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The role of B cells in Multiple Sclerosis and related disorders

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AUTHOR CONTRIBUTIONS

All authors wrote or critically reviewed the manuscript, including data acquisition, analysis and figures, and approved the final version. Members of the Expert Panel of the 27th European Charcot Foundation Annual Meeting who are not authors are listed with their institutional affiliations in the supplementary online table.

POTENTIAL CONFLICT OF INTEREST

The following companies are involved in MS drug development: Actelion (Ac), Alexion (Al), Annexon (An), Atara Bio (AB), Bayer (Ba), Biogen (Bi), Bionure (Bn), Celgene/BMS (Ce), Forward Pharma (FP), GeNeuro (GN), GlaxoSmithKline (GS), Janssen/Johnson-Johnson (JJ), MedDay Pharmaceuticals (MP), Merck (Me), Molecular Stethoscope (Mo), NervGen (NG), Neurona Therapeutics (Ne), Novartis (No), Octapharma (Oa), Roche/Genentech (Ro), Sanofi/Genzyme (Sa), Teva (Te), TG Therapeutics (TG) and Viel Bio (VB). GC has received personal compensation for consulting and speaking fees from No, Te, Sa, Me, Ce, Bi, Ro, FP, MP and Excemed. ABO has served on scientific advisory boards for AB, Bi, Ce, JJ, Me, No, Ro and Sa, and has sponsored research agreements with Bi, Me, No and Ro.

HL received honoraria for lectures from No, Bi, Sa, Me, Ro and MP.

AU has received consultancy fees from Bi, SG, Me, No, Ro and Te and research support from Bi, Me and No.

HPH received fees for serving on steering committees, data monitoring committees and scientific advisory boards from Ba, Bi, Ce, GN, Me, No, Oa, Ro, Sa, Te, TG, VB with approval by the Rector of Heinrich-Heine-University.

XM received speaking honoraria and travel expenses for scientific meetings, has been a steering committee member of clinical trials or participated in advisory boards of clinical trials in the past 3 years with Ac, Al, Ba, Bi, Ce, Me, Excemed, Sa, MP, Multiple Sclerosis International Federation, NG, National Multiple Sclerosis Society, No, Ro, Te and TG.

PSS has received personal compensation for serving on scientific advisory boards, steering committees, independent data monitoring committees or speaker honoraria for Bi, Me, No, Te, GS and Ce.

RH received honoraria and grant support from No, Sa, Bi, Te, Me, JJ and Ro.

SLH currently serves on the Scientific Advisory Board of Symbiotix, An, Bn, and Mo and on the Board of Trustees of Ne. SLH also has received travel reimbursement and writing assistance from Ro and No for CD20-related meetings and presentations.

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Abstract

The success of clinical trials of selective B-cell depletion in patients with relapsing multiple sclerosis (RMS) and primary progressive MS (PPMS) have led to a conceptual shift in the understanding of MS pathogenesis, away from the classical model in which T cells were the sole central actors, and towards a more complex paradigm with B cells having an essential role in both the inflammatory and neurodegenerative components of the disease process. The role of B cells in MS was selected as the topic of the 27th Annual meeting of the European Charcot Foundation. Results of the meeting are presented in this concise review which recaps current concepts underlying the biology and therapeutic rationale behind B-cell directed therapeutics in MS, as well as proposes strategies to optimise the use of existing anti-B-cell treatments and provide future directions for research in this area.

INTRODUCTION

From 21 to 23 November 2019, the 27th Annual Meeting of the European Charcot Foundation was held in Baveno, Italy. “The role of B cells in Multiple Sclerosis (MS)” was selected as this year’s theme. The meeting gathered 500 on-site delegates and provided an opportunity for scientists, clinicians, industry leaders, patients and other healthcare experts to review existing evidence on the mechanisms of action of B cells in MS and other neuroinflammatory conditions such as neuromyelitis optica spectrum disorder (NMOSD), and discuss current and emerging therapeutic strategies of treatments targeting B cells.

