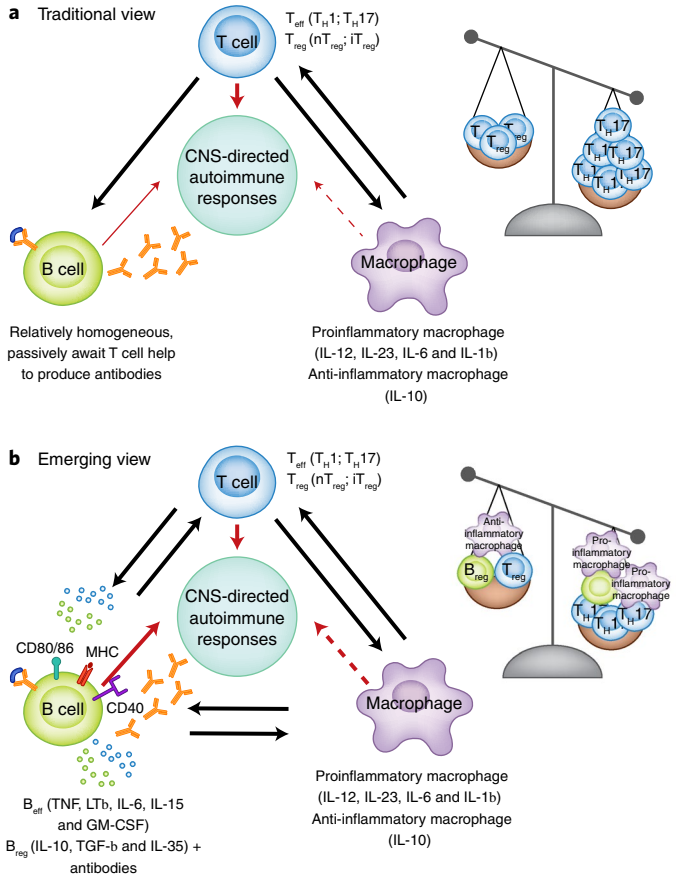

Fig. 1 | An evolving view of cell-subset contributions to MS pathophysiology. a, The traditional view. T cells are central players in MS immunological pathophysiology and the regulation of CNS-directed autoimmunity. An imbalance between proinflammatory type 1 helper T cells (T_H1) and T_H17 effector T cells (T_{eff}) and T_{reg} cells underlies new MS attacks. Myeloid cells, as the main APCs, shape T cell responses. In turn, differentiated T cells can shape myeloid cell responses. B cells, for the most part, are a relatively homogenous and passive population. They await the help of T cells to differentiate into antibody-secreting plasmablasts and plasma cells. Any B cell contribution to MS pathophysiology is largely considered to reflect their potential to produce CNS-autoreactive antibodies. **b**, The updated view. Results of aCD20 therapy in MS highlight a more central role of B cells in new MS attacks, which appears not to be antibody dependent. Antibody-independent roles of B cells, in part mediated through elaboration of distinct cytokines, can manifest as either proinflammatory effector B cells (B_{eff}) or anti-inflammatory regulatory B cells (B_{reg}). These cells can activate (B_{eff}) or downregulate (B_{reg}) proinflammatory responses of both T cells and myeloid cells. Bidirectional interactions among functionally distinct B cells, T cells and myeloid cells, and the consequences of such interactions, underlie the development of new MS attacks. nT_{reg}, natural T_{reg}; iTreg, induced T_{reg}; DMF, dimethyl fumarate.

roles during the selection of B cells in the bone marrow, whereas the CD40–CD40L receptor–ligand pair, the major histocompatibility complex (MHC) and T_{reg} cells[28,29] are considered important for control of autoreactive B cells in the periphery[27]. Through analysis of self-reactive antibody profiles, deficiencies in both central and peripheral B cell tolerance have been documented in people with SLE, rheumatoid arthritis and type 1 diabetes[30–32]. In contrast, B cells from people with MS appear to display abnormalities only in peripheral tolerance[29,32]. In this regard, the tolerance abnormality observed in MS B cells may plausibly be a consequence of the dysfunction of T_{reg} cells. In MS, both the frequency and the immunosuppressive function of T_{reg} cells have been reported to be lower than those in healthy control individuals[33,34]. Questions remain as to whether and how peripheral tolerance of B cells can be restored in people with MS. Some individuals with MS who are undergoing selective B cell depletion experience durable quiescence of their MS disease activity even as their B cells (largely naive) reemerge, thus suggesting that a form of tolerance may have been achieved. In contrast, B cells reconstituting after less selective depletion with anti-CD52 therapy—which primarily targets T and B cells—in MS commonly manifest as a breach in tolerance and the development of secondary (typically antibody-mediated) autoimmune diseases[35]. Development of this form of secondary autoimmune disease might reflect earlier reconstitution of autoreactive B cells in the face of relatively delayed reconstitution of T_{reg} cells, thereby resulting in a breach in peripheral tolerance.

