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Review Article

B-Cell-Directed Therapies: A New Era in Multiple Sclerosis Treatment

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ABSTRACT: Multiple sclerosis (MS) is a chronic autoimmune demyelinating disease of the central nervous system (CNS) that often progresses to severe disability. Previous studies have highlighted the role of T cells in disease pathophysiology; however, the success of B-cell-targeted therapies has led to an increased interest in how B cells contribute to disease immunopathology. In this review, we summarize evidence of B-cell involvement in MS disease mechanisms, starting with pathology and moving on to review aspects of B cell immunobiology potentially relevant to MS. We describe current theories of critical B cell contributions to the inflammatory CNS milieu in MS, namely (i) production of autoantibodies, (ii) antigen presentation, (iii) production of proinflammatory cytokines (bystander activation), and (iv) EBV involvement. In the second part of the review, we summarize medications that have targeted B cells in patients with MS and their current position in the therapeutic armamentarium based on clinical trials and real-world data. Covered therapeutic strategies include the targeting of surface molecules such as CD20 (rituximab, ocrelizumab, ofatumumab, ublituximab) and CD19 (inebilizumab), and molecules necessary for B-cell activation such as B cell activating factor (BAFF) (belimumab) and Bruton's Tyrosine Kinase (BTK) (evobrutinib). We finally discuss the use of B-cell-targeted therapeutics in pregnancy.

RÉSUMÉ : Les traitements ciblant les lymphocytes B dans la sclérose en plaques : nouvelle ère thérapeutique en vue. La sclérose en plaques (SP) est une maladie auto-immune chronique démyélinisante du système nerveux central (SNC) qui aboutit souvent à une grande incapacité. Le rôle des lymphocytes T dans la physiopathologie de la maladie a déjà été mis en évidence dans des études antérieures, mais les bons résultats des traitements ciblant les lymphocytes B ont suscité de l'intérêt pour le rôle de ces derniers dans l'immunopathologie de la maladie. Aussi présenterons-nous dans l'article de synthèse des données probantes qui font ressortir l'action des lymphocytes B dans les mécanismes d'évolution de la SP, depuis la maladie elle-même jusqu'aux éléments immunobiologiques des lymphocytes B potentiellement associés à la SP. Dans la première partie, il sera question des théories existantes sur le rôle fondamental que jouent les lymphocytes B dans le milieu inflammatoire du SNC, dans la SP, à savoir i) la production d'autoanticorps; ii) la présentation d'antigènes; iii) la production de cytokines pro-inflammatoires (activation de voisinage); iv) le rôle du virus d'Epstein-Barr. Dans la seconde partie, nous présenterons un résumé des médicaments qui ciblent les lymphocytes B chez les patients atteints de la SP, et discuterons de leur place dans l'arsenal thérapeutique de la maladie d'après les résultats d'essais cliniques et des données réelles. Les stratégies thérapeutiques traitées dans l'article porteront notamment sur la prise pour cible des molécules présentes à la surface des lymphocytes telles que la CD20 (par le rituximab, l'ocrélizumab, l'ofatumumab ou l'ublituximab) et la CD19 (par l'inébilizumab), ainsi que sur la prise pour cible des molécules nécessaires à l'activation des lymphocytes B tel que le facteur d'activation des lymphocytes B (BAFF, en anglais) (par le bélimumab), et à l'inhibition de la tyrosine-kinase de Bruton (par l'évobrutinib). Enfin, il sera question de l'emploi des traitements ciblant les lymphocytes B chez les femmes enceintes.

Keywords: Multiple sclerosis; B cells; Monoclonal antibodies; CD20; CD19

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Introduction

Multiple sclerosis (MS) is a chronic autoimmune demyelinating inflammatory disease of the central nervous system (CNS), in the majority of cases gradually leading to progressive, severe disability if left untreated. MS is the leading cause of non-traumatic disability among young adults in the developed world. It is most often diagnosed between 20 and 40 years of age and affects women and men at a ratio of approximately 2:1.[1–3] The clinical course of MS can be characterized as (i) clinically isolated syndrome (CIS), (ii) relapsing-remitting (RRMS), (iii) primary progressive (PPMS), or (iv) secondary progressive (SPMS). Each of the above MS categories can be further subcategorized as either active or inactive, based on both the clinical relapse rate and MRI findings (new T2 lesions and/or active, gadolinium-enhancing lesions-GdELs). Further, progressive forms can be subcategorized as actively progressive or stable.[4]

Significant progress in understanding MS pathophysiology has been accomplished in the past decades. Two hundred and thirty-

