Neurotherapeutics (2013) 10:34–43
DOI 10.1007/s13311-012-0164-3

REVIEW

Humoral-Targeted Immunotherapies in Multiple Sclerosis

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Published online: 4 December 2012
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Summary The continuous improvements of our understanding of the pathophysiological changes that occur in multiple sclerosis (MS) have translated into many novel therapeutic agents at different stages of development. These agents target more specifically the innate or the adaptive immune response. We will review agents available or under development that target the humoral pathways of the adaptive immune response. As such, humoral targeted immunotherapies that are being developed for MS are discussed herein: rituximab, ocrelizumab, and ofatumumab show promise as B-cell depleting agents. Other agents, such as atacicept were suspended during development in MS due to increased inflammatory activity *versus* the placebo. Although most agents were tested in relapsing-remitting forms of MS, rituximab and ocrelizumab have both been studied in progressive MS, whereas ocrelizumab only is currently moving forward in primary progressive MS trials. We provide an overview of agents available and under development that target the humoral response and include their mechanisms of action, safety profiles, and results of clinical trials.

Keywords Novel therapeutics · B-cell · Monoclonal antibodies · Humoral therapies · Relapsing-remitting · Progressive

Introduction

Genetic, epidemiologic, and pathologic studies support the hypothesis that the neurologic manifestations of multiple sclerosis (MS) arise, at least in part, from immune-mediated demyelination [1, 2]. Advances in the field of basic immunology, along with accumulating results from clinical trials targeting B cells in MS and other autoimmune diseases, rejuvenated interest in antibody-dependent and antibody-independent B-cell role in MS and its animal models.

The contributions of B-lineage cells and their secreted products to central nervous system (CNS) inflammatory diseases are thought to occur beyond their differentiation into plasmacytes and their ability to produce antibodies. They also function as antigen-presenting cells, contribute to T-cell activation and produce effector cytokines that are considered modulators of the local immune environment. Recent evidence in MS also suggests a role in formation and maintenance of new lymphoid foci within the CNS [3].

The identification of chronically activated B-cells in the meninges of patients with MS further points to the potential for B-lineage cells chronically residing in the CNS to act as antigen-presenting cells for T-cells and may contribute to the propagation of local disease-relevant immune responses [4, 5]. The presence of isolated cerebrospinal fluid (CSF) oligoclonal bands (OCBs) and increased intrathecal immunoglobulin (Ig) IgG synthesis compared to serum in MS suggests plasmacyte activation to specific antigens within the CNS [6]. The presence of OCBs, increased free light chains, and increased intrathecal IgM synthesis in MS CSF have been reported to correlate with more aggressive forms of MS and worse outcomes in a few studies [7, 8]. Of note, rituximab trials showed that depleting peripheral B cells was not associated with changes in CSF IgG levels, IgG index, or OCB pattern [9, 10].

B cells also influence the immune response through expression of distinct profiles of accessory molecules and/or production of an array of effector cytokines, including immune regulatory cytokines (such as interleukin [IL]-10), polarizing cytokines (such as IL-4), and lymphoid tissue-organizing cytokines (such as tumor necrosis factor [TNF]α and Leukotrienes) [11, 12]. In earlier experimental autoimmune encephalomyelitis studies of B-cell depletion, animals

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depleted of B cells failed to remit [13], an effect that was attributed to the role of IL- 10 from B cells in regulating the expression of the autoimmune disease [14].

The regulatory B-cell subsets (Bregs), described in both animals and humans, delineate the importance of B-cell subsets that could either induce or inhibit immune responses, and account for the variable effects that targeting B cells may have *in vivo* [15]. Several abnormalities in B-cell cytokine regulation, including impaired capacity to produce the down-regulatory cytokine IL-10 [11], as well as the tendency to produce the pro-inflammatory cytokines TNFα and LT [16], have been described in patients with MS. The latter has been suggested to contribute to abnormal "bystander" T-cell activation in patients with MS, providing a conceivable mechanism of action to explain why B-cell depletion, with consequent decreases in T-cell activation (effects that may be relevant both in the periphery and in the CNS), results in diminished new MS activity [16]. Furthermore, depleting B cells resulted in decreased numbers of T cells in the CSF of treated patients [9, 10]. Another important B-cell function emerged as they contribute to the formation and maintenance of new lymphoid follicles. These follicle-like structures of chronically activated B cells are found in the meninges of MS patients where ectopic germinal centers reside [4].

Herein, we provide an overview of treatments targeting the humoral response in MS, with specific focus on recent clinical trials of B-cell-depleting agents. Among these agents, a majority of monoclonal antibodies with various specificities has emerged.

