CD20 Therapies in Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis - targeting T or B cells?

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HIGHLIGHTS

- Recent therapeutic approaches for MS focus on CD20+ B cells yet emerging evidence indicates these approaches also target T cells.
- EAE in animals is used to identify mechanisms and generate therapies for translation to MS, a uniquely human disease.
- The original assumption of the role of B cells was as a source of pathogenic antibodies yet many more functions have been revealed.
- B cell therapies in EAE do not reflect the efficacy in people with MS
- The efficacy of CD20 therapy in MS may be removal of the proposed aetiological agent Epstein-Barr virus.

ABSTRACT

MS is widely-considered to be a T cell-mediated disease although T cell immunotherapy has consistently failed, demonstrating distinct differences with experimental autoimmune encephalomyelitis (EAE) in which T cell therapies are effective. Accumulating evidence has highlighted that B cells also play key role in MS pathogenesis. The high frequency of oligoclonal antibodies in the CSF, the localization of immunoglobulin in brain lesions and pathogenicity of antibodies originally pointed to the pathogenic role of B cells as autoantibody producing plasma cells. However, emerging evidence reveal that B cells also act as antigen presenting cells, T cell activators and cytokine producers suggesting that the strong efficacy of anti-CD20 antibody therapy observed in people with MS may reduce disease progression by several different mechanisms. Here we review the evidence and mechanisms by which B cells contribute to disease in MS compared to findings in the EAE model.

KEY WORDS: multiple sclerosis; animal models; experimental autoimmune encephalomyelitis; therapeutics; B cells; CD20; Epstein-Barr virus

ABBREVIATIONS: CNS - central nervous system; EAE - experimental autoimmune encephalomyelitis; MS - multiple sclerosis, PwMS - Person with MS, EBV - Epstein-Barr virus.

1. INTRODUCTION

Multiple sclerosis (MS) is a chronic immune-mediated neurodegenerative disease of the central nervous system (CNS) characterized by inflammation, demyelination and axonal loss. The most widely used model to study such CNS damage as well as for drug development for MS is experimental autoimmune encephalomyelitis (EAE) (Baxter 2007; Gold et al., 2006, Kipp et al., 2012). EAE is a spectrum of experimentally induced immune-mediated neurological diseases of the CNS (Kipp et al 2012), induced in many species. It is well known that the mode of induction, animal species and strains of rodents heavily influence the course of disease and observed immunological and pathology in the CNS (Kipp et al 2012, Baker and Amor 2013). Most current drugs available for MS are broad spectrum immunosuppressant/immunomodulatory agents, although in recent years more specific therapies using cell-depleting antibodies have been designed. Amongst the most successful of these antibody treatments are the B cell-targeted treatments (Kappos et al. 2011; Brück et al., 2013). Despite the apparent efficacy of B cell depletion there is much to be learned about how B cells contribute to the regulation and pathogenesis of MS, given the balance between B regulatory and B effector cell functions as shown both in animals and MS. In addition, broad B cell-targeted depletion carries risks, notably associated infections. This indicates that despite being very

effective, the depletion regimen could be better targeted to remove pathogenic B cells while leaving the protective and regulatory arm intact.

2. B CELLS AND AUTOANTIBODIES IN EAE

Until the last decade MS has been widely-considered a white matter disorder, and thus EAE has been commonly induced following immunization with myelin antigen such as myelin basic protein (MBP). However, clinical and pathological features of MS are usually better represented when EAE is induced using total CNS tissues (Sospedra et al., 2005) suggesting that autoimmunity to other myelin components may be essential to model chronic relapsing neurological disease, demyelination and axonal damage and secondary progressive disease. Currently, the most widely used autoantigen for induction of EAE is the minor protein myelin oligodendrocyte glycoprotein (MOG), which present on the outer membrane of oligodendrocytes (Schluesener et al., 1987, Linington et al., 1988; Amor et al., 1994). Similar to EAE induced with other autoantigens, the clinical course and pathology of MOG-induced EAE is heavily depend on the nature of the MOG antigen and mouse strain [Table 1].

