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B-Cell Therapies in Multiple Sclerosis

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B cells play a vital function in multiple sclerosis (MS) pathogenesis through an array of effector functions. All currently approved MS disease–modifying therapies alter the frequency, phenotype, or homing of B cells in one way or another. The importance of this mechanism of action has been reinforced with the successful development and clinical testing of B-cell-depleting monoclonal antibodies that target the CD20 surface antigen. Ocrelizumab, a humanized anti-CD20 monoclonal antibody, was approved by the Food and Drug Administration (FDA) in March 2017 after pivotal trials showed dramatic reductions in inflammatory disease activity in relapsing MS as well as lessening of disability progression in primary progressive MS. These and other clinical studies place B cells at the center of the inflammatory cascade in MS and provide a launching point for development of therapies that target selective pathogenic B-cell populations.

B cells are considered major contributors to multiple sclerosis (MS) pathogenesis, a role that has taken on renewed importance with the advent of B-cell-depleting therapies. The majority of MS lesions contain infiltrating B cells with antibody deposition (Lucchinetti et al. 2001). Although animal models of MS have existed for decades, a key limitation was their inability to adequately recapitulate MS pathology. A startling finding was the ability of antimyelin antibodies to replicate MS-like lesions when cotransferred with myelin-reactive T cells (Genain et al. 1995; Hauser 2015). As our understanding of the mechanisms of MS pathology increases, it has become clear that the central role of B cells in MS is likely mediated through a number of effector functions.

Antibodies from cerebrospinal fluid (CSF)–infiltrating plasmablasts and plasma cells produce oligoclonal bands (OCBs) (Obermeier et al. 2008; von Büdingen et al. 2010), a subset of which are reactive against myelin and other central nervous system (CNS) antigens (Warren et al. 1994; Genain et al. 1999) as well as other ubiquitous intracellular self-antigens (Brändle et al. 2016; Winger and Zamvil 2016). B-cell-containing germinal centers in meningeal follicle-like structures, described in the CNS of secondary progressive MS (SPMS) patients (Serafini et al. 2004; Magliozzi et al. 2007), may be an additional source of OCB production. Some data also indicate that patients have higher serum titers of pathogenic antimyelin oligodendrocyte glycoprotein (MOG) antibodies compared to controls (Zhou et al. 2006). B cells in the CSF, CNS parenchyma, and meninges are clonally expanded, class-switched, and somatically hypermutated (Owens et al. 1998; Qin et al. 1998; Baranzini et al. 1999; Colombo et al. 2000; Ritchie et al. 2004; von Büdingen et al. 2012),

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indicating antigen-driven stimulation likely occurs both in the CNS and periphery (von Büdingen et al. 2012; Bankoti et al. 2014; Palanichamy et al. 2014a; Stern et al. 2014).

B cells also play an important function as antigen-presenting cells (APCs). In animal models, major histocompatibility complex (MHC) II–expressing antigen-reactive B cells are crucial for CNS autoimmunity, and this effect is independent of soluble antibody production (Molnarfi et al. 2013). B cells that enter the CNS recruit other inflammatory cells, including $CD4^+$ T cells and monocytes (Lehmann-Horn et al. 2015). B cells in MS patients express increased levels of costimulatory molecules (Genç et al. 1997; Aung and Balashov 2015), in particular in the CSF (Fraussen et al. 2016), which functions to increase the stimulation of antigen-reactive T cells. Memory B cells from MS patients may have an enhanced ability to stimulate myelin-reactive T cells (Harp et al. 2010). In addition, B cells in MS patients secrete increased levels of proinflammatory interleukin (IL)-6 and granulocyte macrophage colony-stimulating factor (GM-CSF) (Barr et al. 2012; Li et al. 2015), which is correlated with increased proinflammatory T helper (Th)17 cells in MS. Depletion of B cells in MS animal models results in reduced myelin-reactive Th1 and Th17 cells (Weber et al. 2010).

CD20 DEPLETION THERAPIES: DAWN OF A NEW TREATMENT ERA IN MS

Targeted depletion of $CD20^+$ B cells has proven to be an extremely effective method of suppressing inflammatory activity in MS. Several different anti-CD20 monoclonal antibodies (mAbs) have been developed for MS treatment, including rituximab, ocrelizumab, and ofatumumab, which are described below in further detail. CD20 is a cell-surface molecule that functions as an ion channel and is expressed on most B-cell subsets, including pre-B cells, immature, mature, and memory B cells. CD20 is not, however, expressed on pro-B cells, or on plasmablasts and plasma cells. Consequently, anti-CD20 mAbs typically do not cause a reduction in serum immunoglobulin (Ig)G levels because of the sparing of plasma cells, although modest reductions in IgM levels can occur (Hauser et al. 2008).