Monoclonal antibodies (MABs) are produced from an immortalized unique murine clonal cell line [17]. MABs can be divided into 3 main groups: 1) those that inhibit processes involved in MS progression, such as leukocyte migration into the CNS, such as natalizumab, 2) those that are cytolytic such as rituximab, ocrelizumab, ofatumumab, and alemtuzumab, and 3) a group of MABs and recombinant proteins that target cytokines, chemokines, complement, and their receptors such as daclizumab, ustekinumab, atacicept, tabalumab, eculizumab, and secukinumab [18]. There are numerous available MABs that are currently Food and Drug Administration (FDA) approved for the treatment of various autoimmune diseases and lymphomas. Natalizumab is the only FDA-approved MAB for MS treatment. Several others are in different stages of development for MS. Daclizumab, natalizumab, and alemtuzumab are described in detail in chapters 6, 8, and 10, respectively, and will not be addressed in this chapter.

Initial use of murine MABs in MS patients was dampened by the development of antibodies against the murine protein, especially when used repeatedly, thereby limiting their potential in MS [19]. To decrease MAB immunogenicity, chimeric antibodies were made by cloning the murine antigen-binding domains onto a human IgG framework [20]. Chimeric antibodies were further refined by cloning the complementary determining regions into a human variable chain backbone, which rendered them less immunogenic.

Rituximab

Rituximab is a glycosylated IgG1 chimeric MAB directed against CD20, a cell surface antigen expressed on pre-B cells and B cells, but not on stem cells or fully differentiated plasma cells [21]. The Fab domain of rituximab binds to the CD20 antigen on B lymphocytes and the Fc domain recruits immune effector cells that result in B-cell death. Rituximab depletes B cells by antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity, and by inducing apoptosis through cross-linking membrane CD20 [22]. Another recent hypothesis is that binding of rituximab IgG molecules to B cells could potentially generate decoy sacrificial immune complexes that attract and bind Fc gamma receptor effector cells, and therefore decrease recruitment of effector cells and reduce inflammation and tissue damage [23]. It has been reported that B-cell depletion in relapsing-remitting multiple sclerosis (RRMS) reduces proliferation and pro-inflammatory cytokines (Th1 and Th17) responses of both CD4+ and CD8+ T cells [16].

Rituximab is FDA approved for treatment of follicular non-Hodgkin lymphomas, chronic lymphocytic leukemias, and refractory rheumatoid arthritis, and in combination with steroids for Wegener's granulomatosis and microscopic polyangiitis.

Open-Label Studies of Rituximab

The first study in MS was an investigator-initiated open-label trial of rituximab (given as 4 weekly infusions of 375 mg/m^2) as add-on therapy in 30 patients with RRMS who had evidence of breakthrough disease despite standard treatment with either interferon β-1a (IFNB)-1a or glatiramer acetate (GA), with at least 1 gadolinium-enhancing (Gd+) lesion at baseline [24]. The primary endpoint was change in number of Gd+ lesions on 3 post-treatment *versus* 3 pre-treatment brain magnetic resonance imaging (MRI) scans. Treatment with rituximab was associated with an 88 % reduction in the mean number of Gd+ lesions at weeks 12, 16, and 20 after treatment compared to pre-treatment ($p<0.0001$). Twenty-five of 30 participants had a reduction of 50 % or more in the number of Gd+ lesions. Annualized relapse rate (ARR) during the 52-week study was 0.23 [24].

In another open-label study, 26 patients with RRMS were enrolled in a 72-week multicenter, re-treatment trial with rituximab [25]. Subjects received 2 courses of rituximab 6 months apart. Each course consisted of 2 doses of 1 g, administered 2 weeks apart from one another [25].

The primary outcome measure was the safety of rituximab, as determined by adverse events (AEs) and the total number of Gd+ lesions to assess safety during the 72-week trial. Secondary clinical outcome measures included number of relapses per patient during the study and MRI change from baseline in the total number of Gd+ and new T2 lesions, and cumulative volume of T2 brain lesions [25].

Phase II Placebo-Controlled Trial of Rituximab in RRMS

A 48-week, randomized, placebo-controlled, multicenter trial of rituximab in RRMS enrolled 104 participants [26]. The patients were randomly assigned in a 2:1 ratio to receive rituximab or placebo. They were stratified with respect to previous treatment with IFNB-1A or glatiramer acetate (either no treatment or discontinuation of medication >6 months previously *versus* treatment within the previous 6 months), and baseline disability according to the EDSS score (≤2.5 *vs* >2.5). Patients received rituximab 1 g twice 2 weeks apart (n=69), or a placebo (n=35) at week 0 and week 2 and no repeated infusions thereafter. Baseline demographics, clinical characteristics, and use of prior disease-modifying therapies were overall well-balanced between treatment groups. However, at baseline, the proportion of participants without Gd+ lesions was greater in the placebo than the rituximab group (85.7 % *vs* 63.8 %, respectively), which was possibly biased against finding a treatment effect [26].