Table 1: Outcome of MOG-induced EAE in WT and B cell deficient mice

Antigen	Mutation or treatment	EAE Course	Antibody or Bell requirement	Reference
(m)recMOG	Biozzi ABH mice	Chronic relapsing		Amor et al 1994
	Biozzi mice + MOG antibodies	Increased EAE severity Augmented demyelination	Yes	Morris-Downs et al 2002
(m)SCH	Biozzi mice + Rituximab	No effect on EAE	No	D Baker - Unpublished
(m)MOG$^{35-55}$	Biozzi ABH	Monophasic chronic	No	Amor et al., 2005
(m)recMOG	WT C57BL/6	Chronic EAE	No	Lyons et al.,1999
	µMT C57BL/6	No EAE		
	CD20+ cell depletion	*Not known*		
(r)MOG	WT C57BL/6	Chronic EAE	No	Oliver et al., 2003
	µMT C57BL/6	Chronic EAE		
	CD20+ cell depletion	*Not known*		
(h)recMOG	WT C57BL/6	Chronic	Yes	Oliver et al., 2003
	µMT C57BL/6	No EAE		
	B cell depletion	*Not known*		
(m)MOG$^{35-55}$	WT C57BL/6	Chronic	No	Lyons et al., 1999
	Mouse specific CD20+ cell depletion	Severe or reduced EAE depends on timing of CD20 depletion	Yes	Matsushita et al., 2008

MOG - myelin oligodendrocyte glycoprotein, m – mouse; h – human; recMOG – recombinant MOG; WT – wild type; µMT - Knock-out mice that lack functional B cells.

Importantly, the majority of studies, including those focusing on the role of B cells, makes use of the C57BL/6 mice since most transgenic or mutant mice are bred on this background. Notably, the

majority of EAE studies in this mouse strain uses mouse MOG^{35-55} peptide. The disease is monophasic and B cells or pathogenic antibodies to MOG do not play a role (Oliver et al 2003). Such pathogenicity of the protein is also influenced by the protein conformation as well as the presence of post translational modifications that may better reflect proteins in their native form in the myelin membranes (Smith et al 2005; de Graaf et al 2012).

The role of pathogenic autoantibodies was indicated following the demonstration that circulating autoantibodies to myelin in EAE induce myelin damage *in vitro* and *in vivo* (Apple and Borstein 1964). This is supported by studies showing that titres of antibodies to myelin are high during chronic stages of EAE when demyelination was most pronounced, and that some MOG antibodies, notably those that fix complement, augment EAE (Linington et al., 1989, Morris-Downes et al., 2002; Piddlesden et al., 1993, Menon et al., 1997, Mead et al., 2002; Hundgeburth et al., 2013). In addition, MOG antibodies have functional effects on oligodendrocyte inducing stress, physiological and morphological changes (Duvanel et al., 2004; Marta et al., 2005) and have recently been shown to trigger T cell activation by opsonisation of endogenous antigen (Kinzel et al., 2016). It is clear that pathogenic antibodies, possibly secondarily to damage, are generated by MS (Willis et al. 2015) and can cause demyelination and pathology when injected in to animals (Linington et al., 1988, Morris-Downes et al., 2002)). However, it appears that autoreactive T cells are necessary for the clinical disease to develop. It was shown in animals studies that T cells initiate the blood-brain barrier disturbances that facilitate entry of B cells and immunoglobulins into the CNS. Once in the CNS autoantibodies bind to oligodendrocytes and myelin, activate complement and induce demyelination that subsequently enhances inflammation and increases EAE severity.

3. B CELL AS IMMUNE MODULATORS

Emerging evidence using B cell-deficient mice has revealed a much more complex role for B cells in antigen presentation, cytokine production and immune regulation of EAE [Figure 1]. While early studies showed that depletion of IgM from birth prevented B cell maturation and subsequent EAE induction (Willenborg and Prowse, 1983), recent evidence for B cells in EAE comes from studies using the MOG EAE model in C57BL/6 following genetic ablation and depletion using anti-CD20 antibodies [Table 1]. These studies suggest that B cells are necessary for EAE induced with recombinant MOG protein but not MOG^{35-55} EAE, revealing the critical impact of the source and nature of immunogens (Weber et al. 2010). This difference in EAE induction can be explained by the role of B cells to differentially present MOG protein and peptide (Weber et al., 2010). While genetic ablation of MHC class II antigens on B cells abrogates antigen presentation of MOG to T cells and thus inhibits EAE induction (Molnarfi et al., 2013), antigen presentation by B cells and thus EAE induction requires dendritic cells (Parker et al 2015). Next to the requirement of MHC class II