Anti-CD20 therapies lead to rapid and near complete depletion of circulating $CD20^+$ B cells, but with limited penetration of lymphoid tissues (Kamburova et al. 2013). Although rituximab does not efficiently cross the blood–brain barrier (BBB), it eliminates B cells in the CSF (Cross et al. 2006) and CNS perivascular space (Martin et al. 2009) without any detectable effect on the IgG index or oligoclonal bands (Cross et al. 2006; von Büdingen et al. 2016). In addition, $CD20^+$ B cells are absent in the CNS perivascular space months after rituximab treatment (Martin et al. 2009). Following anti-CD20 depletion, repopulating B cells in the peripheral blood are comprised of primarily naïve and immature B cells with fewer memory B cells and plasmablasts (Duddy et al. 2007; Hauser et al. 2008; Palanichamy et al. 2014b). Memory B cells appear to remain suppressed for at least 1–2 years following a single course of rituximab treatment (Roll et al. 2006; Palanichamy et al. 2014b). In addition, data indicate that reconstituted B cells following anti-CD20 therapy produce less proinflammatory GM-CSF, tumor necrosis factor (TNF)-α, and lymphotoxin (LT)α, and increased anti-inflammatory IL-10 (Duddy et al. 2007; Li et al. 2015).

Although recognized as a protein expressed by B cells, CD20 is also expressed at lower levels in 3%–5% of $CD3^+$ T cells in the peripheral blood of healthy individuals (Palanichamy et al. 2014b; Schuh et al. 2016); these $CD3^+$ $CD20^{dim}$ cells are possibly increased in MS patients (Palanichamy et al. 2014b). $CD3^+$ $CD20^{dim}$ T cells are also slightly more prevalent among $CD8^+$ T cells compared to $CD4^+$ T cells (Palanichamy et al. 2014b), and secrete more interferon (IFN)-γ, tumor necrosis factor (TNF)-α, IL-17, and IL-4 than $CD3^+$ $CD20^-$ T cells (Schuh et al. 2016). In MS patients, and in other neurologic diseases, $CD20^+$ $CD3^+$ T cells are present at similar frequencies in CSF and peripheral blood (Schuh et al. 2016). CD20-expressing T cells are rapidly depleted by rituximab (Cross et al. 2006), but begin repopulating sooner than B cells (Palanichamy et al. 2014b; Schuh et al. 2016). Despite these

interesting findings, the biologic relevance of $CD20^+$ T cells in MS remains unclear. Anti-CD20 treatment also significantly alters T-cell function beyond direct depletion of CD20-expressing T cells. Proliferation and proinflammatory IFN-γ and IL-17 production of $CD4^+$ and $CD8^+$ T cells are markedly reduced following rituximab treatment in MS patients (Bar-Or et al. 2010). In addition, some data suggest that regulatory T cells are increased following rituximab treatment of systemic lupus erythematosus (SLE) patients (Vallerskog et al. 2007). Taken together, it appears clear that anti-CD20 mAb produces profound quantitative and qualitative changes in both the humoral and cellular arms of the adaptive immune system and are the basis for their therapeutic efficacy in MS.

RITUXIMAB

Rituximab was initially developed for non-Hodgkin's lymphoma and was the first anti-CD20 mAb to be tested in MS. Rituximab is a mouse–human chimeric IgG1 antibody that eliminates circulating B cells via complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC). In a phase II double-blind placebo-controlled trial of relapsing remitting MS (RRMS) patients, a single course of rituximab (1 g given twice, 2 weeks apart) reduced newly enhancing lesions by more than 90%, which was sustained at 48 weeks with a greater than 50% reduction in relapse rates (Hauser et al. 2008). In a small study of 26 RRMS patients treated with two courses of rituximab, relapses were reduced by more than 80% after 18 months (Bar-Or et al. 2008). RRMS patients who experienced breakthrough disease activity on glatiramer acetate (GA) or IFN-β therapy experienced a 74% reduction in contrast-enhancing lesions following a single course of rituximab treatment, an effect that was evident as early as 12 weeks (Naismith et al. 2010). Given the impressive results of early clinical trials, rituximab has been used for years as an off-label therapy for MS patients. A retrospective analysis of over 550 RRMS patients treated with 500–1000 mg rituximab every 6 to 12 months experienced an annual relapse rate of 0.044 or the equivalent of one relapse every 23 years (Salzer et al. 2016).