The understanding of the role of B cells in MS has evolved substantially in recent years, shifting from the classical model (T cells being central players) to a mechanism in which the interplay between B- and T cells is a central feature of the disease pathogenesis.¹ This shift was mostly driven by the success of clinical trials of selective B-cell depletion in patients with relapsing MS (RMS) and also primary progressive MS (PPMS) indicating that B cells are essential contributors to immune responses involved in MS. This changed the MS treatment landscape substantially: B-cell therapies represent a significant conceptual advance in treating all forms of MS and in understanding the biology of this complex disease and will hopefully lead to development of even more selective, effective, and safe therapeutics.

A wide range of topics were discussed at the meeting, including but not limited to the role of intrathecal antibodies in demyelinating diseases, therapeutic experience with anti-CD20 monoclonal antibodies, approaches to monitor efficacy and safety of B-cell directed therapies. This concise review recaps current concepts underlying the biology and therapeutic rationale behind B-cell directed therapeutics in MS and proposes future directions that could impact today’s unmet need, preventing and treating MS progression.

IMPACT OF B CELLS ON THE PATHOPHYSIOLOGY OF MS

B cells as immunomodulators in MS

Though T cells are widely considered to be major contributors to inflammatory demyelination in MS, growing evidence suggests a significant role for B cells in disease pathogenesis. Both antibody-dependent and independent mechanisms are thought to underlie B-cell mediated central nervous system (CNS) injury in MS. In addition to antibody secretion by plasmablasts and plasma cells, B-cell functions implicated in pathogenesis include (i) antigen presentation to T cells and driving autoprolieration of brain-homing T cells (presumably by memory B cells), (ii) production of pro-inflammatory cytokines and chemokines that propagate inflammation, (iii) production of soluble toxic factors contributing to oligodendrocyte and neuronal injury, (iv) contribution to the formation of ectopic lymphoid aggregates in the meninges, and (v) providing a reservoir for Epstein-Barr (EBV) virus infection.²⁻⁶ These B cell actions may contribute to both MS relapses and disease progression.

The importance of B cells in MS is underscored through clinical trials revealing that anti-CD20 monoclonal antibodies are highly effective in limiting new relapsing disease activity.⁷⁻¹⁰ Of note, this therapy does not directly target plasma cells, nor does it appear to significantly impact the abnormal cerebrospinal fluid (CSF) antibody profile.⁷ Peripheral B cells of MS patients exhibit aberrant pro-inflammatory cytokine responses, including exaggerated lymphotoxin- α , tumour necrosis factor (TNF)-alpha, interleukin (IL)-6 and granulocyte macrophage-colony stimulating factor (GM-CSF) secretion. B-cell depletion results in significantly diminished pro-inflammatory responses of CD4+ and CD8+ T cells as well as myeloid cells.^{11, 12} It is noteworthy that a small proportion of circulating T cells express CD20 and these are also depleted with anti-CD20 therapy; though, since anti-CD19 therapy also seemed effective in MS, the robust effects of anti-CD20 in MS are not likely to be exclusively mediated by removal of CD20-expressing T cells.¹³

In addition, B cells have the capacity to produce anti-inflammatory cytokines such as transforming growth factor (TGF)- β 1, IL-35, and IL-10.¹ In mice with experimental autoimmune encephalomyelitis (EAE), gut-derived immunoglobulin A (IgA)+ B cells are mobilised to the CNS where they attenuate neuroinflammation through expression of IL-10.¹⁴ Studies in MS patients indicate that their B cells are deficient in IL-10 production compared to healthy controls, which may imply that B cells of patients are less capable of downregulating immune responses. Consistent with such a role, MS patients who are infected with parasites harbour higher frequencies of IL-10 expressing B cells and appear to have less disease activity than uninfected MS patients.¹⁴

B cell trafficking in the CNS

Molecular analysis of B-cell populations in the CNS and periphery of MS patients points to bi-directional trafficking of B cells. Relatively little is known about the molecular mechanisms that underlie human B-cell migration into, and retention within, the CNS.^{3, 15} Ex vivo studies found that B-cell migration across the blood-brain barrier (BBB) endothelial cells is significantly inhibited by blocking antibodies to the adhesion molecules ICAM-1