B cells and antibody responses within the CNS in MS

Early studies implicating B cell antibody responses and CNS injury in MS have come from pathologic studies of perivascular MS lesions, which have identified antibodies bound to myelin fragments within phagocytic cells[36,37]. The presence of abnormally elevated immunoglobulin synthesis rates and a typical oligoclonal band (OCB) pattern of immunoglobulins in the cerebrospinal fluid (CSF) are hallmarks of MS and an indication that immunoglobulin is being abnormally produced within the CNS (referred to as intrathecal production). Immunoglobulin G (IgG) OCBs can be found in most patients with MS. Immunoglobulin M (IgM) OCBs are present in approximately 30–40% of patients[38], and their presence has been associated with more active disease[39] and potentially with therapeutic responses to B cell–directed therapy[40]. The findings suggesting intrathecal immunoglobulin production are supported by somatic hypermutation analysis of B cells and plasma cells, which has demonstrated a restricted number of expanded clones

within the CNS in people with MS[41–44]. Such cells isolated from CSF from patients with MS have been found to generate the antibodies that make up their OCBs[45]. Serial CSF studies have suggested the persistence of the same clones within the CNS in individual patients over time[46], and the same B cell and plasma cell clones can be shared among different CNS subcompartments (CSF, parenchyma and meninges) of the same patient[47]. More recent somatic hypermutation studies have demonstrated that, in individual patients, identical B cell clones can be shared between the CNS and the periphery[48–51]. These studies provide evidence of bidirectional trafficking of distinct B cell clones (both into and out of the CNS) and in fact suggest that much of the clonal expansion of these B cells occurs in the deep cervical lymph nodes rather than in the CNS. Indeed, the traditional view that the CNS is 'immunologically privileged' (including specialized endothelial cells of the blood–brain barrier restricting immune cell trafficking into the CNS) has evolved to recognize that normal immunological surveillance can involve ongoing low-level immune cell trafficking across additional and molecularly distinct barriers (blood–leptomeningeal and blood–choroidal interfaces), and that the CNS also has a system of lymphatic egress that appears to involve drainage into deep cervical lymph nodes[52–54]. Intriguingly, B cell contributions to MS relapses may possibly involve not only B cells residing in peripheral lymphoid tissues but also B cells that dynamically traffic into and out of the CNS, potentially present CNS antigens in deep cervical lymph nodes and trigger new waves of CNS-targeted inflammation. Relatively little is known about the molecular mechanisms involved in B cell trafficking both in and out of the CNS[17,55–57]. Levels of the B cell and/or plasma cell chemoattractants CXCL10, CXCL12 and CXCL13 are elevated in the CSF in people with MS[57] and, among these, CXCL13 levels have been suggested to predict an optimal response to B cell–depletion therapy[58]. When and how B cells enter the CNS across distinct interfaces, how they migrate between the CNS subcompartments, what determines their egress from the CNS and the roles that they might play on both sides of the CNS barriers are topics of active investigation.

Although the same B cell and plasma cell clones tend to persist in the CNS of a given patient over time, they differ among patients[43,46]. Furthermore, and in spite of considerable efforts, defining specific CNS-reactive antibodies within the CSF that are shared across individuals with MS has been elusive[59]. Thorough examination of the immunoglobulin making up the CSF OCBs in MS has identified antibodies that primarily recognize ubiquitous intracellular self-proteins, thus suggesting that the OCBs might be generated as a response to dead-cell debris rather than being primary perpetrators of the injury[60,61]. Similarly, establishing pathogenic roles of circulating CNS-reactive antibodies in MS has been elusive. Early studies using solid-phase detection assays, such as enzyme-linked immunosorbent assays (which do not reliably detect antibodies to potentially more relevant conformational epitopes), have suggested that circulating antibodies against the myelin antigens myelin basic protein and myelin oligodendrocyte glycoprotein (MOG) might be involved in early MS disease mechanisms; however, these findings have not been confirmed in subsequent studies[62]. When measured in the appropriate assays, the presence of circulating anti-MOG in a subset of patients with CNS inflammatory demyelinating disease might actually identify patients who do not have MS[63,64]. Circulating antibodies specific to KIR4.1 (an ATP-sensitive inward rectifying potassium channel expressed primarily by glial cells) have been reported in approximately half of adults with MS[65] as well as in children with CNS inflammatory demyelination[66], though their presence does not appear to be associated with a particular clinical phenotype. Other studies have not reproduced the original results[67,68], although there were several technical differences in the methods used, thus highlighting the challenge of using different techniques and the importance of direct assay comparisons[69]. The difficulty in establishing the disease relevance of circulating CNS-reactive antibodies in people with MS contrasts with the situation in other CNS inflammatory conditions in which specific CNS-reactive antibodies are strongly implicated in disease pathophysiology, including antibodies to aquaporin-4 in neuromyelitis optica spectrum disorders and to the *N*-methyl-D-aspartate receptor in autoimmune encephalitis.