Transition from natalizumab to another disease-modifying therapy (DMT) has been notoriously challenging in MS given the risk for rebound disease activity following discontinuation of natalizumab. In a retrospective review of a Swedish registry, outcomes were compared for natalizumab-treated RRMS patients who were transitioned after John Cunningham (JC) virus seroconversion to either fingolimod or rituximab. Relapses and contrast-enhancing lesions occurred in 17.6% and 24.2% of fingolimod-treated patients, respectively, versus 1.8% and 1.4% of rituximab-treated patients, respectively (Alping et al. 2016). Despite these compelling data, rituximab ultimately did not proceed to phase III testing in MS because of complex factors, including patent life, other economic considerations, and the development of ocrelizumab.

OCRELIZUMAB

Ocrelizumab is a humanized anti-CD20 IgG1 mAb that leads to depletion of $CD20^+$ B cells via ADCC and CDC activity, but with a greater role of the former mechanism because of a higher affinity for FcγRIII receptors on natural killer (NK) cells. In a phase II trial, contrast-enhancing lesions in RRMS patients were reduced by 89% and 96% with 600 mg and 2000 mg ocrelizumab, respectively, compared to placebo at 24 weeks (Kappos et al. 2011). The annual relapse rate over 24 weeks was reduced by 86% and 73% with 600 mg and 2000 mg ocrelizumab, respectively, compared to placebo. More than 99% of B cells in the peripheral blood were depleted through 24 weeks for both doses of ocrelizumab (Kappos et al. 2011). Given the largely similar results with the two ocrelizumab doses, the lower dosage of 600 mg (300 mg given twice over 2 weeks with subsequent redosing given as a single 600 mg dose every 6 months) was chosen for the subsequent pivotal trials.

When ocrelizumab was tested in RRMS in two large phase III trials (termed OPERA I and II) a 46%–47% reduction in the annualized relapse rate was observed at 96 weeks compared to

44 μg IFN-β-1a administered three times weekly (Hauser et al. 2017). Moreover, confirmed sustained disability progression at 12 and 24 weeks was 40% lower in the ocrelizumab-treated patients. $CD19^+$ B cells remained undetectable through 96 weeks. The clinical benefits of ocrelizumab were paralleled by a 95%–97% reduction in contrast-enhancing lesions, a 97%–98% reduction in new or enlarging T2 lesions after week 24, and a higher proportion with improved brain volume loss compared to IFN-β-1a (Hauser et al. 2017). Forty-eight percent of ocrelizumab-treated patients achieved no evidence of disease activity ([NEDA] defined as no clinical relapse or disability progression and no radiological activity) compared to 25%–29% in the IFN-β-treated patients (Hauser et al. 2017). The percent of patients achieving NEDA on ocrelizumab surpasses all other DMTs, including 33% for fingolimod, 37% for natalizumab, and 32%–39% for alemtuzumab.

In open-label extension studies of MS patients who received four courses of ocrelizumab, clinical and radiographic disease activity remained silent through 18 months after the last dose (Kappos et al. 2017). These effects mirror the prolonged suppression of memory B cells after a single course of rituximab (Roll et al. 2006; Palanichamy et al. 2014b), suggesting possible resetting of the immune system long after anti-CD20 treatment. In contrast to natalizumab-treated MS patients, patients treated with rituximab and ocrelizumab do not develop rebound inflammation following B-cell reconstitution. Given these effects, it is intriguing to consider that a limited induction period (e.g., 2–3 years) of anti-CD20 treatment followed by surveillance might be an effective way to treat MS. Further study will be needed to determine whether this is a viable paradigm of disease control.

OFATUMUMAB

Ofatumumab is another anti-CD20 IgG1 mAb that binds a distinct epitope of CD20 compared to rituximab and ocrelizumab. Similar to rituximab, the mechanism of action of ofatumumab involves CDC more than ADCC. In a small clinical trial of 38 RRMS patients, several different doses (100, 300, or 700 mg) of intravenous ofatumumab led to significant reductions in contrast-enhancing lesions and new or enlarging T2 lesions at 8–24 weeks (Sorensen et al. 2014). There was a modest reduction in relapses in the first 24 weeks for the low and medium doses, but no patients experienced any relapses at 6–12 months for any dose of ofatumumab. Although B-cell depletion was complete within 1 week of infusion of all doses, B-cell reconstitution began at 3–4 months in the low-dose group; B-cell depletion was persistent in the high dose at 24 weeks (Sorensen et al. 2014). Ofatumumab's formulation was subsequently modified to subcutaneous injections administered every 4 to 12 weeks. In a double-blind placebo-controlled trial of RRMS patients, subcutaneous ofatumumab was tested at doses of 3, 30, 60 mg every 12 weeks or 60 mg every 4 weeks (Bar-Or et al. 2014). A 65% reduction in new contrast-enhancing lesions was observed at all doses, with a greater than 90% reduction in enhancing lesions at 1–3 months. B cells were depleted and reconstituted in a dose-dependent manner, but B-cell repletion was not seen when dosing was given every 4 weeks (Bar-Or et al. 2014). Phase III testing of ofatumumab in RRMS is in progress.

