# B cells in multiple sclerosis — from targeted depletion to immune reconstitution therapies

*Maria T. Cencioni*[1], *Miriam Mattoscio*[1], *Roberta Magliozzi*[1,2], *Amit Bar-Or*[3] *and Paolo A. Muraro*[1] ✉

Abstract | Increasing evidence indicates the involvement of B cells in the pathogenesis of multiple sclerosis (MS), but their precise roles are unclear. In this Review, we provide an overview of the development and physiological functions of B cells and the main mechanisms through which B cells are thought to contribute to CNS autoimmunity. In MS, abnormalities of B cell function include pro-inflammatory cytokine production, defective B cell regulatory function and the formation of tertiary lymphoid-like structures in the CNS, which are the likely source of abnormal immunoglobulin production detectable in the cerebrospinal fluid. We also consider the hypothesis that Epstein–Barr virus (EBV) is involved in the B cell overactivation that leads to inflammatory injury to the CNS in MS. We also review the immunological effects — with a focus on the effects on B cell subsets — of several successful therapeutic approaches in MS, including agents that selectively deplete B cells (rituximab, ocrelizumab and ofatumumab), agents that less specifically deplete lymphocytes (alemtuzumab and cladribine) and autologous haematopoietic stem cell transplantation, in which the immune system is unselectively ablated and reconstituted. We consider the insights that these effects on B cell populations provide and their potential to further our understanding and targeting of B cells in MS.

Multiple sclerosis (MS) is an immune-mediated disease of the CNS of unknown aetiology. Strong evidence supports an association between immune gene polymorphisms and susceptibility to MS[1]. MS has been associated with inflammatory T helper ($T_H$) cell profiles and T cells play key roles in adoptive transfer models of demyelinating disease; therefore, historically, the emphasis of MS research has been on T cells. However, evidence has accumulated, especially over the past 10–15 years, that strongly implicates the involvement of B cells in the pathogenesis of MS. Indeed, some of the most effective approved therapies for MS target B cells. However, the precise role of B cells in the evolution of the disease remains unclear and a greater understanding of their roles is needed to refine therapeutic approaches.

In this article, we review what is known about the involvement of B cells in MS pathophysiology and examine the mechanisms of action of B cell-targeted therapies, the effects of pan-lymphocyte-depleting therapies on B cells and the effects of the immune reconstitution strategy autologous haematopoietic stem cell transplantation (AHSCT) on B cells in the treatment of MS. We consider how these therapeutic effects will inform us about the involvement of B cells, with the potential for the refinement of therapeutics.

[1]*Department of Brain Sciences, Faculty of Medicine, Imperial College London, London, UK.*

[2]*Department of Neurology, University of Verona, Verona, Italy.*

[3]*Center for Neuroinflammation and Experimental Therapeutics and Department of Neurology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA.*

✉*e-mail: p.muraro@imperial.ac.uk*

https://doi.org/10.1038/s41582-021-00498-5

## B cell basics

### *B cell development*

B cells progress through a number of developmental stages (Supplementary Figure 1). They develop from the lymphoid progenitor lineage, which derives from haematopoietic stem cells in the bone marrow. Following the productive rearrangement of the immunoglobulin heavy chain (that is, the production of a DNA sequence that is translated into a full-length and correctly structured protein), pro-B cells proliferate and rearrange their immunoglobulin κ light chain as they develop into immature B cells[2]. These immature B cells pass through a negative selection process that removes autoreactive clones[3]. Selected B cells then leave the bone marrow and evolve through transitional (T1 and T2) B cell stages into mature follicular and marginal zone B cells within the peripheral lymphoid organs (the spleen, lymph node and tonsils)[4–8].

In the marginal zone and lymphoid follicles, mature B cells differentiate into memory B cells, plasmablasts and plasma cells, which are the immunoglobulin-producing

**Key points**

- Accumulating neuropathological, serological and immune cellular evidence strongly suggests that B cells are involved in the pathophysiology of multiple sclerosis (MS).
- Specific B cell subsets seem to be involved in MS as antigen-presenting cells and pro-inflammatory cytokine-producing cells; other B cell subsets serve as anti-inflammatory regulatory cells.
- The persistently active infection of B cells with Epstein–Barr virus could lead to CNS damage; however, the causative role of the virus in MS remains controversial.
- Treatments that target B cells are effective in MS, which strongly suggests the involvement of B cells in disease pathophysiology.
- Autologous haematopoietic stem cell transplantation is known to have regenerative effects on T cells and limited evidence indicates that the treatment leads to repopulation with predominantly naive B cells.
- The effects of therapies on B cell subsets provide insight into the roles of B cell populations in disease; further immunological studies are required to improve our understanding of these roles.

cells. Here, further negative selection mechanisms remove clones that have dysfunctional B cell receptor (BCR) rearrangements or a high affinity for self-antigens, which could initiate autoimmune disease[9]. A balance between cell proliferation and programmed cell death ensures the homeostasis of circulating memory B cells and bone marrow-resident, long-lived plasma cells[9].

### *Essentials of B cell function*

B cells play various roles (Supplementary Figure 1) in mediating humoral immunity and take part in cellular immunity in the adaptive immune system and at the interface of the innate and adaptive immune systems. BCRs and Toll-like receptors expressed by B cells recognize the components of pathogens. Antigenic stimulation and T cell-derived signals promote B cell activation and differentiation into plasma cells that produce protective immunoglobulins. However, plasma cells can also produce autoantibodies that can be pathogenic and associated with autoimmune disease.

Memory B cells participate in the development of cellular immunity in various capacities. First, they can serve as antigen-presenting cells (APCs) for T cells, whereby antigen and the major histocompatibility complex (MHC) expressed by B cells deliver the so-called 'signal 1' required for T cell activation. Second, as part of their APC role, B cells express co-stimulatory molecules that engage with appropriate co-stimulatory receptors on T cells to deliver the so-called 'signal 2' required for effective T cell activation. Co-stimulatory signals are important for the differentiation of $CD4^+$ $T_H1$ and $T_H2$ cells[10]. Third, activated B cells release a range of cytokines, including IL-6, tumour necrosis factor (TNF) and granulocyte–macrophage colony-stimulating factor (GM-CSF), that can increase T cell and myeloid cell activation and further contribute to B cell proliferation and differentiation.

### *B cell-mediated immune regulation*

B cells can regulate humoral and cellular immunity through the release of anti-inflammatory cytokines, including IL-10, transforming growth factor-β (TGFβ), granzyme B and IL-35 (REFS[11,12]). Various regulatory B ($B_{reg}$) cell subsets have been described that can suppress inflammatory immune responses. $CD19^+CD24^{Hi}CD38^{Hi}$ transitional B cells (henceforth referred to as transitional B cells) and $CD19^+CD24^+CD27^+$ B cells (the human equivalent of murine B10 cells) are the primary producers of IL-10. An in vitro study has shown that $CD19^+CD24^+CD27^+$ B cells are more efficient than transitional B cells at suppressing $CD4^+$ T cell proliferation and the expression of IFNγ and IL-17 (REF.[13]). The same study demonstrated that both subsets of $B_{reg}$ cells release IL-10 and TNF and yet $CD19^+CD24^+CD27^+$ B cells express higher amounts of TGFβ1 and granzyme B, suggesting that the mechanism of immunosuppression differs between the subsets[13].

In one experimental model of autoimmune arthritis, mice that were deprived of IL-10-producing regulatory B cells exhibited worse arthritis than wild-type animals; the numbers of inflammatory $T_H1$ and $T_H17$ T cells were increased in B cell-deficient mice and the number of $Foxp3^+$ T regulatory cells was reduced[14]. These findings illustrate the protective effect of IL-10-producing $B_{reg}$ cells. This protective role has also been demonstrated in experimental autoimmune encephalomyelitis (EAE), a model of CNS autoimmunity that is commonly used to study MS[15,16]. In this study, a deficiency of TGFβ1-producing B cells exacerbated the susceptibility to EAE; this effect depended on failure of myeloid dendritic cell activation to be downregulated, which in turn increased the encephalitogenic responses of $T_H1/T_H17$ cells[17].

