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Learning from other autoimmunities to understand targeting of B cells to control multiple sclerosis

David Baker[1], Gareth Pryce[1], Sandra Amor[1,2], Gavin Giovannoni[1]*, Klaus Schmierer[1]*

1. BartsMS, Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London E1 2AT, United Kingdom.

2. Pathology Department, Free University Amsterdam, 1081 HV Amsterdam, The Netherlands

*Joint senior authors

Running head: Targeting B cells to control autoimmunity

Corresponding author: Prof. David Baker; BartsMS, Blizard Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, 4 Newark Street, London E1 2AT. Tel: +442078822485; Fax: +442078822180; Email: david.baker@qmul.ac.uk

ABSTRACT

Although many suspected autoimmune diseases are thought to be T cell-mediated, the response to therapy indicates that depletion of B cells consistently inhibits disease activity. In multiple sclerosis, it appears that disease suppression is associated with the long-term reduction of memory B cells, which serves as a biomarker for disease activity in many other CD20+ B cell depletion-sensitive, autoimmune diseases. Following B cell-depletion, the rapid repopulation by transitional (immature) and naive (mature) B cells from the bone marrow masks the marked depletion and slow repopulation of lymphoid tissue-derived, memory B cells. This can provide long-term protection from a short treatment cycle. It seems that memory B cells, possibly via T cell stimulation, drive relapsing disease. However, their sequestration in ectopic follicles and the chronic activity of B cells and plasma cells in the central nervous system may drive progressive neurodegeneration directly via antigen-specific mechanisms or indirectly via glial–dependent mechanisms. Whilst unproven, Epstein-Barr virus may be an aetiological trigger of multiple sclerosis. This infects mature B cells, drives the production of memory B cells and possibly provides co-stimulatory signals promoting T cell-independent activation that breaks immune tolerance to generate autoreactivity. Thus, a memory B cell centric mechanism can integrate: potential aetiology, genetics, pathology and response to therapy in multiple sclerosis and other autoimmune conditions with ectopic B cell activation that are responsive to memory B cell depleting strategies.

KEYWORDS. Autoimmunity; multiple sclerosis, memory B cells, relapse, neurodegeneration

ABBREVIATIONS. CNS central nervous system; EBNA Epstein-Barr virus antigen; EBV Epstein-Barr virus; latent membrane protein LMP; MS multiple sclerosis;

INTRODUCTION. Multiple sclerosis [MS] is widely-considered to be an autoimmune, demyelinating disease of the central nervous system [CNS]. This creates a neurodegenerative environment that leads to the accumulation of significant motor, sensory and cognitive disabilities [Compston & Coles 2002]. The disease affects about 2,500,000 people worldwide and tends to be commoner in high-income countries with healthcare systems willing to pay the significant costs for therapy [Compston & Coles 2008; Hartung et al. 2015]. It is widely assumed that the adaptive-immune arm generates lesions following entry of leucocytes into the CNS [Compston & Coles 2002]. These are associated with blood-brain barrier dysfunction, oligodendrocyte death and the generation of demyelinated tracts within the white and grey matter [Compston & Coles 2002; Giovannoni et al. 2017a; Thompson et al. 2018]. As oligodendrocytes do not express major histocompatibility complex class II antigens *in vivo* [Lee & Raine 1989] they will not be targeted directly by CD4 T cells, but could be targeted by CD8 T cells that are the predominant T cell phenotype in the parenchyma of MS lesions [Hoftberger et al. 2004]. Furthermore, oligodendrocytes may be damaged by the action of soluble mediators from T and B cells [Buntinx et al. 2004; Baker et al. 2017a, Lisak et al 2012]. However, other factors such as tissue hypoxia due to inflammation could cause or contribute to demyelination [Martinez Sosa & Smith 2017]. Demyelinated nerves are vulnerable to degeneration due to: energy deficits, excitotoxicity, toxic ion accumulations and actions involving innate-immune and astroglial responses that may drive progressive neurodegeneration (Figure 1) [Compston & Coles 2002; Heidker et al. 2017; Giovannoni et al. 2017a]. Elements of these processes may not readily respond to the same disease modifying treatment that blocks relapsing attacks [Compston & Coles 2002; Giovannoni et al. 2017a]. However targeting B cells may to be of value for inhibiting both relapsing and progressive MS.

Targeting B cells inhibits MS. Animal models of MS have created an emphasis on the action of CD4, Th17 T cells as the major pathogenic mediator of MS [Awasthi et al. 2008; Hiltensperger & Korn 2017]. However, these views fail to adequately accommodate the pathology of MS, where macrophages, CD8 T cells and B cells predominate over CD4 T cells [Lassmann & Bradl 2017; Eggers et al 2017]. More importantly the CD4-centric view of MS does not adequately explain responses to therapy [Baker et al. 2017a]. It has been found that marked CD4 T cell depletion and inhibition of Th1/Th17-related cytokines have so far only exhibited marginal efficacy in MS that is no better than beta interferon treatment [van Oosten et al. 1997; Segal et al. 2008; Vollmer et al. 2011; De Stefano et al. 2014; Havrdova et al. 2016]. However, B cell depletion has consistently shown a high level of efficacy at controlling lesions in relapsing MS [Dooley et al. 2016; Baker et al. 2017a; Baker et al. 2017b, Baker et al. 2017c; Lehmann-Horn et al. 2017] and active, primary progressive MS [Montalban et al. 2017] (Table 1). This is strongly supported by numerous double-blind phase II and phase III trials of: chimeric, humanized and human CD19 or CD20 depleting monoclonal antibodies [Hauser et al. 2008; Kappos et al. 2011; Castillo-