antigen expression of T cell costimulatory molecules expressed by B cells, such as CD80/CD86 and CD40, also plays a crucial role in EAE (Mann et al., 2007; Ray et al., 2011). While mice (in this case B10.PL) in which B cells lack co-stimulatory CD80/CD86 molecules develop EAE, they fail to recover due to a delay in IL-10 production and mobilization of Foxp3 regulatory T cells (Mann et al., 2007). In addition, B cells produce pro-inflammatory and anti-inflammatory cytokines that impact on EAE (Molnarfi et al., 2013) [Table 2].

Table 2. B cell production of cytokines during EAE

Cytokine	Produced via	Effect mechanism	Effect on EAE	Reference
LT1α2β	FDCs and CXCL13	Follicle formation	Increased inflammation	Magliozzi et al.,2004, 2007
TNF	LT1α2β	Increased IFN-γ and inflammation	Increased severity	Menrad et al.,2007, Matejuk et al.,2002
IL-6	Innate receptors	Increased ICAM and VCAM expression. Increased T cell infiltration	Increased severity	Okuda et al,1998, Eugster et al,1998
IL-10	CD40 and B7	Increased Tregs, decreased inflammation	Reduced severity	Bettelli et al.,1998, Fillatreau et al.,2002 Matsushita 2008
IL-35	TLR4 and CD40	Decreased APC function	Reduced severity	Shen et el.,2014

Abbreviations: APC – antigen presenting cell; IL – Interleukin; Tregs – regulatory T cells, TLR - Toll-Like receptor, FDCs - Follicular Dendritic Cells, CXCL13 - Chemokine CXC-motif Ligand 13,

B cells secrete lymphotoxin α1-β2 (LTα1β2) essential for lymph node expansion and development of tertiary lymphoid tissue, which are sometimes present in the CNS during EAE (Lassmann et al., 2011). LTα1β2 is observed in the CNS during EAE in SJL mice (Magliozzi et al., 2004, Columba-Cabezas et al., 2006) and clinical disease is prevented with antibodies to LTα1β2 receptor, due to inhibition of CXCL13 important for lymphoid follicle development (Columba-Cabezas et al., 2006). Antigen activated B cells also produce TNF, a key cytokine in EAE (Matejuk et al., 2002). Another B cell-derived cytokine is interleukin-6 (IL-6), it is increased during EAE and has an impact on EAE pathogenesis (Barr et al., 2012). A mechanism by which IL-6 may contribute to MS pathogenesis is by regulating T cell infiltration into the CNS since expression of ICAM-1 and VCAM-1 are absent in IL-6 deficient mice (Eugster et al., 1998). Additionally, B cells also produce IL-10 as well as IL-35; cytokines that reduce EAE severity. Such regulatory B cells (Bregs) from mice in EAE remission, produce IL-10 in a CD40 dependent manner and transfer of these Bregs into mice with EAE induces recovery (Fillatreau et al., 2002). Thus, IL-10 producing Bregs are important for disease recovery. Furthermore, IL-10-producing B cells play an important role in EAE initiation by down-regulating the conditions leading to T cell CNS infiltration (Matsushita et al., 2008, 2010). Finally, IL-35 producing B

cells also control EAE severity since mice lacking these B cells develop exacerbated EAE. IL-35 regulates APC functions of B cell that together with IL-10 induces differentiation of plasma cells. Mice in which B cells lack IL-35 have stronger antigen presentation capabilities, this is associated with stronger inflammatory responses and more severe EAE (Shen et al., 2014).