(intercellular adhesion molecule-1) and VLA-4 (leukocyte very late antigen-4).^{16, 17} Activated leukocyte cell adhesion molecule (ALCAM; CD166) on human and mouse brain endothelial cells has also been assigned a role in transmigration across the BBB. ALCAM promotes the recruitment of pro-inflammatory B cells across the BBB and blood-meningeal barrier. Blocking ALCAM reduced disease severity in animals affected by a B-cell-dependent form of EAE, and the proportion of ALCAM+ B cells was increased in the peripheral blood and within brain lesions of MS patients.¹⁸

These mechanisms raise the prospect of novel therapeutic targets for limiting CNS B-cell infiltration and could predict how therapies currently developed to target T-cell migration, such as anti-VLA-4 antibodies, may impact B-cell trafficking. B-cell retention within the chronically inflamed CNS may be mediated by both immune and brain cells. For example, Th17 cells known to be present in the CNS of both MS patients and in EAE models have been shown to induce robust tertiary lymphoid tissue formation within the brain meninges in EAE, where these B-cell rich immune aggregates were associated with local demyelination.¹⁹ Activated glial cells (such as astrocytes) within the inflamed MS CNS may also secrete factors that support B-cell survival and persistence within the CNS.^{20, 21}

B cells in the MS CNS compartment

Neuropathological studies provided evidence for a significant contribution of B cells in the CNS of MS patients. B cells in the inflammatory infiltrates are more abundant in MS - particularly in patients with active disease - in comparison to other inflammatory brain diseases. B cells are mainly located in the meninges and in the large perivascular spaces around the cerebral ventricles. In early and active lesions, CD20+ B cells dominate and may have pro-inflammatory functions, while at later stages plasma cells with possible anti-inflammatory functions increase in number.²²

Within the brain there is a local production of cytokines, which support homing, survival and functional activation of B cells.²³ The intrathecal production of these cytokines is stimulated by the pro-inflammatory environment in the MS lesion and their action is controlled by shedding of their receptors (B-cell maturation antigen [BCMA], transmembrane activator and CAML interactor [TACI]) from the surface of B cells, by gamma-secretase.²⁰ Shedded survival receptors, liberated into the CSF, may become promising biomarkers for disease activity.²⁴

Prominent B-cell rich inflammatory aggregates with features of tertiary lymph follicles reside in the meninges of MS patients, and especially within deep cortical sulci. Their abundance correlates with the amount and size of cortical lesions, with the degree of neurodegeneration in the cortex and the accrual of disability.^{25, 26} Meningeal infiltrates are the source of cytokines and chemokines in the CSF and correlate topographically with the presence and size of cortical lesions, the degree of neurodegeneration in the cortex, and the liberation of neurofilament light (NFL) protein into the CSF which is an established biomarker for neurodegeneration.^{27, 28}

To date, meningeal inflammation can be detected – at least in part - by high-resolution magnetic resonance imaging (MRI) since it is associated with blood-meningeal barrier

impairment, visualised by gadolinium enhancement. Through direct correlation between MRI and pathology it was shown that meningeal enhancement is associated with inflammation and the presence of cortical lesions.²⁹ Similarly, in EAE mouse models meningeal enhancement discloses areas of meningeal inflammation.³⁰ Some data describe an association between meningeal enhancement in MRI and the degree of cortical atrophy, but this has to be validated in larger patient cohorts.³¹

Autoantibody involvement in MS and NMOSD

The importance of antibody-producing B cells and the potential role of autoantibodies in MS has been a topic of interest for many years. The establishment of reliable biomarkers for diagnosis, prognosis and treatment of MS has proven to be very difficult. Autoantibodies are formed against different CNS cell types, including neurons, oligodendrocytes and astrocytes, and even immune cells, however none of them have been validated so far for clinical use in MS.^{32, 33}

Many active MS lesions are characterised by deposition of immunoglobulin G (IgG) and activated complement products.³⁴ Oligoclonal IgG bands (OCBs) are clonally expanded antibodies produced intrathecally and one of the few biomarkers in CSF included in the diagnostic criteria of MS.³⁵ Their presence remains relatively stable over time, although it has been shown that some therapeutic interventions could mildly affect OCB production.^{36, 37} OCBs as well as the presence of intrathecal immunoglobulin M (IgM) synthesis have some prognostic value in MS at the time of diagnosis.^{38–42} Some OCB antibodies recognise conformational epitopes of ubiquitous intracellular proteins, indicating that part of the OCB response may occur secondary to tissue damage.⁴³ OCB production and expanded intrathecal plasmablast clones can be observed even at the earliest prodromal stages of MS as revealed in MS-discordant monozygotic twin pairs where the clinically unaffected co-twin may show CSF changes of ‘subclinical neuroinflammation’.⁴⁴