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three genetic variants have been identified as risk factors for MS, 32 of which refer to the major histocompatibility complex family (MHC).[5] Prominent among the many risk variants, MHC Class II DR15 molecule entails mechanistically relevant susceptibility to the disease rather than just being a genetic marker.[6] Additional genetic variants associated with the disease refer to other genes of the immune system, such as genes involved in T-cell activation and proliferation (IL-2, IL-7R), tumor necrosis factor-alpha (TNF-α)-related pathways, and vitamin D metabolic pathways (GC, CYP24A1).[3,7–11]

In MS, myelin is phagocytosed by CD68-positive macrophages, while immune cells including B and T cells seem to be activated in the periphery and to express adherence molecules that enable them to cross blood-brain barrier (BBB) in order to participate in the formation of MS lesions. Accumulating evidence suggests an important contribution of $CD4^+$ T cells to disease pathophysiology.[12] Often being present from the beginning and increasing in quantity as disease progresses, axonal degeneration is regarded as a correlate of disability progression.[13]

MS was long considered mainly T-cell-mediated; however, intrathecal IgG synthesis,[14] a hallmark of MS, supports B-cell involvement.[15,16] The T-cell-dominated view of MS pathogenesis was further challenged by the remarkable efficiency of $CD20^+$ B-cell depletion in eliminating inflammatory activity in patients with MS. In this review, we aim to shed light on the key role B cells play in the pathogenesis of MS and present current advances in MS treatment strategies based on promising and effective B-cell-targeted therapeutic regimens.

B Cells in MS Pathology

Histological studies of active MS lesions have demonstrated that B cells can reside in the perivascular space and the CSF but also within the parenchyma.[17] Moreover, ectopic lymphoid follicles are found primarily in the intrameningeal spaces.[18] and are associated with subpial cortical demyelination in patients with SPMS.[19,20] In addition, four histopathological patterns have been proposed for the classification of acute MS plaques. Type I lesions (15% of MS patients) are characterized by a T-cell and activated microglia inflammatory environment without immunoglobulin deposition and complement activation. On the contrary, type II lesions (58% of MS patients) develop in an inflammatory milieu with immunoglobulin production and complement activation. Demyelination in type III lesions (26% of MS patients) is accompanied by oligodendrocyte apoptosis without immunoglobulin deposition or complement activation. Finally, in type IV lesions (rare; 1% of MS patients) inflammatory modulators result in non-apoptotic death of oligodendrocytes in the white matter surrounding the plaque due to metabolic disorganization processes.[21] However, it is important to note that IgG deposits in MS histopathological specimens are not specific for MS[22] and that no disease-characterizing autoantibodies have been defined to date. Nevertheless, pattern II has been linked to better response to plasma exchange.[23]

Potential Roles of B Cells in MS Pathophysiology

Several studies have explored potential roles of B cells in the development of MS: antibody production, antigen presentation, and secretion of pro- and anti-inflammatory mediators are three prominent research directions that have been explored.[24,25] In addition, clear epidemiological associations of B-lymphotropic Epstein-Barr virus (EBV) infection to MS have led to the explorations of its pathophysiological relevance.[26]

Autoantibodies

Autoreactive B cells that escape peripheral tolerance checkpoint selection could target antigens of the CNS and cause autoimmune inflammation; however, no consistent B-cell antigen that is specific for MS and that causes demyelination has been identified to date despite numerous attempts. Intrathecal oligoclonal bands, a hallmark of MS diagnosis found in up to 95% of patients,[27,28] are not specific for MS (found also in e.g. meningitis and subacute sclerosing panencephalitis) and have been found to target intracellular antigens in patients with MS.[29,30] In addition, detection of intrathecal IgM synthesis has been associated with onset of relapses and a more aggressive disease course.[31] Similarly to antibodies of oligoclonal bands, B cells of MS lesions have been found to target intracellular antigens.[30] Antibodies previously thought to be present in MS such as antibodies against myelin oligodendrocyte glycoprotein (MOG) rather characterize a distinct disease entity (MOG-antibody disease) that encompasses pediatric acquired demyelinating syndrome, recurrent optic neuritis, acute disseminated encephalomyelitis, and neuromyelitis optica without anti-aquaporin four autoantibodies. A minority of MS patients harbor antibodies against a variety of antigens (some of them cell surface proteins) such as contactin-2,[32] OMGP,[33] and other peptide and lipid antigens.[34,35]

It must be noted that autoantibodies can also be responsible for the activation and chemotaxis of CD4+ T cells. The opsonization of myelin antigens, even at low concentrations, enhances the presentative competence of resident antigen-presenting cells (APCs), such as macrophages and dendritic cells, leading to increased recruitment of effector T cells and, consequently, aggravation of the disease severity, as explained in more detail below.[36–38]

