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A Case for Regulatory B Cells in Controlling the Severity of Autoimmune-Mediated Inflammation in Experimental Autoimmune Encephalomyelitis and Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is considered to be a T cell-mediated autoimmune disease that results in the presence of inflammatory lesions/plaques associated with mononuclear cell infiltrates, demyelination and axonal damage within the central nervous system (CNS). To date, FDA approved therapies in MS are thought to largely function by modulation of the immune response. Since autoimmune responses require many arms of the immune system, the direct cellular mechanisms of action of MS therapeutics are not definitively known. The mouse model of MS, experimental autoimmune encephalomyelitis (EAE), has been instrumental in deciphering the mechanism of action of MS drugs. In addition, EAE has been widely used to study the contribution of individual components of the immune system in CNS autoimmunity. In this regard, the role of B cells in EAE has been studied in mice deficient in B cells due to genetic ablation and following depletion with a B cell-targeted monoclonal antibody (mAb) (anti-CD20). Both strategies have indicated that B cells regulate the extent of EAE clinical disease and in their absence disease is exacerbated. Thus a new population of “regulatory B cells” has emerged. One reoccurring component of regulatory B cell function is the production of IL-10, a pleiotropic cytokine with potent anti-inflammatory properties. B cell depletion has also indicated that B cells, in particular antibody production, play a pathogenic role in EAE. B cell depletion in MS using a mAb to CD20 (rituximab) has shown promising results. In this review, we will discuss the current thinking on the role of B cells in MS drawing from knowledge gained in EAE studies and clinical trials using therapeutics that target B cells.

Keywords

multiple sclerosis; experimental autoimmune encephalomyelitis; B cell; immune regulation; central nervous system; autoimmunity

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1. Introduction

In recent years, B cells or B lymphocytes have emerged as an active component of both the innate and adaptive immune responses. Although all B cells have the capacity to present antigen and produce immunoglobulins (Ig), there are specialized subsets of B cells that are anatomically located according to function. B cells develop in the bone marrow and migrate to the periphery to complete their maturation process (Browning, 2006). B cells with innate functions that can be activated in a T cell-independent manner reside in the peritoneal and pleural cavities (B1 cells) and in the marginal zone of the spleen. Both B cell subtypes produce polyreactive IgM known as natural antibodies because their Ig sequences tend to be germline encoded. These natural IgM are reactive to bacterial components providing an innate front line defense against pathogens (Hardy, 2006). In addition, B1 B cells are active participants in mucosal immunity by producing IgA (Kantor and Herzenberg, 1993). Marginal zone B cells are located at the border of the white and red pulps of the spleen, where they can encounter blood borne pathogens and rapidly respond to antigenic challenge involving macrophages and dendritic cells (Viau and Zouali, 2005). Marginal zone B cells exhibit a preactivated phenotype and are not thought to recirculate, but upon encounter with antigens rapidly migrate into the follicles and proliferate and can differentiate into antibody secreting plasma cells in the absence of T cell help (Fagarasan and Honjo, 2000). Both B1 and marginal zone B cells can undergo antibody class switching (Zandvoort and Timens, 2002). Follicular B cells are largely responsible for the adaptive humoral response and migrate throughout the secondary lymphoid organs where they encounter antigens and following interaction with T cells at the boundary of the B cell follicle and the T cell zone, become activated, undergo proliferation and can differentiate into plasmablasts (Browning, 2006; Garside et al., 1998). These short-lived extrafollicular plasma cells exist to provide an immediate response pathogens. This reaction is followed by migration of B cells into the follicle where the germinal center is formed and the B cells undergo rapid proliferation, isotype class switching and differentiation into either plasma cells or memory B cells (Jacob et al., 1991; Liu et al., 1991). These T cell-dependent responses can lead to the generation of hypermutated high affinity antibodies (Browning, 2006). With the progression of the immune response, long-lived plasma cells migrate to the bone marrow and secrete antibody for weeks or months, while the memory B cells invoke a rapid and vigorous antibody response upon a second exposure to the same antigen (Blink et al., 2005; Hargreaves et al., 2001).

Although historically B cells have been viewed as the cellular source of Ig it is now clear that B cells are active participants in determining the nature of the immune response. B cells produce cytokines important in determining the nature of the immune response and like T cells can become polarized (Harris, 2000; Lund et al., 2005). In particular, B cell production of IL-10 has been implicated in controlling the extent of immune responses associated with CNS autoimmunity (Fillatreau et al., 2002; Matsushita et al., 2010; Matsushita et al., 2008).

It is becoming ever more important to understand the role of the various B cell subsets in inflammatory disorders since a variety of B cell therapeutics have been developed for autoimmune disorders such as MS, but have had varied success in ameliorating disease symptoms (Dalakas, 2008). For example, rituximab, a B cell depleting strategy, has demonstrated efficacy in a number of autoimmune diseases, including MS (discussed in detail below). In contrast, a Phase II clinical trial in relapsing MS using atacept, a recombinant fusion protein targeted to block the activity of BLYS and APRIL, TNF family cytokines that promote B cell proliferation, maturation and survival was terminated early due to increased disease activity as compared to placebo (<http://clinicaltrials.gov/ct2/show/NCT00642902>). These studies highlight the importance of

understanding B cell functions in health and disease in order to predict the effectiveness of specific B cell therapeutics.