In addition to identifying relevant target antigens of B-cell and antibody responses in MS, understanding the repertoire of pathogenic B cells and how they differentiate, as well as their location in the CNS and peripheral immune system, have become central issues in MS pathogenesis.⁴⁵ Some immunophenotyping studies have focused on alterations in composition of B-cell subsets. Rituximab in rheumatoid arthritis (RA) for example effectively depletes B cells and skews the B-cell compartment. Repopulation occurred mainly with naïve mature and immature B cells. Patients whose RA relapsed on return of B cells tended to show repopulation with higher numbers of memory B cells.^{46–48} In patients with MS, restoration of regulatory B cells was observed following cladribine and alemtuzumab treatment, suggesting that these cells might serve as surrogate markers for disease activity.^{49, 50} However, these are preliminary findings that will require confirmation. Standardised multisite cytomics data could provide further understanding of treatment impact on MS immunophenotype and pave the way toward monitoring B cells to personalise treatment.⁵¹

Pathogenic autoantibodies

Several approaches for antigen hunting in MS have been conducted. Antibodies such as aquaporin 4 (AQP4) and myelin oligodendrocyte glycoprotein (MOG) have helped to classify and define subgroups previously included under the umbrella of MS but are now identified as distinct diseases with different prognostic and therapeutic implications (Table 1).⁵² Despite the vast effort that has been expended over the last decades in the field, the pursuit for the antigen(s) in MS is still open.

The binding of pathogenic AQP4-specific autoantibodies to astrocytes is a key event in the formation of neuromyelitis optica (NMO) lesions. This has been well documented in animal models, and is supported by the pathology of NMO in humans.^{53–55} NMO is an inflammatory demyelinating disease of the CNS caused by binding of pathogenic IgG autoantibodies to AQP4. Astrocyte damage and downstream inflammation require NMO-IgG effector function to initiate complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC).^{56–58} The discovery of AQP4 as a biomarker marked a breakthrough in the understanding of the pathogenesis of the disease.

MOG is expressed on the outermost layer of CNS myelin sheaths and on the extracellular surface of oligodendrocytes.⁵⁹ While the pathogenic role of anti-MOG antibodies in EAE is undisputed, the exact role of anti-MOG antibodies in MS patients has been controversially discussed over decades.³³ It has been shown that myelin-specific MS antibodies cause oligodendrocyte loss and demyelination in organotypic cerebellar slices and display seminal features of active MS lesions. Myelin-specific antibodies may play an active role in MS lesion formation through CDC mechanisms.^{60, 61} Typical MS cases are largely anti-MOG negative.^{62, 63} In a small trial, initial detection of serum anti-MOG and anti-myelin basic protein (MPB) antibodies has shown to be correlated with early conversion from clinically isolated syndrome to definite MS.⁶⁴ Analysis of pathogenic antibodies could thereby be of value to estimate individual risk of early relapse. However, the association between anti-MOG antibodies and progression to MS has not been reproduced in other trials.⁶⁵

With the use of different cell-based immunoassays more recently⁶⁶, anti-MOG antibodies could be identified in a subset of inflammatory demyelinating diseases of the CNS clinically and pathologically distinct from MS and AQP4 antibody seropositive NMOSD, defined as MOG antibody-associated disorder (MOGAD). MOGAD phenotypes are varied and range from classical NMO to acute disseminated encephalomyelitis (ADEM) and cortical encephalitis.^{67–69} The diagnosis depends on using a reliable, specific and sensitive assay of the antibody.⁷⁰ Clinical and imaging features of MOG-associated syndromes overlap with AQP4 antibody NMOSD but can be usually distinguished from MS: in particular, the silent lesions typical of MS that progressively increase lesion volume are rare in MOGAD.⁷¹

THERAPEUTIC DEPLETION OF B CELLS IN MS

Different therapeutic approaches are under investigation aiming to improve prognosis, prevent relapse and minimise the extent of disability. Most MS therapies alter the frequency, phenotype or homing of B cells in one way or another.