Of the 104 participants, 96 (92.3 %) completed 24 weeks, and 79 (76 %) completed 48 weeks, including 84.1 % in the rituximab group and 60 % in the placebo group. The primary endpoint was the sum of the number of Gd+ lesions on serial MRI brain scans at weeks 12, 16, 20, and 24. Secondary outcome measures were the proportion of patients with relapses; the ARR; the total number of new Gd+ lesions observed on serial MRI brain scans at weeks 12, 16, 20, and 24 and the change of T2 lesion volume from baseline [26].

Rituximab-treated participants demonstrated a significant reduction in total Gd+ lesion counts at weeks 12, 16, 20, and 24 when compared to placebo recipients (intent-to-treat analysis; $p<0.001$). During the first 24 weeks, those receiving rituximab had a mean total Gd+ lesion count of 0.5, compared with 5.5 in those receiving placebo, a relative reduction of 91 %. Beginning at week 12, rituximab significantly reduced the number of Gd+ lesions at each subsequent study visit compared with placebo ($p=0.003$ to <0.001). Rituximab-treated patients also showed a significant reduction in the volume of T2-lesions detected on MRI from baseline to week 24 ($p=0.008$) and from baseline to week 36 ($p=0.004$) when compared to patients who received placebo [26].

Clinical relapses were significantly reduced in the rituximab group compared with placebo at week 24 (14.5 % *vs* 34.3 %; $p=0.02$) and week 48 (20.3 % *vs* 40.0 %; $p=0.04$). Placebo recipients were at more than double the risk (with a relative risk=2.31) at week 24 and 1.90 at week 48. ARR was lower in the rituximab-treated participants compared with placebo at 24 weeks (0.37 *vs* 0.84; $p=0.04$), and 48 weeks (0.37 *vs* 0.72; $p=0.09$) [26].

Phase II/III Trial of Rituximab in Primary Progressive MS

None of several clinical trials in progressive MS has reported a benefit of the numerous tested promising agents. A 2-year phase II/III multicenter randomized double-blind trial tested the effect of rituximab *versus* placebo in participants with primary progressive multiple sclerosis (PPMS) [27]. The patients were randomized 2:1 to rituximab and a placebo, and hierarchically stratified similar to the randomized control trial previously described in RRMS with regard to prior therapy, but divided into different EDSS scores of ≤4.0 *versus* ≥4.0. There were 439 participants who received either 2 infusions of 1000 mg of rituximab or 2 placebo infusions (separated by 2 weeks) intravenously, every 24 weeks for a total of 4 treatment courses through 96 weeks [27].

The mean baseline EDSS score was 4.8 (more than 50 % of the participants had an EDSS score of 4.0 or more). Half of the participants were males. Median age at baseline was 51 years. There were 84.4 % and 82.5 % of the participants who completed 96 weeks, respectively, in the placebo and rituximab groups [27].

The primary efficacy endpoint in this study was the time-to-confirmed disease progression (CDP) defined by an increase of at least 1.0 point from baseline if the EDSS score was between 2.0 and 5.5 inclusive or an increase by at least 0.5 point if the EDSS score was less than 5.5 sustained for 12 weeks. The difference in time to CDP between treatment arms was not statistically significant ($p=0.144$, stratified log-rank test). Kaplan-Meier estimates for the proportion of participants with CDP at 96 weeks were 38.5 % for the placebo and 30.2 % for rituximab (a 24 % reduction in the risk of progression in rituximab recipients). Stratified hazard ratio was 0.77. For secondary imaging endpoints, rituximab-treated patients had significantly less T2-lesion volume increase than the placebo, with a median increase of 302.95 mm^3 for rituximab-treated participants *versus*

809.50 mm^3 for placebo recipients ($p<0.001$). Brain volume change was similar in both groups ($p=0.62$) [27].

Compared with placebo, rituximab-treated participants had less worsening in the timed 25-foot walk at weeks 48 ($p=0.04$), 96 ($p=0.076$), and 122 ($p=0.015$). Other MS functional composite items showed no difference between the groups. Pre-planned subgroup analysis showed that rituximab-treated participants who were less than 51 years of age (hazard ratio, 0.52; $p=0.01$) or participants who had Gd + lesions at baseline (hazard ratio, 0.41; $p=0.007$) were less likely to experience CDP compared with the placebo. No gender effect was noted [27]. Table 1 summarizes both randomized clinical trials with rituximab.