2. B cells and MS

While many studies have suggested a role for autoreactive antibodies in MS, their specificity is up for debate (Archelos et al., 2000). B cells have been detected in CNS lesions and the cerebrospinal fluid (CSF) of MS patients, and are often found to be clonally expanded in the CSF (Baranzini et al., 1999; Colombo et al., 2000; Monson et al., 2005a; Owens et al., 2001; Owens et al., 1998; Owens et al., 2003; Qin et al., 2003; Qin et al., 1998). In addition, oligoclonal Ig is often present in the CSF of MS patients, which is not observed in patients suffering from most other neurological disorders (Holmoy, 2009; Martin and McFarland, 1995). These combined data suggest that an antigen-driven response within the CNS leads to the development of one or more B cell clones secreting a specific antibody subset. However, only recently it has been shown that B cells present in the CSF of patients can be the source of oligoclonal banding (Obermeier et al., 2008). This connection between B cells in the CNS and production of oligoclonal banding was determined through the correlation of the Ig proteome with B cell transcription (Obermeier et al., 2008), suggesting that intrathecal antibody production is relevant to the development of MS pathology. Oligoclonal banding patterns were found to be distinct between patients, suggesting that while a B cell specific response occurs, it is unique to the individual (Obermeier et al., 2008). Characterization of Ig from the CSF of MS patients has revealed antibody specificities against myelin components such as myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG); the oligodendrocyte proteins 2', 3'-cyclic-nucleotide 3'-phosphodiesterase type I (CNPase), glial fibrillary acidic protein (GFAP) and transketolase as well as axons (Fraussen et al., 2009; Kolln et al., 2006; Lambracht-Washington et al., 2007; Lovato et al., 2008; McLaughlin and Wucherpfennig, 2008; Villar et al., 2005; von Budingen et al., 2008; Zhang et al., 2005a; Zhang et al., 2005b). Axonal reactivity was demonstrated to be specific for the glycolytic enzymes triosephosphate isomerase (TPI) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Kolln et al., 2006). In a subsequent study, it was shown that the CSF antibodies were able to inhibit GAPDH, but not TPI, activity, suggesting a mechanism for neurodegeneration (Kolln et al., 2010). However, recently, antibody clones isolated from the CSF of MS patients were unable to bind specific myelin proteins, indicating that CSF antibody specificity may be more complex than previously appreciated (Owens et al., 2009). In addition to the CSF, evidence for antigen-driven production of Ig has also been found within CNS plaques (Baranzini et al., 1999; Owens et al., 1998; Smith-Jensen et al., 2000), and Ig with CNS specificities has also been detected in the serum of MS patients (Archelos et al., 2000; Cross et al., 2001). Thus it remains unknown the extent to which Ig plays a role in the pathogenesis of MS, but the presence of Ig self-reactive to a variety of cellular components in the CSF, brain and serum of MS patients suggests that it is playing a pathogenic role or at least is an indication that T:B cell interactions are a prominent feature of the disease.

3. Historical perspective of B cells in experimental autoimmune encephalomyelitis (EAE)

The understanding of the pathological processes leading to MS has been advanced by the development of an animal model known as EAE, as expertly reviewed in (Baxter, 2007; Wekerle, 2008). EAE in mice mimics the inflammatory infiltrate, the neurological paralytic symptoms and demyelination observed in MS. While there are arguable limitations, recent work has demonstrated that the EAE model has been critical in dissecting the many roles that B cells play in regulating MS (Steinman and Zamvil, 2006). This has led to critical

insights into B cell function in human pathogenesis and a focus on the development of B cell therapeutics.

Initial studies that sought to determine the role of B cells in EAE used injections of anti-IgM antibodies starting at birth to deplete B cells in rats prior to EAE induction. In these early studies, depletion of B cells prevented the induction of EAE (Willenborg and Prowse, 1983). However, EAE could be induced if the animal also received MBP-specific antiserum (Willenborg et al., 1986). Similarly, in the mouse, anti-IgM treated animals were also refractory to EAE induction following immunization with MBP antigen/complete Freund's adjuvant (CFA) (Myers et al., 1992). However, EAE could be induced in 33% of B cell depleted mice by the adoptive transfer of MBP-specific encephalitogenic T cells, an efficiency that was increased by the simultaneous administration of anti-MBP antibodies (Myers et al., 1992). A pathogenic role for Ig was more definitively demonstrated by the acceleration/enhancement of disease in both a mouse and rat model of EAE by the administration of a monoclonal antibody (mAb) specific for MOG (Schluesener et al., 1987). These studies indicated that B cells were functioning as antigen presenting cells (APC) facilitating the priming of MBP-specific T cells and that anti-MBP was playing a role in the induction and/or progression of EAE clinical disease. These conclusions were recently supported in more recent studies using a transgenic mouse expressing a MOG-specific TCR on the SJL/J (H-2^s) background, which exhibit a relapsing-remitting EAE disease course (Pollinger et al., 2009). A high rate of spontaneous EAE was observed in mice that was associated with a strong MOG-specific B cell response, and deposits of Ig and complement in CNS lesions (Pollinger et al., 2009). Furthermore, pathogenesis of the MOG-specific Ig was demonstrated by its enhancement of the severity of suboptimal EAE. Finally, depletion of B cells with an anti-CD20 mAb reduced the incidence of spontaneous EAE (Pollinger et al., 2009). These cumulative studies highly suggest that B cells are active drivers of EAE disease, and by extrapolation, MS.