Trivino et al. 2013; Sorensen et al 2014; Hauser et al. 2017; Montalban et al. 2017; Agius et al 2017]. There have been few studies reported concerning the mechanism of action of ocrelizumab. However, this treatment maintains the relative absence of peripheral blood CD19+ B cells by repeated six-monthly infusions [Kappos et al. 2011; Avasarala 2017]. Greater insight can be gained from examining the impact of rituximab, which also depletes CD19+ for at least 6 months [Palanichamy et al. 2014]. This has been used extensively in MS and notably in other autoimmune diseases [Piehl & Hillert 2018]. These conditions include: rheumatoid arthritis [Leandro et al. 2006; Nakou et al. 2009], systemic lupus erythematosus [Anolik et al 2007; Vital et al. 2011], chronic inflammatory demyelinating polyneuropathy [Dalakas et al. 2009], neuromyelitis optica [Kim et al 2011; Cohen et al. 2017], myasthenia gravis [Lebrun et al. 2016; Muto et al. 2017], autoimmune haemolytic anaemias [Reynaud et al. 2015], thrombocytopenic purpuras [Godeau 2013], bullous skin disorders such as pemphigus vulgaris [Wang et al. 2015], type 1 diabetes mellitus [Pescovitz et al. 2009], and Sjögrens syndrome [Verstappen et al. 2017]. Whilst many of the above autoimmune conditions have soluble autoantibodies as major effector molecules, this may not be the case in MS. This is in part because a consistent pathogenic autoantibody has been yet to be found in MS [Srivastava et al. 2012; Van Haren et al. 2013; Navas-Madroñal et al. 2017]. However, as the CNS is relatively inaccessible compared to the peripheral tissues, study of B cell targeting in other autoimmune conditions may provide valuable insight into the mechanisms of action of treatments, and possibly disease, as they may be occurring in MS.

The bulk of peripheral blood B cells are derived from the bone marrow. Whilst T cell subpopulations feature heavily in the definition of the mechanisms of actions of treatments, B cells are often presented as a single, amorphous, CD19+ B cell population. These often rapidly repopulate to reach the normal range within 3-6 months of depletion [Cohen et al. 2012; Palanichamy et al. 2014; Baker et al. 2017b; Baker et al. 2017c]. The repopulation of the CD19+ population may trigger re-treatment decisions for CD20-depletion and has been used to support the rationale for administering ocrelizumab at 6 monthly intervals [Hauser et al. 2008, Hauser et al. 2017, Baker et al. 2017a]. There is a view that B cell depletion is effective in MS by simply blocking antigen presentation by B cells to T cells [Baker et al. 2017a]. Thus, the observation that CD19+ B cells repopulate quickly compared to the long-term depletion of T cells, can be interpreted to suggest that T cells are the essential drivers of disease activity [Cohen et al. 2012, Baker et al. 2017c]. However, until the complexity of B cell biology is appreciated, it is impossible to dissect-out how these treatments work at a cellular level in MS.

There are two major compartments where the B response is formed and resides (Figure1). These consist of the bone marrow and the secondary lymphoid organs [Pabst 2018]. B cells are generated from CD34+ stem cells in the bone marrow from pro- and pre-B cells that

form immature [CD10+, CD19+, CD38+, CD27-] cells, which can rapidly enter the blood following immune-depletion [Thompson et al. 2010; Baker et al 2017c]. These cells, sometimes described as transitional cells, are followed by repopulation with mature [naive] B cells [CD10, CD19+, CD38+, CD27-] that also arise from the bone marrow [Baker et al. 2017c]. These mature cells populate the secondary lymphoid organs, where they encounter their target antigen, differentiate and expand in lymphoid follicles (Figure 1). It appears that these B cell subsets repopulate the peripheral blood following immunodepletion and serve to give the impression of rapid CD19+ B cell repopulation and normalisation of response following immunosuppressive treatments [Cohen et al. 2012; Palanichamy et al. 2014; Baker et al. 2017c]. Lymphoid tissue and notably bone marrow may not be purged as effectively as peripheral blood by the therapeutic antibodies. This is possibly due to more limited access of immunoglobulin and cellular antibody-dependent cellular cytotoxicity components to tissues and the fact that these organs (bone marrow harbours about 12% of the lymphocytes and spleen and lymph nodes about 55% of the lymphocytes) contain substantially more lymphocytes than the blood (2% of lymphocytes) at any one time [Trebel 1974; Pabst 2018]. This is clearly seen in CD52-humanised mice [Turner et al. 2013], non-human primates [van der Windt et al. 2010; Marco et al. 2013] and humans [Dilly et al. 1986] treated with alemtuzumab. This may in part explain the rapid hyper-repopulation of immature B cells following alemtuzumab treatment [Thompson et al. 2010; Baker et al. 2017c]. Furthermore, it may account for the apparent lack of efficacy of alemtuzumab in many people switching treatment from fingolimod [Willis et al. 2017]. There it is thought that fingolimod may sequester pathogenic lymphocytes in lymphoid tissue and thus avoid alemtuzumab-induced depletion [Willis et al. 2017]. Similarly, the depleting response to rituximab, which involves complement fixation, is less marked in the bone marrow than in peripheral blood [Leandro et al. 2007; Nakou et al. 2009]. This again allows the stereotyped, CD19+ B cell repopulation of the blood by immature and mature B cells [Baker et al. 2017c, Ceronie et al. 2018]. This however, serves to mask the long-term depletion of CD19+ memory B cells [Thompson et al. 2010; Palanichamy et al. 2014; Baker et al. 2017c]. This is important because it appears that all agents that inhibit active relapsing MS, physically or functionally deplete cells within the memory B cell populations to prevent them from entering the CNS [Dooley et al. 2016; Baker et al. 2017a; Ceronie et al. 2018; Li et al. 2018]. Furthermore, this seems to occur in a hierarchical fashion that reflects clinical efficacy of the drugs [Dooley et al. 2016; Baker et al. 2017a; Ceronie et al. 2018; Li et al. 2018]. The mechanism(s) by which these cells may facilitate disease development requires further elucidation. However, it may relate to their antigen presenting function to T cells or via their production of cytokines and other factors that they can promote demyelination and nerve loss [Lisak et al. 2012; Li et al. 2015; Baker et al. 2017a; Lisak et al. 2017; Li et al. 2018]. These memory cells are probably generated within the peripheral, secondary lymphoid tissue and this may help explain durability of the efficacy of B cell deleting therapies [Cohen et al. 2012; Baker et al. 2017a; Ceronie et al. 2012].