Clinical Safety and Tolerability of Rituximab

Data on rituximab clinical safety is available from initial [28] and subsequent trials in lymphomas [29–31], which confirmed the previously described trials. The most common side effects of rituximab are infusion-related reactions that present as fever, chills, rigors, hypotension, and general flu-like symptoms. These typically occur during the first infusion, with the majority classified as mild or moderate, and typically abating in both frequency and intensity with repeated infusions. Clinical trials in rheumatoid arthritis have shown that these common infusion-related reactions can be decreased by premedication with intravenous glucocorticoids prior to the start of rituximab infusion [32]. If needed, starting the infusion at 50 mg/hour and slowing the rate of infusion during several hours may be helpful as well. The rate can be increased by 50 mg/h every half hour, if well tolerated. Rarely, severe or even fatal infusion-related reactions have occurred in lymphoma patients. Rituximab did not appear to otherwise increase the rate of serious infections in most studies [28–30]. Nevertheless, cases of progressive multifocal leukoencephalopathy have been reported in patients receiving rituximab for lymphoma, rheumatoid arthritis (RA), and lupus, who were receiving concomitant immunosuppressive drugs. No progressive multifocal leukoencephalopathy case has been reported so far in patients with MS, or in patients, with other conditions receiving rituximab as a monotherapy, who had not been exposed to prior immunosuppression.

Safety in MS Trials

All 3 trials of rituximab monotherapy in RRMS and PPMS showed concordance with a side effect profile similar to that previously reported in RA and other autoimmune disorders. Infusion-associated reactions were the most common accounting for ≥10 % of drug-related AEs in MS patients receiving rituximab. These infusion-associated reactions are known to be associated with cytokine release syndrome during B-cell lysis [24–27, 31]. More than 90 % of AEs occurring with the first infusion were mild or moderate, whereas 7 % were grade 3 and none were grade 4. There was no difference in infusion-related AEs in rituximab-treated patients when compared to a placebo with successive infusions. In the phase II trial in RRMS [26], serious AEs were reported in 3 rituximab recipients: 1) ischemic coronary artery disorder (n=1), 2) malignant thyroid neoplasm (n=1), and 3) acute and progressive MS symptoms (n=1). Severe AEs were reported in 14.3 % of placebo and 13 % of rituximab recipients. A total of 5.7 % placebo participants and 4.3 % of rituximab recipients withdrew from the study due to AEs. The incidence of any infections was similar in the placebo (71.4 %) and rituximab (69.6 %) groups. Nasopharyngitis, upper respiratory tract infections, urinary tract infections, and sinusitis were the most common infections (≥10 %) in rituximab recipients. Urinary tract infections (14.5 % rituximab *vs* 8.6 % placebo) and sinusitis (13.0 % *vs* 8.6 %) were more common in the rituximab recipients. No opportunistic infections were reported. The RRMS trial was short (48 weeks), and as such is not informative for long-term safety.

A longer phase II/III trial in PPMS conducted for a 96-week duration showed infection-related serious AEs to be

Table 1 Summary of rituximab randomized control trials in MS*

Study	Number of patients enrolled	Dose	Primary outcome measures	Secondary outcome measures	Results
HERMES (double blinded, 48 weeks)	104 (69 Drug: 35 placebo) RRMS patients	1g 2 doses *vs* placebo	Summation of all Gd+ lesions	ARR, new Gd+ lesions, change in T2-lesion volume from baseline	Total and new Gd+ lesions, ARR, T2-lesion volume
OLYMPUS (96 weeks)	439 (292 Drug: 147 placebo) PPMS patients	1g 2 doses every 24 weeks *vs* placebo	Time to CDP	Change in T2 volume from baseline, change in brain volume	No difference in time to CDP; some benefit to patients <51 years with baseline Gd+ lesions

ARR=annualized relapse rate; CDP=confirmed disease progression; Gd+=gadolinium enhancing; MS=multiple sclerosis; PPMS=primary progressive multiple sclerosis; RCT=randomized control trials; RRMS=relapsing-remitting multiple sclerosis

*See Hauser et al. [26] and Hawker et al. [27]

higher in the rituximab group than in the placebo group (4.5 % *vs* <1.0 %). The majority of these infection-related serious AEs occurred in patients aged ≥55 years. Three participants died during the trial (1 from each group with pneumonia, and 1 from the placebo group) due to cardiopulmonary failure [27]. There was no evidence for increased incidence of infection or other AE in participants with immunoglobulin levels below the lower limit of normal, although this group is probably too small to be conclusive. Trials are thus limited in how they inform us as to potential severe AEs occurring with long-term use of rituximab, especially in older patients.