B Cells as Antigen-Presenting Cells

B cells are efficient APCs and express MHC class II and costimulatory molecules, such as CD40, CD80, and CD86.[25,39] They can capture soluble and membrane-tethered antigens via their B-cell receptor (BCR) and present them to T cells in an up-to-a 10.000 times more efficient way compared to myeloid APCs.[40] Evidence from experimental autoimmune encephalitis, a rodent model simulating "efferent" MS pathophysiology, opposes the hypothesis that the antigen-presenting function of B cells is central to the pathophysiology of MS. Specifically, MOG-specific B cells may initiate CNS inflammation and, consequently, the symptomatic onset of the disease, but do not affect either the proliferation or the molecular profile (i.e. secreted cytokines, activation markers) of MOG-specific T cells in the spleen and the draining lymph nodes.[37]

On the other hand, a recently published report focusing on human leukocyte antigen (HLA)-DR15, which is the major genetic risk factor for MS, addresses how the immunopeptidomes presented by both DR15 allomorphs, DR2a and DR2b, on different APCs in the thymus, peripheral blood, and brain – including B cells – could affect autoimmune T cells. The results showed that DR2a and DR2b immunopeptidomes on B cells are significantly skewed toward HLA-DR-self peptides (HLA-DR-SPs) – compared to monocytes – which are consequently presented to autoreactive $CD4^+$ T cells. These T cells responded robustly to individual and pooled HLA-DR-SPs in MS patients, compared to healthy

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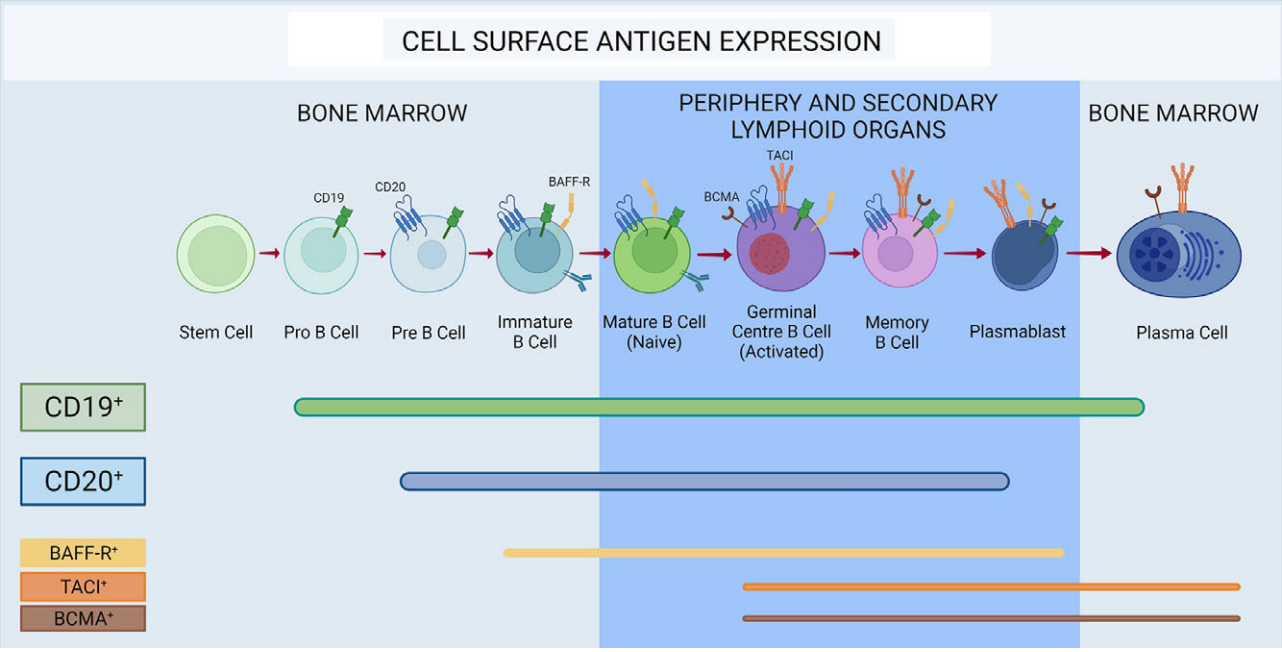