Memory B cell responses are slowly formed in secondary lymphoid tissue. Naïve (mature B cells) upon encountering antigen, differentiate into germinal centre cells that form plasmablasts or memory B cells (Figure 1) [Burrows et al. 1993]. Memory B cells have the capacity for self-renewal, but may also form plasmablasts as a precursor to the formation of mature, antibody-secreting, plasma cells [CD19-, CD20-, CD27+, CD138+] (Figure 1) [Burrows et al. 1993]. Memory B cells exit germinal centres to reside in the marginal zone and may enter the circulation [Zandvoort et al. 2001; Steiniger et al. 2005]. Plasma cells and their precursors exit germinal centres and rapidly migrate to the bone marrow (Figure 1) [Costes et al. 1999, Paubst 2018]. There they lose their migratory response to chemokines and become long-lived [Hammarland et al. 2012; Paubst 2018]. In addition to secreting antibody, plasma cells also produce cytokines and exhibit antibody-independent functions [Shen & Fillatreau 2015; Fillatreau 2018]. Secreted cytokines such as interleukin ten may promote regulatory cell activity but may be produced to support B cell growth and differentiation [Shen & Fillatreau 2015, Banchereau et al. 1994]. Other B cells subsets, including memory B likewise produce cytokines that may exhibit regulatory and pro-inflammatory activity [Banchereau et al. 1994; Li et al. 2015].

Decreases of circulating memory B cells have been observed following splenectomy, which is sometimes used to inhibit autoimmunity [Kuwana et al. 2002; Wasserstrom et al. 2008; Rosado et al. 2013; Lee et al. 2013]. This supports the concept that peripheral, secondary lymphoid tissue is an important site for memory B cell formation [Carsetti et al. 2006; Mamani-Matsuda et al. 2008; Cameron et al. 2011]. In people receiving rituximab prior to splenectomy, it is evident that there is destruction of the normal follicular architecture and loss of memory cells in spleens to leave plasma cell aggregates [Cioc et al. 2008; Audia et al. 2011]. This is supported by studies examining lymph node and tonsil biopsies in humans [Anolik et al. 2007; Cioc et al. 2008] and studies in non-human primates [Schroder et al. 2003], despite memory B cells in the spleen appearing to be more susceptible to rituximab-induced depletion than in the lymph nodes [Egawa et al. 2007]. With time there is recovery of lymphoid architecture and the formation of germinal centres. However, in long-term treatment responders, these follicles may remain less-well organised years after treatment [Anolik et al. 2007]. Although the spleen may be an important site for memory B cells formation, it is important to note that sufficient lymphoid capacity is available elsewhere. As such splenectomy does not stop the development of MS [Matsui et al. 2005; Lee et al. 2013].

In contrast to the rapid repopulation of naive B cells from the bone marrow, memory B cells generate slowly in Western societies [Morbach et al. 2010, DuChamp et al. 2014]. Indeed, it can take many years for the memory B cell pool to be populated, as seen during development [Zandvoort et al. 2001, Morbach et al. 2010, DuChamp et al. 2014; Cooles et

al. 2016]. This is also evident in long-term responders to CD20-depletion, where persistent depletion of memory B cells can occur (Table 2) [Anolik et al. 2007]. This is also consistent with the observation that it takes 2-5 years for memory B cells to colonize the marginal zone in the spleen [Zandvoort et al. 2001]. Until this occurs a child is unable to initiate rapid secondary humoral responses comparable to adults and is it therefore of interest that young children seldom develop MS [Compston & Coles 2002; Zandvoort et al. 2001]. In addition to slow repopulation of memory B cells, there may also be also less of a drive for B cell development after depleting-antibody treatment than in ontogeny, as immunity to childhood-infections may be maintained [Pescovitz et al. 2011; McCarthy et al. 2013; Cho et al. 2017]. This is because the highly-active, immune-depleting therapies for MS probably leave the long-lived plasma cell pool relatively intact. This means that pre-existing vaccine-responses and immunity do not need to be regenerated, unlike that occurring of immunoablative haematopoietic stem cell transplantation [Brinkman et al. 2007]. This is because plasma cells do not express CD20 and are therefore resistant to killing by rituximab or ocrelizumab [Leandro 2013]. In addition, plasma cells also express low levels of deoxycytidine kinase that is phosphorylated by cladribine to kill lymphocytes [Ceronie et al. 2018]. Whilst alemtuzumab can deplete lymphoid follicles in lymph nodes, it is evident that plasma cells, which have the potential to become CD52 negative as seen in myeloma, may remain [Westerman et al. 2005; Dilly et al. 1986]. Furthermore, if alemtuzumab does not access and act in the bone marrow that effectively [Turner et al. 2013], it will not target the long-lived plasma cells that reside within the bone marrow.