Anti-Chimeric Antibodies

Some patients developed human anti-chimeric antibodies (HACA) in response to rituximab. The assay used for measuring these antibodies may not have been optimal in terms of sensitivity and consistency. In the phase II RRMS trial, HACA to rituximab were present in 16 of 65 participants (24.6 %) at week 48 and in none who received the placebo [26]. In the PPMS study, HACA to rituximab was present in 20 of 286 (7 %) rituximab recipients and in 9 of 143 (6.3 %) of placebo recipients at some point during treatment or safety follow-up [27]. There was no apparent association between HACA positivity and the type or severity of AEs or efficacy response at weeks 24, 36, or 48, but the study may not have been designed to answer this specific question [26].

Pharmacodynamics of Rituximab in MS Trials

The clinical trials of rituximab in MS and other disorders offered ample data on the pharmacodynamics of rituximab [24–27, 31]. Because rituximab depletes CD20+ B cells, CD 19 expression was used as a measure of circulating B cells. Peripheral B cells were depleted by more than 95 % from baseline within 2 weeks of rituximab infusions. After the first course of rituximab (2 doses), very low levels of B cells were maintained through to 24 weeks. At week 48, CD19 cells returned to 30.7 % by week 48 and 34.5 % by week 72 of their baseline values [25, 26]. As expected, rituximab treatment rapidly depleted circulating B cells, followed months later by preferential reconstitution of naive B cells, in both RRMS clinical trials. The reconstituted B-cells were mostly CD19+ CD27- naïve B cells (mean, 51 % of baseline) rather than CD19+, CD27+, memory B cells (mean, 14 % of baseline) implying that naïve B cells recover faster than memory B cells. This raises the possibility that rituximab therapy could, to some extent, "reset" the immune response. Absolute counts of CD3+ T-cell subsets (CD4 and CD8), CD14+ monocytes, or CD3-/CD56+ NK cells were not appreciably altered by rituximab in the circulation of treated patients in RRMS cohorts [25], but more recent data at our center indicates a transient 25 % decrease in CD4 and CD8+ T cells during the first 3 months after the first round of rituximab therapy. The recovery of peripheral B cells in patients with MS was variable and showed no association with return of disease activity. This dissociation was also noted in RA trials [25, 26, 33].

It is important to recognize that the doses used in clinical trials are based on treatment with maximum tolerated doses to reach the warranted outcome. Recent evidence in the biochemical response of rituximab, ofatumumab, and alemtuzumab in chronic lymphocytic leukemia indicates that this may not necessarily be the most appropriate approach [34, 35]. The *in vitro* experiments with ofatumumab and rituximab suggest that host effector mechanisms that support CD-20 monoclonal mediated lysis can be saturated at high B-cell burdens. Only a fraction of available complement was required to kill cells with CD20 monoclonal antibodies, and this could be tuned by titrating the concentration of the MABs. Consequently, maximal B-cell killing was achieved with intermediate MAB concentrations, whereas high concentrations promoted lower overall killing. Therefore, MAB therapies that rely substantially on effector mechanisms are subject to exhaustion, including complement. As such, it is possible that the effectiveness of these drugs may benefit from lower, more frequent dosing schemes optimized to sustain and maximize killing by cytotoxic immune effector systems. An open-label investigation with a single low dose of 100 mg infusion of rituximab adequately depleted peripheral B cells for at least 6 weeks in 12 patients with RRMS [36]. This data suggests the need for more trials to identify the minimally effective dose in MS.

Ocrelizumab

Ocrelizumab is a novel humanized MAB against CD20 constructed with recombinant DNA techniques and is designed to selectively target CD20+ B cells. Compared to rituximab, ocrelizumab binds to a different but overlapping epitope of the extracellular domain of CD20 [37]. As compared to rituximab, ocrelizumab is associated with increased antibody-dependent cell-mediated cytotoxicity and reduced complement-dependent cytotoxic effects *in vitro*, which is postulated to make ocrelizumab a more effective drug by modulating tissue-dependent mechanisms of pathogenic response [38].

Ocrelizumab was first tested in MS in a phase II randomized, placebo-controlled, multicenter trial, summarized in Table 2 [38]. This trial enrolled 220 patients with RRMS who had 2 or more relapses within 3 years before screening, 1 of which had to be within the year prior to enrollment. Other inclusion criteria included baseline EDSS between 1.0 and 6.0 and evidence of active inflammation noted by 6 or more T2 lesions on MRI or 2 relapses in the year prior to screening [38].