## B cells in multiple sclerosis

### *B cell abnormalities*

Several abnormalities in the function of peripheral blood-derived B cells have been identified in MS (Supplementary Fig. 1). Abnormalities in the cytokine profiles of naive and memory B cells have been observed in patients with MS[18]. In particular, activated B cells from patients with MS produce excessive amounts of the cytokines TNF, lymphotoxin-α, IL-6 and GM-CSF[18–20]. The details of the mechanisms that underlie B cell cytokine dysregulation, including transcriptional and epigenetic regulation, and the research progress and challenges in this area have been reviewed elsewhere[21].

In addition to, and independent of, inflammatory cytokines, B cells in patients with MS produce some as yet unidentified secretory factors that are toxic to oligodendrocytes[22] and neurons[23]. Furthermore, one study has shown that memory B cells can drive the spontaneous proliferation of self-reactive $CD4^+$ T cells from people with MS, an observation that provides fascinating insight into the complex interplay between B cells and T cells[24].

Several studies have also indicated the dysfunction of $B_{reg}$ cells in MS. In vitro, B cells that derive from people with MS exhibit deficient IL-10 production when stimulated with CD40L in comparison with B cells from matched healthy controls[25]. In people with MS, IL-10-producing $B_{reg}$ cells are reduced during disease relapse compared with in remission[26] and this reduction is associated with a reduction in the ratio of naive to memory $B_{reg}$ cells, suggesting an enrichment of functional $B_{reg}$ cells in the naive subset. Similarly, low numbers of transitional $B_{reg}$ cells have been observed during

MS relapse when compared with numbers of these cells during remission, suggesting that a reduction in B cell-dependent regulation is involved in inflammatory clinical disease[27]. Finally, a study published in 2020 demonstrated that, in addition to producing low levels of IL-10, transitional B cells from patients with MS are defective in the suppression of $T_H1$ cell effector functions[28].

Taken together, the evidence suggests that, within the B cell pool, the memory subset harbours most of the potentially pathogenic MS-associated cells. However, deficiencies in protective (anti-inflammatory or regulatory) B cells in other subsets could be equally important in the pathophysiology of MS and further investigation is needed to fully understand the contributions of different B cell populations.

### *Neuropathological evidence*

Abnormalities in the quantity and quality of immunoglobulins in the cerebrospinal fluid (CSF) are classic pathological features of MS. In >90% of patients with MS, the CSF is positive for immunoglobulin G (IgG) oligoclonal bands, a long-known and still useful diagnostic biomarker[29]. Immunoglobulin M (IgM) oligoclonal bands are detected less frequently but are associated with a worse disease course[30] and therefore have prognostic value[31]. In a key study, a comparison of the immunoglobulin transcriptomes of B cells with the corresponding immunoglobulin proteomes in patients with MS demonstrated that intrathecal B cells are the source of immunoglobulin oligoclonal bands in the CSF[32].

B cells have long been recognized as a subset of infiltrating cells in brain and spinal cord lesions in MS. Initially, these cells were identified mainly in perivascular cuffs and were rarely seen to spread into the parenchyma[33–35]. Subsequently, B cell accumulation within the meningeal and perivascular immune cell infiltrates has been demonstrated[36] (FIG. 1). These abnormalities are accentuated in a subgroup of patients who have a high level of brain inflammation, extensive and active subpial grey matter demyelination, and a rapidly progressive clinical disease course, suggesting that B cell accumulation causes or contributes to the worse clinical course[36–43]. In particular, meningeal inflammation has been associated with a gradient of neuronal, astrocyte and oligodendrocyte loss from the surface inwards, accompanied by microglial activation in subpial grey matter lesions that is greatest in the most external cortical layers and lower in the inner layers close to the white matter[44]. Changes in mRNA levels in the meninges were similar to changes in protein levels in the CSF, suggesting that immune infiltrates in the subarachnoid space — in particular those that are rich in B cells — release inflammatory and/or cytotoxic mediators into the CSF, creating an intracerebral milieu that sustains chronic compartmentalized inflammation and also directly mediates or exacerbates cortical pathology and disease progression[45,46].

Furthermore, a study of brain biopsy samples from patients with a recent diagnosis of MS showed that actively demyelinating cortical lesions that contain perivascular immune infiltrates are common in early MS and are associated with meningeal inflammation[47]. Autoantibodies to myelin oligodendrocyte glycoprotein (MOG) are also present in these lesions, suggesting that myelin is disrupted around axons in acute MS lesions[48]. Studies of MS lesion subtypes[49] suggest that antibody-dependent mechanisms are crucial in MS pathogenesis in a subgroup of patients (type II pattern). However, this pattern could be common to all plaques and just reflect a specific stage of development[50] and B cells could contribute to MS pathogenesis not only by producing antibodies but also through their capacity to release inflammatory factors and their antigen-presenting function. Subsequent studies have shown that autoantibodies to MOG are detectable in a subset of people with MS but are also associated with a wide spectrum of CNS disorders for which the term MOG spectrum disorders has been proposed[51].

Analysis of the immunoglobulin variable region gene repertoire of B cells that were isolated from MS lesions and the CSF of people with MS revealed high levels of clonally expanded memory B cells with extensive somatic mutations, which indicates antigen-driven B cell activation rather than a random bystander response[52–54]. Furthermore, the same antigen-experienced clones can be found in meningeal immune cell infiltrates, the CSF and perivascular parenchymal lesions[55], suggesting that the same initial B cell activation and clonal expansion event led to the invasion of different areas throughout the CNS. Indeed, high-throughput sequencing of IgG heavy chain variable region repertoires in patients with MS[56] has demonstrated that B cell clones are bidirectionally exchanged between the CNS and the periphery and that affinity maturation occurs on both sides of the blood–brain barrier[56].

In two studies published in 2020, single-cell transcriptomic approaches were used to investigate abnormalities in CSF and blood leukocytes in MS[57,58]. The first study identified various T cell subset changes in the CSF of patients with MS, including the enrichment of T follicular helper cells, which support B cell function. This observation suggests that high levels of T follicular helper cells in the CNS could promote B cell maturation and accumulation[57]. In the second study, single-cell RNA sequencing of paired CSF and blood samples from people with MS and healthy controls[58] revealed the differential expression of several genes that encode cytokines and chemokines and the activation of nuclear factor κB signalling and cholesterol synthesis pathways in memory B cells in MS. Furthermore, single-cell immunoglobulin sequencing revealed clonally expanded, somatically hypermutated IgM and IgG1 B cells in the CSF that were associated with acute inflammation[58]. Taken together, these data indicate a skewing of CNS B cells towards an inflammatory phenotype and provide evidence that cross-talk with T cells promotes the differentiation of B cells to plasmablasts and plasma cells as well as the production of abnormal immunoglobulins.

### *Tertiary lymphoid-like structures*

The studies described above have revealed the presence and accumulation of B cells and plasmablasts in the chronically inflamed CNS, possibly through the selective recruitment of differentiated B cells.