These slow repopulation characteristics may in part account for the long-term treatment effects seen with immune-reconstitution [induction] therapies such as: alemtuzumab, cladribine and haematopoietic stem cell therapy. This probably occurs with rituximab and ocrelizumab as it is clear that there are treatment effects that extend for some time beyond the six-monthly dosing used with CD20-depletion [Bar-Or et al. 2008; Baker et al. 2017a]. Long-term studies, show that the memory B cell populations will numerically repopulate to pre-treatment levels, although their repertoires are different [Cooles et al. 2016; Anderson et al. 2012; Eggers et al. 2017]. However, monitoring of peripheral blood B memory cells CD19+, CD27+ may exhibit biomarker activity. Indeed this has been used to determine retreatment of many other autoimmune diseases including: neuromyelitis optica [Kim et al 2011; Kim et al. 2013; Cohen et al. 2017] and myasthenia gravis [Lebrun et al. 2016; Muto et al. 2017]. This has allowed reduced dosing requirements to maintain remission [Kim et al. 2013; Lebrun et al. 2016; Cohen et al. 2017]. Similar activity has been seen in non-neurological conditions where activity has been associated with memory B cells, within notably the Ig class-switched memory population [Anolik et al. 2007; Colucci et al. 2016]. Memory B cell repopulation was associated also with disease activity in systemic lupus erythematosus [Anolik et al. 2007; Vital et al. 2011], idiopathic thrombocytopenia purpura [Becerra et al. 2015] and rheumatoid arthritis [Leandro et al. 2006; Nakou et al. 2009;

Trouvin et al. 2015], where people often relapsed within 4 months of memory B cell repopulation [Leandro et al. 2006, Trouvin et al. 2015]. However, disease relapse can occur without apparent peripheral blood CD19+ B cells, indicating that that the triggers driving disease occur elsewhere [Trouvin et al. 2015]. This many not be surprising as the peripheral blood compartment contains only a small proportion of the total lymphocyte pool [Trepel 1974; Paubst 2018]. Thus, it remains to be established whether the peripheral blood B cell pool has biomarker activity in MS. However, it is clear that this activity is not evident through analysis of the total CD19+ B cell population or the T cell subsets in MS [Kousin-Ezewu et al. 2014; Baker et al. 2017b]. If there is biomarker activity that predicts disease activity in MS, this may be used for clinical advantage. This is because in other CD20-responsive autoimmune conditions, memory B cell numbers are monitored to inform re-treatment to help maintain remission from disease [Kim et al. 2013, Cohen et al 2017].

B cells in advanced [progressive] multiple sclerosis. It is evident that MS often consists of relapsing elements that responds well to blockade by peripheral immunity and neurodegenerative components that respond poorly to inhibition of peripheral immunity [Coles et al. 1999; Giovannoni et al 2017a]. This has generated the concept that relapsing and advanced MS are distinct disease entities. However, this arbitrary distinction was created by the pharmaceutical industry to generate "orphan drug" status allowing treatments to enter the MS market. This was reinforced by health care systems that rationed access of high-cost treatments to people with progressive MS on grounds of cost-effectiveness [Giovannoni et al. 2017a]. However, this two stage process is not supported by biology or the clinical process [Giovannoni et al. 2016; Giovannoni et al. 2017a]. It is simply part of a single disease continuum that evolves as damage accumulates, ageing continues and neurological reserve is exhausted [Tutuncu et al. 2013; Giovannoni et al. 2016; Weideman et al. 2017; Giovannoni et al. 2017a]. As such, both adaptive-immune inflammation [Luchetti et al. 2018] and neurodegeneration [De Stefano et al. 2014; Matute-Blanch et al 2018] are present from the beginning to the end of MS, whether it is classified as relapsing or advanced MS [Lublin et al. 2014; Giovannoni et al. 2016; Giovannoni et al. 2017a]. Therefore, elements of progressive MS, notably active disease, will clearly respond to peripheral immunomodulation [Hawker et al. 2009; Lublin et al. 2014; Montalban et al. 2017, Muraro et al. 2017]. This is provided that the studies are long-enough to detect change and the outcome system has sufficient neuronal reserve to respond [Weideman et al. 2017; Giovannoni et al. 2017a]. As such, lower limb reserve is lost quicker than hand and head function [Montalban et al. 2017; Giovannoni et al. 2017a; Giovannoni et al. 2017b]. This perhaps accounts for the failure of treatments to slow loss of lower limb mobility [Coles et al. 1999]. Thus, while it may take a few months for lesion formation in relapsing MS to respond, it may take two to three years for progressive MS to adequately respond to treatment [Giovannoni et al. 2017a, Giovannoni et al. 2017b].

Peripheral blood B cell numbers do not necessarily predict disease activity or progression [Wurth et al. 2017]. Similarly, total peripheral CD19 B cells and T cells subset levels do not necessarily predict relapse activity [Kousin-Ezewu et al. 2014; Baker et al. 2017b]. Never the less peripheral immunosuppression does influence advanced MS, as clearly seen following haematopoietic stem cell therapy that replaces all or large parts of the peripheral adaptive-immune system [Burt et al. 2015; Atkins et al. 2016; Muraro et al. 2017]. The relative failure of immunosuppressive agents to rapidly halt progressive disease [Coles et al. 1999], has created alternative focus on pathogenic mechanisms. These include: ionic imbalances, excitoxicity, mitochondrial dysfunction and glial/innate-inflammatory responses that support/damage oligodendrocytes, axons, neurons and synapses (Figure 1) [Trapp & Stys 2009; Mahad et al. 2015; Liddelow et al. 2017; Campbell & Mahad 2018; O'Loughlin et al. 2018]. However, it is important to appreciate that current disease modifying treatments largely do not induce immunomodulatory effects via activity on immune cells within the CNS. Therefore, it remains to be seen whether immune-inhibition of B cell activity will inhibit progressive MS.