Though the pathogenic role of immunoglobulin present in the CNS and the relevant antigenic specificities of B cells involved in MS remain unclear, CNS-compartmentalized B cells might contribute to the MS process independently of antibody production. In contrast to classical perivascular MS lesions, which typically contain few B cells and/or plasma cells, meningeal immune cell collections (meningeal inflammation) can be B cell or plasma cell predominant[16,20]. These 'B cell–rich' structures (occasionally recapitulating the features of ectopic lymphoid structures seen in other chronic inflammatory conditions[70]) can also contain T cells, follicular dendritic cells and stromal cells[16,20]. Autopsy findings of more substantial meningeal inflammation are associated with more aggressive MS course before death[20] and more severe cortical pathology involving loss of oligodendrocytes and neurons as well as microglial activation[18,20]. This form of cortical injury, which appears to be unique to MS, develops in the most superficial part of the cortex subjacent to the meninges (referred to as 'subpial' cortical injury) and is now considered an important contributor to the pathology of progressive MS. Of interest is the possibility that meningeal inflammation, and particularly B cells, may contribute to propagation of the subpial cortical injury in MS. In this regard, soluble products of B cells isolated from people with MS but not controls) can be toxic to both oligodendrocytes and neurons in vitro[71,72]. This toxic effect persists even when immunoglobulin is removed from the B cell–soluble products, thus suggesting a potentially important antibody-independent function of B cells in the CNS inflammatory injury in MS. The observation that the same B cell clones are maintained over time within the CNS in people with MS suggests that these clones are fostered by factors in the local environment. These factors may include the B cell–survival factor BAFF, secreted by astrocytes[17,73,74], as well as BAFF-independent mechanisms that might support not only B cell survival but also their activation and contribution to propagating CNS inflammation and injury[75].

Antibody-independent functions of peripheral B cells in MS

The most compelling implication of the antibody-independent roles of B cells in MS has come from the robust and rapid decrease in new relapsing MS disease activity observed after B cell depletion with anti-CD20 (aCD20) therapy[10–15,76]. Plasmablasts and plasma cells (the actual antibody-secreting cells) express little or no CD20 and hence are not directly depleted by aCD20 therapy. Given the relatively long half-life of any antibodies already secreted, the rapid decrease in new disease activity after aCD20 therapy would seem unlikely to reflect removal of pathogenic antibodies. Indeed, direct assessment of the CSF in patients with MS before and after aCD20 treatment has revealed no major changes in the abnormal immunoglobulin levels, synthesis rates or OCB number and pattern[77,78] at a time at which patients benefit from significantly less relapsing disease activity. Together, these observations suggest a key role of B cells in triggering new MS relapses and also indicate that such a role would probably reflect one or more antibody-independent functions of B cells, such as antigen presentation, T cell activation and/or cytokine production (Fig. 1b).

B cells as antigen-presenting cells and modulators of T cell responses in MS

Compared with professional antigen-presenting cells (APCs; for example, dendritic cells and tissue-resident macrophages), which specialize in linear epitope presentation, B cells can recognize