Treatment of RMS

CD20 is a transmembrane ion channel protein expressed on the surface of pre-, immature, mature, and memory B cells. Several anti-CD20 monoclonal antibodies, each reacting with different epitopes of CD20, have been developed for RMS treatment including rituximab, ocrelizumab, ublituximab and ofatumumab which are further detailed in Table 2 and Figure 1. Anti-CD20 antibodies spare plasma cells (which do not express CD20), and their critical therapeutic target in MS are thought to be memory B cells.⁷² In contrast, atacicept, a recombinant fusion protein of the extracellular domain of TACI and the human IgG1 Fc domain (TACI-Ig) does target plasma cells, though tends to spare memory B cells. Of note, atacicept treatment resulted in dose-dependent exacerbations of MS disease activity, which may reflect its limited impact on pro-inflammatory memory B cells and potentially the removal of anti-inflammatory plasma cells.⁷³

Administration of rituximab markedly reduced MRI evidence of MS disease activity and diminished the clinical relapse rate.⁷ Ocrelizumab, a newer humanised anti-CD20 monoclonal antibody, was approved by the Food and Drug Administration (FDA) in March 2017 after the pivotal OPERA trials revealed dramatic effects on all key clinical and MRI outcomes versus interferon (IFN)- β -1a in RMS.⁹ Ofatumumab, a fully human anti-CD20 monoclonal antibody administered by subcutaneous injection at home, recently completed successful clinical trials in RMS.⁷⁴ Phase III testing of ublituximab, another anti-CD20, in RMS is currently in progress.

Anti-CD20 therapies rapidly and almost completely deplete circulating CD20+ B cells but have only limited effects in secondary lymphoid organs. Since the CD20 antigen is absent on the earliest B-cell precursors, stem cells and pro-B cells in the bone marrow, and also on plasmablasts and plasma cells responsible for immunoglobulin production, B-cell repletion and pre-existing humoral immunity are largely preserved. These factors likely account for the favourable overall safety profile of anti-CD20 monotherapy. Anti-CD20 monoclonal antibodies cross the BBB poorly; they do partially reduce B-cell numbers in the CSF though without a detectable effect observed so far on CSF IgG synthesis or OCBs.⁷⁵ Small-molecule therapies are being explored for their B-cell modulatory actions and could be beneficial due to higher BBB penetration and higher flexibility in treatment initiation and discontinuation. Reduction in enhancing lesions with evobrutinib, a Bruton's tyrosine kinase (BTK) inhibitor, has recently been shown in a Phase II trial.⁷⁶

Treatment of progressive MS

PPMS, which affects 10–15% of MS patients, has been a notoriously difficult form of MS to recognise and to treat.^{77–82} Rituximab was tested in PPMS patients in the Phase 2/3 OLYMPUS trial and failed to meet the primary endpoint; however, the trial may have been underpowered and a positive trend was evident; subgroup analyses suggested that younger patients particularly those with inflammatory lesions may have responded favorably.⁸³ These results provided the rationale for the investigation of ocrelizumab in PPMS in the Phase 3 ORATORIO trial. This was the first trial to show positive results in PPMS, persisting over a duration of up to 6.5 years in open-label extension observations, albeit with modestly favourable effects on the primary endpoint.^{10, 84} No evidence of progression, a novel

composite endpoint tested post-hoc, was also achieved more frequently in patients treated with ocrelizumab compared to placebo patients.⁸⁵ Ocrelizumab is the first and only approved treatment for PPMS and recommended as first-line therapy in theECTRIMS-EAN (European Committee for Treatment and Research in Multiple Sclerosis-European Academy of Neurology) guideline.⁸⁶ Still, the need for more effective therapies in PPMS remains.

A pilot trial with intrathecal rituximab in progressive MS did not show a convincing effect on the clinical course of MS or CSF biomarkers including NFL.⁸⁷ Treatment was well tolerated but not without risks: i.e. a case of low-virulent bacterial meningitis was reported.