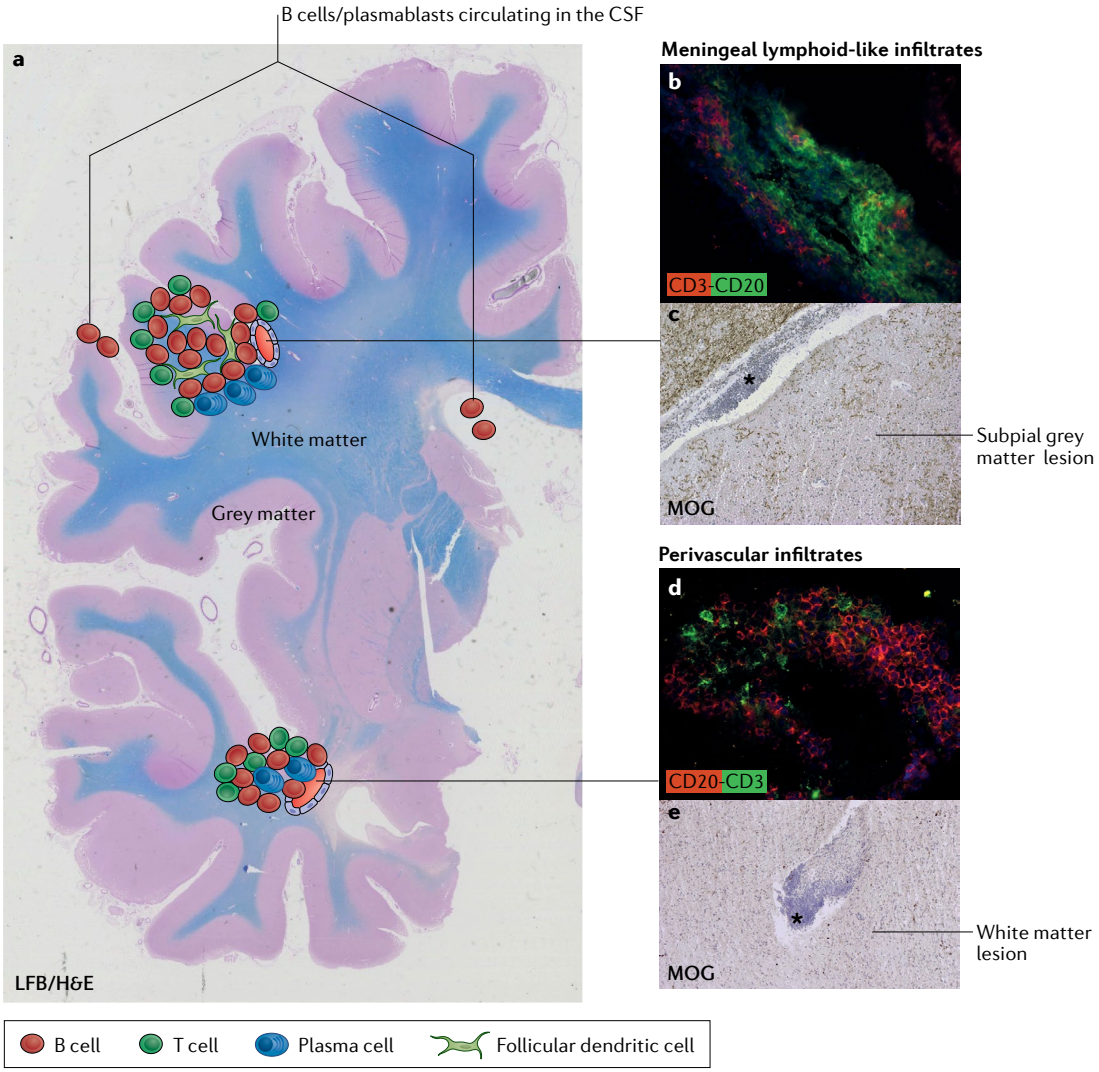



Fig. 1 | **B cell inflammatory infiltrates in multiple sclerosis. a** | Immune infiltrates accumulate in meningeal lymphoid-like structures (top) and perivascular regions (bottom) and CD20$^+$ B cells and plasmablasts are present in the cerebrospinal fluid (CSF). **b** | Immunostaining of a post mortem brain section from an individual with multiple sclerosis shows that CD20$^+$ B cells and CD3$^+$ T cells accumulate in meningeal lymphoid structures. **c** | Immunostaining for myelin oligodendrocyte glycoprotein (MOG), a marker of myelin in white and grey matter, suggests that infiltration of CD20$^+$ B cells and CD3$^+$ T cells into the meninges (asterisk) is associated with subpial cortical grey matter lesions. **d** | Immunofluorescent staining shows that CD20$^+$ B cells and CD3$^+$ T cells accumulate in perivascular inflammatory infiltrates. **e** | Immunostaining for MOG suggests that infiltration of CD20$^+$ B cells and CD3$^+$ T cells into the perivascular spaces (asterisk) is associated with white matter demyelination. H&E, haematoxylin and eosin; LFB, Luxol fast blue.

The long-lived plasma cells that result can persist in the CNS in so-called survival niches that are similar to ectopic follicle-like structures found in chronically inflamed target organs in other autoimmune diseases[59]. Their appearance resembles secondary lymphoid organs and they are therefore referred to as tertiary lymphoid organs[60]. The function of these structures is unknown but some evidence suggests that they act as local sites of antigen presentation and lymphocyte activation and differentiation, including B cell maturation steps such as immunoglobulin gene hypermutation and class switch recombination (reviewed in detail elsewhere[59]). Chemokines that are known to support the formation of such germinal centres, such as CXC-chemokine ligand 13 (CXCL13), CXCL10, lymphotoxin α, IL-6 and IL-10, are present at high levels in the CSF of patients with MS, which is consistent with the formation of tertiary lymphoid-like structures in the leptomeninges[46].

### *The Epstein–Barr virus hypothesis*

The cause of B cell overactivation in MS is poorly understood. One study has demonstrated that genetic variation in B cell activating factor (BAFF) causes its abnormal expression, leading to excess B cell activation in MS[61]. Lymphotropic viruses are also a potential cause of B cell activation — in the context of MS, the Epstein–Barr virus (EBV) is the most strongly implicated and the most extensively investigated virus. A discussion of all viral associations is beyond the scope of this Review but we illustrate the principle with the hypothesis

that EBV drives B cell overactivation in people who develop MS (FIG. 2).

EBV is a human gamma herpesvirus that infects primary B cells and epithelial lineage cells. Approximately 90% of the world's adult population is seropositive for EBV. Infection is usually benign and asymptomatic, though it can cause infectious mononucleosis syndrome and, far less commonly, it can evolve into lymphoproliferative disorders[62]. EBV typically persists throughout life in host memory B cells, normally in a latent, inactive state or, less frequently, in a lytic, replicative state, depending on the combination of viral (latent and lytic) gene expression and host (immune surveillance and epigenetic) factors. Strong epidemiological data implicate EBV as a factor in the risk of developing MS, and serological positivity for EBV increases the risk of developing MS[63,64]; however, the role of EBV infection in the pathogenesis of MS is controversial and the mechanism unknown.

Studies in post mortem brain tissue from people with MS indicate that EBV reactivation can cause $CD8^+$ T cell cytotoxicity against infected B cells and plasma cells in tertiary lymphoid-like follicles and acute lesions[65–67]. In addition, changes in cytokines that are involved in immune surveillance against the virus, such as the overexpression of IFNα, have been detected in active lesions and are associated with the presence of EBV latent proteins[68]. However, in other studies, EBV has not been detected in CNS tissue from patients with MS, bringing into question the notion that EBV acts directly as a pathogen within the target organ[69–71]. The discrepancies in these studies could be partially explained by technical differences in tissue preservation that might alter the quality of proteins and RNA[72]. Alternatively, the control of EBV replication by the immune system could be dysfunctional within the CNS, a hypothesis supported by evidence of low titres of antibodies to EBV in the CSF of people with MS[70,73]. However, in a transcriptomic study published in 2020, the interrogation of RNA sequencing libraries for human viral transcripts, including EBV, in B cells from the CSF of people with relapsing–remitting MS, no viral transcripts were found[58]. This study included a relatively small number of patients ($n = 12$) and therefore confirmatory studies are needed.

Several studies have been conducted to characterize T cell reactivity to EBV in the blood. EBV antigen-specific $CD4^+$ T cells are detectable in the blood of patients with MS and these cells have the potential to cross-react with myelin antigen and produce inflammatory cytokines[74]. $CD8^+$ T cells specific to EBV lytic antigens are also increased in patients with active MS[75]. Studying EBV antigen-specific T cell responses in the CNS is more difficult because of the limited access to tissue, but the phenotypes and functional profiles of T cells in post mortem brain tissue and in vitro cell co-culture experiments in one study showed that $CD8^+$ T cells from CSF derived from patients with MS could recognize autologous EBV-infected B cells[76]. EBV-specific $CD8^+$ T cells express programmed cell death protein 1 (PD1), which has a central role in immune tolerance by inducing the apoptosis of cytotoxic T cells, and the expression of PD1 in $CD8^+$ T cells is higher in patients with stable MS than in those with inflammatory-active MS. These observations suggest that the suppression of cytotoxic responses against viruses, including EBV, is associated with the attenuation of inflammation and, accordingly, CNS disease remission[77]. Furthermore, immunohistochemical analysis of post mortem brain tissue has demonstrated that the expression of PDL1, the main receptor for PD1, is higher in MS lesions than in control tissue from individuals without CNS disease and this expression co-localized with astrocyte or microglia/macrophage cell markers[78]. Taken together, these observations suggest that the PD1–PDL1 axis modulates cytotoxic T cell responses against EBV in MS and could therefore be implicated in the pathogenesis of MS and/or be harnessed therapeutically.