Figure 1: *Expression of cell surface antigens throughout B-cell maturation. CD19 is expressed in all stages of B-cell development, with the exception of stem cells and the majority of plasma cells. CD20 is not present on plasma cells, most plasmablasts, pro-B cells, and stem cells. BAFF-receptor (BAFF-R) is expressed on both immature and mature B cells in the germinal center, as well as memory B cells and late plasmablasts. Transmembrane activator and CAML interactor (TACI) and B-cell maturation antigen (BCMA) are expressed on germinal center B cells, memory cells, and antibody-secreting cells.*

donors, suggesting that DR2a and DR2b could jointly shape an autoreactive T-cell repertoire in MS.[41]

B Cells as a Source of Cytokines

Physiologically, B cells can be a source of both proinflammatory and anti-inflammatory (regulatory) cytokines. B cells of RRMS patients however feature a profile that is skewed towards an abnormally hyperactive proinflammatory response. In mice, high levels of B-cell-secreted IL-6 can foster the differentiation of Th17 cells, while preventing the generation of T regulatory cells.[42–44] In MS patients, B-cell production of lymphotoxin alpha (LT-α), TNF-α, and granulocyte macrophage-colony stimulating factor (GM-CSF) appears elevated, forging a chronically inflammatory milieu within the CNS.[19,25,45] At the same time, anti-inflammatory, regulatory cytokines that are produced by B cells, such as IL-35,[46] but also TGF-β1 and IL-10, are instrumental in controlling inflammation in experimental models of MS.[47]

EBV in MS

Among the infectious factors examined, the B-lymphotropic EBV has been shown to confer increased risk of developing MS, via, as yet, unclear mechanisms.[48] Ninety-six percent of the general population is positive for IgG antibodies against EBV (indicating past infection), while in MS patients this percentage is almost 100%.[26] Moreover, a prospective cohort study of 955 incident MS patients showed a 97% of EBV (but not other viruses) seroconversion before development of the disease, significantly higher compared to controls.[49] Studies and experiments have shaped four major theories about EBV's role in the pathogenesis of MS: the cross-reactivity hypothesis,[50] the bystander damage hypothesis,[51] the αβ-crystallin hypothesis,[52] and the EBV-infected autoreactive B-cell hypothesis.[53] Cellular and CSF findings however only partially match the pathophysiology of MS as far as the first three hypotheses are concerned. One important finding is the recent demonstration of molecular mimicry between EBV transcription factor EBNA1 and CNS protein glial cell adhesion molecule (GlialCAM), leading to the production of cross-reactive antibodies with higher affinity towards an intracellular GlialCAM epitope.[54] The fourth hypothesis postulates that EBV-infected autoreactive B cells accumulate in the target organ and orchestrate the disease by producing antibodies and stimulating T cells due to a defect in their elimination by the antiviral $CD8^+$ T cells. Moreover, the EBV anti-apoptotic protein BHRF1, produced by both latently and lytically infected cells, inhibits B-cell apoptosis,[55] resulting in immortalization of the autoreactive B cells it infects. In support of this hypothesis, substantial EBV persistence in B and plasma cells as well as meningeal B-cell lymphoid follicles of all MS cases examined was reported,[56] but could not be reproduced in multiple independent replication studies.[57–60] Overall, the epidemiological associations remain; however, no underlying biological mechanism has been consequently supported by experimental data.

Anti-B-cell Agents as a Therapeutic Strategy

Most anti-B-cell agents are monoclonal antibodies (mAbs); however, small molecules have also emerged as promising agents and have better CNS penetrance (Table 1). B-cell-depleting antibodies can be categorized in 1st-, 2nd-, or 3rd-generation. 1st-generation monoclonal antibodies (mAbs) can be either fully murine (suffix: -omab) or chimeric (65% human, suffix: -ximab), while 2nd-generation ones can be humanized (>90% human, suffix: -zumab) or even fully human (suffix: -mumab). 3rd-generation mAbs consist of a modified Fc region, chimeric, or humanized. Immunogenicity in theory ranges from higher in 1st-generation mAbs to lower in 2nd- and 3rd-generation ones.[61]

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Table 1: A summary of medicines targeting B cells that have been used in MS