Although the above studies suggest a role for B cell antigen presentation and Ig production in EAE/MS, an alternative role for B cells in the regulation of disease severity has also been demonstrated. This was first demonstrated in the B10.PL (H-2^d) mouse that was rendered genetically deficient in B cells by disruption of the Ig μ heavy chain transmembrane exon (μ MT) (Kitamura et al., 1991). Without the expression of the IgM heavy chain, B cells cannot complete their developmental program and thus no mature B cells are present in the periphery. When EAE was induced in B10.PL μ MT mice by immunization with the MBP immunodominant peptide Ac1-11, the animals exhibited a similar incidence and severity of EAE as was observed in the wild-type controls (Wolf et al., 1996). However, unlike the controls, the B cell-deficient mice failed to undergo spontaneous recovery and exhibited a chronic disease course (Wolf et al., 1996). This was one of the first studies to demonstrate that B cells play an important role in the regulation of the nature and/or extent of the immune response. The chronic disease exhibited by the B cell-deficient mice was only discernable because wild-type B10.PL mice undergo spontaneous recovery. Susceptibility of μ MT mice to EAE induction was subsequently confirmed in the C57BL/6 MOG₃₅₋₅₅ peptide EAE mouse model in two independent studies (Fillatreau et al., 2002; Hjelmstrom et al., 1998). However, the confirmation of the altered disease course in the μ MT mice was only obvious in the study in which the wild-type animals exhibited recovery (Fillatreau et al., 2002). However, a dependency on B cells for the induction of EAE in the C57BL/6 mouse has been demonstrated and is dependent upon the source and nature of the MOG immunogen. While both C57BL/6 wild-type and B cell-deficient mice were susceptible to EAE following immunization with MOG₃₅₋₅₅, in contrast, only wild-type mice were susceptible to EAE induced with recombinant MOG (rMOG) protein (Lyons et al., 1999). Although the species source of the rMOG in this study was not indicated, subsequent studies demonstrated that the B cell dependency only occurred when human rMOG was used.

Induction of EAE following immunization with rMOG from mouse or rat was shown to be B cell-independent (Fillatreau et al., 2002; Oliver et al., 2003). Although the species difference is not completely clear, immunization with either human or rat rMOG lead to the development of antibodies that bound mouse MOG (Oliver et al., 2003) and MOG-specific antibody generated to human rMOG was pathogenic (Lyons et al., 2002). Interestingly, we reported that the relapsing-remitting disease course in (B10.PLxSJL/J)F₁ B cell-deficient mice was not altered as compared to wild-type control mice (Dittel et al., 2000). These cumulative data indicate a complex role for B cells in the pathogenesis and regulation of CNS autoimmunity and that the experimental outcome is highly dependent upon the EAE model used. Table 1 summarizes the reported literature in which B cell-deficient or – depleted mice were used for EAE.

4. Mechanisms of regulatory B cell function

Although a role for regulatory B cells has now been demonstrated in disease processes other than EAE, many of the mechanistic studies examining regulatory B cell function have been conducted using EAE models. The first such study demonstrated a role for B cell production of the anti-inflammatory cytokine IL-10 and expression of CD40 for their regulatory function **in EAE** (Fillatreau et al., 2002). Although not shown in the former study, CD40-stimulated B cells from **human** have been shown to produce IL-10 (Duddy et al., 2004). Interestingly, in relapsing and remitting MS (RRMS), CD40 ligation of naïve B cells from treatment-naïve patients resulted in a significant increase in IL-10 production as compared to naïve B cells from healthy donors (Harp et al., 2010). These data are important because they demonstrate that B cells from MS patients do not have an intrinsic defect in the ability to produce IL-10. A link between CD40-stimulation and IL-10 production was further elucidated in CD19-deficient mice, which exhibited EAE with increased severity accompanied by significantly increased levels of IFN- γ and decreased levels of IL-10 production in the CNS during EAE (Matsushita et al., 2006). Splenic B cells isolated from mice with EAE followed a similar cytokine production trend following stimulation with either MOG + anti-CD40 or anti-CD40 alone (Matsushita et al., 2006). Thus the increased severity of disease in CD19^{-/-} mice was attributed in part to alterations in cytokine production by B cells (Matsushita et al., 2006). One caveat to the above studies is that EAE induction by active immunization requires the emulsification of antigen in CFA, which is known to contain ligands for toll-like receptors (TLR). The impact of TLR signaling on B cells during EAE was recently investigated and it was found that MyD88, an adaptor protein used by most TLR, expression by B cells was required for recovery from EAE (Lampropoulou et al., 2008). Furthermore, signaling through either TLR4 (LPS) or TLR9 (CpG) induced the expression of IL-10 in B cells, but only TLR2/4 expression by B cells regulated recovery from EAE (Lampropoulou et al., 2008). The LPS induced IL-10 production by B cells was able to reduce cytokine production by dendritic cells, but did not have a direct effect on T cell proliferation (Lampropoulou et al., 2008). While these data provide a mechanistic explanation for how B cells regulate recovery from EAE via IL-10 production, it is not clear how relevant they are to MS, which has not been definitively linked to bacterial infections. In this regard, a recent study demonstrated that peripheral blood B cells from MS relapsing-remitting patients with and without disease modifying treatments following stimulation through TLR9 (CpG) produced significantly less IL-10 (Hirotsani et al., 2010). Although these data suggest that bacterial infections in MS patients result in decreased IL-10 production, the question of whether and how infectious agents play an active role in MS is still not resolved. In EAE and MS, pathogenic T cells express proinflammatory cytokines, the best studied of which are T cells that express IL-17 and the quintessential Th1 cytokine IFN- γ . T cells that produce Th2 cytokines such as IL-4 are unable to induce EAE. Thus it is possible that infections that drive “Th2-like” immune responses could protect from MS (Sewell et al., 2003). In this regard, helminth infections are