Although knowledge of the relationship between EBV and MS is incomplete and, in some areas, controversial, a model that includes EBV provides a plausible hypothesis to explain B cell overactivation in MS. The relationship of EBV with MS has been reviewed in more detail elsewhere[79].

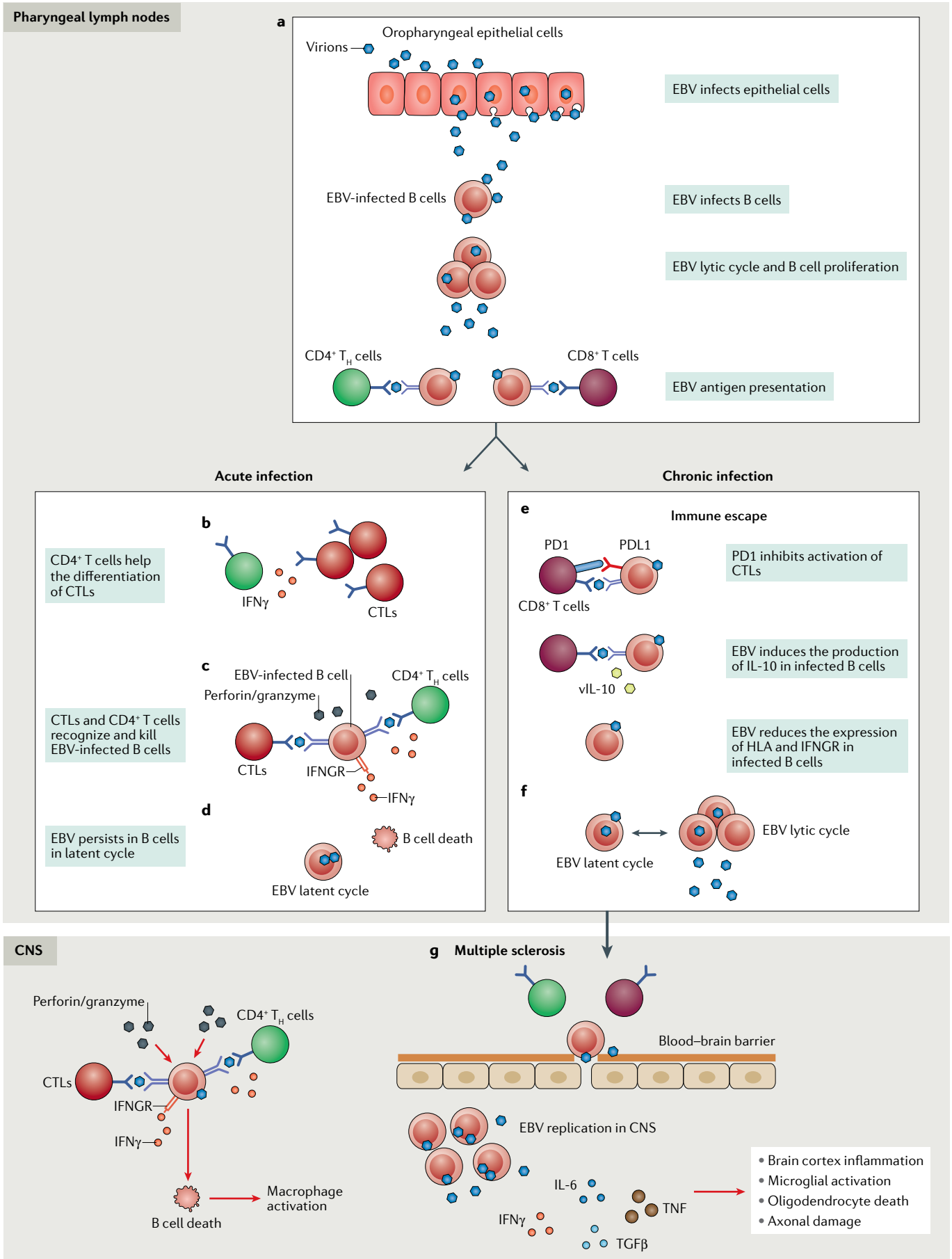
## B cell-targeted therapy in MS

The involvement of B cells in the pathogenesis of MS has long been suggested by immunoglobulin abnormalities, including oligoclonal bands, in the CNS. However, only in recent years have treatments that target B cells, including rituximab, ocrelizumab, ofatumumab, atacicept and Bruton tyrosine kinase (BTK) inhibitors, been tested in MS in clinical trials and two of these treatments have been approved — ocrelizumab and ofatumumab. Below, we summarize the key results from the pivotal trials of these treatments and discuss the immunological effects of these therapies on B cell subsets and B cell-related biomarkers. Disease-modifying treatments for MS that do not specifically target B cells but might also have effects on B cell function (for example, dimethyl fumarate, teriflunomide, IFNβ, glatiramer acetate, natalizumab and fingolimod) have been reviewed elsewhere[80,81] and are therefore not discussed here.

### *Rituximab*

Rituximab is a mouse–human chimeric anti-CD20 monoclonal antibody that is licensed for the treatment of some haematological cancers (non-Hodgkin B cell lymphoma and chronic lymphocytic leukaemia), rheumatoid arthritis and granulomatosis with polyangiitis. CD20 is expressed in cells of the B cell lineage, including pre-B cells, naive B cells and memory B cells. The mechanisms by which rituximab depletes B cells include complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC)[82–84].

***Summary of clinical evidence.*** Pivotal studies of the intravenous administration of rituximab in MS include a phase I open-label trial in relapsing–remitting MS[85], a phase II study in relapsing–remitting MS[86] and a phase II/III study in primary progressive MS[87]. Phase I/II studies have also been done to investigate the intrathecal administration of rituximab in secondary progressive MS[88,89]. The trials demonstrated that treatment with rituximab reduced relapses in relapsing–remitting MS


Pharyngeal lymph nodes
a
Oropharyngeal epithelial cells
Virions
EBV infects epithelial cells
EBV-infected B cells
EBV infects B cells
EBV lytic cycle and B cell proliferation
CD4+ TH cells
CD8+ T cells
EBV antigen presentation
Acute infection
Chronic infection
b
CD4+ T cells help the differentiation of CTLs
IFNγ
CTLs
c
EBV-infected B cell
CD4+ TH cells
Perforin/granzyme
CTLs and CD4+ T cells recognize and kill EBV-infected B cells
CTLs
IFNGR
IFNγ
d
EBV persists in B cells in latent cycle
B cell death
EBV latent cycle
e
Immune escape
PD1
PDL1
PD1 inhibits activation of CTLs
CD8+ T cells
EBV induces the production of IL-10 in infected B cells
vIL-10
EBV reduces the expression of HLA and IFNGR in infected B cells
f
EBV latent cycle
EBV lytic cycle
CNS
g Multiple sclerosis
Perforin/granzyme
CD4+ TH cells
CTLs
IFNGR
IFNγ
B cell death
Macrophage activation
Blood–brain barrier
EBV replication in CNS
IL-6
IFNγ
TNF
TGFβ
• Brain cortex inflammation
• Microglial activation
• Oligodendrocyte death
• Axonal damage