PROGRESSIVE MULTIPLE SCLEROSIS

Despite major breakthroughs in the treatment of RRMS, primary progressive MS (PPMS), which affects 10%–15% of MS patients, has been a notoriously difficult form of MS to treat. Initial PPMS trials testing IFN-β-1a, IFN-β-1b, GA, and fingolimod all failed to meet their end points (Comi 2013; Montalban et al. 2017). Rituximab was tested in PPMS patients in a large randomized placebo-controlled trial; however, there was no change in time to confirmed disease progression sustained for 12 weeks (CDP12), which was the primary end point (Hawker et al. 2009). There was nonetheless a trend toward increased efficacy with rituximab (30.2% with CDP12 vs. 38.5% for placebo). In preplanned subgroup analyses, patients with contrast-enhancing lesions and/or less than 51 years of age showed a delay in CDP12 (Hawker

et al. 2009). In addition, rituximab-treated patients showed a reduction in T2 lesion volume, but no overall brain volume change compared to placebo (Hawker et al. 2009). In light of these findings, rituximab was not approved for PPMS, but continued to be used as an off-label therapy for certain patients. In a retrospective analysis of 67 PPMS as well as 198 SPMS patients treated with rituximab, the annualized relapse rates were 0.015 and 0.038, respectively, and contrast-enhancing lesions occurred in 4% and 1%, respectively (Salzer et al. 2016). Given that relapses and magnetic resonance imaging (MRI) contrast enhancement are less likely to occur in progressive MS, these findings are perhaps not surprising. In the same cohort of patients, the expanded disability status scale (EDSS) increased by 1.0 and 0.5 in PPMS and SPMS, respectively (Salzer et al. 2016).

Intravenous administration of rituximab leads to CSF concentrations that are approximately 1000-fold less than the plasma concentration (Petereit and Rubbert-Roth 2009). Thus, it seems plausible that progressive MS is driven by compartmentalized CNS inflammation that is resistant to intravenous anti-CD20 treatment, but might be amenable to intrathecal (IT) administration. IT rituximab leads to near complete depletion of B cells in the CSF and blood (Komori et al. 2015; Svenningsson et al. 2015) as well as reductions in certain IT cytokines (Studer et al. 2014). However, IT B-cell depletion was surprisingly transient (approximately 3 months), which was attributed to suboptimal CD20 saturation, partial ADCC killing, and poor CDC depletion of B cells in the CSF relative to the blood (Komori et al. 2015). In addition, markers for IT T-cell depletion and axonal injury did not change, and the trial was ultimately halted as part of the stopping criteria for failing to meet adequate biomarker goals of efficacy (Komori et al. 2015). The OCB pattern and IgG index were also unaffected in an SPMS patient who underwent two treatments of IT rituximab (Studer et al. 2014). That IT antibody production is unaffected by such potent interventions as intracranial radiation (Tourtellotte et al. 1980a), IT cytarabine (Tourtellotte et al. 1980b), and autologous hematopoietic stem-cell transplantation (Saiz et al. 2001; Nash et al. 2003) is a testament to the difficulty of targeting the inflammatory process in the CNS and meninges.

A major advance in the treatment of PPMS occurred with the testing of ocrelizumab in a phase III randomized, placebo-controlled trial (Montalban et al. 2017). In this study, 732 PPMS patients (all younger than age 56, no history of relapses, EDSS of 3–6.5, and positive OCBs/IgG index in CSF) were randomized 2:1 to ocrelizumab (300 mg intravenous given 2 weeks apart every 24 weeks) or placebo for at least 120 weeks. There was a significant difference in the CDP12, the primary outcome of the study, with a 24% reduction in ocrelizumab-treated patients; there was also a 25% reduction in the CDP24 and a 29.3% reduction of worsening in the timed 25-ft walk in ocrelizumab-treated patients (Montalban et al. 2017). There was also significant radiographic improvement in total lesion volume (3.4% decrease in ocrelizumab vs. 7.4% increase in placebo) and brain volume loss (0.9% decreased loss in ocrelizumab, 1.09% increased loss in placebo) (Montalban et al. 2017).

