Published in final edited form as:

Curr Treat Options Neurol. 2013 June ; 15(3): 259–269. doi:10.1007/s11940-013-0232-y.

B-cell Targeting Agents in the Treatment of Multiple Sclerosis

Tiffany J. Braley, M.D., M.S. and

Assistant Professor of Neurology, Holtom-Garrett Program in Neuroimmunology, Department of Neurology, University of Michigan

Benjamin M. Segal, M.D.

Holtom-Garrett Professor of Neurology, Director, Multiple Sclerosis Center, Department of Neurology, University of Michigan

Opinion statement

The aims of this article are to discuss the potential role of B lymphocytes in the pathogenesis of multiple sclerosis (MS) and in the mechanisms of action of approved and emerging disease modifying therapies. Over the last few years, significant progress has been made in the introduction of novel pharmacologic treatments that reduce the frequency of clinical exacerbations and radiological lesion formation in relapsing remitting MS. The mechanism of action of a number of these disease modifying therapies (DMT) implicates B cells in the pathogenesis, as well as in the regulation, of MS. Recent advances in MS therapeutics indicate that B lymphocytes can act as critical mediators, as well as regulators, of disease activity in MS. Further research into B-cell subset trafficking patterns, functional activities and interactions with other immune cells in the context of neuroinflammation is likely to inform the development of future generations of DMT.

Keywords

Multiple sclerosis; B cells; Disease modifying therapy; Treatment; Interferon; Glatiramer acetate; Natalizumab; Fingolimod; Teriflunomide; Rituximab; Ocrelizumab; Atacicept

INTRODUCTION

Multiple sclerosis (MS) is a chronic, autoimmune disease of the central nervous system (CNS) that results in inflammation, demyelination, and axonal damage in the optic nerves, brain and spinal cord. MS is estimated to affect 400,000 people in the U.S., and is the leading cause of non-traumatic neurological disability among young adults. Eighty-five percent of patients initially present with a relapsing-remitting (RR) course, characterized by defined episodes of neurological dysfunction, separated by periods of clinical stability. When untreated, the majority of individuals with RR disease transition to a secondary progressive (SP) phase in which relapses decrease in frequency and are replaced by a gradual, insidious accumulation of disability. A primary-progressive subtype (PPMS) occurs in a small percentage of patients, who experience a steady decline in neurological function from onset, without an antecedent RR stage. Although there is no cure for MS, dramatic advances have been made in the development of novel DMT that reduce the frequency of clinical relapses and suppress the formation of inflammatory demyelinating lesions by targeting immune pathways. The success of these DMT corroborates the widely held belief that MS is an autoimmune syndrome. Paraclinical findings of B cell dysregulation in MS

AUTHOR OF CORRESPONDENCE: Benjamin M. Segal, M.D., 4013 Biomedical Science Research Center, 109 Zina Pitcher Place, SPC 2200, Ann Arbor, MI 48109, Ph: 734-615-5635, Fax: 734-615-7300, bmsegal@med.umich.edu.

Disclosure Tiffany J. Braley declares that she has no conflict of interest.

patients, as well as the impact of DMT on B cell function, support a role of that subset of lymphocytes in the pathogenic process.

A PUTATIVE ROLE OF B-CELLS IN MS PATHOGENESIS

A role for B cells in MS pathogenesis has long been suggested by the finding of monoclonal antibodies in the cerebrospinal fluid (CSF), that are not present in the peripheral circulation, of the vast majority of afflicted individuals (so called oligoclonal bands or OCBs). The detection of OCBs in the CSF of patients presenting with a first clinically isolated syndrome is an independent risk factor for the development of definite MS [1]. Nonetheless, B cells are not a prominent component of the perivascular infiltrates in MS lesions. This apparent paradox has been resolved, at least in part, by the discovery of lymphoid follicles in the meninges of individuals with MS [2]. These follicles are composed of proliferating B cells, antibody producing plasma cells, helper T cells and follicular dendritic cells. Intriguingly, the follicles have been found in proximity to underlying cortical plaques, suggesting that they release soluble factors that initiate CNS damage [3]. Although meningeal lymphoid follicles have primarily been identified in autopsy specimens from persons with progressive MS, it is possible that nascent immune cell aggregates develop in the RR stage but are easily disrupted during the dissection and processing of CNS tissues.