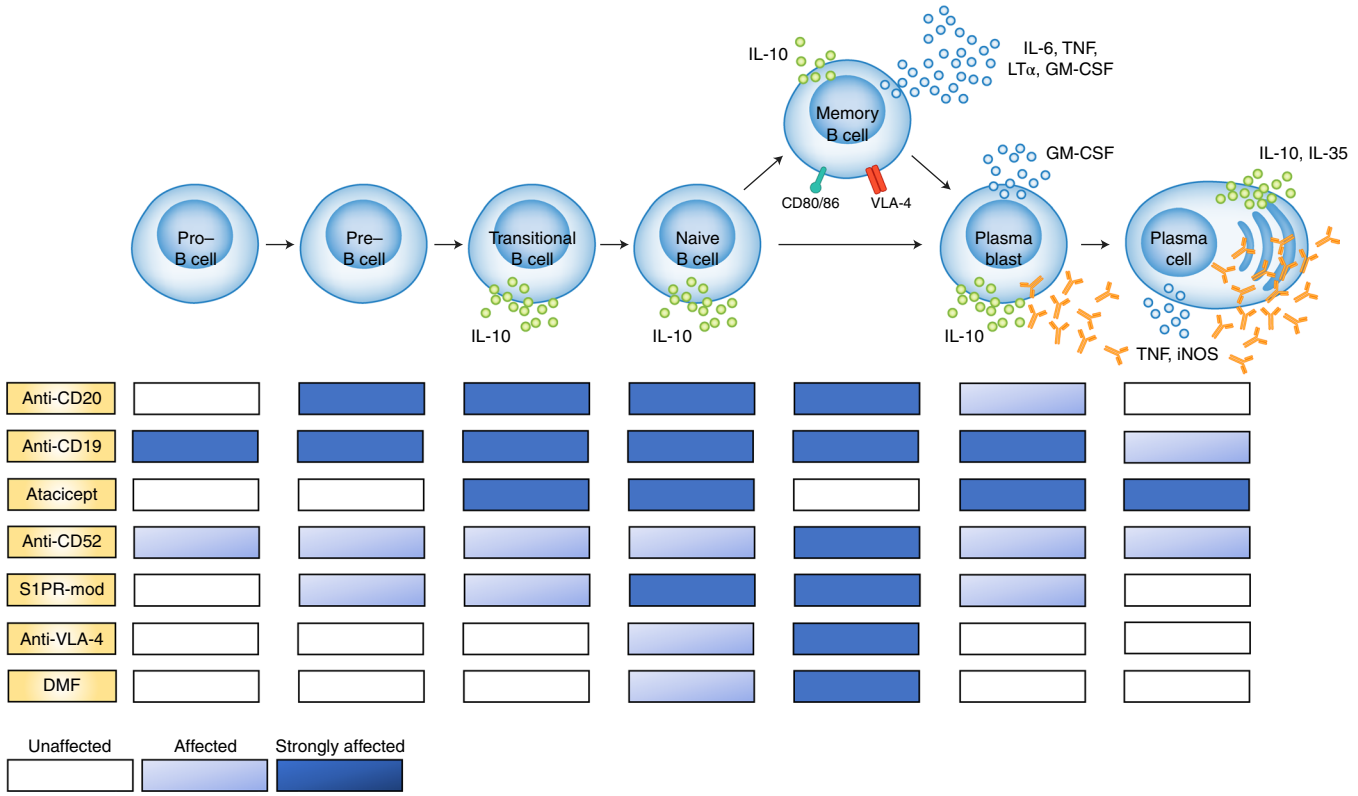

Fig. 2 | Different therapies applied in MS preferentially target distinct cell stages along the B cell lineage. Schematic representation of the stages of B cell maturation and differentiation, highlighting predominant cytokine responses identified for different subsets. Therapies used or considered in patients with MS are shown in orange rectangles at left. Blue rectangles indicate B cell–lineage stages thought to be affected by the different treatments (additional information in Table 1). Atacicept, which is the only one of these therapies that exacerbates rather than limits MS attacks, is also the only one that relatively spares memory B cells. These results from in vivo patient exposures to various immunological interventions support the view that key pathogenic B cells involved in the development of new MS attacks reside in the memory B cell pool. iNOS, inducible nitric oxide synthase.

three-dimensional 'conformational' epitopes[79,80]. They are more efficient at presenting protein antigens and appear to be the main source of APCs when antigen levels are low[80,81]. B cells are particularly effective APCs when they recognize the same antigen as T cells[79,80,82]. Such 'cognate' interactions appear to be important for both the activation of effector T cells and the generation of T_{reg} cells[83,84]. The APC function of B cells has been highlighted in many disease contexts including infectious diseases and transplantation and autoimmune diseases[82,85–88]. In the context of CNS inflammatory disease, the potential APC function of B cells is supported by findings in EAE experiments in which B cell–specific MHC-II-knockout mice have been found to be resistant to recombinant MOG-induced disease[82,89]. Knocking out MHC-II on only B cells results in total abrogation of anti-MOG production in this highly B cell–dependent model of EAE, whereas injecting these mice with anti-MOG only partially recapitulates EAE severity. Together, these findings suggest an MHC-II-dependent but antibody-independent role of B cells in EAE. Crossing MOG B cell receptor–specific mice with MOG TCR–specific mice substantially increases the incidence of spontaneous EAE[82], thus suggesting that enhancing cognate interactions between B cells and T cells can overcome CNS tolerance.

As part of APC–T cell interactions, the integration of co-stimulatory signals plays a key role in defining the T cell response. Of the more than 20 co-stimulatory molecule–receptor pairs identified to date[90], CD80/86 and their T cell–activating binding partner CD28 are among the best characterized. In the resting state, a subset of human memory B cells express CD80 (ref. [91]). Both CD80 and CD86 can be induced by various B cell–activating stimuli (both innate and adaptive) in vitro[91,92]. In vivo, selective knockout of CD80 and CD86 expression by B cells has been associated with decreases in both primary and secondary T cell responses[93]. Human B cells also stimulate T cells through both CD80 and CD86 (refs [91,94]), and in MS, the frequency of circulating $CD80^+$ B cells is abnormally elevated in patients with active disease[95].

In addition to expressing co-stimulatory molecules, B cells express co-inhibitory molecules involved in downregulating the responses of effector T cells. For example, the ligand PD-L1, expressed by B cells, protects against EAE by downregulating T cell responses through its receptor, PD-1 (ref. [96]). B cell expression of the ligand GITRL, another co-inhibitory molecule, directly induces T_{reg} cell differentiation through its receptor, GITR[97]. In mice, memory B cells can be divided into two subsets, $PD\text{-}L2^+CD80^+$ and $PD\text{-}L2^-CD80^-$ B cells, independently of their antibody isotype[98]. Double-positive B cells rapidly become antibody-secreting cells but cannot induce a germinal-center reaction, whereas double-negative B cells can induce a germinal-center reaction, but very few of them can become antibody-secreting cells[98]. These findings provide an initial basis for defining functionally distinct B cell subsets according to their different profiles of co-stimulatory- or co-inhibitory-molecule expression. Whether this annotation will prove useful in humans, and how different B cell subsets defined by co-stimulatory and co-inhibitory molecules might contribute to CNS inflammatory diseases including MS, remains to be explored.