◀ Fig. 2 | **Model of the involvement of Epstein–Barr virus in the pathogenesis of multiple sclerosis.** This model is based on the hypothesis that the Epstein–Barr virus (EBV) causes the activation of infected B cells and their targeting by T cell responses. During primary infection, EBV is transmitted orally and replicates in the oropharyngeal epithelium with a rapid expansion of the viral load (part **a**). The EBV then colonizes the pharyngeal lymph nodes and infects B cells where it persists for the life of the host as asymptomatic latent infection (latent cycle). The virus produces infectious virions with intermittent reactivation of the lytic cycle that drives the expansion of infected $CD27^+CD70^+$ memory B cells. The balance between the latent and lytic cycle depends on the ability of the immune system of the host to generate an appropriate immune response to the EBV infection and the EBV to escape the immune system. The EBV-specific cytotoxic T cell response, including both $CD4^+$ and $CD8^+$ T cells, reduces the expansion of latently infected B cells. Upon the recognition of EBV-infected B cells, $CD4^+$ T helper ($T_H$) cells differentiate and sustain the proliferation and differentiation of cytotoxic $CD8^+$ T cells (CTLs; part **b**) and contribute to the elimination of EBV-infected cells by producing interferon-γ (IFNγ) and perforin/granzyme (part **c**). The T cell response controls the infection, but EBV can remain in a latent state in memory B cells for life (part **d**). If the T cell response does not effectively resolve the EBV infection owing to either insufficient CTL activity (which can result from the overactivity of immune checkpoints such as PD1) or to the ability of the virus to evade the immune system (which it does by producing viral IL-10 (vIL-10) that downregulates cell-surface HLA and modulates the immune response or by inhibiting IFNγ receptor (IFNGR) expression on EBV-infected B cells (part **e**)), EBV persists and can reactivate (part **f**). EBV reactivation induces the proliferation of memory B cells, which shed EBV virions and produce inflammatory cytokines. Some evidence suggests that EBV is present in activated memory B cells within follicle-like structures in the leptomeninges in multiple sclerosis (part **g**). The reactivation of the virus in EBV-infected B cells attracts T cells that enter the CNS across the blood–brain barrier and generate an immune response against EBV without resolving the infection. EBV induces B cell proliferation and the production of inflammatory mediators (tumour necrosis factor (TNF), IL-10, transforming growth factor-β (TGFβ) and IL-6) that cause microglial activation and neuronal injury.

and attenuated the worsening of disability in a subset of patients with primary progressive MS.

***Immunological effects.*** After the administration of rituximab, a dose-dependent depletion of circulating $CD20^+$ B cells rapidly ensues and persists for several months. A small subset of $CD20^+CD3^+$ T cells is also depleted by rituximab in patients with MS[90]. The function of this T cell population is unknown but they have been proposed as contributors to the pathogenic process in MS, in which case their depletion could partly account for the action of CD20-targeted therapies[90].

The patients with relapsing–remitting MS who received rituximab in the phase I trial exhibited a near-total B cell depletion (~99.8%) in the circulation by week 2 that was sustained at week 48 (REF.[85]). The reconstitution of B cells was slow and reached a mean of only 34.5% of baseline levels by week 72. Most of the re-populating B cells were naive ($CD27^-$). Serum levels of IgM, IgA and IgG were tested in some treated patients and did not decrease below the lower limit of normal by week 72 (REF.[85]).

Similarly, in the phase II trial, patients with relapsing–remitting MS who were treated with rituximab exhibited a rapid and near-complete (>95%) depletion of circulating B cells at 2 weeks after the first course of treatment and substantial depletion persisted until week 24; B cells reconstituted to ~30% of baseline levels by week 48. Serum levels of immunoglobulin were measured at baseline, at 24 weeks and 48 weeks after treatment, and at the time of any relapse; levels were below the lower limit of normal in 7.9% of patients who received rituximab compared with 3.0% of patients who received placebo[86]. The median serum levels of IgM, IgG and IgA across all participants remained above the lower limit of normal for the duration of the trial but individual levels of IgM were abnormally low in 22.4% of patients who received rituximab and in 8.6% of patients who received placebo. The minimal effect on serum immunoglobulin levels is explained by the fact that rituximab and other CD20-targeted treatments do not directly deplete plasma cells because these cells do not express CD20 (except a small population of $CD20^+$ plasmablasts)[91]. However, long-term treatment with anti-CD20 agents can cause hypogammaglobulinaemia and the attendant increased risk of severe infections (reviewed elsewhere[92]). Overall, the beneficial effects of B cell depletion achieved with rituximab and without a reduction in plasma cell number or in the levels of circulating antibodies supports the notion that B cell subsets play a causative role in inflammation in MS.

As discussed above, abnormalities of B cells in MS include a propensity to produce excess pro-inflammatory cytokines, such as TNF, lymphotoxin, IL-6 and GM-CSF, and insufficient anti-inflammatory cytokines (IL-10). The effect of rituximab is thought to be mediated by various cell-dependent mechanisms, including the suppression of APC function, the depletion of pro-inflammatory memory B cells, a decrease in cytokine production, and possibly the prevention of lymphoid neogenesis[93]. In more detail, B cell depletion with rituximab affected B cell–T cell interactions in patients with MS in whom the proliferation of $CD4^+$ and $CD8^+$ T cells and the T cell expression of IFNγ and IL-17 in T cells were reduced[19,94]. Anti-CD20 therapy in patients with MS also decreased pro-inflammatory myeloid cell responses, attributable to the depletion of GM-CSF-producing B cells[20]. Myeloid cells remained less pro-inflammatory even when the B cell population reconstituted, possibly reflecting changes in STAT5 and STAT6 signalling and in GM-CSF to IL-10 cytokine ratios in the reconstituted B cells[20].

The analysis of B cells in people with MS who had received rituximab demonstrated that the repopulating B cells were enriched in transitional and mature naive B cell subsets with a high expression of activation markers but repopulation patterns varied at the individual level[95]. Further studies into B cell compartment reconstitution after rituximab treatment for neuromyelitis optica spectrum disorders and peripheral autoimmune conditions have confirmed the predominance of naive and $B_{reg}$ cells over memory B cells in the repopulated B cell pool[96,97]. However, B cell depletion with CD20 antibodies caused the exacerbation of $MOG_{35–55}$ peptide-induced EAE[98,99] and this exacerbation was associated with an increased production of pro-inflammatory TNF by $CD11b^+$ APCs, suggesting that the depletion of $B_{reg}$ cell subsets could accentuate disease by removing the $B_{reg}$ control of inflammatory cells[99]. These contrasting observations raise the concern that the total B cell depletion that affects the subsets of $B_{reg}$ cells could be ineffective or even counter-productive in some clinical settings.

Though CD20 antibodies efficiently deplete circulating B cells, they do not efficiently cross the blood–brain

barrier and, therefore, upon intravenous administration, they are expected to be less effective at depleting CNS-resident memory B cells, which are the origin of abnormal intrathecal immunoglobulin production in MS. However, in one study in patients with MS who were receiving intravenous rituximab, both B cell and T cell numbers in the CSF were significantly reduced after therapy[100]. This effect indirectly suggests that B cells support T cell recruitment into the CNS through mechanisms that take place in the peripheral lymphoid system. This mechanism could involve a contribution of B cells to the activation and proliferation of pathogenic T cells through antigen presentation and/or the release of pro-inflammatory cytokines. The notion of a cytokine-mediated effect is supported by data from studies in EAE in which the depletion of IL-6-producing B cells ameliorated disease as well as from studies in patients with MS in whom rituximab treatment reduced the production of IL-6 and IL-17 (REF.[101]).

In a trial of intrathecal administration of rituximab[102], levels of BAFF — which regulates B cell survival, development and differentiation, the size of the peripheral B cell pool and production of immunoglobulins — were drastically decreased in the CSF, whereas levels in the serum increased. $CD20^+$ and $CD19^+$ cells were depleted in the periphery but no significant change in the levels of these cells was seen in the CSF, possibly because B cell counts were too low to enable the detection of changes[102].

### *Ocrelizumab*

Ocrelizumab is a humanized monoclonal antibody that is approved for the treatment of active relapsing–remitting MS and primary progressive MS. Like rituximab, it selectively targets CD20, which is expressed on pre-B cells and mature naive and memory B cells but not on lymphoid stem cells or plasma cells[103]. Ocrelizumab binds with high affinity to the large extracellular loop of CD20, a different but overlapping epitope from that to which rituximab binds[84]. Ocrelizumab induces B cell depletion via similar mechanisms to rituximab, including ADCC (to a greater extent than rituximab) and CDC (to a lesser extent than rituximab)[82,104].