4. B CELLS AND AUTOANTIBODIES IN MS

B cells, plasma cells, plasmablasts and antibodies directed to oligodendrocytes, myelin and neurons are detectable in peripheral blood and the cerebrospinal fluid (CSF) of people with MS (pwMS) (Olsson et al., 1990; van Noort et al., 2006; Amor et al., 2014). In the CSF oligoclonal IgG bands (OCBs), present in 95% pwMS, are an established diagnostic tool for MS. That some immunoglobulins may play a role in disease is reflected by the close associate of lesions to CSF flow, such as ventricular and subpial lesions and that OCB-negative patients are characterized by less global and regional brain atrophy (Ferreira et al 2014). Further supports comes from the study showing that clonally expanded plasma cells from the CSF induce myelin damage *in vitro,* thus implicating intrathecal IgG in MS pathogenesis (Blauth et al., 2015). In addition the CNS immunoglobulins and complement deposits are present on disintegrated myelin sheath and demyelinating lesions (Raine et al., 1999, Lucchinetti et al., 2000; Storch et al., 1998), indicating that like EAE CNS reactive antibodies mediate demyelination and neuronal damage [Figure 2]; findings that have been confirmed in vitro studies (Zhou et al., 2006; Blauth et al., 2015). In addition, the finding of B cell follicle-like structures as well as chemokines and cytokines that support B cell development all provide support for a key role of B cells, plasma cells and autoantibodies in MS pathogenesis. Like EAE, B cells extracted from pwMS and stimulation with CD40L and IL-4 have been shown to present myelin specific antigens to autoreactive T cells (Harp et al., 2008) indicating that B cells have the capacity for antigen presentation in MS also.

In support of a role of B cells in MS, B cell follicles have been reported in the meninges of people with secondary progressive MS (pwSPMS; Serafini et al., 2004), although this is controversial since follicles are not present in all pwSPMS and MS cohorts (Peferoen et al 2007). In pwMS in which follicles are observed the presence of the ectopic follicles were more pronounced in pwMS showing earlier clinical symptoms and earlier death than the negative group (Magliozzi et al., 2007). The number of these B cells characterized by up regulated chemokine receptors to CXCL13 (Corcione et al., 2004) were significantly higher in the CSF as compared to blood. Together with LT1α2β, CXCL13 has been found in the CSF and MS brain tissue in active lesions (Corcione et al., 2004, Krumholz et al., 2006) and may thus contribute to disease severity by aiding inflammation. In support of this, B cell follicles and meningeal infiltrates have been associated with cortical grey matter lesions widely considered to contribute to cognitive decline in MS (Magliozzi et al., 2007). How these follicles arise

is as yet unknown, although several studies suggest strong association with EBV widely considered to be an aetiological agent in MS.

There is also clinical evidence for regulatory B cell functions in MS (Fillatreau et al., 2002, Shen et al., 2014). Recently a subset of IL-10 producing B cells has been identified in blood of healthy controls and pwMS (Iwata et al., 2011) in which production is lower in pwMS and then restored or increased via therapy (Ozenci et al., 2000). As in rodents production of IL-10 in human B cells increases following treatment with CD40L, lipopolysaccharide (LPS) or CpG. Together the gathered data on the role for B cells in MS and EAE demonstrates its involvement in antigen presentation, cytokine production and T cell activation. This abundant evidence demonstrates that the role of B cells in MS pathology is not merely antibody production. Therefore targeting B cell in the treatment of MS has been a rational approach towards improving the MS pathology.

5. B CELL THERAPIES

Many current therapies for MS also inhibit or modify B cell function. For example the cytostatic agents mitoxantrone, cyclophosphamide preferentially kill B cells due to their high basal proliferation rate (Fox 2004), alemtuzumab depletes T and B cells (Ruck et al 2015) and glatiramer acetate reduces B cells numbers in subset of pwMS (Rovituso et al 2015). Likewise, fingolimod decreases memory B cells but increases naive B cells (Claes et al 2014) while dimethyl fumarate is reported to also reduce B cells numbers (Spencer et al 2015). In contrast, natalizumab increases circulating B cells while IFN-β treatment increases CD19, CD24, CD38 B cells in peripheral blood, although whether B cells in the CNS are affected has not been reported. Cladribine is a highly effective depleting agent of peripheral B cells, compared to T cells (Mitosek-Szewczyk et al 2013) and through targeting dividing and non-dividing lymphocytes inhibits lesion formation in both relapsing MS and progressive MS (Rice et al 2000; Giovannoni et al. 2010). It is the only MS disease-modifying agent that is CNS penetrant, with an immunomodulatory action that is active in the CNS, which has the capacity to target plasma cells and can inhibit oligoclonal band protein formation (Sipe et al. 1994). Whilst efficacy in relapsing MS has been reported (Giovannoni et al., 2010), trials in progressive MS were too short to determine efficacy in progression (Rice et al. 2000). Unfortunately, due to probably unfounded concerns on safety, oral cladribine was withdrawn (Pakpoor et al., 2015). In summary, although studies clearly reveal the multifunctional roles of B cells in MS several therapeutic approaches can be applied to modulate B cell functions.