Pro- and anti-inflammatory cytokine-defined B cells in MS

B cells are now recognized for their abilities to both enhance and downregulate local immune responses according to their distinct cytokine-secretion profiles, thereby affecting both health and

disease[88,99,100]. Circulating B cells from untreated people with MS exhibit an abnormal balance between pro- and anti-inflammatory cytokine responses[100–107]. B cells (particularly memory B cells) from individuals with MS, compared with healthy control individuals, can be activated to produce abnormally high amounts of the cytokines TNF, LTα, IL-6 and GM-CSF[101–103,105,106]. B cell IL-6 is particularly involved in the generation of type 17 helper T cell (T_H17) responses[88] and is considered pathogenic in both MS and EAE[104]. Selectively knocking out IL-6 from B cells results in decreased T_H17 responses and diminished EAE severity[82,104]. GM-CSF-producing B cells, described in both humans and mice[88,106,108–110], are particularly proinflammatory and may be pathogenic. People with MS exhibit increased frequencies and responses of GM-CSF-expressing B cells, which belong to the memory B cell pool, coexpress particularly high levels of both TNF and IL-6 (but not the cytokine IL-10), and efficiently enhance myeloid cell proinflammatory responses in a GM-CSF-dependent manner[106]. Anti-CD20-mediated B cell depletion in people with MS results in decreased inflammatory responses of both T cells (in a manner that appears to be partly dependent on B cell TNF, LTα and IL-6)[103,104] and myeloid cells (in a manner dependent on B cell GM-CSF)[106]. In addition to providing a plausible therapeutic mode of action of B cell depletion in MS, these observations highlight the antibody-independent contributions of cytokine-expressing proinflammatory B cells as well as the importance of B cell–myeloid cell–T cell interactions (Fig. 1) in the peripheral immunological cascades that contribute to new inflammatory-disease relapses[100].

The B cell profile that reconstitutes after aCD20 therapy is biologically different from the profile of B cells in untreated individuals with MS[102,104,106]. In most patients, reconstituting B cells are $CD27^-$ (naive) and produce less proinflammatory cytokines (TNF, LT, IL-6 and GM-CSF) but higher levels of IL-10 (refs [102–104,106]), which appear to be associated with a persistent decrease in both T cell and myeloid-lineage proinflammatory responses during the reconstitution phase[103,104,106]. Of interest is whether reconstituting naive B cells after aCD20 treatment not only might lose their proinflammatory propensity but also might be able to exert a regulatory function (discussed below), thereby limiting new relapses. This prospect of 'durability' of a treatment effect that might persist even after B cells reconstitute requires further examination in MS. Whether monitoring the return of naive versus memory B cells will be helpful in guiding retreatment decisions with aCD20 therapy in MS remains to be seen. In any event, the opportunity to compare the biological-response profiles of reconstituting B cells in individuals who do or do not develop new disease relapses during B cell reconstitution should provide important insights into the contributions of functionally distinct B cells to MS disease activity.

B cells can also downregulate immune responses through the secretion of anti-inflammatory cytokines (including IL-10, TGF-β and IL-35), thereby limiting different stages of CNS inflammation[99]. In mice, selectively knocking out IL-10 from B cells results in more severe EAE[111], and adoptive transfer of in vitro–induced IL-10-producing B cells suppresses EAE in an IL-10-dependent manner[112–114]. Inducing EAE in IL-10-reporter mice has implicated draining lymph nodes (rather than the spleen or CNS) as the sites where $IL\text{-}10^+$ B cells regulate disease-relevant immune responses[115]. CNS inflammation in EAE can also be regulated by IL-10-secreting B cells that are induced through alteration of the gut microbiome[116]. Different stimuli induce IL-10 production from B cells, including engagement of TLRs, CD40, microbiota and cytokines[112,114,117]. In humans, both naive and memory B cells are capable of producing IL-10 in a context-dependent manner[101,118–120]. Human $CD27^-$ (naive) B cells, but not $CD27^+$ (memory) B cells, produce IL-10 after engagement with isolated CD40 (refs [102,103,105,107,121]), a response that has been found to be abnormally deficient in B cells of people with MS[102,107]. In contrast, B10 cells, described as IL-10-expressing B cells that are induced within the $CD27^+$ memory pool by stimulation through TLR4 and TLR9, suppress TNF production by monocytes through an IL-10-dependent mechanism[119]. Unexpectedly, B10 cells have been found to increase after ex vivo stimulation of B cells in several human autoimmune diseases including MS[119]. TGF-β1 production by B cells has been shown to limit the induction phase of CNS inflammation in EAE through downregulation of APC function and decreased responses of encephalitogenic T_H1 and T_H17 cells[122]. In turn, a role of anti-inflammatory IL-35-producing B cells has been suggested in the recovery phase of both EAE and experimental autoimmune uveitis[123,124]. In these contexts, IL-35-expressing B cells inhibit proinflammatory immune responses either directly through IL-35 (ref. [123]) or indirectly through induction of IL-10-producing B cells[124]. Notably, although they are referred to as B cells, the anti-inflammatory $IL\text{-}10^+$ and $IL\text{-}35^+$ cells in several of the above studies have been found to exhibit plasma cell or plasmablast markers, thus highlighting previously unappreciated antibody-independent functions of antibody-secreting cells. Plasma cells may also support proinflammatory responses, such as those through secretion of TNF and inducible nitric oxide synthase, independently of antibody secretion[125].