B-cell therapy in paediatric MS

Paediatric MS is characterised by more prominent inflammatory activity but better capability to compensate brain damage. Therefore it should be treated early and efficiently.⁸⁸ A considerable experience with rituximab exists in many immune-mediated disorders of children and adolescents. Rituximab has also been found effective in open-label trials in paediatric patients with MS.^{89, 90} Clinical data with ocrelizumab and ofatumumab in children/adolescents are still lacking. Anti-CD20 therapy may represent an attractive option in paediatric MS but safety issues such as the still incompletely known potential long-term risks should be kept in mind.

Monitoring of B cells

Studies in experimental animal models reveal that anti-CD20 therapy efficiently depletes peripheral B cells, while a subset of CD27+ B cells persists in secondary lymphoid organs.⁹¹ B-cell repletion starts in bone marrow and spleen, followed by blood. The reappearing B cells in animals possess an enhanced capacity to recognise and present autoantigen. Of interest is whether monitoring B cells, in particular memory B cells, in the peripheral blood of MS patients may be useful for assessing the individual benefit-risk of therapy and personalising treatment accordingly. High inter- and intra-individual variability in B-cell repopulation is seen after anti-CD20 depletion therapy in patients and repopulation of memory B cells is not proportional to repopulation of CD19+ cells. In NMO, monitoring CD19+CD27+ memory B cells (instead of total B cell counts) has been found to be a more reliable marker for relapses, though cut-offs to identify early re-populators are not yet validated and the extent to which such an approach may be relevant to MS remains to be defined.⁹² It is also possible that the beneficial effects of anti-CD20 on clinical and MRI disease activity persist for some time even after B-cell repletion occurs.^{7, 93} Until more is known about the pharmacodynamics of the various anti-CD20 therapies, as an initial schedule, adherence to the dosing regimens used in the clinical trials seems prudent.

Monitoring CD4+ T cells after depletion therapy in RA patients showed a correlation between clinical improvement and CD4+ count decrease.⁹⁴ This could be explained by memory B-cell-driven autoprolieration of CD4+ T cells.⁹⁵

CONCLUSIONS AND FUTURE PERSPECTIVES

The demonstration that B cells play a central role in MS pathogenesis led directly to the discovery that their depletion in peripheral blood is a highly successful therapeutic strategy.

⁹⁶ Still, several important questions and challenges exist on the role of B cells in MS and the optimal clinical approach to treatment.

Depletion of B cells by anti-CD20 antibodies is mediated through several molecular mechanisms including CDC, ADCC and antibody-dependent cellular phagocytosis.⁹⁷ B-cell depletion produces outstanding control of clinical relapses and focal inflammatory MS disease activity, but benefits against progressive MS are only partial. This could be due to inefficient depletion of CNS B-cell populations, especially in progressive MS, due to CNS compartmentalisation of the B-cell response in progressive disease and inefficient transit of anti-CD20 monoclonal antibodies across the BB. In this regard OCBs in CSF, which are believed to be secreted by long-lived plasma cells that do not express CD20, also appear to be largely unaffected by B-cell depleting interventions.⁷⁵

The extent and duration of optimal depletion is not yet fully known but is likely to be partial and depends on the type of anti-CD20 therapy (different CDC or ADCC activities) and dose in combination with individual host factors such as genetics. Lymph node B cells are not fully depleted by anti-CD20 therapy, and this could also provide an ongoing source of peripherally maintained disease activity. Uncertainties on the application of anti-CD20 therapies in medical practice include when to initiate treatment (early treatment might be more effective) and optimal dosing as well as duration (development of biomarkers to guide the need for continued therapy).

In addition, post-marketing surveillance is essential to fully uncover the effects on long-term disability and safety and will be essential to help position anti-CD20 therapies within the greater context of available MS disease modifying therapies. The generally favourable safety profile of anti-CD20 therapy likely results from the large B-cell reservoir remaining even after repeated and chronic administration. Nevertheless, the long-term risk of infection or other adverse outcomes remains an important consideration given the profound and sustained depletion of circulating B cells that is the hallmark of these agents.