Focal inflammatory infiltrates in the meninges and the perivascular spaces appear to produce soluble factors that can induce demyelination and neurodegeneration, either directly or indirectly [Figure 1, Figure 2] [Lock et al. 2002; Lisak et al. 2012, Lisak et al. 2017; Magliozzi et al. 2018]. It is clear that CD20-depletion inhibits relapsing MS, without any major influence on antibody levels, consistent with the lack of expression of CD20 by plasma cells and a peripheral activity that blocks relapsing attacks [Cross et al. 2006]. A peripheral action is supported by the observation that limited amounts [<0.1%] of parenteral antibody enters the CNS [Rubenstein et al. 2003]. In addition, intrathecal CD20-depleting antibody largely fails to deplete B cells within the CNS, until the disease modifying effect occurs following leakage of the antibody into the circulation to kill peripheral B cells [Cross et al. 2006; Piccio et al 2010; Komori et al. 2016; Topping et al. 2016]. Nevertheless, intrathecal production of oligoclonal antibodies is a characteristic feature in people with MS [Link & Huang 2006]. Detection of immunoglobulin G and activated-complement proteins in close proximity to actively demyelinating lesions indicates the involvement of antibody-mediated effector mechanisms [Lucchinetti et al. 2000]. Peripherally-produced antibodies contribute to the pathology of MS in some people as seen by the benefit of plasmapheresis and immunoadsorption [Keegan et al. 2005; Faissner et al. 2016; Lehmann-Horn et al. 2017]. However, in MS there is also central antibody production observed by the detection of oligoclonal bands of immunoglobulins, which can be produced by parenchymal and cerebrospinal fluid B cells [Obermeier et al. 2008; Eggers et al. 2017]. B cells are recruited during neuroinflammation, such as in response to chemokines, such as CXCL13 [Kowarik et al. 2012]. These may form B cell aggregates and ectopic B cell follicles that develop, notably in the leptomeninges, in people with MS [Serafini et al. 2004; Magliozzi et al. 2007; Magliozzi et al. 2013]. Ectopic follicles have

been suggested to associated with grey matter demyelination (Figure 2), cortical atrophy and important in disease progression [Serafini et al. 2004; Magliozzi et al. 2007; Magliozzi et al. 2013]. Ectopic follicles have been observed also in many autoimmune conditions, such as: systemic lupus erythematosus, rheumatoid arthritis, type 1 diabetes mellitus, myasthenia gravis and Sjögrens syndrome [Corsiero et al. 2016; Jones & Jones 2016]. Individuals with these autoimmune conditions often appear to show some response to CD20-depleting antibodies also [Anolik et al 2007; Vital et al. 2011; Leiper et al. 2011; Muto et al. 2017, Nakou et al. 2009, Hamad et al. 2016; Lebrun et al. 2016, Verstappen et al. 2017]. However, treatment responsiveness autoimmunity is not always universal, as occurs in MS also [Hawker et al. 2009; Leiper et al. 2011; Hamad et al. 2016]. Never the less, this suggests further that these diseases are likely to have some overlapping pathogenic mechanisms.

In MS, there is evidence of somatic hypermutation and clonal expansion of B cells, suggestive of antigen-driven affinity maturation within the CNS [Qin et al. 1998; Govarts et al 2009; Obermeier et al. 2011; Beltran et al. 2014; Eggers et al. 2017]. These may also be derived from precursors found in the periphery [Govarts et al 2009; Eggers et al. 2017]. However, analysis of antibody specificity often shows no consistent myelin-directed autoimmunity and evidence has not been reproducible [Srivastava et al. 2012; Willis et al. 2015; Brändle et al. 2016; Navas-Madroñal et al. 2017]. These antibodies are often targeted to cytoplasmic proteins [Zhang et al. 2005; Willis et al. 2015; Brändle et al. 2016]. This suggests that the autoantibodies may be formed secondary to damage rather than being of primary significance. However, antibodies purified from cerebrospinal fluid can induce pathogenic effects *in vitro* and when transferred into animals [Zhang et al. 2005; Elliot et al. 2012; Blauth et al. 2015; Agahozo et al. 2016]. This indicates that pathogenic antibodies do occur within the CNS during MS. However, antibody-specificity could be of limited significance and it is possible that B cells can drive neurodegeneration via indirect effects. As such, immunoglobulins could simply, chronically, activate microglia via Fc receptor-binding activity [Okun et al. 2010]. During MS, Fc receptors are upregulated on microglia [Ulvestad et al. 1994]. Furthermore, immunoglobulin and immune complexes activate microglia to induce cytokine production, phagocytosis, oxidative bursts and cytotoxicity [Ulvestad et al. 1994b; Coggeshall, K.M., 2002; Teeling et al. 2012]. This may stimulate down-stream neuropathic mechanisms (Figure 1). As such, activated microglia may secrete cytokines such as interleukin one, tumour necrosis factor and complement components that may stimulate pathogenic astrocytes to secrete toxic moieties [Liddelow et al. 2017]. These may promote excitoxicity and block lactate that can contribute to redox and energy deficits that are neurotoxic (Figure 1) [Bolanos 2016]. Likewise astrocytes can produce growth factors that drive damage by microglial cells [Vainchtein et al. 2018]. Thus by targeting B cells and antibody production within the CNS, it may be a possible treatment

for progressive disease. This may complement inhibition of peripheral B cell responses to limit relapsing MS and methods of neuroprotection and repair of demyelinated nerves.