Mechanisms underlying B cell cytokine dysregulation

Given the ability of cytokine-expressing B cells to modulate immune responses, and the identification of abnormalities in these functions in MS and other disease conditions, elucidating the molecular mechanisms underlying human B cell cytokine regulation is of considerable interest. Prior work has shown that the transcription factors STAT5 and STAT6 reciprocally regulate human B cell expression of GM-CSF and IL-10 (ref. [106]). Abnormally increased phosphorylation of STAT5 and STAT6 in the B cells of individuals with MS is associated with elevated production of GM-CSF and diminished production of IL-10, whereas dual STAT5–STAT6 signaling blockade during activation reverses the abnormal cytokine-response profile of MS B cells[106]. Epigenetic mechanisms such as histone modifications, DNA methylation and regulation by noncoding RNAs (microRNAs and long noncoding RNAs)[126] may also contribute to the control and regulation of B cell responses and their potential roles in both disease initiation and propagation[127]. To date, most studies assessing epigenetic effects on B cells in disease have focused on their antibody-related functions, with implications of abnormalities in conditions such as SLE[128]. Several microRNAs have been found to be dysregulated in the B cells of patients with MS[105,129]. In particular, miR-132, which is overexpressed by MS B cells, has been found to enhance the secretion of TNF and LTα by targeting sirtuin-1 expression, whereas in vitro addition of the sirtuin-1 agonist resveratrol normalizes the exaggerated proinflammatory cytokine expression of MS B cells[105]. Future work will assess whether sirtuin-1 affects proinflammatory-cytokine programs of MS B cells through its effects on chromatin function, transcriptional repression through histone deacetylation and/or alterations in histone and DNA methylation. To date, defining master transcriptional regulators and unique surface markers of B cells with proinflammatory or anti-inflammatory potential has been elusive, thus possibly reflecting a greater capacity for functional plasticity. A better understanding of functionally distinct B cells and their roles in humans may facilitate the development of future therapies, including cell-based approaches, that either more selectively target or harness B cell responses for treatment of autoimmune diseases including MS.

Reassessing therapeutic targeting of B cells in MS

The aforementioned discoveries that B cells from untreated individuals with MS exhibit abnormal proinflammatory response profiles and can overactivate proinflammatory T cells and myeloid cells provide a plausible explanation for the therapeutic mode of action underlying the ability of aCD20 antibodies to robustly

Table 1 | Treatments used or considered in MS and the disease-implicated B cell responses that they affect