B cells are “professional” antigen presenting cells. Therefore they could promote neuroinflammation and consequent demyelination by mediating the activation of encephalitogenic T cells- either within the CNS or in the periphery. They also have the potential to secrete anti-myelin antibodies that fix complement and/ or stimulate microglia and infiltrating myeloid cells via engagement of Fc receptors. Activated myeloid cells, in turn, release toxic factors and directly phagocytose the myelin sheath. A pathogenic role of autoantibodies in inflammatory demyelination is supported by the observation that transgenic expression of a myelin-specific immunoglobulin increases the susceptibility of laboratory mice to spontaneous encephalomyelitis [4]. Although there is conflicting data in the literature, a number of investigators have reported high titer and/ or high affinity anti-myelin antibodies in patients with MS or in individuals at risk for development of MS [5–10]. It has also been proposed that antibodies reactive against the axonal protein neurofascin contribute to neuronal dysfunction in secondary progressive MS [11]. Most recently, Srivastava, et al. found that antibodies specific for KIR4.1, a potassium channel predominantly expressed on glial cells, were elevated in the sera of individuals with MS by comparison to individuals with other neurological diseases or healthy controls [12]. The pathophysiological significance of the anti-KIR4.1 antibodies remains to be determined. B cells also express lymphotoxin which could drive the formation of lymphoid follicles within the CNS [13].

In contrast to their pro-inflammatory activities, under certain circumstances B cells have the ability to suppress immune responses. Mice that are genetically deficient in B cells fail to remit following the presenting episode of experimental autoimmune encephalomyelitis (EAE), which is widely used as an animal model of MS [14]. A rare subset of murine splenic B cells that express the cell surface phenotype $CD1d^{high}CD5^{+}CD19^{high}$ play a protective role in some models of EAE [15]. These so called B10 cells suppress the development of encephalitogenic T cells by secreting the immunosuppressive cytokine interleukin (IL)-10 [16]. Individuals with secondary progressive MS have been reported to be deficient in $CD5^{+}$ B cells, which might be analogous to murine B10 cells [17]. A regulatory role of IL-10 has long been established in EAE, and implicated in MS [18–20]. Several research groups claim that B cells from MS patients have a diminished capacity to produce IL-10 [21,22]. Beyond suppressing neuroinflammation, studies in a virus-mediated

model of MS indicate that B cells can promote repair via secretion of antibodies that stimulate remyelination and axonal outgrowth [23–25].

MODULATION OF B CELLS BY FDA-APPROVED DMT

At present, nine US Food and Drug Administration (FDA) approved therapies are used in the clinical setting to decrease disease activity in relapsing forms of MS. Each of these agents has been reported to alter B cell activity in the EAE model and/ or in persons with MS.

Interferon β

Recombinant interferon-beta (IFN β) has been used as a DMT in MS for 20 years. Type I interferon was initially entertained as a disease modifying agent based on the presumption that MS is caused by a virus, a hypothesis that has since been widely discounted. Nonetheless, numerous Phase 3 placebo controlled clinical trials have demonstrated that IFN β therapy decreases annualized relapse rate (ARR) by approximately 30% and new lesion formation by approximately 65%, across different doses and routes of administration [26,27]. As with many medications that have been proven efficacious, the specific mechanism of action of IFN β is now a matter of speculation, though an immunomodulatory effect is generally accepted. Among its many pleiotropic functions in this regard, IFN β can modify B cells. Ramgolam, et al. found that B cells from untreated RRMS patients were compromised in their ability to activate antigen specific T cell proliferation following incubation with recombinant IFN β *in vitro* [28]. This defect in antigen presentation was associated with downregulation of the costimulatory molecules, CD80 and CD40. Furthermore, B cells exposed to IFN β secreted reduced quantities of IL-23 and IL-1 β , and enhanced quantities of IL-12 and IL-27, which could, collectively, curtail the differentiation of encephalitogenic Th17 cells. It is possible that IFN β impairs the antigen presenting and/ or T cell polarizing properties of B cells without affecting their ability to produce immunoglobulins. Hence, Comabella and colleagues found that effective IFN β therapy in MS was associated with diminished T cell proliferative recall responses, but unaltered humoral responses, against Epstein Barr virus [29].