Drug	Type	Target molecule	Clinical trials	Pregnancy	Dosing
Rituximab	Chimeric IgG1	Large extracellular loop of CD20 molecule	Phase 1; Phase 2 (Hermes); Phase 3 (Olympus). *Not FDA-approved*	Last infusion 3.5 months prior to conception	Intravenous dose of 2 × 1000 mg in a 2-week period
Ocrelizumab	Humanized glycosylated IgG1	Large extracellular loop of CD20 molecule	Phase 2; Phase 3 (OPERA I & II, ORATORIO). *FDA-approved*	Last infusion 6 months prior to conception	Intravenous doses of 600 mg twice a year
Ofatumumab	Fully human IgG1	Large & small extracellular loop of CD20 molecule	Phase 2b (MIRROR); Phase 3 (ASCLEPIOS I & II). *FDA-approved*	N/A	20 mg/0.4 mL once per week for the first 3 weeks, once a month thereafter
Ublituximab	Glycoengineered chimeric IgG1	Large extracellular loop of CD20 molecule	Phase 2 (NEDA); Phase 3 (ULTIMATE I & II). *Not yet FDA-approved*	N/A	N/A
MEDI-551	Glycoengineered, humanized, fucozylated IgG1κ	CD19	Phase 1; Phase 2/3 (N-MOmmentum). *FDA-approved*	N/A	Intravenous infusions of 300 mg every 6 months.
Atacicept	Human recombinant fusion protein (Fc IgG + TACI)	BAFF-APRIL	Phase 2 (ATAMS & ANOS). *Failed*	N/A	–
Evobrutinib	Small molecule drug	BTK	Phase 2; Phase 3. Not FDA-approved	N/A	75 mg dose daily

Anti-CD20 mAbs

CD20 is a 33-37kDa transmembrane protein, which spans the membrane four times, thus consisting of two extracellular loops and intracellular C- and N-termini. Although some T cells with CD20 surface expression can be found in all lymphatic organs, are often CD8-positive, can be myelin-specific,[62–64] and may correlate positively with disease severity,[65] CD20 serves a more important role on B cells. The molecule is not expressed throughout the entirety of the B-cell line of differentiation, but only in pre-B cells and mature B cells, with stem cells and the majority of antibody-secreting cells being CD20-negative (Figure 1).[66–68] Physiologically, CD20 plays a key role as regulator of calcium influx in the signaling pathways that lead to B-cell differentiation into antibody-secreting plasma cells[69] and its presence on the surface of most, but not all, B cells makes it an attractive target for monoclonal antibody-based therapy. B cells targeted by anti-CD20 monoclonal antibodies are eliminated via three main mechanisms: programmed cell death / apoptosis, complement-dependent cytotoxicity (CDC), or antibody-dependent cellular cytotoxicity (ADCC) processes.[70] Evidence from animal studies shows that anti-CD20 antibody-mediated B-cell depletion may be incomplete in lymph node germinal centers.[71] Moreover, cerebrospinal fluid B cells seem to be less affected than peripheral B cells by intravenous rituximab (the first anti-CD20 monoclonal antibody) administration, although the drug itself can be detected in a very low concentration (up to 1000 times lower than in the periphery) behind the BBB.[72–75] Of note, the limited access of anti-CD20 mAbs to the CNS due to their relatively high molecular weight could, at least to some extent, be overcome with intrathecal (IT) administration of anti-CD20 mAbs. The four main antibodies evaluated for anti-CD20 MS therapy are analyzed below.

Rituximab

Rituximab is a 1st-generation chimeric monoclonal antibody (IgG1), engineered by fusing a murine Fab with a human Fc domain.[61] Its elimination half-time is estimated at around 20 days;[76] it may, however, vary according to sex, body weight, and renal function.[77] Rituximab depletes B cells via ADCC and CDC and has been found to be extremely effective in patients with RRMS. A landmark 48-week, phase 2, double-blind, placebo-controlled study convincingly highlighted the efficacy of rituximab monotherapy in reducing gadolinium-enhanced lesions in patients with RRMS (n = 104).[78] In addition, a retrospective observational study of 808 patients with RRMS revealed absence of rebound disease activity upon rituximab cessation,[79] whereas rebound activity has been reported with natalizumab[80] and fingolimod cessation.[81,82]

In regard to progressive forms, a phase 3 (n = 439 PPMS patients), double-blind and placebo-controlled trial concluded in 2009 that CD20+ B-cell depletion can slow disease progression in a subgroup of younger patients with PPMS, particularly those with inflammatory lesions (GdELs), as rituximab-treated patients had less increase in T2 lesions and confirmed disease progression was delayed in the subgroup with GdELs.[83] Overall, however, the study was negative. Moreover, a large observational, retrospective study from the Swedish MS registry included 822 patients (557 RRMS, 198 SPMS, and 67 PPMS) and confirmed both rituximab's safety as well as its efficacy in reducing GdELs; GdELs went from 26.2% (pretreatment) to 4.6% in the pooled post-treatment cohort.[84] Interestingly, disability remained constant in RRMS patients but increased in SPMS and more so in PPMS patients. The question of whether disability progression differs in treated and untreated patients was tackled by a retrospective cohort study of 88 SPMS patients. This study resulted in significantly lower Expanded Disability Status Scale scores ($p < 0.001$) and delayed disease progression ($p = 0.02$) in the rituximab-treated group in comparison to the matched control group.[85] It should be noted however that the rituximab-treated group included more patients with radiologic activity, which may have driven the difference between the two groups.