***Summary of clinical evidence.*** In two essentially identical randomized phase III trials (OPERA I and OPERA II)[105], ocrelizumab was shown to be more effective than subcutaneously administered IFNβ1a in patients with relapsing–remitting MS. The clinical benefits of ocrelizumab treatment were also observed in patients with primary progressive MS in the ORATORIO study, a phase III, placebo-controlled trial[106]. Based on these trials, ocrelizumab has been approved for the treatment of relapsing–remitting MS and primary progressive MS.

***Immunological effects.*** As with rituximab, the radical depletion of B cells occurs within 2 weeks after the first ocrelizumab infusion[85,105]. Preliminary analysis of blood from a small group of participants in the OPERA I trial indicated that a small number of B cells remain detectable in the peripheral circulation after treatment with ocrelizumab, characterized by a memory class-switched profile with a low diversity of BCRs[107]. CD20-expressing T cells, which one study has suggested make up nearly 20% of all CD20-expressing cells (including B cells), are efficiently depleted in addition to B cells[108], suggesting that the clinical efficacy of ocrelizumab might not only be mediated by effects on B cells[109]. The longitudinal analysis of the CSF before and after ocrelizumab treatment has been presented in abstract form and reveals reductions in the CSF levels of B cells, T cells and CXCL13 (REF.[110]).

Although plasma cells are not directly targeted by ocrelizumab, data from the pivotal trials of ocrelizumab and their extensions reveal that, as with rituximab[92], immunoglobulin levels can be reduced during long-term treatment (mean 5.5 years) and could be associated with serious infections, particularly if IgG levels are low[111]; this risk warrants further investigation. Detailed studies of B cell reconstitution in patients with MS who have been treated with ocrelizumab are awaited.

The mechanisms that mediate the clinical effects of ocrelizumab in primary progressive MS are unknown, particularly as many other therapeutics have failed in this form of the disease. Most speculation has focused on the possibility that $CD20^+$ B cell (and T cell) depletion could dampen the 'smouldering' inflammatory activity in primary progressive MS. However, some investigators have[21] suggested that the mechanisms are not solely anti-inflammatory but also involve a reduction of neurotoxic factors produced by B cells[22,23].

### *Ofatumumab*

Ofatumumab is a fully human monoclonal antibody that binds to a small-loop epitope of CD20 close to the cell surface and induces both CDC and ADCC even when the expression of CD20 is low[112,113].

***Summary of clinical evidence.*** Completed clinical trials of ofatumumab in patients with MS are the phase IIb double-blind MIRROR study[114] and the phase III trials of ofatumumab versus teriflunomide ASCLEPIOS I and II[115]. In August 2020, ofatumumab was approved by the FDA for the treatment of relapsing forms of MS, including clinically isolated syndrome, relapsing–remitting MS and active secondary progressive MS.

***Immunological effects.*** The pivotal trials demonstrated that ofatumumab depletes B cells in a dose-dependent manner; B cell counts were between <2% and 25% of baseline levels at week 12. The time to onset of repopulation was lengthened by a higher dose of the drug. At the last follow-up time point (132 weeks), the repletion of $CD19^+$ B cells to the normal reference range had occurred in 64–74% of patients across the ofatumumab treatment groups[114]. However, detailed information about the effects of ofatumumab on B cell populations is awaited.

### *Atacicept*

Atacicept is a human recombinant fusion protein that comprises the ligand portion of a receptor for B lymphocyte stimulator and a proliferation-inducing ligand, which are important cytokines for B cell maturation, proliferation and survival[116]. In contrast to anti-CD20

therapies, atacicept affects only mature naive B cells and B cells in the late stages of development, including antibody-secreting cells, and spares B cell progenitors and memory B cells[117–119]. Consequently, the survival of short-lived plasma cells and serum immunoglobulin levels are reduced with atacicept treatment as documented in healthy volunteers and patients with systemic lupus erythematosus or rheumatoid arthritis[120–122].

All trials of atacicept in MS have been halted because an unexpected increase in disease activity was observed during treatment[116]. One plausible suggested reason for the observed effect is that the preferential diminishing of naive B cells and plasmablasts and the relative sparing of memory cells, which are the probable disease-promoting B cell subset in MS, exacerbated an imbalance of pro-inflammatory cytokines[21].

### *Bruton tyrosine kinase inhibitors*

Bruton tyrosine kinase (BTK) is critically involved in B cell receptor signalling and, consequently, in B cell activation, and activates myeloid cells via the Fc receptor. The observation of abnormal BTK activity in autoimmune diseases has prompted the development of BTK inhibitors as potential therapeutics[123]. The oral BTK inhibitor evobrutinib has been tested in MS in a phase II trial[124]. The treatment reduced MS lesion activity and the study met the primary MRI endpoint but significant effects were not seen on key secondary endpoints, including the annualized relapse rate and change in disability.

The effects of BTK inhibitors on lymphocyte subsets in this trial were not reported but the immunological effects of evobrutinib have been studied in the EAE mouse model. Multiple effects on B cells were observed, including the inhibition of activation, cytokine release and APC function[125].

Overall, BTK inhibition is an attractive strategy for the therapeutic targeting of B cells without their depletion. Therapeutics in this class could become an important alternative for patients whose immunoglobulin levels are reduced by B cell depleting therapy. Further trials of BTK inhibitors are expected to investigate the clinical efficacy of these drugs.

## Non-specific lymphocyte depletion

Some highly effective treatments for MS deplete all lymphocytes and consequently affect B cell numbers and functions. In the sections below, we discuss the effects that these therapies have with a focus on their effects on B cells.

### *Alemtuzumab*

***Summary of clinical evidence.*** Alemtuzumab is a humanized monoclonal CD52 antibody approved for the treatment of active relapsing–remitting MS. Its approval was based on findings that it reduces relapse rates to a greater extent than IFNβ in treatment-naive patients with active MS (demonstrated in the CAMMS232 and CARE-MS I trials)[126,127] and in patients who experienced a relapse while on previous treatment (demonstrated in the CARE-MS II trial)[128]. Treatment with alemtuzumab also reduced the sustained accumulation of disability progression[126,128]. The durability of efficacy has been demonstrated over 5 years of follow-up without continuous treatment[129,130]. The efficacy of alemtuzumab has largely been attributed to the long-lasting drug-induced T cell lymphopenia and the homeostatic changes it induces[131,132].

***Immunological effects.*** Alemtuzumab induces the rapid depletion of CD52-expressing leukocytes (mainly T cells and B cells, with transient effects on neutrophils, monocytes, eosinophils, basophils and natural killer cells) through antibody-dependent[133] and complement-dependent cytolysis[134], followed by reconstitution that starts within a few days after each treatment cycle. After a single course of alemtuzumab, B cell counts recover to the lower limit of normal after ~8 months, whereas T cell subsets do not normalize for up to 3 years[135].

At 1 month after treatment, transitional type 1 immature B cells ($CD19^+CD23^-CD27^-$) constitute the majority of the B cell pool and the levels of BAFF increase and remain high until at least 12 months after treatment[136]. BAFF is critical for the transition of immature B cells into mature naive B cells ($CD19^+CD23^+CD27^-$), which account for most of the B cell population at 3 months after treatment and ultimately hyper-populate the peripheral circulation; their levels reach 165% of baseline levels at 12 months after treatment. By contrast, the repletion of memory B cells is slow and their levels reach only 25% of baseline at 12 months after treatment[136]. Levels of $CD19^+CD24^{hi}CD38^{hi}$ $B_{reg}$ cells increase substantially at 6 months and 9 months after treatment compared with their pre-treatment levels, suggesting that B cell regulation is preferentially restored[27].

In comparison with B cells, T cells reconstitute more slowly — $CD8^+$ T cells return to baseline levels by 12 months after treatment and $CD4^+$ T cells remain below baseline levels at 12 months. During the repopulation phase, the proportion of the memory T cell population that are T regulatory cells ($CD4^+CD25^{high}$ T cells) is higher than before treatment[137,138] and the proportion of $T_H17$ and $T_H1$ cells is lower[138]. The findings of a study published in 2019 suggest that a population of pro-inflammatory T cells that express CD20 and are present at high levels in the blood and CSF of patients with MS is depleted after treatment with alemtuzumab[139].