Epstein-Barr Virus and memory B cells. The demographics and disease development patterns are well studied in MS. Whilst MS is genetically and geographically restricted [Compston & Coles 2002; Compston & Coles 2008], it appears that essentially everybody who develops MS, is infected with Epstein-Barr Virus [EBV], compared to the general population where most (~95%) people have been infected [Laurence & Benito-Leon 2017, Burnard et al. 2017; Moreno et al. 2018]. Furthermore, age of EBV-infection is a risk factor for disease development [Ascherio & Munger 2015]. Changes in life-style and health innovation within Western societies has led to a trend for EBV infection in adolescence rather than childhood, such that it appears to shape the B cell-repertoire differently [Morbach et al. 2010, Berron-Ruiz et al. 2016], cause infectious mononucleosis due to immune destruction of EBV-infected cells [Dunmire et al 2018] and result in a longer life span. This may have allowed the prevalence of MS to increase over recent time [Kingwell et al. 2013]. Although controversial [Burnard et al. 2017], many studies suggest that EBV may be an important aetiological trigger of MS that can be shown to infect pwMS before diagnosis [Ascherio & Munger 2015, Laurence & Benito-Leon 2017].

Infection of naive/mature B cells with EBV occurs via CD21, sometimes CD35, and HLA-DR, which contribute to viral load and drives the expansion of B cells [Agostini et al. 2018]. In addition, transcription factor-related products of the EBV virus have been linked to activity of autoimmune susceptibility loci [Harley et al. 2018]. Furthermore, it is of interest that many susceptibility loci which associate with MS and other autoimmune diseases, including those often associated with T cell function, are involved in B cell function [Steri et al. 2017; Baranzini & Oksenberg 2017; Harley et al. 2018]. The common involvement of genetic variants in autoimmunity [Harley et al. 2018] suggests a commonality of disease mechanisms and perhaps the value of common treatments between these diseases. In addition, there may be some antigen-specificity within the individual autoimmune conditions that probably links to the Human leucocyte antigen associations with these diseases [Compston & Coles 2002, Bodis et al. 2018, Harley et al. 2018].

Several hypotheses have been put forward to explain how EBV can trigger T cell autoimmunity, such as through molecular mimicry or production of HspB5 (alpha B crystallin)-specific T cells to target stressed oligodendrocytes [van Noort et al. 2012, Laurence & Benito-Leon 2017]. However, B cell pathways may be involved [Baker et al. 2017a, Kreft et al. 2017]. It has also been suggested that EBV may be present in immune and glial cells within the CNS [Serafini et al. 2004, Hassani et al. 2018, Veroni et al. 2018; Moreno et al. 2018]. These could be targeted by the B cell immune response and this could be facilitated by the expression of MS susceptibility, major histocompatibility complex

alleles [Kreft et al. 2017]. Alternatively, lesions may be formed when CNS-extravasated B cells are targeted by CD8 cytotoxic T cells, which form the major T cell subset in lesions in MS [Serafini et al. 2017, van Nierop et al 2017]. However, this is unlikely to be a general mechanism of pathology in MS, as EBV is not always detected in MS [Moreno et al. 2018]. However, EBV-free, autoreactive, memory B cells entering the CNS may be descended from memory B cells that matured while containing episomal EBV enabling autoimmunity [Laurence & Benito-Leon 2017]. It has also been suggested that peripherally-infected B cells may not be adequately controlled by CD8 T cells that could facilitate the development of autoimmunity by B or T cells [Baker et al. 2017a, Pender et al 2017, Laurence & Benito-Leon 2017].

In some instances EBV infection can create lymphomas, such as Burkitts and Hodgkins lymphomas [Thorley-Lawson 2015]. Immortalization of B cells is facilitated by the viral production of Epstein-Barr virus nuclear antigen (EBNA) one, EBNA2 and the production of latent membrane protein one (LMP1) and two [Thorley-Lawson 2015]. These latter proteins, notably LMP1 can be frequently found in CNS from people with MS [Moreno et al. 2018]. These mimic the signalling of CD40 co-stimulation (LMP1) and the B cell receptor (LMP2), promote B cell survival and have the capacity to activate nervous-antigen specific cells in the absence of neural antigen and T cell help [Thorley-Lawson 2015]. Memory B cell formation due to EBV infection is particularly evident following haematopoietic stem cell therapy, where EBV infection drives memory B cell proliferation [Burns et al. 2015]. This is in part due to activity of EBNA3 and other EBV products that suppresses differentiation into plasma cells and drives memory cell production and latent virus infection [Minamitani et al. 2015; Styles et al. 2017]. Viral production and lytic infection occurs following differentiation of memory cells into plasma cells [Laichalk et al. 2005;]. 2017]. Therefore, memory B cells become a viral reservoir where viral adaptations and latency promote escape from immune surveillance by antibody and cytotoxic T cells [Thorley-Lawson 2015, Ressing et al. 2015]. Whilst both LMP1 and LMP2 are usually produced together and regulate each other [Thorley-Lawson 2015], dysregulation of this may create the capacity for expansion of autoreactive B cells that become T cell/co-stimulation independent. This may be facilitated as CD40-related MS susceptibility alleles reduce CD40 expression, which perhaps reduce the activity-requirements of available LMP1 [Smets et al. 2018]. This process through creating memory cells would allow the individual to more quickly respond to infectious elements creating survival advantage for the host and supporting the co-evolution of humans and virus. This could create a survival-advantage from being infected with EBV. However, this would be at the consequence of development of some cancers as well as autoimmune diseases. As such, EBV also links to the development of autoimmunity in: systemic lupus erythematosus, rheumatoid arthritis, Sjögrens syndrome and some other, CD20-treatment responsive, diseases that are associated with ectopic B cell follicles in tissues [Ascherio & Munger 2015, Dittfeld et al. 2016, Draborg et al. 2016; Harley et al.