Drug name		Effects on B cells	Effects on T cells	Effects on myeloid cells	References
IFN-β		↓mBs in blood, IL-12, CD40, CD80; ↑tBs in blood, IL-10, TGFβ; no change in OCB	↓ central mTs; ↓ IFN-γ, IL-17, GM-CSF, IL-2; ↑ T_{reg} and T_{reg} function, CTLA-4, Fas	↓ IL-12, ROS, TNF, MMPs; ↑ IL-10, BDNF, TIMPs	145–156
Glatiramer acetate		↓nBs, mBs and pBs in blood; ↓LTα, IL-6, ICAM1, immunoglobulin production; ↑ IL-10	↓ IFNγ, TNF, IL-2, helper T cell survival; ↑ T_{reg} function, cytotoxic function of $CD8^+$ T cells, IL-10, BDNF; no change in major T cell subsets	↓ IL-12, NO, TNF; ↑ IL-10, IL-1RA, phagocytosis	150,157–174
Mitoxantrone		↑ B cell apoptosis; ↓ B cells (particularly mBs) in blood; ↓ LTα; ↑ IL-10	↑ T cell apoptosis (especially $CD8^+$); ↓ proliferation of T cells; ↑ IL-4, IL-5; no effect on T_{reg} function	↓ macrophage-mediated myelin degradation	102,175–179
Teriflunomide		↓ B cells in blood, proliferation of B cells; effective humoral immune responses to neoantigen generally mounted in teriflunomide-treated patients	↓ $CD4^+$ T cells, proliferation of T cells; no adverse effects of teriflunomide on the cellular memory response to recall antigens	↓ IL-6, IL-8, MCP-1	180–184
Dimethyl fumarate		↓ nBs and mBs (especially mBs) in blood; ↓ GM-CSF, IL-6, TNF ↑ tBs	↓ nTs, mTs (especially $CD8^+$ T cells; mTs); ↓ IFN-γ, IL-17, GM-CSF, TNF, IL-22	↓ miR-155, antioxidant expression, IL-6, TNF, IL-10; dimethyl fumarate cytotoxicity in monocytes in vitro	139–142,185–193
Daclizumab		↓ B cells in both blood and CSF (especially mBs); B cell production of normal neutralizing antibodies after vaccination challenge	↓ T cells in both blood and CSF; decreased responses to CMV and EBV antigen in vitro	No obvious changes in absolute counts of different myeloid compartments	194–197
Natalizumab		↓ B cells, plasma cells in CSF, IgG and IgM synthesis; ↑ pBs, mBs and $CXCR3^+$ B cells in blood; no change in OCB	↓ $\%T_{reg}$ in blood; ↓ T cells in CSF; ↑ T cells (especially effector mTs in blood); ↓ IL-17, IFN-γ	↓ miR-155, antioxidant expression	188,198–209
S1PR modulator	**On treatment**	↓ nBs, mBs, TNF, CD80 and HLA-DR in blood; ↑ tBs in blood, IL-10, TGFβ; no change in OCB	↓ T cells (especially central mTs); ↓ IFN-γ, IL-17, GZMB; ↑ $CD39^+$ T_{reg}, $CD56^+$ T cells, TCF-1	↓ IL-1β, IL-6, TNF, miR-155, antioxidants HMOX1 and OSGIN1	136–138,188,203,210–215
	Reconstitution off treatment	↓ tBs in blood; ↑ nBs and mBs in blood	↓ T_{reg}/T_H17 ratio to baseline; nTs, effector mTs remaining lower than on treatment	NA	216
Alemtuzumab	**On treatment**	↓ B cells in blood	↓ T cells ↑ $\%T_{reg}$	↓ Number of DCs; ↓ DC IL-23, GM-CSF	217–219
	Reconstitution off treatment	↑ B cells (especially tBs and nBs) in blood	↓ IFN-γ, IL-17; ↑ T cells (T_{reg} and mTs with active phenotype reconstituted first); ↑ IL-10, TGF-β, IL-4; delayed reconstitution of $CD4^+$ T cells	NA	220–224
Anti-CD20	**On treatment**	↓ pBs, tBs, nBs and mBs in blood; ↓ B cells in CSF; no change in OCB	↓ $CD20^{dim}$ T cells; 50% decrease in T cells in CSF; ↓ IFN-γ, IL-17, proliferation of T cells; no change in other blood T cell counts	↓ IL-12, IL-6 ↑ IL-10	77,102–104,106,130,225–227
	Reconstitution off treatment	↓ GM-CSF, IL-6, TNF, LTα; ↑ tBs, nBs, IL-10	↓ IFN-γ, IL-17, proliferation of T cells	↓ IL-12, IL-6; ↑ IL-10	102–104,106
Atacicept[a]		↓ tBs, nBs, plasma cells and IgM in blood; no changes in circulating mBs and IgG; ↑ IL-15	NA	NA	228–231

[a]Atacicept exacerbates MS disease activity. B cell–phenotype data after Atacicept treatment acquired from other autoimmune diseases (SLE and rheumatoid arthritis). BDNF, brain-derived neurotrophic factor; CMV, cytomegalovirus; CSF, cerebrospinal fluid; HMOX1, heme oxygenase 1; ICAM, intracellular adhesion molecule; mBs, memory B cells; MCP-1, monocyte chemoattractant protein 1; miR, microRNA; MMPs, matrix metalloproteinases; mTs, memory T cells; NO, nitric oxide; nBs, naive B cells; nTs, naive T cells; OSGIN1, oxidative stress induced growth inhibitor 1; pBs, pre-B cells; tBs, transitional B cells; TIMPs, tissue inhibitors of metalloproteinases; DCs, dendritic cells; ROS, reactive oxygen species; S1PR, sphingosine-1 phosphate receptor; IL-1RA, IL-1 receptor agonist; NA, not available.Data are from refs. [77,102–104,106,130,136–142,145–231].