5.1 CD20 targeted therapy

Three anti-CD20 antibodies rituximab, a chimeric human/mouse IgG1 antibody; ocrelizumab, a humanized antibody, and ofatumumab, a full recombinant human IgG1 antibody have been used in

clinical trials for MS (Hauser et al. 2008; Kappos et al. 2011; Sorensen et al. 2014). All antibodies deplete cells expressing CD20, a surface antigen present on maturating B cells, from pre-B cells to plasmablasts, precursors of plasma cells [Figure 3], as well as a population of T cells. The first study of rituximab, conducted in 5 people with primary progressive MS (pwPPMS), showed that most peripheral blood B cells were depleted until 14 months (Monson et al., 2005). The depletion of CSF B cells was less efficient. A phase I open study, designed to assess the drug safety and tolerability in RRMS did not reveal serious adverse effects (Bar-Or et al.,2008). Clinical trials that followed showed that B cell depletion effectively suppressed MS disease activity (Hauser et al., 2008, Hawker et al., 2009, Kappos et al., 2011). In a double-blind placebo-controlled phase II trial, Rituximab reduced newly formed CNS lesions and relapses in relapsing remitting MS (RRMS) (Hauser et al., 2008). Studies with ocrelizumab, reduced gadolinium-enhancing lesions in RRMS by 89% compared to placebo and Interferon β (Kappos et al., 2011) and inhibited relapsing MS and lesion formation in phase III trails (Hauser et al. 2015). Ocrelizumab has shown some positive results in PPMS in the phase III trial (Montalban et al. 2015). Here, the study focused on younger people with active disease and closer to progressive onset of MS, based on the responder profile of an earlier trial in PPMS with rituximab (Hawker et al. 2009). Lastly, ofatumumab, an approved drug for chronic lymphocytic leukemia is expected to be far less antigenic than rituximab. This was exmained in RRMS, revealing a greater than 99% reduction in newly formed lesions in RRMS compared to controls. No increase in adverse effects was noticed in treated compared to controls (Sorensen et al., 2014). While CD20 targeting therapies reduce MS lesions and clinical relapses the immunoglobulin levels are not significantly affected (Hauser et al., 2008), not surprisingly since plasma cells do not express CD20 and are therefore not depleted. This suggests that CD20+ cell depletion interferes with antigen presentation, T cell activation or cytokine production [Figure 4]. The preclinical findings in EAE showing that B cells polarize T cell differentiation were confirmed in pwRRMS treated with anti-CD20 antibody treatment, emphasizing the APC function of B cells in RRMS (Bar-Or et al.,2010). The number of CSF T cells also decreased following treatment in the majority of RRMS patients (Cross et al., 2006).

5.2 $CD20^+$ T cells

In addition to the effect on B cell function and thereby indirectly affecting T cells, emerging evidence shows that anti-CD20 antibody therapy also directly affect $CD20^+$ T cells. Interestingly, evidence for the direct T cell effect was suggested in early Rituximab studies for treatment of rheumatoid arthritis (Wilk et al., 2009) who also reported $CD20^+$ T cells in peripheral blood of healthy people and those with rheumatoid arthritis (Wilk et al., 2009). $CD20^+$ cells comprise both CD8 and CD4 T cell subtypes and are depleted with $CD20^+$ B cells during Rituximab therapy (Wilk et al., 2009). Data from RRMS