2018]. However, these would have limited evolutionary-selective pressure as autoimmune diseases are largely of adult-onset [Compston & Coles 2002]. Importantly, historically the onset of autoimmunity would occur at an age long after childbirth and perhaps not long prior to the natural life-span, avoiding negative selective pressures. However, definitive evidence for EBV exhibiting a causal link in MS is lacking [Burnard et al. 2017]. The increased prevalence and association between EBV in MS and other autoimmunities may only be a consequence of chronic B cell activation rather than a cause. Thus, the aetiological involvement of EBV in MS, and above ideas, must remain as speculation until disease activity can be shown to respond to viral inhibition. This may be some way off, as an effective EBV-control method has yet to be adequately developed [Cohen 2018].

In addition, although EBV may be associated with the development of MS, other environmental agents, such as cytomegalovirus may also drive the B cell responsiveness in other individuals [Varani et al. 2007]. The involvement of viruses such as EBV, may explain why such autoimmune diseases are uniquely human and why animals do not often adequately model them [Baker & Amor 2015]. By challenging the current view that B cells may simply help pathogenic T cells via antigen presentation, to consider T-independent, B cell pathology or that T cells may actually serve to help pathogenic B cells, may lead to better treatments options for MS and other human autoimmune conditions.

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FIGURE LEGENDS

Figure 1. *Role of B cells in the pathogenesis and control of multiple sclerosis.*

B cell subsets develop in different tissue niches. These may be involved in different aspects of multiple sclerosis. **(A)** *The bone marrow niche gives rise to autoimmune B cells and is a major tissue location for long-lived plasma cells. These may not be effectively targeted by many MS therapeutics.* B cells are generated in the bone marrow from haematopoietic stem cells. These subsets rapidly repopulate the peripheral blood, to normalize CD19+ cell levels, following B cell depletion therapies. In the absence of effective T cell regulation these may give rise to autoreactive B cells and secondary B cell autoimmunities as seen following haematopoietic stem cell therapy and alemtuzumab treatment. They also populate the lymphoid tissue. **(B)** *The peripheral lymphoid organ niche gives rise to memory B cells, which is targeted by current MS therapies to control active/relapsing MS.* When naive cells encounter their potential target-antigen they expand in germinal centres, undergo antigen-specific B cell receptor rearrangement and hyper-mutation, and then differentiate to form memory B cells or plasma cells that can produce antibodies. Epstein-Barr Virus (EBV) infected naive B cells, break immunological tolerance and drive the production of memory cells, possibly by mechanisms not requiring T cell help. Whilst the memory B cells may activate pathogenic T cells, relapsing autoimmunity probably develops following entry of memory B cells into the CNS. There they may damage oligodendrocytes and myelin, making nerves vulnerable to acute and chronic damage by a variety of down-stream mechanisms. **(C)** *The CNS niche that needs to be targeted to control advanced (progressive) MS.* Once in the nervous system, B cells form plasma cell niches that produce pathogenic antibody or may activate innate immune cells, via Fc receptors, and glial responses. These cause accumulating tissue/nerve loss, by a variety of different mechanisms. This leads to the chronic neurodegeneration underlying advanced MS, which worsens in the absence of overt relapsing attacks. This is outside the control of peripheral immunosuppression of the adaptive immune response. This is largely not targeted by most current MS therapeutics.

Figure 2. *White and grey matter demyelinated lesions in multiple sclerosis.*

Multiple sclerosis tissue was stained, using diaminobenzidine, to detect myelin [proteolipid protein] loss in [A] An active perivascular lesion in the white matter. Reproduced from Peferoen L, Kattenbelt M, Lodder L, van der Valk P, van Noort JM, Amor S. Demyelinating Disorders of the CNS in Woodroofe N Amor S (Eds) Neuroinflammation and CNS disorders. ISBN 97811184406410. DOI: 10.1002/9781118406557. 2014, pg211-234 with Permission John Wiley & Sons. [B] Sub-pial grey matter lesions.

Table 1: B cell depleting antibodies active in multiple sclerosis

Antibody	Target	Isotype	Reference of use in MS
Inebilizumab	CD19	Humanized IgG	Agius et al. 2017
Rituximab	CD20	Chimeric mouse-human IgG	Hauser et al. 2008
Ublituximab	CD20	Chimeric IgG glycoengineered for ADCC	Lovett-Racke et al. 2017
Ocrelizumab	CD20	Humanised IgG	Hauser et al. 2017
Ofatumumab	CD20	Human IgG	Sorensen et al. 2014
Alemtuzumab	CD52	Humanized IgG	Cohen et al. 2012

ADCC antibody dependent cellular cytotoxicity

Table 2. Induction therapy using CD20-B cell depletion in non-MS autoimmune disease

	Total Memory B cells	Class-switched memory B cells
Healthy Control	30.5 ± 6.9%; *P* = 0.001	18.3 ± 5.8% *P* = 0.001
Responder	6.3 ± 0.9%	3.6 ± 0.5%
Non-Responder	51.1 ± 23.2% *P* = 0.009	42.8 ± 18.1% *P* = 0.036

Peripheral blood numbers stained with CD19, CD27 and IgD to detect memory (CD19+, CD27+) and immunoglobulin class-switched (CD19+, CD27+, IgD-) B cells in people with systemic lupus erythematosus treated with rituximab three to five years earlier in controls clinical responders at 5 years post treatment and clinical non-responders at 3-5 year post treatment. Data reported in Anolik et al. 2007. DOI.10.1002/art.22810. P verses clinical responder.