limit new MS relapses[10–15,76]. Such a view would suggest that selective depletion of B cells (including abnormally proinflammatory memory B cells) may remove their ability to contribute through antibody-independent mechanisms (for example, proinflammatory-cytokine-mediated activation and antigen presentation) to the peripheral cellular immunological cascades involved in triggering new relapses. Notably, a small subset of CD20$^+$ T cells has been described in both the periphery and CNS in patients with MS. Although CD20$^+$ T cells can express proinflammatory cytokines such as IL-17 and IFN-γ and can be effectively removed by anti-CD20 therapy, characterization of these T cells to date has not indicated a particular role in disease activity[130]. Further indirect support that the balance between proinflammatory- and anti-inflammatory-cytokine-producing B cells or plasma cells can contribute to the regulation of CNS inflammation has come from observations that CNS inflammatory disease activity is elevated (rather than diminished) in patients treated in clinical trials of Atacicept, a fusion protein of immunoglobulin and TACI, the receptor for the B cell–survival factors APRIL and BAFF[131–133]. The genetic variant of BAFF that leads to overexpression of this molecule has been implicated in both MS and SLE[134]. Unlike aCD20 therapy (which removes both naive and memory B cells but spares plasmablasts and plasma cells), Atacicept preferentially limits the survival of naive B cells and plasmablasts or plasma cells, but has a lesser effect on memory B cells[135] (Table 1 and Fig. 2), thus reinforcing the view that memory B cells are the relevant disease-promoting subset. Notably, essentially all approved MS therapies (many designed to affect putatively disease-relevant T cell responses) also affect the response profiles of B cells in ways that might be relevant for their therapeutic effects (Table 1 and Fig. 2). For example, in patients with MS treated with fingolimod, a modulator of sphingosine 1 phosphate receptor, decreases have been observed in not only the total numbers of B cells in the circulation but also the proportion of memory B cells (whereas the proportions of regulatory and transitional B cells are increased), with a concomitant decrease in TNF expression and increase in IL-10 expression in circulating B cells[136–138]. Treatment with fingolimod also appears to enhance the migratory ability of regulatory B cells in an in vitro model of the blood–brain barrier[138]. Another example is dimethyl fumarate, the methyl ester of fumaric acid, which preferentially decreases the number of circulating memory B cells (but has a lesser effect on naive B cells and relative sparing of transitional B cells)[139–142]. In vitro, dimethyl fumarate preferentially limits B cell proinflammatory-cytokine expression through inhibition of the transcription factor NF-κB[140,142].

In addition to the strong implication of peripheral B cells in the pathophysiology of MS relapses, their potential to contribute to the pathophysiology of progressive MS has been suggested on the basis of the results of the ORATORIO aCD20 study, the first successful clinical trial in patients with PPMS[76]. Interpretation of the ORATORIO findings, in which aCD20-treated patients with PPMS experienced a modestly less severe trajectory of worsening neurological function than did placebo-treated patients, is not straightforward. First, of note, patients with MS can worsen neurologically over time (i.e., exhibit 'progression' or worsening of disability) both because of repeated relapses that did not fully remit and because of nonrelapsing worsening[3]. The biological processes underlying these two contributors to the progression of disability are not identical; both processes may coexist, and much of the injury to which they contribute might be subclinical (i.e., damage occurs that is not clinically evident yet diminishes CNS reserves). The concept that relapse biology might exist subclinically in individuals meeting the clinical criteria for PPMS is supported by the observation that approximately 25% of these individuals (especially younger ones closer to disease onset) exhibited focal gadolinium-enhancing lesions in brain magnetic resonance imaging at the time of study entry[76]. These lesions represent active perivascular inflammation thought to reflect relapse biology. Hence, all the benefits observed in the ORATORIO PPMS study might reflect aCD20's robust effect on relapse biology. An alternate (and not mutually exclusive) explanation is that CD20-expressing cells may contribute in a different way to nonrelapsing progressive disease. In particular, B cells accumulating as part of CNS-compartmentalized inflammation might contribute through direct toxicity to CNS cells as well as through propagation of inflammatory processes within the CNS[71,72], as noted above. Whether anti-CD20 monoclonal antibodies including ocrelizumab meaningfully access the CNS, and whether the molecular machinery required for B cell killing (e.g., complement-dependent cytotoxicity and antibody-dependent cell-mediated cytotoxicity) is present to enable B cell depletion within the CNS compartment even if aCD20 is present, is not clear.

The success of aCD20 in MS treatment has led to interest in generating other potential B cell–targeting therapies. The monoclonal antibody MEDI-551 targets CD19, which is expressed on a broader range of the B cell lineage than CD20, including plasmablasts and some plasma cells. Treatment with MEDI-551 in MS appears to be safe and has provided hints of efficacy in a recent phase 1 study[143]. The potential use of Bruton's tyrosine kinase inhibitors, which inhibit B cell–receptor signaling, is being explored in MS on the basis of initial studies in other immunologically mediated conditions including SLE[144]. Such use of small molecules that can also access the CNS may prove beneficial through limiting both the peripheral B cell contribution to the immunological cascades involved in MS relapses and the potential contributions of B cells within the CNS to CNS-compartmentalized inflammation implicated in progressive MS biology.

Conclusion

The opposing outcomes of different B cell–targeting therapies in MS underscore the need to better understand how B cells contribute to both regulation of normal autoimmunity and to immunologically mediated diseases including MS. This knowledge gap is particularly important to fill as new approaches to target B cells and their subsets are increasingly being clinically applied. Elucidating the particular B cell populations that contribute to disease processes and developing reliable tools for monitoring them and/or their effects on other disease-relevant immune responses should provide key biomarkers to aid in the optimization of treatment choices and to improve clinical decision-making.

Received: 2 January 2018; Accepted: 9 May 2018;
Published online: 20 June 2018

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Competing interests

A.B.-O. has consulted for multiple entities involved in B cell–targeting therapies and has received consulting fees and/or grant support from Atara Biotherapeutics, Biogen Idec, Celgene/Receptos, Genentech/Roche, GlaxoSmithKline, MAPI, Medimmune, Merck/EMD Serono, Novartis and Sanofi-Genzyme. R.L. and K.R.P. have nothing to disclose.

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