These results beg the question why ocrelizumab met multiple end points in PPMS, whereas rituximab showed a nonsignificant trend toward therapeutic benefit. One important difference is that the ocrelizumab PPMS study was approximately one-third larger than the rituximab study (732 compared to 439 patients, both with 2:1 enrollment into active treatment vs. placebo arms). There were also several differences in patient characteristics, including ocrelizumab patients being younger by an average of 5 years, having a shorter disease duration by 2.6 years, and having a slightly decreased EDSS (0.5 less) (Montalban et al. 2017) compared to rituximab patients (Hawker et al. 2009). In addition, ocrelizumab patients had nearly twice as many contrast-enhancing lesions at baseline compared to rituximab, but both groups ended up with similarly very low numbers of contrast-enhancing lesions at 12 weeks. Despite the rituximab data showing a nonsignificant ($p = 0.14$) trend toward reduced disability (Hawker et al. 2009), the CDP12 data for ocrelizumab and rituximab are fairly similar. Thus, both ocrelizu-

mab and rituximab provide a modest benefit in PPMS but ocrelizumab may be slightly superior. Whereas anti-CD20 therapies offer the first glimmers of hope for the treatment of progressive MS, their effects are somewhat limited and the need for more effective therapies remains.

ADVERSE EFFECTS OF B-CELL DEPLETION

Infusion reactions are the most common side effects of rituximab and ocrelizumab. In the phase II trial of rituximab in RRMS, 93% of rituximab-treated patients experienced grade 1–2 infusion reactions, 7% experienced grade 3, and none experienced grade 4 reactions (Hauser et al. 2008). Of note, no steroid pretreatment was given in these studies. In the ocrelizumab RRMS phase III trials, 34% of ocrelizumab-treated patients experienced infusion reactions, including one patient who experienced bronchospasm with recovery (Hauser et al. 2017). Infusion reactions with ocrelizumab were most often present with the initial dose and were less frequent with subsequent infusions and are likely the result of target cell lysis and effector cell cytokine release following Fc receptor ligation by anti-CD20 mAb opsonized cells. Pretreatment with acetaminophen, antihistamine, and steroids is recommended to mitigate the frequency and severity of infusion reactions. Whereas rituximab is a mouse–human chimeric mAb, ocrelizumab is a more fully humanized mAb, which likely reduces the immunogenicity of the latter. This is reflected by the strikingly small percentage of ocrelizumab-treated patients (0.4%) who develop antidrug antibodies (Hauser et al. 2017) compared to the nearly 30% of rituximab-treated patients (Bar-Or et al. 2008). However, in contrast to late serum sickness reactions mediated by preformed antibodies against administered drugs, early infusion-related reactions are thought to result from cell lysis and cytokine release mechanisms and have no correlation with antidrug antibodies.

Frequencies of infusion-related reactions with the various anti-CD20 antibodies have not been studied in a head-to-head fashion, and differences in protocols (for example, premedication with glucocorticoids in the ocrelizumab and ofatumumab, but not rituximab, studies) make cross-trial comparisons difficult. Ocrelizumab has greater ADCC than CDC activity, whereas the opposite is true for rituximab and ofatumumab. As CDC activity is believed to contribute prominently to infusion reactions (van der Kolk et al. 2001), ocrelizumab might be expected to have a more favorable mechanism of action in this regard.

Because of the profound depletion of circulating B cells, risk of infection is an important consideration in anti-CD20 therapy. In phase III trials, infections were reported in 57%–60% of ocrelizumab-treated RRMS patients compared to 53%–54% of IFN-β-1a patients (Hauser et al. 2017); infections in the phase III PPMS were 71% in the ocrelizumab group and 70% in the placebo group (Montalban et al. 2017). Upper respiratory tract infections and nasopharyngitis were slightly more common in ocrelizumab-treated patients, but there was no difference in the frequency in serious infections in ocrelizumab and IFN-β-1a patients (Hauser et al. 2017; Montalban et al. 2017). Given that clinical trials are relatively short in duration, infection risk after long-term CD20 depletion is unknown. Tuberculosis and hepatitis B reactivation are of particular concern with B-cell depletion, therefore prescreening for these infections is highly recommended. In addition, prescreening for hepatitis C and HIV are also recommended. Patients should undergo required vaccinations at least 6 weeks before anti-CD20 treatment and should not receive live vaccines in the setting of B-cell depletion.