known to induce Th2 immunity (Coffman, 2010). Following chronic infection with the helminth *H. polygyus*, the adoptive transfer of mesenteric lymph node (MLN) CD4⁺ T cells from these mice had a modest effect on reducing EAE disease severity (Wilson et al., 2010). While in contrast, CD19⁺CD23^{hi} MLN B cells were able to dramatically reduce EAE severity (Wilson et al., 2010). Interestingly, the B cell regulation was IL-10-independent. In humans, B cells from helminth-infected patients stimulated via CD40 or with soluble egg antigen, or a TLR2 agonist as a control, suppressed myelin antigen-specific T cell activation in an IL-10-dependent manner (Correale and Farez, 2009; Correale et al., 2008). These cumulative data strongly implicate B cell production of IL-10 as a critical mechanism in their regulatory activity in EAE and MS.

CD40 is a costimulatory molecule expressed on B cells and provides activating signals when engaged by CD40-ligand expressed on activated T cells. This interaction is also characterized by costimulation of the T cells via CD28 following engagement with either B7.1 (CD80) or B7.2 (CD86) expressed by the B cells. This crosstalk between B and T cells plays an important role in immune regulation. To determine whether such crosstalk occurred during EAE, we first eliminated the immune enhancing components of the immunization procedure CFA and pertussis toxin. This was accomplished by inducing EAE in B10.PL μ MT mice by the adoptive transfer of MBP-specific encephalitogenic T cells (Dittel et al., 1999). The B cell-deficient mice were completely susceptible to EAE induction and exhibited a chronic EAE disease course similar to that in which we first described using active immunization (Mann et al., 2007; Wolf et al., 1996). Thus the inclusion of TLR agonists in the EAE induction protocol is not necessary for B cells to exhibit regulatory functions. Furthermore, in this study, we demonstrated that B cell expression of B7 using a CD80/CD86 double knockout mouse (Borriello et al., 1997) was required for recovery from EAE clinical disease (Mann et al., 2007). In addition, in mice lacking B7 expression by B cells, the emergence of IL-10 and Foxp3⁺ T regulatory (Treg) in the CNS was delayed (Mann et al., 2007). These data suggest that regulatory B cells control the severity of EAE via costimulatory crosstalk with a T cell population that we hypothesize to be a Foxp3⁺ Treg. Treg cells are now recognized as a subpopulation of T cells capable of regulating a variety of immune responses including autoimmunity (Levings et al., 2006). They often express high levels of CD25 and their development and function are dependent upon the transcription factor Foxp3 (Fontenot et al., 2003; Hori et al., 2003). A role for B7 in Treg maintenance, function and conversion has been demonstrated (Liang et al., 2005; Paust and Cantor, 2005; Paust et al., 2004; Sansom and Walker, 2006). Adoptive transfer of Treg reduced the severity of EAE and Treg have been shown to accumulate in the CNS of mice with EAE (Kohm et al., 2002; Korn et al., 2007; Mann et al., 2007; McGeachy et al., 2005; O'Connor and Anderton, 2008; O'Connor et al., 2007; Zhang et al., 2004). Thus it is clear that a better understanding of the mechanisms controlling B:T cell crosstalk will be instrumental in developing therapies to modulate either regulatory B cells or Treg for controlling autoimmunity.

In order to develop cell-based B cell therapies the phenotype of the regulatory B cells needs to be elucidated. Given that there are a number of distinct B cell subtypes that all produce IL-10, this is not necessarily straightforward. The identification of a regulatory phenotype in EAE was made possible in mice deficient in CD19 because these mice have B cells but also develop a severe form of EAE (Matsushita et al., 2006). It was subsequently found that these mice lack CD1d^{hi}CD5⁺ B cells (Yanaba et al., 2008) and that their adoptive transfer into mice depleted of B cells prior to EAE induction prevented exacerbated disease observed in their absence (Matsushita et al., 2008). When obtained from IL-10^{-/-} mice, they lost the ability to control the severity of EAE (Matsushita et al., 2008). Further data supporting a role for CD1d^{hi}CD5⁺ B cells in controlling CNS autoimmunity is the more severe EAE observed following their depletion with a mAb that blocks CD22 binding to its ligand

(Matsushita et al., 2010). However, a complicating factor is that this strategy also depleted marginal zone B cells (Matsushita et al., 2010). Besides requiring CD19, the number of CD1d^{hi}CD5⁺ B cells producing IL-10 following stimulation with LPS, PMA, ionomycin in the presence of monensin was dependent upon B cell receptor diversity and MyD88 expression (Yanaba et al., 2009). These data suggest that CD1d^{hi}CD5⁺ B cells are a distinct lineage that could also be present and suppress T cell-mediated inflammation in humans. In support of this is the finding that IL-10 producing B cells in MS patients infected with helminths were shown to express high levels of CD1d (Correale et al., 2008). Figure 1 depicts a model of regulatory B cell phenotype and function.