Although therapies that target humoral immune system cells more broadly than anti-CD20 could possibly offer a higher level of efficacy, especially against progression, a less favourable safety profile could be a consequence, due to a greater degree of elimination of non-circulating B cells, depletion of earlier precursors in the bone marrow or reducing antibody-producing plasma cells. Small molecules that target B-cell signaling (through BTK, PI3 kinase, or Janus kinases), the proteasome involved in plasma cell differentiation, or EBV which infects B cells and is believed to be involved in MS aetiology, may provide novel mechanisms to target B cells, increase the therapeutic effect, and better clarify the humoral immune pathogenesis of MS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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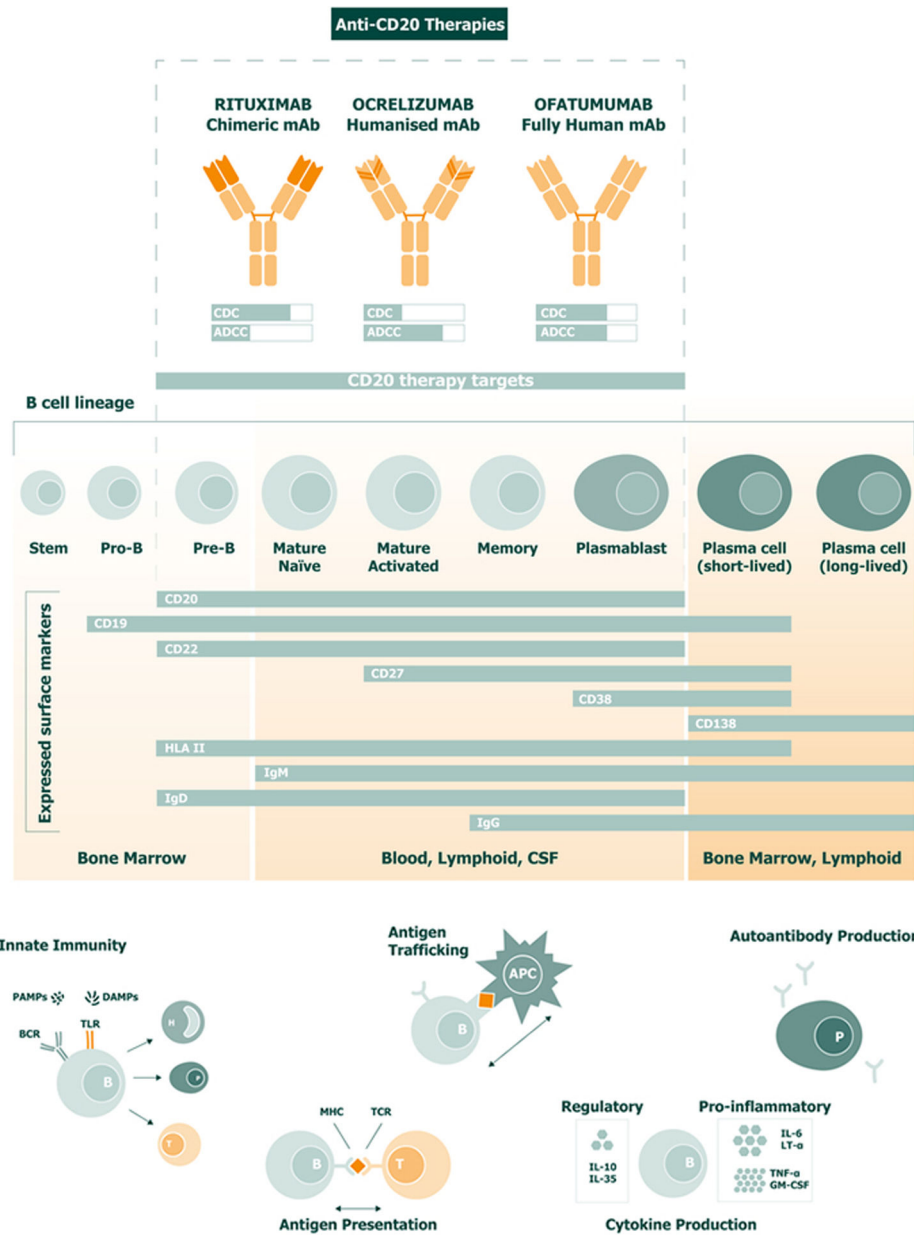


FIGURE 1. ANTI-CD20 MONOCLONAL ANTIBODIES: MECHANISMS OF ACTION AND MATURATION STAGES.