Progressive multifocal leukoencephalopathy (PML) is a particularly feared complication of MS treatment since it was first reported in natalizumab-treated patients (Kleinschmidt-DeMasters and Tyler 2005; Langer-Gould et al. 2005). B cells are a cellular reservoir for JC virus dissemination to the CNS in PML (Durali et al. 2015), but much remains unknown about how or whether B-cell elimination alters PML risk and course. In rituximab-treated rheumatoid arthritis patients, the incidence of PML was estimated at 1 in 25,000 and prior immunosuppression was a significant risk factor (Clifford et al. 2011). Whether or to what extent rituximab con-

tributes to PML risk in rheumatoid arthritis or other rheumatic diseases is unclear; unlike MS, these disorders are PML-associated and rituximab is also administered as an add-on therapy, generally with glucocorticoids and antimetabolites in combination. In retrospective analyses of over 800 MS patients treated with rituximab for an average period of nearly 2 years, no PML cases were reported, despite 83% of patients being JC virus seropositive (Salzer et al. 2016). In addition, no cases of PML were reported in any of the ocrelizumab trials (Kappos et al. 2011; Hauser et al. 2017; Montalban et al. 2017). Nonetheless, the risk of PML associated with long-term B-cell depletion in MS is unknown. In addition, many patients who are JC virus seropositive and previously treated with PML-associated DMTs, such as natalizumab and fingolimod, will likely be transitioned to anti-CD20 therapies, and it is likely that "legacy" cases of PML will be reported in the future. Vigilance for PML in MS patients treated with anti-CD20 mAb is therefore recommended.

There was an unexpected imbalance in the incidence of malignancies observed in the ocrelizumab phase III trials. In the OPERA trials, four malignancies (0.5%) occurred in ocrelizumab-treated patients (including two breast cancer cases) compared to two (0.2%) in the IFN-β-1a arms (Hauser et al. 2017); in the PPMS trial that enrolled 2:1 to either ocrelizumab or placebo, 11 malignancies (2.3%) occurred in ocrelizumab-treated patients (including four breast cancer cases) compared to two (0.8%) in the placebo arm (Montalban et al. 2017). The reason for the malignancy disparity with ocrelizumab is not clear, but there are several suggestions that it may not be clinically significant. First, the trend of increased malignancy, including breast cancer, has fallen in open-label extension studies (Kappos et al. 2017). In addition, the incidence of breast cancer of ocrelizumab-treated patients was not higher than epidemiologic expectations; in other words, breast cancer rates were less than expected in the control arms of the phase III ocrelizumab studies. Last, no changes in the risk of breast cancer or other malignancies has been noted with rituximab, which has been in clinical use since the late 1990s. Further study will be needed to determine whether there is in fact an altered risk of malignancy with ocrelizumab or other B-cell-directed therapies. In the interim, standard breast cancer screening guidelines are recommended for MS patients treated with ocrelizumab.

THE EFFECTS OF OTHER MS DMTs ON B CELLS

The success of anti-CD20 therapies in MS highlights the essential role of B cells in MS. In light of these effects, it is worth reviewing the effects of older MS disease modifying therapies as all impact the function, trafficking, or frequency of B cells (Claes et al. 2015; Longbrake and Cross 2016). The therapeutic efficacy of all DMTs in MS may therefore be a result, at least in part, of their effects on B cells.

Interferons

The IFNs have been shown to reduce the levels of costimulatory factors on B cells (Liu et al. 2001; Ramgolam et al. 2011), thereby decreasing the ability of B cells to simulate T cells. In addition, IFN-β reduces Th17-inducing cytokines and increases anti-inflammatory IL-10 production by B cells (Ramgolam et al. 2011). IFN-β also increases the frequency of transitional and regulatory B cells (Bregs) and reduces the frequency of class-switched memory B cells and plasmablasts (Schubert et al. 2015). These changes may be partly mediated by increased B-cell activating factor (BAFF) levels by IFN-β treatment (Krumbholz et al. 2008a; Kannel et al. 2015). Notably, the therapeutic effect of IFN-β in experimental autoimmune encephalomyelitis (EAE) is B-cell dependent (Schubert et al. 2015).