Given the powerful immune regulation that B cells exert during EAE, the development of therapeutics that harness and induce these mechanisms may be beneficial for the treatment of MS. In this regard, B cell-activating factor (BAFF), a member of the TNF family that regulates B cell maturation and survival, was shown to induce the differentiation of CD1d^{hi}CD5⁺ IL-10 producing B cells in vitro that were able to reduce the incidence of autoimmune arthritis development in mice (Yang et al.). In addition, a recent study showed that naïve B cells treated with a granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-15 fusokine (GIFT15), in vitro, demonstrated immune suppressive regulatory properties during MOG₃₅₋₅₅-induced EAE. Adoptive transfer of the treated B cells following onset of EAE resulted in a rapid and complete resolution of clinical disease and reduction of neuropathology in MOG-induced EAE (Rafei et al., 2009). These “induced regulatory B cells” required expression of MHC class II, STAT-6 and IL-10 for suppressive activity (Rafei et al., 2009). Whether GIFT15 preferentially enriched the regulatory B cell population already present in the starting splenocyte population or induced regulatory properties in naïve B cells needs to be further investigated. The induction of regulatory B cells has been suggested to occur following treatment of MS with glatiramer acetate (GA), a FDA approved drug for the treatment of MS and whose mechanism of action is not completely understood (<http://www.mult-sclerosis.org/Copaxone.html>). In a recent study in mice, it was observed that GA treatment induced the production of IL-10 in B cells and also downregulated their surface expression of B7 molecules (Kala et al., 2010). Transfer of these B cells suppressed MOG₃₅₋₅₅-induced EAE in wild-type recipient mice; but interestingly it was ineffective in controlling the disease in B cell-deficient mice (Kala et al., 2010). When splenic B cells from EAE mice treated with GA (days 1–19) post-MOG₃₅₋₅₅ EAE induction were examined for changes in cytokine production significant decreases in the LPS-stimulated production of IFN- γ and IL-6 were reported while IL-4, IL-13 and IL-10 were all significantly increased (Begum-Haque et al.). These cumulative data suggests that GA has the potential to modulate the regulatory potential of B cells to resolve EAE and that a similar mechanism of disease resolution may be involved in MS patients undergoing this treatment. If so, this mechanism is likely therapeutically relevant because several studies have reported decreased production of IL-10 by B cells from MS patients stimulated through either CD40 or TLR9 (Duddy et al., 2007; Hirotani et al.).

All the above collective studies suggests that regulatory B cells are involved in resolving CNS autoimmunity and that elucidating their presence and function in humans will be important to finding ways to modulate them as therapeutic targets. Modulation of IL-10 production by B cells is an attractive possibility because of its potent anti-inflammatory properties, which includes downmodulating T cell functions (Moore et al., 2001). Mechanisms whereby IL-10 could downregulate autoimmune T cell responses are numerous and include effects on APC function by inhibiting pro-inflammatory cytokine production (ex. IL-1, IL-12, GM-CSF, TNF), chemokine production and expression of antigen presentation proteins (MHC class II, CD80, CD86) (Moore et al., 2001). These effects on APC function result in decreased T cell proliferation and cytokine production (Fig. 1). Moreover, T cells activated in the presence of IL-10 become anergic and fail to produce

effector cytokines (Groux et al., 1996). IL-10 also been shown to act directly on Foxp3⁺ regulatory T cells promoting the maintenance of Foxp3 expression and their regulatory function during inflammation associated with colitis (Murai et al., 2009).

5. B cell depletion therapy in MS (rituximab)

Based on EAE models, B cells have the capacity to promote both pathogenic and protective mechanisms in MS. The extent to which these contrasting roles contribute to MS remains unknown. Clues to the function of B cells during MS are emerging from clinical trials in which B cells were depleted using rituximab that consists of a mAb specific to human CD20. Rituximab was initially widely used for the treatment of non-Hodgkin's lymphoma (Plosker and Figgitt, 2003) and is also approved for the treatment of rheumatoid arthritis. CD20 is a non-glycosylated phosphoprotein expressed by B cells from the pre to memory stage, but not by plasma B cells. Proposed mechanisms of B cell depletion by rituximab include antibody dependent cell mediated cytotoxicity, complement mediated cytotoxicity, induction of apoptosis and phagocytosis (Bielekova and Becker, 2010) (<http://www.gene.com/gene/products/information/oncology/rituxan/moa.pdf>). Clinical trials using rituximab therapy for the treatment of MS have shown promising results (Bar-Or et al., 2008; Hauser et al., 2008). In a phase II, double blind, 48-week clinical trial of a single dose of rituximab in RRMS patients, occurrences of relapse and formation of new gadolinium-enhancing lesions were significantly reduced (Hauser et al., 2008). Similarly, a reduction in new gadolinium-enhancing or T2 lesions was also observed in a longer trial that lasted 72 weeks (Bar-Or et al., 2008). In this later trial, rituximab was administered twice on days 1 and 15. However, in a 96-week trial in primary progressive MS, differences in time to confirmed disease progression were not significantly different between the rituximab and placebo groups (Hawker et al., 2009). However, in patients under 50 years, time to confirmed disease progression was significantly delayed (Hawker et al., 2009). Interestingly, six months after rituximab treatment both T and B cell numbers were reduced in the cerebrospinal fluid (CSF) with relapsing MS, while serum antibodies specific for MOG or MBP were only modestly reduced (Cross et al., 2006). In a follow-up study, this same group showed that the CSF Ig index, IgG concentration and oligoclonal band number were unchanged (Piccio et al., 2010). In a 52-week phase II clinical trial, rituximab has also recently been shown to have efficacy in patients with relapse while taking an injectable disease-modifying agent (interferon or glatiramer acetate) (Naismith et al., 2010). A reduction in the number of gadolinium-enhancing lesions and a significant improvement in the MS functional composite score were observed (Naismith et al., 2010). Collectively, these clinical data clearly demonstrate that B cell depletion therapy is a safe and effective treatment for RRMS.