Differences in the repopulation of T cell and/or B cell subsets, such as different ratios of naive and memory T cell and B cell subsets, are an attractive explanation for differences in clinical efficacy and in the occurrence of secondary autoimmunity among patients after treatment with alemtuzumab. However, a study published in 2020 investigated this possibility and found no evidence for a correlation between clinical outcomes and lymphocyte dynamics[140]. The effects of alemtuzumab on B cells in the CSF are also poorly understood; additional insight could be gained by testing the hypothesis that the long-term clinical effects observed after alemtuzumab treatment are partly mediated by the prevention of B cell-related immune pathology in the CNS compartment, including the formation of tertiary lymphoid-like structures[141].

### *Cladribine*

Cladribine is a synthetic purine analogue (2-chlorodeoxyadenosine) that selectively depletes lymphocytes but spares innate immune cells. The drug can be administered orally or intravenously and the oral preparation is approved for the treatment of highly active relapsing–remitting MS.

***Summary of clinical evidence.*** Oral cladribine was approved for the treatment of highly active relapsing–remitting MS based on the phase III CLARITY trial and its extension study, which showed that cladribine effectively reduced relapses, disability progression and MRI activity when compared with placebo[142–145]. The ORACLE study has also shown that cladribine effectively reduces the rate of conversion to MS in patients with a first demyelinating episode[146,147].

***Immunological effects.*** Cladribine is a pro-drug and requires intracellular phosphorylation to become chemically active. The active metabolite accumulates in the cell, ultimately leading to apoptosis[148,149]. It is thought to act as a short-term immunosuppressant that induces a transient depletion of B cells and T cells that is followed by immune reconstitution[148,150]. Pooled data from CLARITY, CLARITY extension and PREMIERE (Prospective Observational Long-Term Safety Registry of Multiple Sclerosis Patients Who Participated in Cladribine Clinical Trials) indicate that cladribine induces the selective depletion of B cells and T cells. In the lymphocyte-depleted phase after the first treatment cycle, B cell counts were reduced by 70% at 5 weeks, 81–84% at nadir (13 weeks), ~60% at week 24 and ~30% at week 48 (REF.[151]). These reductions were faster and larger than those for T cells, which reduced by ~50% at week 5, predominantly owing to $CD4^+$ T cell depletion — median $CD8^+$ T cell counts never dropped below the reference range, suggesting that this cell subset is relatively resistant to the lymphodepleting effects of cladribine[151]. Each treatment cycle is followed by a gradual lymphocyte repopulation. B cells recovered to numbers within the reference range by week 84, ~30 weeks after the last dose of treatment[150], whereas the recovery of median $CD4^+$ T cell counts to within the normal range was slower and took up to 96 weeks, ~43 weeks after the last dose of cladribine.

The effects of oral cladribine on the composition and functions of B cell subsets in MS are yet to be examined in depth. One cross-sectional study of 40 patients with relapsing–remitting MS who were at the end of the first cycle of alemtuzumab or injectable cladribine treatment showed that the latter induced a marked depletion of class-switched and un-switched memory B cells to levels comparable with those induced by alemtuzumab but without the severe initial lymphopenia associated with alemtuzumab — grade 3 and 4 lymphopenia occurred in 1.8% of patients who received injectable cladribine but in >80% of those who received alemtuzumab[152]. The numbers of immature and mature (naive) B cells at 1 year after treatment was higher in patients who received injectable cladribine than in those who received alemtuzumab, suggesting that cladribine depletes memory B cells most strongly[152]. Interestingly, and providing mechanistic support for these effects, the ratio of deoxycytidine kinase to cytosolic 5′-nucleotidase was reported to be high in B cells in physiological conditions, especially in mature, memory and germinal centre B cells, but not in plasma cells. This observation explains the sensitivity of memory B cells to the effects of cladribine and their substantial and persistent depletion after treatment[152]. B cells remain substantially below their baseline level until the second treatment cycle with cladribine, whereas they reach baseline levels 6 months after each course of alemtuzumab and increase to well-above baseline levels by the end of years one and two after alemtuzumab cycles[153].

Interestingly, cladribine can penetrate the CNS, which is, in principle, desirable for the potential treatment effects on lymphocytes that infiltrate the CNS compartment in MS but, to our knowledge, the effects of oral cladribine on the CNS compartment and the CSF of patients with MS has not been definitively reported. In a study of treatment with an injectable variant of cladribine in 29 treatment-naive patients with relapsing–remitting MS, the CSF of 55% of patients became negative for oligoclonal immunoglobulin bands, suggesting a treatment effect on a biomarker of abnormal B cell and plasma cell activation[154].

## AHSCT and B cells in MS

AHSCT has been used to treat patients with severe autoimmune diseases, usually when the disease is refractory to conventional therapies[155,156]. The treatment involves the mobilization of haematopoietic stem cells, the preservation of these cells and their reinfusion after high-dose immunosuppressive conditioning that is achieved with varying degrees of lymphoid and myeloid ablation, depending on the regimen[157]. This procedure enables immune reconstitution and the changes induced by depletion and repopulation are thought to underlie the durable remission of autoimmune disease.

### *AHSCT for the treatment of MS*

During the past 10–15 years, interest has increased in AHSCT as a treatment option for highly active MS, especially for patients whose disease is refractory to standard therapies[157,158]. The rationale for the treatment strategy is that immunosuppression or ablation will deplete presumably pathogenic immune cells and the subsequent transplantation of autologous bone marrow-derived haematopoietic stem cells will enable the regeneration of a new, tolerant immune system[159]. The intensity of the immunosuppressive conditioning treatment varies depending on the specific protocol and ranges from non-myeloablative[158] to myeloablative[160]. The autologous transplantation mostly provides a form of haematopoietic support but might influence the rate and quality of immune reconstitution in ways that are still poorly understood[161].

AHSCT has stabilized the clinical course of MS in most patients who have undergone the treatment. This effect has resulted from the suppression of inflammatory disease activity, with well-documented beneficial effects on clinical relapses, brain and spinal cord MRI

lesions, and neurological dysfunction quantified by the Expanded Disability Status Scale[162–166]. A meta-analysis of data from 15 studies of AHSCT in any form of MS published between 1995 and 2016 has shown that AHSCT is most likely to offer an advantageous benefit-to-risk profile for patients with aggressive MS who have a relapsing–remitting course and who have not yet accumulated a high level of neurological disability[167].

### *Regeneration of adaptive immunity*

To date, most studies of immune reconstitution after AHSCT in MS have focused on T cells. In one of the first demonstrations of extensive immune regeneration, the expansion of naive T cells in the peripheral circulation was observed in patients with MS 1–2 years after total-body irradiation followed by AHSCT and the repertoire of these T cells was distinct from and more diverse than the pre-treatment repertoire[168]. Subsequent work has shown that a substantial (median 80%) renewal of the $CD4^+$ TCR clonal composition is detectable 12 months after AHSCT and that this renewal is more extensive than that of the $CD8^+$ TCR clonal composition (median 45%)[169].

Longer-term studies of TCR repertoires in blood, combined with extension of the analyses to CSF cells, have revealed that the ablation of pre-therapy T cell clones achieved in the blood at 12 months of follow-up persists after 2 and 4 years of follow-up and is more extensive in the CSF, where it also persists at the same time points, long after the completion of peripheral immune reconstitution[170]. These observations are consistent with the hypothesis that the beneficial effects of AHSCT are mediated by the combined effects of depletion and reconstitution, possibly including a rebalancing of the pro-inflammatory and tolerogenic arms of immunity[171] rather than solely lymphoid ablation. A rebalancing mechanism would explain the long-lasting clinical effects better than would immunosuppression from lymphoid depletion as this mechanism would no longer operate after immune repopulation is complete.