Table 2 Summary of ocrelizumab trials in RRMS*

Study	Patients enrolled	Dose	Primary outcomes	Secondary outcomes	Results
(96 Weeks, data available from first 48 weeks)	220 RRMS	Group 1: 600 mg; group 2: 2000 mg; groups 1 and 2: dose every 24 weeks; group 3: placebo; group 4: IFNB-1a; groups 3 and 4: drug on week 24	Effect on total Gd+ lesions	ARR, proportion of relapse-free patients; total new Gd+ lesions; change in T2 volume, safety, and tolerability	Group 1 (77 %) and group 2 (88 %) had no new Gd+ lesions, overall decrease in ARR; no change in total Gd+ lesions and T2-lesion volumes between groups

ARR=annualized relapse rate; Gd+=gadolinium enhancing; IFNB-1a=interferon β-1a; PPMS=primary progressive multiple sclerosis; RCT: Randomized control trials; RRMS=relapsing-remitting multiple sclerosis

*See Kappos et al. [38]

Eligible patients were randomized to 1:1:1:1 in 1 of the following groups: placebo (n=54), low-dose ocrelizumab 600 mg (n=55), high-dose ocrelizumab 2000 mg (n=55), and open-label intramuscular IFNB-1a (n=54). Patients received 4 treatment cycles (first cycle, days 1 and 15, subsequently at weeks 24, 48, and 72). Patients in the placebo and IFNB groups were offered ocrelizumab after 24 weeks. Brain MRIs (with and without Gd) were done at baseline and every 4 weeks thereafter until week 24 [38].

The primary endpoint was the effect of ocrelizumab on the total number of Gd + lesions observed on brain MRI scans for weeks 12, 16, 20, and 24 *versus* the placebo. Key secondary endpoints included the ARR; proportion of relapse-free patients; total number of Gd + T1 lesions; total number of new Gd + lesions; change in total volume of T2 lesions from baseline to week 24; safety and tolerability of 2 dose regimens of ocrelizumab; and safety of ocrelizumab therapy up to 96 weeks. Data are available from the first 48 weeks [38].

Baseline characteristics were similar in all treatment groups. There was a highly significant difference in both ocrelizumab groups ($p<0.0001$) for total number of Gd+ lesions from weeks 12 to 24 *versus* the placebo. The relative reductions were 89 % (95 % confidence interval [CI], 68-97) for the 600 mg ocrelizumab group, and 96 % (95 % CI, 89-99) for the 2000 mg group compared with the placebo. There were 77 % (600 mg) and 82.7 % (2000 mg) of the ocrelizumab groups that remained free of Gd+ lesions, which was more than in the placebo (35 %) and IFNB-1a (48 %) groups. The total number of Gd+ lesions (both new and persistent) was also lower for both ocrelizumab groups ($p<0.0001$) compared to the placebo. There was no difference between groups at week 24 in total volume of T2 lesions. Compared with a placebo, ARR was given for a duration of 24 weeks resulted in 80 % (95 % CI, 45-99) lower in the 600 mg ocrelizumab group, and 73 % (95 % CI, 29-97) lower in the 2000 mg group. Patients in the placebo and IFNB-1a groups also experienced a dramatic decrease in disease activity after 1 treatment cycle with ocrelizumab. There was no clear dose separation in the intention-to-treat population, although the estimated mean ARR from week 24 to week 48 was lower in the 600 mg group (0.09) (95 % CI, 0.04-0.20) *versus* the 2000 mg group (0.28) (95 % CI, 0.17-0.47) [38]

Clinical Safety and Tolerability

In the phase 3 rheumatoid arthritis trials of ocrelizumab, higher rates of serious and opportunistic infections were seen with ocrelizumab treatment combined with methotrexate *versus* methotrexate alone, especially in patients recruited in Asia on the higher dose (2×500 mg/ 6 months). The reason for this is not clearly understood [20]. Some of these serious infections resulted in death. Even though initial trials in RA were promising [37], the manufacturing companies, Biogen Idec (Weston, MA) and Roche (Basel, Switzerland) on its development in RA due to a lack of improvement in the efficacy and safety of ocrelizumab compared with rituximab in RA and lupus nephritis [39].

Patients enrolled in MS trials, however, were younger and healthier, and were screened for any high risk of infections at baseline. These patients were also not placed on any concomitant immunosuppressant or immune-modulating therapy. Both doses of ocrelizumab were overall well-tolerated. Infusion-related events occurred in patients receiving 2,000 mg (44 %) (95 %, CI, 31-57) and 600 mg (35 %) (95 % CI 22-47) of the first infusion of ocrelizumab compared with a placebo (9 %) (95 % CI, 2-17), which indicated a dose-related event. Infusion-related reactions decreased to rates comparable to the placebo in the second part of the dual infusion. Rates of serious infection-related events were similar for patients receiving either 600 mg (3.4/100 patient-years; 95 % CI, 1.3-9.0) or 2,000 mg (3.5/100 patient-years; 95 % CI, 0.9-14) of ocrelizumab, or a placebo (3.8/100 patient-years; 95 % CI, 0.527). There were no opportunistic infections reported within the first 48 weeks [38].