Anti-CD20 therapy has also been examined in the EAE model. Using anti-mouse CD20 mAb, it was shown that depletion of B cells prior to induction of EAE via MOG₃₅₋₅₅ peptide immunization, the mice exhibited exacerbated disease (Matsushita et al., 2008). These data are consistent with a regulatory role for B cells in controlling chronic disease observed in B cell-deficient mice (Fillatreau et al., 2002; Mann et al., 2007; Wolf et al., 1996). However, the affect on the disease course was dependent on the timing of the B cell depletion. When B cells were depleted just prior to disease onset or after peak disease was reached, no change in disease severity was noted (Matsushita et al., 2008). Of particular interest to MS, is the finding that EAE disease severity was reduced if B cells were depleted shortly after EAE onset (Matsushita et al., 2008). In a similar study utilizing human CD20 transgenic mice, B cell depletion was achieved using an anti-human CD20 mAb (Weber et al., 2010). B cell depletion prior to EAE induction with MOG₃₅₋₅₅ (B cell-independent), as expected, exacerbated EAE (Weber et al.). In contrast, in the same experimental design immunization with rMOG (B cell-dependent) resulted in less severe EAE (Weber et al., 2010). Similar

results were obtained if the B cells were depleted after the onset of EAE clinical disease. Differences in the two outcomes were attributed to a reduction in MOG-specific Th1 and Th17 cells in the rMOG model as compared to a lack of impediment of Th1 or Th17 development in the MOG₃₅₋₅₅ immunized mice (Weber et al., 2010). This study also demonstrated that rMOG, but not MOG₃₅₋₅₅, immunization lead to the generation of antigen-specific B cells capable of efficiently processing and presenting rMOG to transgenic T cells specific for MOG₃₅₋₅₅ (Weber et al.). These cumulative data (Table 1) provide strong evidence for dual roles for B cells in EAE and perhaps MS, with a regulatory B cells dampening the overall severity of disease, and a pathogenic population that drives disease progression.

6. Possible mechanisms of rituximab action in MS

The reduction in gadolinium-enhancing lesions in rituximab treated MS patients as compared to placebo in the absence of changes in myelin-specific serum antibody (Bar-Or et al., 2008; Cross et al., 2006; Hauser et al., 2008; Hawker et al., 2009), suggests that a pathogenic B cell population is being depleted. It further suggests that anti-myelin antibody may not play a significant role in MS progression. One mechanism whereby B cells could drive the progression of MS is via antigen presentation. Antigen-specific B cells are potent APC and efficiently present antigen to activated T cells and prime naïve T cells (Constant, 1999; Lanzavecchia, 1990; Rock et al., 1984). The presence of activated B cells in the meninges of patients with MS suggests a possible role for B cells in triggering autoreactive T cells in the CNS by presenting self-antigens (Corcione et al., 2004; Serafini et al., 2004; Uccelli et al., 2005). The presence of activated B cells was determined by cell proliferation their upregulation of CD80/CD86 expression. B cells also provide help to T cells through the costimulatory molecules CD80 and CD86, and B cells from the CSF of MS patients expressed a higher surface expression of CD80 compared to controls (Sellebjerg et al., 1998). T cells in turn augment B cell activity through costimulatory molecules and secreted cytokines. Crosstalk between B and T cells can potentially lead to expansion of self-reactive immune responses, which is presumed to be involved in MS. Similar MBP epitope specificity between autoantibodies and T cells from MS patients supports this assumption (Wucherpfennig et al., 1997). B cell depletion may limit the activating signals provided by B cells through antigen presentation and costimulation to autoreactive T cells in the CNS; thereby dampening pathogenic immune responses. The observed reduction of B cells paralleled by a decrease in T cells in the CSF post-rituximab treatment supports this hypothesis (Cross et al., 2006). However, a comparison between peripheral and CSF B cells numbers following rituximab therapy in patients with progressive MS indicated that CSF B cells are not depleted as efficiently as their peripheral counterparts (Monson et al., 2005b). This finding was attributed to the highly activated memory and plasma cell phenotypes of the CSF B cells that express little or no CD20 and thus escape depletion (Monson et al., 2005b).