Glatiramer Acetate

GA treatment induces many of the same changes in B cells as the IFNs, including increased anti-inflammatory (IL-10) and reduced proinflammatory (TNF-α, LTα) cytokine levels in EAE (Kala et al. 2010) and MS (Ireland et al. 2014). GA treatment of EAE mice in vivo promotes development of IL-10-producing mono-

cytes (Weber et al. 2007) and induces anti-inflammatory B cells (Kala et al. 2010), leading to reductions in myelin-reactive T-cell responses. Circulating total B cells as well as naïve B cells and plasmablasts are reduced in GA-treated MS patients, although there are conflicting reports on whether memory B cells are affected (Ireland et al. 2014; Rovituso et al. 2014). This effect on B-cell levels may be because of a reduction of BAFF levels in the CNS and BAFF receptor on B cells in GA-treated EAE (Begum-Haque et al. 2010).

Fingolimod, Dimethylfumarate (DMF), and Teriflunomide

Inhibition of sphingosine-1-phosphate (S1P) receptors by fingolimod leads to reduced circulating levels of all B-cell populations, including naïve and memory B cells and plasmablasts. However, transitional/immature and naïve B cells represent a higher proportion of B cells in the peripheral blood of fingolimod-treated MS patients and produce less TNF-α and increased IL-10 and TGF-β (Miyazaki et al. 2014; Chiarini et al. 2015; Blumenfeld et al. 2016). DMF can induce lymphopenia, affecting T cells in particular, but also causes a modest reduction in circulating B-cell levels (Spencer et al. 2015; Lundy et al. 2016). DMF reduces memory more than naïve B-cell levels, reduces mature B-cell survival in vitro, and reduces proinflammatory GM-CSF, IL-6, and TNF-α production by B cells (Chi et al. 2011). DMF also reduces MHC II expression on B cells and reduces the severity of B-cell-dependent EAE (Schulze-Topphoff et al. 2016). Teriflunomide inhibits the proliferation of both B and T cells in vitro without affecting overall lymphocyte survival (Li et al. 2013).

Natalizumab

Natalizumab blocks VLA-4-mediated lymphocyte entry across the BBB into the CNS. VLA-4 is expressed on B cells, in particular at higher levels on memory B cells compared to naïve B cells (Silvy et al. 1997). In EAE, B-cell-specific VLA-4 deficiency leads to reduced disease severity and decreased Th17 and macrophage infiltration in the CNS (Lehmann-Horn et al. 2015). B-cell VLA-4 deficiency can also prevent accumulation of IL-10$^+$ Bregs (Lehmann-Horn et al. 2016). In MS, natalizumab also reduces numbers of B cells in the CSF (Warnke et al. 2015) and has been reported to reduce the IgG index and OCBs transiently in some patients (Harrer et al. 2012; von Glehn et al. 2012; Mancuso et al. 2014; Warnke et al. 2015). Inhibition of B-cell CNS infiltration by natalizumab leads to an increase in peripheral circulating B cells, in particular in pre- and newly produced B cells (Krumbholz et al. 2008b; Zanotti et al. 2012).

Alemtuzumab

Alemtuzumab is an anti-CD52 monoclonal antibody that depletes B cells and T cells via ADCC and CDC. B cells reconstitute earlier than T cells following alemtuzumab treatment and recover by 6 to 7 months (Thompson et al. 2010; Hill-Cawthorne et al. 2012; Cossburn et al. 2013). Immature transitional B cells increase within 1 month of alemtuzumab treatment and, beyond 3 months, mature naïve B cells predominate over memory B cells (Thompson et al. 2010). BAFF levels are increased after 1 month of alemtuzumab, correlating with the surge in immature B-cell development (Thompson et al. 2010).

Mitoxantrone

Mitoxantrone is a potent immunosuppressant that was effective in a mixed population of relapsing and progressive MS patients (Millefiorini et al. 1997; Hartung et al. 2002), but has fallen out of favor because of cardiotoxicity and risk of leukemia from prolonged exposure (Marriott et al. 2010). B cells are preferentially depleted more than other lymphocytes following mitoxantrone treatment (Gbadamosi et al. 2003). Memory B cells are particularly reduced and B-cell production of IL-10 is increased, whereas TNF-α and LTα are decreased (Duddy et al. 2007).

Atacicept: Lessons from an Unsuccessful B-Cell-Directed Therapy

Atacicept is not approved for MS therapy and represents a negative clinical trial, but is none-

theless noteworthy. Atacicept is a recombinant fusion protein of TACI-Fc IgG that binds the cytokines BAFF (also known as BLyS or B-lymphocyte stimulator) and a proliferation-inducing ligand (APRIL), thereby preventing their interaction with surface receptors on B cells. BAFF and APRIL are important for B-cell differentiation, maturation, and survival through interactions with several B-cell receptors, including the shared transmembrane activator and calcium modulator and cyclophilin-ligand interactor (TACI) receptor (Mackay and Browning 2002).