supports the presence of $CD20^{+}$ T cells in the peripheral blood and CSF in pwMS (Palanichamy et al., 2014) and cerebral subventricle white matter samples (Holley et al., 2014). Since CD20 expression is lower on CSF T cells was less noticeable, they were characterized as $CD20^{dim}$ T cells that are both CD8+ and CD4+. Interestingly, CD20-specific antibody predominantly depleted $CD8^{+}$ $CD20^{dim}$T cells although the overall CD4/CD8 ratio remained unchanged (Palanichamy et al., 2014). In the CNS, particularly in active MS lesions, T cells are $CD20^{high}$ and express IL-17 and IFN-γ indicating a more pro-inflammatory profile (Holley et al., 2014) suggesting that CD20-specific immunotherapy may thus modify disease by directly depleting inflammatory $CD20^{high/dim}$ T cells. Furthermore,, it was demonstrated that rituximab/anti-human CD20 had most efficacy in controlling EAE in human CD20 transgenic mice when antibody treatment induced marked T cell depletion (Weber et al. 2010).

5.3 Risks of Infection

Due to the broad B cell target, anti-CD20 antibodies also deplete B cells that regulate immune responses including those controlling viruses. Incidental cases of progressive multifocal leukoencephalopathy (PML) in people treated with rituximab with other immune disorders have been observed (Clifford et al 2011), which suggests a risk of PML development in MS. Furthermore, ocrelizumab development in rheumatoid arthritis and systemic lupus erythematosus was halted because it was linked to serious life-threatening infections (Tak et al 2012). However, development of B cell depletion has a better risk:benefit profile in MS, because of the poor prognosis of MS. Whilst it has similar levels of efficacy to alemtuzumab in relapsing MS (Cohen et al. 2012; Kappos et al. 2011), it does not cause the high incidence of secondary B cell autoimmunities following treatment with alemtuzumab (Cohen et al. 2012, Tuohy et al. 2015) thus making it an attractive new treatment for MS.

6. Novel B cell targeting therapies

Anti-CD19 therapy, being developed for neuromyelitis optica has been suggested as a new treatment for MS target B cells including plasma blasts and short-lived plasma cells (Stüve et al.,2014), thereby reducing the levels of pathogenic antibodies (Stüve et al.,2014). MEDI-551, a humanized anti-CD19 IgG1 antibody has been reported to reduce lesions in RRMS (Aguis et al. 2015). The humanized antibody is able to bind and deplete a broader range of B cells but its working mechanism of depletion is similar to that of anti-CD20. In a humanized CD19 B cell animal model, anti-CD19 treatment could reduce antibody levels because of its ability to deplete plasma blasts (Yazawa et al., 2005). In a CD19 and CD20 transgenic animal model, MEDI-551 more efficiently depletes B cells by depleting pro-B cells, delaying B cell renewal (Herbst et al.,2010). However such approaches might lead to more adverse events such as PML or opportunistic infections. VAY736, is

directed against the B-cell activating factor receptor (BAFF-R) designed to reduce the B cell fostering milieu in the CNS (Kumbholz et al., 2005, Magliozzi et al., 2004). Upon binding to its receptor, BAFF aids B cell survival but it is also involved in B cell development and B cell mediated meningeal follicle formation (Magliozzi et al., 2004). Since BAFF is elevated in MS, targeting the BAFF-R prevents its binding to take place thereby reducing B cell survival and lymphoid follicle-like structures. BAFF-R deficient mice with EAE had fewer mature B cells and increased EAE severity, suggesting a predominant effect on B_{reg} (Sasaki et al., 2004, Kim et al., 2011). Similarly increased disease severity was also shown with atacicepet, a B BAFF-immunoglobulin fusion protein that targets BAFF receptor and A ProlifeRation-Inducing ligand (APRIL). The study was halted after increased inflammatory activity was observed in pwRRMS (Hartung et al., 2010) demonstrating that B cell depletion is not universally a predictable and positive therapeutic effect, as was shown in initial studies in EAE, when B cell depletion could inhibit, do nothing or augment disease (Matsushita et al. 2008).