Apart from costimulation, B cells crosstalk with T cells through secreted cytokines. B cells are potent secretors of proinflammatory cytokines contributing to immune pathology (Duddy et al., 2004). One possible outcome of B cell depletion is curtailed proinflammatory cytokine production in the CNS limiting inflammation. The loss of B cell secreted cytokines may also affect the survival and function of T cells in the CSF. In support of this hypothesis is a study that compared cytokine production by freshly isolated peripheral blood CD19⁺ B cells from MS patients and from age- and sex-matched controls. Following stimulation through the BCR and CD40 in the presence of IFN- γ , B cells from MS patients produced significantly higher levels of lymphotoxin (LT) and TNF- α , and exhibited no difference in the production of IL-10 (Bar-Or et al., 2010). When the same stimulation was performed in the presence of CpG (TLR9 ligand), significant increases in LT and IL-10 were observed

(Bar-Or et al., 2010). Thus the cytokine production profile in MS during an immune response not associated with infection and its accompanying TLR signaling would be proinflammatory. This study also demonstrated that following B cell depletion with rituximab, T cell proliferation and production of IFN- γ and IL-17 was reduced following stimulation with either anti-CD3 or PHA (Bar-Or et al., 2010). These results suggest a link between B cells and the function and survival of pathogenic T cells. This general mechanism encompasses both the enhancement of regulatory B cell function, perhaps by promoting IL-10 production, or by the depletion of self-antigen specific B cells.

7. Additional new therapies in MS targeting B cells

Besides rituximab, there are other immune modulating therapies that target B cells being evaluated for treatment of autoimmune diseases including RA and SLE. Depending on the outcome of these trials, they have the potential to be used for the treatment of MS. Humanized anti-CD20 (ocrelizumab); anti-CD22 (epratuzumab); TRU-015, a single chain polypeptide that binds to CD20 and depletes B cells, and anti-CD19 (MDX-1342) are some examples (Dorner and Burmester, 2008; Levesque, 2009). Several other cell-targeted therapies with effects on B cells for the treatment of MS are also being evaluated (Lim and Constantinescu, 2010). Leukocyte depletion using anti-CD52 antibody (alemtuzumab) reduced the risk of relapse and improved disability in MS in a phase II clinical trial (Coles et al., 2008). These positive clinical results were accompanied by reduced lesion burden and increased brain volume as determined by MRI. CD52 is a glycoprotein expressed on B cells, T cells, monocytes and dendritic cells. Anti-CD52 therapy resulted in rapid lymphopenia with CD4⁺ T cells being the most affected (Coles et al., 2006). Both lymphocytes and monocytes were undetectable in the circulation within an hour of intravenous administration of alemtuzumab. Monocyte and B cell numbers returned to normal three months post-treatment, but the median recovery rate for CD4⁺ and CD8⁺ T cells was 61 and 30 months, respectively (Coles et al., 2006). Further efficacy of alemtuzumab will be determined by an ongoing Phase III clinical trial (<http://care-ms.com>) (Jones and Coles, 2009). One of the main concerns with many of the current therapies is that they are not orally administered. In this regard, cladribine, which has oral bioavailability, in a phase III clinical trial in relapsing MS, demonstrated efficacy with the treatment group exhibiting significantly reduced brain lesions, relapse rates and risk of disability (Giovannoni et al.; Yates). Cladribine is a purine nucleoside analogue that inhibits DNA synthesis and results in the induction of apoptosis due to intracellular accumulation of 2-chlorodeoxyadenosine triphosphate, resulting in lymphopenia (Beutler, 1992). However, in progressive MS, although some MRI measures showed improvement, cladribine did not significantly impact disease progression (Rice et al., 2000).

8. Clinical side effects

Although B cell depletion with rituximab was well tolerated by the majority of patients, some adverse side effects have been observed. A recent publication from the Research on Adverse Drug Events and Reports project found that between 1997 and 2008 57 cases of progressive multifocal leukoencephalopathy (PML) were reported in the literature (Carson et al., 2009). The majority of these patients were being treated for autoimmune disorders. PML is caused by reactivation of latent JC polyoma virus and results in demyelination in the CNS, and is a particularly severe side effect due to its high fatality rate (Carson et al., 2009). In ulcerative colitis, when rituximab was used as a salvage therapy the patient suffered severe exacerbation of disease (Goetz et al., 2007). Interestingly, a complete depletion of mucosal B cells associated with suppression of IL-10 production was noted suggesting a regulatory and not pathogenic role for B cells in colitis. This possibility is supported by the occurrence of ulcerative colitis in a patient with Grave's disease after the second infusion of

rituximab (El Fassi et al., 2008). This patient also had complete deletion of B cells from the colon. Three cases of psoriasis in patients with no known risk for this disease were reported after rituximab treatment for rheumatoid arthritis or systemic lupus erythematosus (Dass et al., 2007). A case of psoriasis arthropathy was also reported after rituximab was used in the treatment of non-Hodgkin's lymphoma (Mielke et al., 2008). In a phase II clinical trial involving previously untreated, early, RRMS, alemtuzumab therapy was associated with the development of additional autoimmune disorders including thyroid-associated events in 23% of patients and to a lesser extent immune thrombocytopenic purpura in 3% of patients (Coles et al., 2008). The incidence of an infection-associated event was 66% (Coles et al., 2008). These data suggest a probable connection between B cell or lymphocyte depletion and susceptibility to some autoimmune diseases in patients treated with anti-CD20 or anti-CD52. A possible cause may be depletion of the regulatory cell populations including regulatory B cells, leading to impaired immune tolerance.