In clinical practice, rituximab is widely used as an off-label treatment for the management of RRMS, as well as active SPMS, as its safety profile is acceptable, well-characterized,[86,87] and the efficacy evident, despite the lack of phase III trials.[88] The drug is

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generally well-tolerated by patients all throughout the MS type spectrum, and the main adverse effects are mild to moderate infusion-related reactions (IRRs), typically with the first dose, as well as mild to moderate infections. No cases of progressive multifocal leukoencephalopathy (PML) due to John Cunningham virus, which is mostly seen with natalizumab treatment,[89,90] have been recorded in MS patients treated with rituximab, and the frequency of PML in non-neurologic patients treated with rituximab seems to range around 1:4000; however, usually these patients have received multiple immunosuppressants.[91,92] Finally, an added advantage of rituximab is its relatively low cost (biosimilars are also available); however, its off-label prescription is complex and time-consuming for physicians. While open questions remain about optimal dosing and frequency strategies, a common tactic is 2 × 500 or 1000 mg, in a 14-day period, and repeat dosing of 500–1000 mg every 6 months or yearly.[61]

Ocrelizumab

Ocrelizumab, an IgG1 immunoglobulin, is a 2nd-generation recombinant humanized anti-CD20 mAb.[93] The drug has a terminal elimination half-time of around 26 days, which is not affected by mild renal or hepatic impairment.[94] Compared to rituximab, ocrelizumab mobilizes *in vitro* lower CDC, but higher ADCC action[95] and as a humanized molecule is expected to be less immunogenic than rituximab with lower titles of neutralizing anti-drug antibodies.[96,97]

In OPERA I (n = 821 patients) and OPERA II (n = 835 patients), two phase 3, double-blind trials published in 2017, ocrelizumab was associated with a lower annualized relapse rate (by 46–47%) and an impressive reduction of the mean number of GdELs (by 94%) over a 96-week time period compared to interferon beta-1a ($p < 0.001$). The drug effectively depleted CD19 B cells (CD19 B cells serve as index of B-cell count in anti-CD20 treatment) within 2 weeks (which is when CD19 cells were measured).[98] ORATORIO, a phase 3, double-blind, placebo-controlled trial, examined ocrelizumab's efficacy in managing PPMS progression. Results from 732 patients revealed that ocrelizumab was associated with lower rates of clinical and MRI progression than placebo. Because in this study the effect was driven by a fraction of PPMS that had evident MRI inflammation, EMA has approved the drug only in inflammatory PPMS, whereas other agencies such as the FDA and Swissmedic have not applied this restriction.[99]

The most common adverse effects of ocrelizumab include mild and manageable IRRs, like pruritus, rashes, throat irritations, and flushing, but their severity and frequency decrease with the number of infusions. Generally, mild to moderate infections occur in 30% of patients, but severe ones are relatively rare. Other adverse events such as extremity pain, diarrhea, and peripheral edema may also occur in rare cases.[100,101]

Ocrelizumab is administered intravenously according to a fixed dosing schedule, as approved based on the phase 3 studies. An initial dose of 600 mg is divided in 2 × 300 mg with a 2-week time interval. Subsequent doses of 600 mg are given in a single infusion once every 6 months.[94] Interestingly, a post hoc analysis from ORATORIO, where patients with lower body weight (and respectively higher ocrelizumab dose per kg) suffered less progression of deficits, prompted a currently ongoing clinical trial that examines the safety and efficacy of higher than standard ocrelizumab doses (1200 mg for body weights <75 kg, or 1800 mg for body weights >75 kg) in PPMS.[102,103]

Ofatumumab

Ofatumumab is a 2nd-generation, fully human IgG1 mAb[104] that depletes circulating CD20 B cells via ADCC[105] and CDC.[106] Two identically designed, double-blind, phase 3 clinical trials, ASCLEPIOS I and II, compared the efficacy of subcutaneously administered ofatumumab to that of oral teriflunomide, the oral pyrimidine synthesis inhibitor. The trials enrolled 1882 patients in total in 1.6 years, and their results indicated a statistically significant advantage of ofatumumab over teriflunomide in suppressing both new relapses and GdEL activity (the latter by 94–97%). Side effects were reported to be mild to moderate and included injection-related reactions, headache, and infections (in 51.6% of patients treated with ofatumumab) such as nasopharyngitis, upper respiratory, and urinary tract infection.[107] Consequently, the FDA approved ofatumumab as a therapy for RRMS, CIS, and active SPMS in the form of an auto-injector pen, while the EMA for relapsing, active MS.[106] Ofatumumab was approved for subcutaneous use at a dose of 20 mg/0.4 mL once per week for the first 3 weeks of treatment and once monthly thereafter.[108]