One patient had a serious adverse event in the 600 mg ocrelizumab group (2 %) (95 % CI, 1.3-2.3) as well as 3 (6 %) (95 % CI, 4.6-6.3) in the 2,000 mg group, 4 (4 %)

(95 % CI, 3.0-4.4) in the placebo group, and 2 (4 %) (95 % CI, 3.0-4.4) in the IFNB-1a group. Serious infections occurred at similar rates in ocrelizumab and placebo recipients. However, there was 1 reported death in a 41-year-old woman in the 2,000 mg group who died in week 14. She had a 10-year history of MS, previously treated with IFNB, and had an inconspicuous course in the trial until week 12. She developed systemic inflammatory reaction syndrome that resulted in multi-organ failure, brain edema, and herniation that lead to her death [38].

Immune Response to Ocrelizumab

As a humanized MAB, ocrelizumab is expected to have lower immunogenicity than chimeric MAB. This may explain the milder infusion-related reactions as compared with rituximab. Yet, a number of patients still developed human antihuman antibodies. It was intriguing to see that at baseline human antihuman antibodies were found in 1 patient in the placebo group and in 1 patient in the 600 mg ocrelizumab group. Baseline human antihuman antibodies were not checked in the interferon group. No patient became seropositive with subsequent treatments. None of the patients in the 2000 mg group developed antibodies [38].

CD19+ peripheral B cells were rapidly and completely depleted in the recipients of ocrelizumab. By week 2 after injection, B-cell counts were reduced by 99.0 % and 99.2 % for both ocrelizumab groups. This persisted until week 24. From weeks 24 to 48, there was no difference in the rate of adverse events across the treatment groups. Serious adverse events had similar rates (1 in the placebo group; 1 and 2 in the 600 mg and 2000 mg ocrelizumab groups, respectively; and 3 in the IFNB-1a group) [38].

Ongoing Trials with Ocrelizumab

Two phase 3 trials of ocrelizumab are currently recruiting patients. The first is a randomized, double-blind, parallel group study to evaluate the efficacy and safety of ocrelizumab in comparison with IFNB-1a in patients with RRMS [40, 41]. The other trial is a randomized, double-blind, parallel group study to evaluate the efficacy and safety of ocrelizumab in comparison with placebo in patients with PPMS [42, 43].

Ofatumumab

Ofatumumab is a human recombinant anti-CD20 antibody. It was FDA-approved in October 2009 for the treatment of chronic lymphocytic leukemia refractory to other treatments. Ofatumumab binds to an epitope different from rituximab and most other anti-CD20 antibodies. It acts more via complement-dependent cytotoxicity than antibody-dependent cell-mediated cytotoxicity when compared to rituximab and ocrelizumab [44]. Ofatumumab demonstrated clinical efficacy in a phase 2 trial in RA patients (n=20) without an increased risk of opportunistic infections. Phase 3 trials are underway in RA [45].

Results of a phase 2 multicenter randomized, double-blind, placebo-controlled trial of ofatumumab in MS was presented in 2010 [46]. There were 38 patients (followed for 24 weeks) who were randomized 2:1 to increasing doses of ofatumumab of 100 mg intravenously (n=8), 300 mg (n=11), or 700 mg (n=7) at weeks 0 and 2 *versus* a placebo (n=12). MRI scans were obtained 1 month prior to enrollment, at baseline, and monthly for 24 weeks thereafter. The mean (SD) cumulative number of Gd + lesions from weeks 8 to 24 after treatment was 0.04 (0.20) in the combined ofatumumab group and 9.69 (24.86) in the placebo group. The estimated relative reduction in the number of Gd+lesion was 99.8 % (90 % CI, 94.7-100.0) ($p<0.001$). At week 24, a dose-dependent B-cell repletion was seen with a mean CD19+ B-cell count reduced by 78 %, 95 %, and 98 % in the 100, 300, and 700 mg groups, respectively. This 24-week study indicated no dose limiting toxicities and no unexpected findings. As with other MABs, ofatumumab can result in infusion-related reactions [46]. Another trial is underway with subcutaneous ofatumumab in RRMS [47].

Eculizumab

Eculizumab is an MAB that targets the complement protein C5 and prevents its cleavage [48]. Protein C5 is the step at which 3 pathways of complement activation converge, and therefore its inhibition interrupts the inflammatory cascade by interfering with formation of anaphylotoxin C5a and formation of cell lysis through C5b-9 [49]. The selective role of targeting C5 preserves the early complement components of C3-mediated activity that is essential for clearance of pathogens and immune complexes [49].

In neuromyelitis optica (NMO)-IgG targets aquaporin-4 channels resulting in selective pathology at cell membranes expressing aquaporin-4 [50, 51]. NMO-IgG binding to aquaporin-4 results in complement-dependent cytotoxicity that in turn launches an inflammatory cascade involving cytokine release, leukocyte infiltration, microglial activation, and myelin loss [51, 52].