Glatiramer acetate (Copaxone®)

Glatiramer acetate (GA), a random polymer of the four amino acids that are most prevalent in myelin basic protein, was initially approved in 1996 [30]. In the US pivotal trial, GA reduced ARR by 29% compared with placebo (ARR 0.59 vs 0.84, respectively) [31]. Although researchers have previously proposed that GA acts by promoting the deviation of myelin-reactive T cells from a Th1 to a Th2 lineage [32,33], or by modulating myeloid cells [34,35], recent publications invoke the induction of regulatory B cells. Two independent laboratories reported that the adoptive transfer of B cells from GA-treated mice into MOG-immunized syngeneic hosts suppresses clinical EAE [36,37]. In both cases the therapeutic response was associated with the emergence of IL-10 producing splenocytes. Kala, et al. also found that transfer of B cells from GA-treated donors blocked the development of myelin-specific Th1 and Th17 effector T cells in contrast to B cells from donors treated with PBS or ovalbumin [36]. Conversely, *in vitro* exposure to GA does not alter the proliferative responses or cytokine secretion by naïve or memory human B cells, obtained either from individuals with MS or healthy volunteers, in response to a range of activating stimuli [38].

Natalizumab (Tysabri®)

Natalizumab is a humanized monoclonal antibody directed against the $\alpha 4$ chain of the adhesion molecule VLA-4 ($\alpha 4\beta 1$ integrin). It received approval by the US FDA for the treatment of relapsing remitting MS in 2004. In a pivotal Phase 3 randomized placebo

control trial natalizumab decreased the ARR by 68% and MRI enhancing activity by 92% [39]. Peripheral blood B cells constitutively express $\alpha 4\beta 1$ integrin and adhere to VCAM-1 on activated endothelial cells [40]. This raises the prospect that their entry from the circulation into the CNS would be blocked by natalizumab. Therefore, it is not surprising that MS patients treated with natalizumab have a higher fraction of B cells in the peripheral blood, and a lower fraction of B cells in the CSF, compared to untreated patients or patients treated with other DMT [41,42]. The absolute number of B cells/ μ l of CSF is even more dramatically reduced by natalizumab consequent to a lowered total CSF white blood count [42,43]. In a recent study, therapeutic responsiveness to natalizumab correlated with reductions in intrathecal immunoglobulin synthesis and in the frequency of CD5⁺ B cells and CD38⁺ plasmablasts [44]. Post-treatment diminution of IgM index was particularly striking.

Fingolimod (Gilenya®)

Fingolimod, an oral, sphingosine-1-phosphate receptor modulator, was introduced to the clinic as a DMT in 2010. It inhibits the egress of lymphocytes from lymph nodes, thereby preventing their entry into the circulation and CNS. In a 12 month double blind study, RR MS subjects on fingolimod exhibited significant decreases in annualized relapse rate relative to those on intramuscular IFN β [45]. Although encephalitogenic T cells are generally assumed to be its primary therapeutic target, administration of fingolimod results in a significant depletion of circulating B cells. Yet it has little impact on B cells frequency or intrathecal immunoglobulin levels in the CSF [41]. Therefore, if the mechanism of action of fingolimod in ameliorating MS does involve B cells, it most likely mediates those therapeutic effects in the periphery. This is consistent with preclinical data suggesting that regulatory B cells are most efficacious during the initiation, as opposed to the perpetuation, of autoimmune demyelinating disease [16].