Figure1.

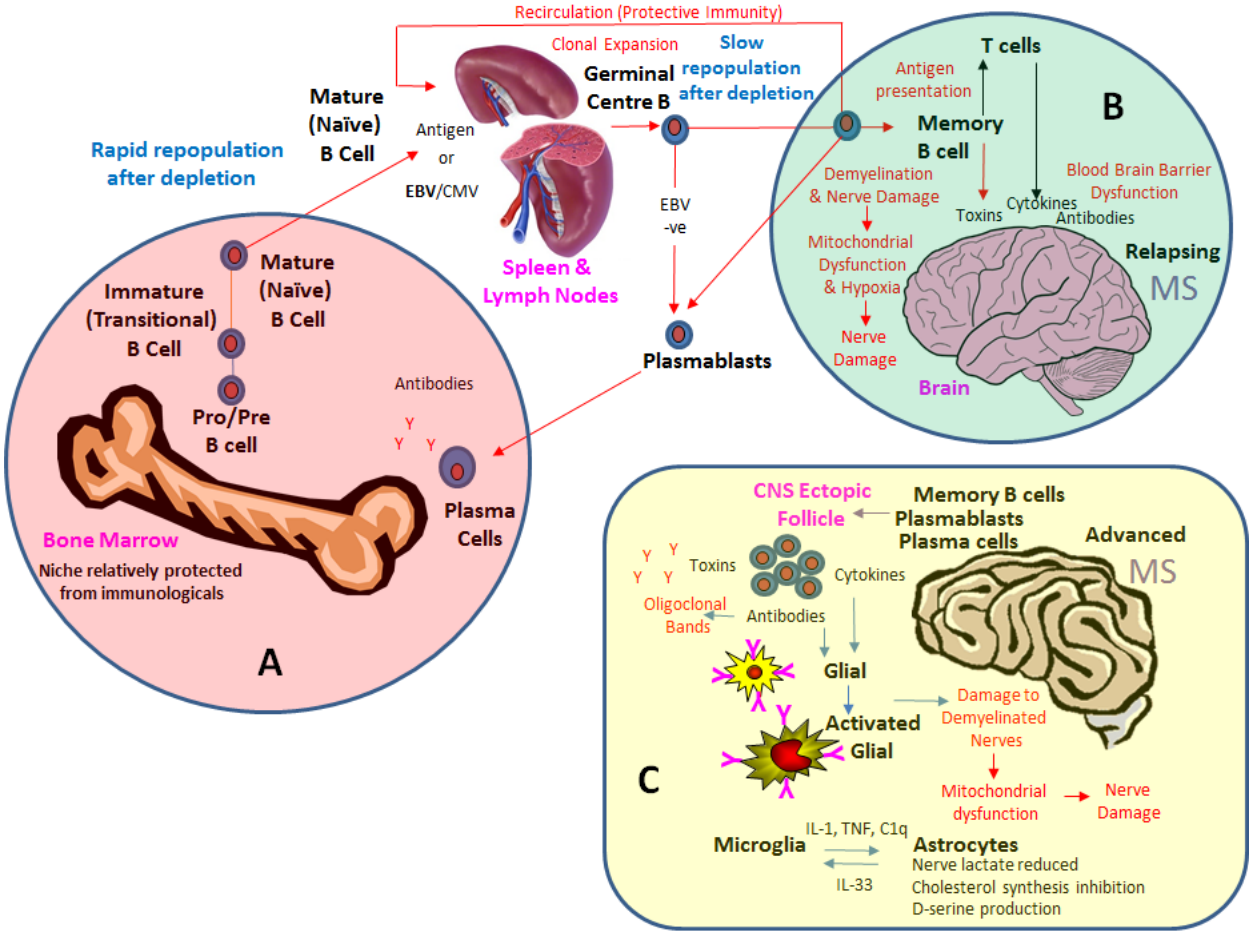
Recirculation (Protective Immunity)
Clonal Expansion
Slow repopulation after depletion
Germinal Centre B
Mature (Naïve) B Cell
Antigen or EBV/CMV
Rapid repopulation after depletion
Spleen & Lymph Nodes
EBV -ve
Plasmablasts
T cells
Antigen presentation
B
Memory B cell
Demyelination & Nerve Damage
Blood Brain Barrier Dysfunction
Toxins
Cytokines
Antibodies
Mitochondrial Dysfunction & Hypoxia
Relapsing MS
Nerve Damage
Brain
Immature (Transitional) B Cell
Mature (Naïve) B Cell
Pro/Pre B cell
Antibodies
Plasma Cells
Bone Marrow
Niche relatively protected from immunologicals
A
CNS Ectopic Follicle
Memory B cells
Plasmablasts
Plasma cells
Advanced MS
Toxins
Cytokines
Oligoclonal Bands
Antibodies
Glial
Activated Glial
Damage to Demyelinated Nerves
Mitochondrial dysfunction
Nerve Damage
C
Microglia
IL-1, TNF, C1q
IL-33
Astrocytes
Nerve lactate reduced
Cholesterol synthesis inhibition
D-serine production

Figure 2.