9. Concluding remarks

The role of B cells in the development of a protective adaptive immune response is well established. It is clear that B cells produce antibodies both dependently and independently of T cell help. Antigen-specific B cells are also known to be potent APC and cell-cell interactions between B and T cells are well described. Newer to our knowledge of B cell functions is their ability to modulate or regulate the extent of immune responses. Although this review has concentrated on EAE and MS, similar regulatory roles described for these diseases have been reported in other disease models. A recurring theme in B cell regulation is the production of IL-10, a cytokine with potent anti-inflammatory properties. It is of interest that this aspect of B cell biology is only recently becoming noteworthy, since the production of IL-10 by B cells was reported as early as 1990 (O'Garra et al., 1990). It is now clear that B cell IL-10 production is an important regulator of the extent of inflammatory immune responses. Since the major B cell subtypes have been shown to produce IL-10 under a variety of stimuli, the specific B cell subpopulations that regulate particular inflammatory conditions remains unknown. It is likely, that multiple B cell subtypes will have similar regulatory mechanisms and the prominent subtype will depend on the nature of the inflammatory condition (Figure 1). Also not known are the cellular targets of the B cell-derived IL-10. In addition to IL-10 production, B cells have the capacity to regulate T cell functions via direct cell-cell interactions (Figure 1). Given that multiple T and B cell subtypes exist much still needs to be learned regarding how interactions between the various lymphocyte subpopulations regulates immune responses. The advent of B cell depletion therapy has provided much needed insight into the functions of B cells in a variety of human diseases. Although much still needs to be learned, clinical trials using B cell-targeted drugs have solidified B cells as potent regulators of immune responses.

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Figure 1. Possible mechanisms of regulatory B cell functions in MS

APC (dendritic cell depicted in the figure) present antigen and provide co-stimulation to myelin-specific T cells resulting in their activation and subsequent upregulation of CD40L, proliferation and cytokine production. Binding of CD40L to CD40 on regulatory B cells drives IL-10 production and upregulation of B7 (B7.1 (CD80) and/or B7.2 (CD86)). Binding of antigens to the BCR or microbial components to TLR, also induce IL-10 production by regulatory B cells. IL-10 then acts on the APC (dendritic cell or B cell) to dampen their antigen presentation capacity leading to impaired proliferation and cytokine production by myelin-specific T cells. Regulatory B cells also crosstalk with Treg through B7 and facilitate their function and/or mobilization to the CNS. Treg then suppress myelin-specific T cell function and survival. Inhibition of myelin-specific T cell functions results in downmodulation of inflammation and remittance from a MS episode.

Table 1

Summary of Disease Outcomes in Murine Models of EAE Accompanied by B Cell Depletion

Murine Strain	B cell depletion strategy	EAE induction protocol	EAE clinical disease	Reference
Lewis rat	anti-IgM	Immunization with spinal cord homogenate (SCH) or MBP	No disease	Willenborg and Prowse, 1983
(BALB/cxSJL)F ₁ mouse (BALB/cxSJL)F ₁ mouse	anti-IgM anti-IgM	Immunization with SCH Adoptive transfer of SCH-primed CD4 T cells	No disease Reduced incidence	Myers et al., 1992 Myers et al., 1992
B10.PL mouse	Genetic ablation	Immunization with MBP _{Ac1-11}	No change in disease onset and severity, but mice did not recover	Wolf et al., 1996
C57BL/6 mouse	Genetic ablation	Immunization with MOG ₃₅₋₅₅	No change in disease onset, severity or recovery (note controls did not recover)	Hjelmstrom et al., 1998
C57BL/6 mouse	Genetic ablation	Immunization with rMOG	No disease	Lyons et al., 1999
C57BL/6 mouse	Genetic ablation	Immunization with MOG ₃₅₋₅₅	No change in disease onset and severity. No mention of recovery status	Lyons et al., 1999
(B10.PLxSJL/ J)F ₁ mouse	Genetic ablation	Immunization with MBP _{Ac1-11}	No change in disease onset, severity or relapses	Dittel et al., 2000
C57BL/6 mouse	Genetic ablation	Immunization with MOG ₃₅₋₅₅ or mouse rMOG	No change in disease onset and severity, but mice did not recover	Fillatreau et al., 2002
C57BL/6 mouse	Genetic ablation	Immunization with rat rMOG	No change in disease onset, severity or recovery (note controls did not recover)	Oliver et al., 2003
C57BL/6 mouse	Genetic ablation	Immunization with human rMOG	No disease	Oliver et al., 2003
B10.PL mouse	Genetic ablation	Adoptive transfer of MBP-specific CD4 T cells	No change in disease onset and severity, but mice did not recover	Mann et al., 2007
C57BL/6 mouse	anti-CD20: before EAE induction	Immunization with MOG ₃₅₋₅₅	No change in disease onset, but disease was more severe	Matsushita et al., 2008
C57BL/6 mouse	anti-CD20: before EAE onset	Immunization with MOG ₃₅₋₅₅	No change in disease	Matsushita et al., 2008
C57BL/6 mouse	anti-CD20: at peak of disease	Immunization with MOG ₃₅₋₅₅	Rapid recovery and reduced cumulative disease	Matsushita et al., 2008
C57BL/6 mouse	anti-CD22: multiple injections starting before EAE induction	Immunization with MOG ₃₅₋₅₅	Increased disease severity	Matsushita et al., 2010
C57BL/6 mouse	anti-CD22: multiple injections starting after EAE onset	Immunization with MOG ₃₅₋₅₅	No change in disease	Matsushita et al., 2010
C57BL/6 human CD20 transgenic mouse	anti-CD20: before EAE induction or after onset	Immunization with MOG ₃₅₋₅₅	Increased disease severity	Weber et al., 2010
C57BL/6 human CD20 transgenic mouse	anti-CD20: before EAE induction or after onset	Immunization with rMOG	Decreased disease severity	Weber et al., 2010