Ublituximab

Ublituximab is a 3rd-generation anti-CD20 glycoengineered chimeric IgG1 mAb that exerts its action primarily via ADCC, which is facilitated by defucosylation of its Fc region and thereby increased affinity for FcγRIIIa.[61] A 48-week, placebo-controlled, phase 2 trial of ublituximab in 45 RRMS patients established that 150 mg iv on day 1 and 450–600 mg on day 15 and week 24 were able to efficiently deplete B cells within 4 weeks (which is when B cells were measured); moreover, 74% of patients achieved no evidence of disease activity status (NEDA), that is had no relapses, no radiological disease activity, and no progression of disability. Similarly to the other CD20 agents, adverse effects comprised mild to moderate IRRs and upper respiratory infections, influenza, nasopharyngitis, sinusitis, and fungal infections.[109] In follow-up, two double-blind, phase 3 trials [ULTIMATE I (NCT03277261) and II (NCT03277248)] will assess ublituximab's efficacy and safety compared to teriflunomide in 880 patients with RRMS.[110,111]

Anti-CD19 mAbs

CD19 belongs to the Ig superfamily and along with CD21, CD82, and CD225 contributes to the formation of a multimolecular signal-transduction complex that ultimately leads to the activation of PI-3 kinase.[112] Compared to CD20, CD19 is expressed on B cells of earlier developmental stages as well as in more antibody-secreting cells and is thus an appealing therapeutic target (Figure 1).[113] In addition to having a broader expression during B-cell stages of development and differentiation, CD19, unlike CD20, is selectively expressed on B cells and not T cells.[62] A phase 1 study assessing the pharmacokinetic (intravenous and subcutaneous) profile of inebilizumab, a humanized afucosylated IgG1κ anti-CD19 mAb,[114] has been conducted in patients with relapsing MS with positive results,[115] but no phase III trials for MS are currently known to be underway.

Atacicept

Atacicept is a human recombinant fusion protein, consisting of a human IgG Fc portion and the extracellular domain of TACI receptor that binds both BAFF and a proliferation-inducing ligand (APRIL).[116] The drug therefore competes for BAFF and APRIL binding with native TACI, which is both membrane-bound and soluble,[117] as well as, to a lesser extent, with the other receptors

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of the BAFF-APRIL system (BAFF-R and BCMA).[118,119] After improving rheumatoid arthritis and systemic lupus erythematosus (SLE),[120] atacicept was tried in MS.

Subcutaneous atacicept was evaluated in a 36-week, phase 2, double-blind, and placebo-controlled trial in 255 patients with relapsing MS. The trial was prematurely terminated when an increase in inflammatory disease activity was noticed despite immunoglobulin and naïve B-cell decrease, which led to the suspension of every atacicept trial in MS.[119,121,122] Another 36-week, phase 2, double-blind, and placebo-controlled atacicept trial in 34 patients with unilateral optic neuritis as clinical isolated syndrome also showed disease exacerbation, with a significantly higher proportion of patients converting to clinically definite MS compared with placebo.[123] As atacicept effectively depletes naive B cells and induces a transient but marked increase in memory B cells (especially class-switched ones),[117,124,125] possible reasons why atacicept aggravated MS include elimination of regulatory naïve B cells and enhancement of pathogenic memory B-cell function.[126,127]

Belimumab

Belimumab is a human IgG1λ recombinant monoclonal antibody directed against BAFF that prevents BAFF from interacting with its three receptors on the surface of B cells, thereby reducing B-cell survival, differentiation, and antibody production.[128,129] Interestingly, belimumab administration does not result in overt immunosuppression.[130] While being moderately effective and FDA-approved for the treatment of SLE since 2011, it failed in myasthenia gravis,[131] a disease mediated by pathogenic autoantibodies.[132] A phase 2, open-label trial of subcutaneous belimumab in addition to ocrelizumab (standard dose) in 40 patients with RRMS was scheduled to start within 2021.[130]

Evobrutinib

Evobrutinib is a small molecule drug that binds permanently to and deactivates Bruton's Tyrosine Kinase (BTK). BTK is an integral part of the BCR signaling cascade that affects B-cell activation and is essential for B-cell maturation and their ultimate, terminal differentiation into memory or plasma cells. Of interest, BTK is involved in the entry of B cells into follicular structures. Knockout or absence of BTK results in lack of B-cell activation, moreover almost complete lack of peripheral B and plasma cells and low circulating immunoglobulin.[133–136] Importantly, about 75% of the CNS cells that express BTK are microglial, while BTK expression levels in the brain increase after demyelination.[137] As evobrutinib can bypass the BBB and enter the CNS, it can affect microglial cells and B cells within the CNS.