Eculizumab is approved for the treatment of paroxysmal nocturnal hematuria and has been studied in atypical cases of hemolytic uremic syndrome [53–57]. There is evidence that severity of attacks is related to complement-mediated cell injury in sera of patients with NMO [58]. This led to an open-label study of eculizumab in NMO [59]. Fourteen

subjects received eculizumab at a dose of 600 mg each week for 4 weeks, then 900 mg at the fifth week, and then 900 mg every 2 weeks for 48 weeks by intravenous infusion. Primary outcome measures include reduction of median ARR in NMO as compared to relapse rate prior to starting therapy, and safety in NMO patients. Secondary outcome measures include pharmacokinetics of the drug in the blood and CSF, as well as improvement of quality of life, visual function, and walking time. The study was completed, but data at the time of preparation of this chapter were not published [60].

Eculizumab has a black box warning for increased incidence of meningococcal infection with its use. A meningococcal vaccine is recommended 2 weeks prior to starting therapy, even though it is not entirely preventive as patients in trials who were vaccinated still developed meningococcal meningitis, but they were successfully treated. In patients with paroxysmal nocturnal hematuria most common eculizumab-related side effects included headache, nasopharyngitis, back pain, and nausea. Patients with hemolytic uremic syndrome reported hypertension, upper respiratory tract infection, diarrhea, headache, anemia, vomiting, nausea, urinary tract infection, and leucopenia [60, 61].

Atacicept

Many growth factors and interleukins that promote B-cell differentiation and survival have been identified within MS lesions, including TNF-α, interleukin (IL)-1, IL-2, IL-4, IL-6, and IL-10 [62]. Two important members of the TNF-α family are BLyS (B-lymphocyte stimulator), also called the B-cell activating factor of TNF family (BAFF) and a proliferation-inducing ligand (APRIL); CD256. BAFF and APRIL were recognized as the lead players in B-cell survival and proliferation [62, 63]. Both APRIL and BAFF were found to be upregulated in patients with MS [64, 65].

Atacicept is a human recombinant fusion protein that contains the extracellular ligand binding domain of the transmembrane activator and calcium modulator and cycophilin ligand interactor (TACI) receptor and a modified Fc portion of human Immunoglobulin [66]. TACI receptors bind to both APRIL and BAFF, and therefore atacicept neutralizes all forms of APRIL and BAFF inhibiting their effects on B-cell survival and function. Atacicept acts on mature B cells and plasma cells, but spares B-cell progenitors and memory cells, and therefore does not result in generalized depletion of B cells [64, 65]. Atacicept was an attractive candidate to target pathogenic B lymphocytes in MS. Unfortunately, the development of this agent had to be discontinued for MS due to clinical trial findings that suggested unexpected increased in disease activity, which is described as follows.

Atacicept was tested in patients with RRMS and clinically isolated syndrome presenting with optic neuritis in 2 separate trials. Both trials were suspended due to an unexpected increase in brain MRI lesions and inflammatory activity in the treated group [67–69]. The reasons underlying increased inflammatory activity in MS patients are not clear. Atacicept may affect regulatory rather pathogenic pathways, thereby interfering with immune protective functions.

Other Monoclonal Antibodies in MS

Several other MABs targeting the humoral responses that are used for other autoimmune disorders may increase the risk to develop demyelinating events. Infliximab, which targets TNF-α, was reported in 2 RRMS patients to increase Gd+ lesions, and therefore it was not tested an further in clinical trials [70].

Conclusion

Emerging therapies in MS are continuously evolving and target novel mechanisms of action. Monoclonal antibodies such as rituximab, daclizumab, ocrelizumab, and alemtuzumab appear to be promising interventions but their long-term safety profiles remain to be defined. The success of B-cell depleting therapies, especially the rapid onset (within weeks of starting therapy) of the prevention of new T2 and Gd+ lesions on MRI highlighted the role of B cells in relapsing forms of MS; their effect of B-cell depletion on disease progression in progressive forms of MS is being studied. The outcome in MS of other therapies that were initially considered to be promising candidates for MS (such as atacicept), but ultimately worsened the course of MS, emphasizes the challenges of developing new MS agents targeting the humoral immune system based on animal model data.

Acknowledgments Dr. Lulu is a recipient of the Sylvia Lawry fellowship award from the National Multiple Sclerosis Society and fellowship training award from Biogen-Idec. Dr. Waubant is funded by the National Multiple Sclerosis Society, National Institutes of Health, and the Nancy Davis Foundation, and is receiving support for ongoing trials from Roche, Sanofi Aventis, and Biogen Idec, and has also provided 3 educational lectures for Teva and Biogen Idec, along with being an ad hoc consultant for Actelion, Chugai, and Sanofi Aventis.

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

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