Abbreviations - ADCC: antibody-dependent cell-mediated toxicity; APC: antigen-presenting cell; BCR: B cell receptor; CDC: complement-dependent cytotoxicity; CSF: cerebrospinal fluid; DAMP: damage-associated molecular pattern molecule; GM-CSF: granulocyte macrophage-colony stimulating factor; IL: interleukin; mAb: monoclonal antibody; MHC: major histocompatibility complex; LT- α: lymphotoxin-α; PAMP: pathogen-associated molecular pattern molecule; TCR: T cell receptor; TLR: toll-like receptor; TNF-α: tumour-necrosis factor-alpha

TABLE 1.**CLINICAL SPECTRUM OF DEMYELINATING DISEASES**

| | MS | AQP4-Ab+ | MOG-Ab+ |
|---|---|--|---|
| | CNS-directed T cells, B cells | CNS-directed Ab | CNS-directed Ab |
| Target | Myelin? | Water channel expressed on astrocytes | Surface of oligodendrocytes and myelin in CNS |
| Clinical presentation | Brain - short TM - ON Chronic progressive | NMO - ON - LETM Relapsing | ON - TM - ADEM - cortical encephalitis Monophasic or relapsing |
| Onset age | 20–50 years | 30–60 years | 10–40 years |
| OCB positivity | >90% | 11–20% | 0–11% |
| CSF cell count⁹⁸ | Mononuclear +/- Polymorphonuclear - | Mononuclear ++ Polymorphonuclear + | Mononuclear + Polymorphonuclear +/- |
| CSF protein | - | + | ++ |
| Cytokine profile⁹⁸ | IFN γ - IL-6 - IL-2 +++ | IFN γ - IL-6 +++ IL-2 - | IFN γ + IL-6 +++ IL-10 - |
| Presence of MOG-IgG Ab⁹⁹ | 3–10%. More frequent in children | Very rare | Yes |
| Presence of AQP4-IgG Ab⁹⁹ | Very rare | Yes | Very rare |
| Pathological features³⁴ | Confluent demyelination pattern; CD8+ dominant, complement activation, axonal injury | Oedema, necrosis, AQP4 and GFAP loss, complement | Perivenous demyelination, CD4+ dominant, no complement deposition, cortical demyelination |

Ab: antibody; ADEM: acute disseminated encephalomyelitis; AQP4: aquaporin 4; CNS: central nervous system; CSF: cerebrospinal fluid; GFAP: glial fibrillary acidic protein; IFN γ : interferon gamma; IL-2: interleukin-2; IL-6: interleukin 6; IL-10: interleukin-10; LETM: longitudinally extensive transverse myelitis; MOG: myelin oligodendrocyte glycoprotein; MS: multiple sclerosis; NMO: neuromyelitis optica; OCB: oligoclonal band; ON: optic neuritis; Th17: T helper 17; TM: transverse myelitis

TABLE 2.**B-CELL-TARGETED THERAPIES IN MS**

| | Mechanism of action | Compound | Structure and route of administration | Efficacy | Development status in MS |
|-------------|--|-----------------|--|---|--|
| RMS | Anti-CD20 monoclonal antibodies | Rituximab | Chimeric IV | High efficacy in the Phase 2 HERMES trial ⁷ | Phase 2 Used off-label in MS |
| | | Ublituximab | Chimeric/ glyco-engineered IV | Phase 2 ¹⁰⁰ | Phase 3 (ULTIMATE I and II) underway |
| | | Ocrelizumab | Humanised IV | High efficacy in the Phase 3 OPERA I and II trials ^{9, 101, 102} | Approved for RMS |
| | | Ofatumumab | Human SC | Phase 2 MIRROR ¹⁰³ Phase 3 ASCLEPIOS I and II ⁷⁴ | Phase 3 finished. Pending approval by agencies |
| | Bruton's tyrosine kinase (BTK) inhibitor | Evobrutinib | Oral | Phase 2 ⁷⁶ | Phase 2; Phase 3 underway |
| PPMS | Anti-CD20 monoclonal antibodies | Ocrelizumab | Humanised IV | ORATORIO ¹⁰ | Approved for PPMS |

IV: intravenous; PPMS: primary progressive multiple sclerosis; RMS: relapsing multiple sclerosis; SC: subcutaneous