Active RR- and PPMS is inhibited by peripheral B cell depletion with CD20-depleting antibodies, which may also be active in the CNS due to blood-brain barrier disturbances (Bonnan et al. 2014, Hauser et al. 2015; Montalban et al. 2015). The thought that central B cell activity my drive progressive MS has led to the idea of targeting B cells via local administration (Bonnan et al. 2014; Svenningsson et al. 2015). Yet, intrathecal administration fails to induce marked B cell depletion within the CNS and simply drains from the natural flow of CSF into the circulation and depletes peripheral B cells (Komori et al. 2016, Topping et al. 2016). Therefore, it remains to be established whether intraventricular CD20 B cell depletion would have merit in targeting CNS-B cells or whether novel plasma cell depleting antibodies (Moreau et al. 2014) will be necessary to target ectopic B cell follicles in the CNS.

7. Future perspectives and concluding remarks

Immunodepleting agents, especially if used early after diagnosis, can induce long-term No-evidence of Disease Activity (NEDA; Giovannoni et al. 2015) in MS (Tuohy et al. 2015). For many years MS has been thought to be a T cell mediated disease, largely due to similarities with EAE (Baker & Amor 2014). With the knowledge that antibodies and B cells play a significant role in MS, B cell targeted therapies have been developed and have been found to have marked inhibitory activity on active MS. Success in treating relapsing and progressive MS by B cell therapy, has been hailed as the beginning of the end of the neurodegeneration of progressive MS (Steinman and Zamvil 2016), however this remains to be established especially as inhibiting peripheral autoimmunity does not appear to stop EAE (Al-Izki et al. 2011) or MS following haematopoitic stem cell therapy (Burt et al. 2015). Even though there has been much focus on T cell autoimmunity in MS, it is clear that the highly effective agents can target B cells. Although the aetiology of MS is unknown, it is clear that

genetics and environmental factors contribute to susceptibility to MS. One consistent feature of MS is the potential role of Epstein-Barr virus (EBV) as a trigger of disease activity (Pakpoor et al. 2013). Given that EBV readily infects B cells another mechanism by which B cell depletion may operate removal of this aetiological trigger of relapsing MS (Pakpoor et al. 2013).

It is clear that broad B cell targeted depletion carries risks, notably associated with risks of infection. However, compared to drugs of similar efficacy such as natalizumab and alemtuzumab the adverse events may even be favourable. Yet, there is much to be learned about how B cells contribute to the regulation and pathogenesis of autoimmunity given the balance between B regulatory and B effector cell functions as shown EAE and MS. Irrespective of this it appears that depletion of CD20+ B cells offers promise as a disease modifying therapy of MS and it will probably gain approval for treatment of MS.

Conflict of Interests

The authors declare no conflict of interest

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Legends

Figure 1. The multifunctional roles of B cells in EAE and MS. A) B cells act as antigen presenting cells that activate autoreactive T cells. **B)** B cells mature to plasma cells that produce antibodies including CNS reactive autoantigens. **C)** Mature B cells process antigens, and once activated produce pro- and anti-inflammatory cytokines important in (D) the development of tertiary lymphoid tissue. **E)** B cells also harbor Epstein-Barr Virus (EBV) thought to a key aetiological agent in MS.

Figure 2. Antibody functions in MS and EAE. The primary effect of pathogenic antibodies is induction of tissue damage. Anti-myelin antibodies produced by plasma cells **(1)** attach to myelin leading to opsonisation **(2).** Macrophages bearing FcR bind to antibody on myelin **(3).** Alternatively, macrophages bind immunoglobulin via FcR receptors **(4)** that then bind to myelin inducing antibody dependent cell mediate cytoxicity. Antibodies on myelin **(5)** trigger complement activation leading to the formation of membrane attack complex (MAC) and cell death **(6).**

Figure 3. B cell development. CD19 is highly expressed throughout B cell development but not on terminally differentiated plasma cells. CD20 is expressed on the majority of B cell subtypes but is down-regulated in plasmablasts. B cell activating factor receptor (BAFF-R) is expressed later in development from immature B cells to plasmablasts.

Figure 4. Effect of anti-CD20 antibody in MS. Anti-CD20 antibody binds to and depletes CD20+ cells, including CD20+ pro-inflammatory CD4 and CD8 cells, involved in CNS inflammation and demyelination.

Reg B - Regulatory B cells, Mϕ - Macrophage CTL: Cytotoxic T cell