Teriflunomide (Aubagio®)

Teriflunomide is an oral pyrimidine synthesis inhibitor that selectively and reversibly inhibits dihydroorotate dehydrogenase (a key mitochondrial enzyme involved in de novo pyrimidine synthesis), thereby exerting a cytostatic effect on proliferating T and B lymphocytes. It was approved by the FDA for management of RR MS in September, 2012. Teriflunomide is the principal active metabolite of leflunomide, an approved treatment for rheumatoid arthritis. In a recent phase 3 clinical trial in RR MS, two separate doses of teriflunomide (7 mg or 14 mg daily) were associated with a 31% relative relapse rate reduction compared to placebo [46]. The 14 mg dose also resulted in significant reductions in disability progression over the 2-year duration of the trial, and both doses were associated with a reduction in the formation of new T2 or enhancing MRI lesions. Neither dose of teriflunomide demonstrated clinical superiority when compared to subcutaneous interferon beta-1a in a separate phase 3 clinical trial [47]. Results of a phase 2 pilot study indicate a role for teriflunomide as a safe, adjunctive therapy in subjects with relapsing forms of MS who have breakthrough disease activity while on a stable dose of interferon-beta [48].

The current understanding of teriflunomide's effects on B-cells has, in large part, been extrapolated from studies of its parent compound, leflunomide. Leflunomide directly suppresses B cell proliferation and cell cycle progression *in vitro* by blocking pyrimidine synthesis. Consequently, leflunomide inhibits antibody production by B cells in response to a range of stimuli, including LPS [49]. B cell suppressive effects have also been observed *in vivo*. Hence, in a hamster-to-C57BL/6 nude mouse heart transplantation model, leflunomide prevented T cell-independent induction of xenoantibodies and graft rejection [50]. Similarly, a novel derivative of leflunomide inhibited the production of autoantibodies in an experimental model of alloreactive B-T cell interaction [51]. Finally, the administration of

teriflunomide mitigated paralysis in the Dark Agouti rat model of EAE, in association with a decrease in the number of infiltrating B cells, as well as T cells and myeloid cells, in the gracile fascicle [52].

EMERGING THERAPIES

In addition to current DMT that are approved for use in MS, experimental agents that specifically target B cells have recently been subject to investigation. Phase 2 clinical trials have shown some of these agents to be therapeutically beneficial in MS. However, one agent in particular paradoxically exacerbated disease.

Rituximab and Ocrelizumab

The most direct evidence for a role of B cells as effector cells in MS pathogenesis comes from trials of monoclonal antibodies directed against CD20, a B cell specific surface molecule. CD20 is expressed on pre-B cells and mature B cells. It is not expressed on antibody secreting plasma cells. Rituximab, a genetically engineered chimeric monoclonal anti-CD20 antibody, induces a rapid, long-lasting depletion of circulating B cells that lasts approximately 6–9 months. Administration of rituximab has been shown to be therapeutically effective in non-Hodgkin's lymphoma [53], rheumatoid arthritis [54], and, more recently, in MS [55–57]. In a phase 2 clinical trial of rituximab in relapsing-remitting MS, subjects in the treatment arm experienced a significant reduction in clinical relapses and in the formation of new or enhancing MRI lesions when compared to subjects in the placebo arm [55]. Interestingly, patients with progressive MS might also benefit from the depletion of CD20-positive cells, particularly if there is evidence of ongoing neuroinflammatory activity at the time of treatment initiation. Hawker and colleagues found that rituximab treatment significantly delayed the time to confirmed disease progression in a subset of primary progressive MS patients who had gadolinium enhancing lesions on baseline MR imaging of the brain [57]. The benefit was most dramatic in subjects aged < 51 years. In an independent case series, the Expanded Disability Status Score (EDSS) of three patients with secondary progressive MS stabilized during treatment with rituximab following a dramatic neurological decline over the previous 1–2 years [56]. The mechanism by which rituximab mediated B cell depletion ameliorates MS remains to be elucidated. Although several groups have reported lower B cell counts in the CSF after rituximab administration, CSF IgG index, IgG concentration and oligoclonal band number were unaffected [58,59]. CSF T cell counts are also reduced (although not as profoundly) by rituximab and correlate with a proportional decrease in CSF levels of the lymphoid chemokine CXCL13 [58]. Treatment of transgenic mice that express human CD20 with rituximab reduced clinical EAE severity in association with diminished myelin-reactive T cell proliferation and IL-17 production [60]. Collectively, these observations serve as proof of principle that rituximab can indirectly alter the activities of encephalitogenic T cells, most likely by skewing the pool of antigen presenting cells with which they interact.