Evobrutinib was the first BTK inhibitor (BTKI) to be tested as a monotherapy in relapsing MS.[138] In a double-blind, phase II trial ($n = 267$), evobrutinib was tested against placebo and dimethyl fumarate. The results showed that patients who received 75 mg of daily evobrutinib had significantly fewer GdELs during weeks 12 through 24 than those who received placebo (1.69 ± 4.69 against 3.85 ± 5.44, $p = 0.005$), while adverse effects were minimal (e.g. nasopharyngitis, alanine aminotransferase, and aspartate aminotransferase level elevation).[139,140] Evobrutinib is now being advanced to phase III evaluation, along with several other BTKI (some of them with reversible BTK binding); fenebrutinib, ibrutinib, and tolebrutinib.[141]

While CD19/20 B-cell depletion has shown tremendous efficacy in reducing clinical and radiological MS activity, it raises several safety concerns on humoral deficiency with long-term usage in addition to a reduced response to vaccination.[86,142,143] These disadvantages could possibly be avoided with inhibition of B-cell activation and maturation with small molecules such as BTKIs.[136,144] In contrast with antibody-based B-cell depletion, BTKIs do not destroy or lastingly minimize the frequency of peripheral B cells, but seem to prevent the development of pathogenic B cells.[145] Their effect on disease activity does not seem to be as impressive as that of anti-B-cell antibodies, and they cannot control the pathogenic properties of B cells as rapidly; however, they are smaller in size, can penetrate the CNS, target microglia, and might therefore have a better effect on disability progression.[146]

B-Cell-Targeted Therapies and Pregnancy

As MS largely affects female patients with childbearing potential, the utilization of B-cell-targeted therapies in women of childbearing age deserves special mention.[147,148] Rituximab-associated B-cell depletion persists long after the drug's elimination, which occurs approximately 3 months after the last infusion. Thus, conception can be considered safe 3 months after the last infusion without significant risk of fetal exposure. But even if a woman conceives before rituximab's effective elimination, IgG1 subclass mAbs cannot cross the placenta barrier during the first trimester, resulting in low chance of fetal exposure.[149] Importantly, rituximab administration and concurrent B-cell depletion have not been linked to increased risk of adverse pregnancy outcomes compared with the expected incidence in population.[150] Also, infants breastfed under anti-CD20 treatment had normal B-cell counts, and no negative impact on health and development was attributed to breastfeeding in the 1-year follow-up period.[151] Although data regarding ocrelizumab administration in this population group are limited, it is reasonable to apply the same principles as with rituximab. One additional advantage of CD20 depletion in terms of family planning is that discontinuation of therapy is not associated with a rebound phenomenon, as has been observed with natalizumab. In that regard, a cohort study regarding the safety of anti-C20 mAbs rituximab and ocrelizumab during the last 12 months before or during pregnancy concluded that the drugs are effective in controlling disease in women with RRMS, during and partly after pregnancy. However, B-cell monitoring is essential both for the newborn and for the mother after delivery, and larger studies are required to assess their safety profile and to establish the best time to restart the therapy after delivery.[152] Recent recommendations suggest prioritization of MS management and conception postponement in cases of highly active MS and contraception for up to 4 months after ocrelizumab administration.[153]

Conclusion

The therapeutic criterion underlines that B cells not only participate in the pathogenesis of MS but can act as the orchestrators of the inflammatory processes. As shown by clinical trials and real-world data, B-cell-targeting agents (in particular CD20-depleting agents) have established a new era in MS therapeutics and immunotherapy in general, considering their remarkable efficacy and safety profile. Long-term safety, especially increased risk of infection with slowly but gradually decreasing total serum immunoglobulin levels, remains a significant concern that has a limiting effect on anti-CD20 usage in clinical practice. Regular monitoring of immunoglobulin levels (e.g. before each follow-up infusion) can help timely detection of a decrease and lowers the risk of infection due to associated immunodeficiency.[154–156]

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Future studies will further inform on long-term effects of CD20-targeting medications, on the use of oral BTKI agents and determine the new therapeutic algorithm that will likely move more towards induction rather than escalation.

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