In a phase II trial, RRMS patients treated with weekly injections of atacicept experienced relapses at twice the rates compared to the placebo group, resulting in early termination of the study (Kappos et al. 2014). There was no difference between the treatment groups in the number of contrast-enhancing lesions, the primary end point of the study. In another phase II study of patients with a first event of optic neuritis (clinically isolated syndrome), twice as many atacicept-treated patients converted to clinically definite MS compared to placebo, again leading to early termination of the study (Sergott et al. 2015). From a biologic perspective, atacicept leads to partial depletion of B cells, with mature B-cell counts reduced by up to 60%–70% and significant reductions in IgM, IgA, and IgG levels (Kappos et al. 2014). After cessation of atacicept, relapse-rates normalized to those of placebo-treated patients, which was paralleled by the recovery of normal B-cell and immunoglobulin levels (Kappos et al. 2014).

These studies beg the question why partial B-cell depletion from atacicept worsened clinical MS activity. As outlined above, all the currently approved MS therapies skew B cells toward an anti-inflammatory/regulatory phenotype (IFNs, GA, fingolimod, DMF, mitoxantrone) or preferentially affect memory B cells (IFNs, fingolimod, DMF, natalizumab, alemtuzumab, mitoxantrone). Thus, there is increasing evidence that targeting the frequency, phenotype, or trafficking of memory B cells in particular is a key factor in effective MS immunotherapies (Baker et al. 2017). BAFF-treated B cells were skewed toward a regulatory phenotype and produced more IL-10, an effect that is abrogated by TACI-Fc (Yang et al. 2010), the same molecule as atacicept. In addition, TACI is preferentially expressed on memory B cells and plasmablasts (Baker et al. 2017), which may serve to explain the relative increase in memory B cells that occurs within weeks of a single dose of atacicept (Tak et al. 2008). It therefore seems likely that the worsening of MS activity that occurred with atacicept treatment resulted from a relative increase in memory B cells and/or reductions in Breg cells.

NEW DIRECTIONS IN B-CELL-TARGETED THERAPIES IN MULTIPLE SCLEROSIS

The clinical success of anti-CD20 mAbs and the failure of atacicept point to the promise as well as challenges of developing B-cell therapies for MS. Numerous studies using different approaches of targeting B cells in MS are in various stages of development. Ublituximab is yet another anti-CD20 mAb that has shown excellent B-cell depletion using a rapid infusion protocol. MEDI-551 is an anti-CD19 mAb that is designed to lead to more complete B-cell depletion as it targets $CD19^+$ $CD20^-$ pro-B cells, plasmablasts, and plasma cells. In phase I testing, MEDI-551 was well tolerated and led to prolonged B-cell depletion (Agius et al. 2015). Daratumumab is an anti-CD38 mAb approved for the treatment of multiple myeloma, a plasma cell malignancy, and is being considered for MS treatment. Additional therapies targeting the BAFF pathway, including anti-BAFF and anti-BAFF-R (VAY736) are underway. Small molecules targeting B-cell signaling, including ibrutinib (Bruton's tyrosine kinase inhibitor) and idealisib (PI3 kinase inhibitor) may provide novel mechanisms of targeting B cells and possibly other cells involved in immune pathogenesis.

There is no doubt that anti-CD20 therapies in MS have ushered in a new era in our understanding and treatment of relapsing and progressive MS. Never before have we appreciated the extent to which B cells contribute to MS pathogenesis, likely through myriad effector pathways both outside and within the CNS. DMTs like ocrelizumab offer a significant opportunity to shut down all metrics of inflamma-

tion in a substantial portion of RRMS patients. For the first time, a treatment for PPMS, albeit with modest effects, is now available. Numerous questions remain regarding anti-CD20 therapies, including how to optimize strategies with respect to treatment initiation (i.e., might earlier treatment be even more effective?) and dosing (can we develop biomarkers to guide the need for continued therapy?), as well as to determine effects on long-term disability (especially with respect to risk for SPMS) and long-term safety (including risk of malignancy, PML, or other adverse outcomes). Increased understanding of the antigenic targets of B cells, their phenotypes, and their interactions with T cells, the innate immune system, and various cells of the CNS, will hopefully provide unique opportunities for even more targeted, safe therapies in MS, and especially for development of more effective interventions to prevent and treat the progressive neurodegenerative phase of the disease.

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