Ocrelizumab is a recombinant humanized anti-CD20 monoclonal antibody. As it is fully human, ocrelizumab is expected to be less immunogenic than rituximab following repeated exposures. In a recent phase 2 clinical trial [61], two different doses of ocrelizumab were compared to placebo or interferon beta-1a. At week 24, the number of gadolinium enhancing lesions was 89–96% lower in the ocrelizumab groups than in the placebo group. Furthermore, exploratory analyses indicated that both doses of ocrelizumab were better than interferon beta for gadolinium enhancing lesion reduction. Several phase 3 clinical trials of ocrelizumab are currently under way for the treatment of both relapsing and progressive forms of MS.

Atacicept

Administration of the DMT discussed above results in the amelioration of MS in association with suppression of B cell activity. However, converse outcomes were obtained following treatment of relapsing patients with atacicept, a novel B cell targeting agent. Atacicept is a fully human recombinant fusion protein composed of the extracellular binding portion of the TACI (Transmembrane Activator and CAML [calcium-modulator and cyclophilin ligand]-Interactor) receptor and a modified Fc portion of human IgG. It binds, and thereby neutralizes, B-Lymphocyte stimulator (BLyS) and a proliferation-inducing ligand (APRIL), two cytokines that are produced by a wide variety of cell types and regulate B cell maturation, function, and survival [62]. BLyS and APRIL are both particularly important for the survival of long-lived, antibody producing bone marrow plasma cells [63]. Blockade of BLyS and APRIL indirectly affects mature B cells and short lived plasma cells by inhibiting B cell survival after the T1 transitional stage, while sparing B cell progenitors and memory B cells [62]. Consequently, serum immunoglobulin levels and frequencies of mature and total circulating B cells are reduced by the administration of atacicept [64,65]. On the basis of the putative role of B cells and antibodies in MS pathogenesis, atacicept appeared to be a promising candidate as a novel DMT in MS. However, a Phase II trial of atacicept in relapsing MS was terminated after an increase in disease activity was observed in the atacicept groups compared to placebo (clinicaltrials.gov/ct2/show/NCT00642902). The mechanism underlying this unexpected outcome is speculative. It suggests an ameliorative effect of mature B cells and/ or plasma cells on MS disease activity, possibly via secretion of immunomodulatory or neuroprotective antibodies [24,25,66] or cytokines, such as IL-10 and TGFβ [67,68].

CONCLUSIONS

A role of B cells in MS has long been suspected due to the occurrence of monoclonal B cell expansion and antibody production in the CNS of the majority of afflicted individuals. This suspicion is supported by the B cell modulating effects of DMT that are currently used in the clinic to reduce the frequency of relapse and new lesion formation. Recent clinical trials with more specific immunomodulators have confirmed a disease promoting effect of CD20⁺ B cells, both in relapsing MS and in progressive MS with inflammatory activity. Unexpectedly, relapsing MS was exacerbated by the administration of a chimeric fusion protein that targets mature B cells and plasma cells and reduces serum Ig levels. Collectively, these observations reflect a complex network of B cell effectors and regulators in MS. The effect of B cells on the disease course might vary based on subset lineage and/ or stage of development. A deeper understanding of B cell subset trafficking patterns, functional activities and interactions with other immune cells in the context of neuroinflammation will be necessary to guide the development of future generations of DMT.

Acknowledgments

Benjamin M. Segal has served as a consultant for Biogen Idec and Bristol-Myers Squibb, has received grant support from Teva Pharmaceuticals and Biogen Idec, and has had travel/accommodations expenses covered/ reimbursed by Biogen Idec and Bristol-Myers Squibb.

ABBREVIATIONS

MS	multiple sclerosis
DMT	disease modifying therapies

CNS	central nervous system
CSF	cerebrospinal fluid
RR	relapsing-remitting
SP	secondary-progressive
PP	primary progressive
OCBs	oligoclonal bands
IL	interleukin
EAE	experimental autoimmune encephalomyelitis
Ig	immunoglobulin
TACI	Transmembrane Activator and CAML [calcium-modulator and cyclophilin ligand]-Interactor
BLys	B Lymphocyte stimulator
APRIL	a proliferation inducing ligand

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