The effects of AHSCT on the B cell compartment in MS have not been investigated in the same depth as the effects on T cells. Given that AHSCT protocols are similar across autoimmune diseases and no major differences in immune reconstitution are expected between patients with different autoimmune diseases, we can gain insight from studies of AHSCT for diseases in which B cells were previously known to be pathogenically relevant such as systemic lupus erythematosus and systemic sclerosis. In both of these diseases, the proportions of naive B cells in the blood were increased after AHSCT[172–174]. In addition, one study of patients with systemic sclerosis showed that the proportion and absolute numbers of IL-10-producing $CD24^{high}CD38^{high}$ $B_{reg}$ cells were increased after AHSCT and higher numbers of these cells were associated with more complete clinical responses[173]; this observation is relevant to the mechanisms of immune tolerance as it suggests that the effects of AHSCT on $B_{reg}$ cells are clinically relevant and a part of the mode of action.

Current knowledge of the effects of AHSCT on B cells specifically in MS comes only from a mass cytometry study of immune cell reconstitution after AHSCT[175]. The analysis of blood samples from participants in the HALT-MS trial revealed that B cell numbers are fully reconstituted at 1 year and that, in comparison with baseline before the start of the AHSCT procedure, the proportion of B cells that are circulating naive B cells is high at 1 year and remains high at 2 years after transplantation[175]. These findings are consistent with trends described in earlier studies in which conventional flow cytometry was used[176,177]. For the mass cytometry study[175] as well as for the previously mentioned studies of TCR repertoire[169,170], the low rate of treatment failure in the HALT-MS trial meant that statistical power was not sufficient to make comparisons that would provide conclusive information about the clinical correlations of B cell (or indeed any immune) reconstitution features.

In early studies of AHSCT in MS, the qualitative and quantitative changes of oligoclonal IgG bands in the CSF and serum were analysed. The included samples were obtained after relatively short follow-up periods and these studies demonstrated the persistence of oligoclonal IgG in the CSF[177–179]. In one study, CSF oligoclonal bands persisted in the CSF of two patients at 4 and 6 years after transplantation, indicating that immunoglobulin-producing plasma cells persisted despite high-intensity conditioning and long-term disease remission[180]. However, in the HALT-MS trial, the number of CSF oligoclonal bands and CSF levels of IgG were substantially reduced at 2 years after AHSCT[181]. In summary, the available evidence suggests that immune-depleting conditioning followed by AHSCT

Box 1 | **Suggested studies of B cells in autologous haematopoietic stem cell transplantation**

Several experiments can be conducted to improve our understanding of how autologous haematopoietic stem cell transplantation (AHSCT) affects B cells in multiple sclerosis (MS), as follows:

- Detailed multi-parametric flow cytometry for analysis of B cell subsets in blood and cerebrospinal fluid (CSF), including naive and memory B cells, transitional B regulatory cells, and plasma cells.
- Single-cell RNA sequencing studies of B cell populations in blood and CSF before and after treatment.
- Analysis of B cell receptor immunoglobulin heavy and light chains in peripheral blood B cell subsets and in B cells present in CSF; this approach could benefit in the near future from single-cell genomic and transcriptomic technologies.
- Functional studies of selected B cell subsets, such as those that activate myeloid cells and produce granulocyte–macrophage colony-stimulating factor and regulatory, IL-10-producing B cells, to compare those in patients who have undergone AHSCT with those in untreated patients.
- Rigorous studies of oligoclonal bands in the CSF and serum after long-term follow-up (≥5 years) periods after AHSCT and comparison with pre-therapy oligoclonal bands in a sufficiently large number of patients to conclusively address the question of whether oligoclonal bands persist and whether oligoclonal bands that are present after long-term follow-up are the same or different.
- Studies of the correlation between immunoglobulin M (IgM) oligoclonal bands, in addition to conventional IgG oligoclonal bands, with clinical outcomes, including after B cell-targeted treatments. IgM oligoclonal bands have been associated with an active inflammatory disease phenotype in relapsing–remitting MS and primary progressive MS and could have prognostic value[31,183,184].
- Studies of Epstein–Barr virus reactivation, which is frequently observed after AHSCT even in the absence of MS activity[185], alongside the characterization of Epstein–Barr virus-specific immune responses and B cell repertoire analyses, as outlined above.


Fig. 3 | **Effects of multiple sclerosis treatments on lymphocyte levels and B cell markers.** Bar charts and pie charts provide semi-quantitative indications of the proportions of cell subsets in the population. Charts in the left column indicate pre-therapy levels in patients with multiple sclerosis. Oligoclonal bands (OCBs) are shown as present (+) or absent (–). Levels of other soluble B cell markers and T helper ($T_H$) cells are shown as increased (↑) or decreased (↓). Question marks indicate unknowns. AHSCT, autologous haematopoietic stem cell transplantation; BAFF, B cell activating factor; $B_{reg}$, B regulatory; CSF, cerebrospinal fluid; CXCL13, CXC-chemokine ligand 13; $T_{reg}$, T regulatory.

in patients with MS leads to peripheral reconstitution of a predominantly naive B cell population. Limited observations indicate that oligoclonal bands persist in the CSF for at least 2 years, albeit at lower levels, and underscore the need for further study. Given that the disease stabilizes in the vast majority of patients with MS who undergo AHSCT, the persistence of plasma cells in the periphery and CNS, probably together with some pro-inflammatory B cells, is counterbalanced by the regeneration of a regulatory and/or less pro-inflammatory population of B cells. The rebalancing effects proposed for B cells could work together with the demonstrated effects on T cells and natural killer cells in mediating restored tolerance and account for the high efficacy of the treatment strategy.

### *Increasing our understanding*

Further studies are needed to characterize the effects of AHSCT on the B cell compartment and to improve our understanding of their importance in the mode of action of this treatment strategy. We suggest a variety of experiments (BOX 1), all of which could involve comparisons before and after AHSCT and comparison with the effects of other treatments, including targeted B cell depleting therapies such as ocrelizumab or rituximab. These studies should be accompanied by clinical and

MRI monitoring during follow-up and should include the provision for sample collection if MS relapses occur in order to capture clinical–immunological correlations.

The strategies outlined will be included in the mechanistic studies in the Best Available Therapy Versus Autologous Hematopoietic Stem Cell Transplant for Multiple Sclerosis (BEAT-MS) and in the Autologous Stem Cell Transplantation versus Alemtuzumab or Ocrelizumab in Relapsing Remitting MS (STAR-MS) trials, in which AHSCT is compared with high-efficacy standard therapy (including B cell depleting agents)[182]. In parallel with studies of other components of adaptive and innate immunity, this type of research has the potential not only to improve our understanding of the mode of action of AHSCT but also to conclusively demonstrate the critical involvement of immune abnormalities in the pathogenesis of MS and help define their causes.

## Conclusions

Several lines of evidence strongly support the involvement of B cells in the pathogenesis of MS beyond abnormal antibody presence within the CNS of patients. First, pathological studies of human tissue have demonstrated B cell infiltration, particularly in tertiary lymphoid-like structures in the meninges. Second, studies of B cells in the peripheral blood and CSF of patients with MS have demonstrated abnormal inflammatory cytokine production by B cells and excess levels of B cell-relevant chemokines. Third, B cell-depleting therapy (either specifically targeted to B cells or not) is effective in MS and the immunological effects of these therapies are providing insight into the roles of different lymphocyte subsets (FIG. 3).

Further studies to examine how the clinical course and response to treatment relate to the levels and activity of key B cell subpopulations and molecules involved in B cell activation and regulation could definitively establish and characterize the role of B cells in MS pathogenesis. The improved knowledge will provide rich opportunities to optimize treatments for MS by more specifically targeting pathogenic mechanisms and sparing protective B cell functions. As different mechanisms could be operating in different forms and stages of MS and in different individuals with MS, the efficacy of treatment strategies that are more selective than the current options might ultimately require stratified or personalized medicine approaches. Until this evolution becomes feasible, less selective B cell-targeted treatment strategies are likely to remain the most effective options for most patients.

Published online 1 June 2021


1. Sawcer, S., Franklin, R. J. & Ban, M. Multiple sclerosis genetics. *Lancet Neurol.* **13**, 700–709 (2014).
2. Hardy, R. R. & Hayakawa, K. B cell development pathways. *Annu. Rev. Immunol.* **19**, 595–621 (2001).
3. Brink, R. & Phan, T. G. Self-reactive B cells in the germinal center reaction. *Annu. Rev. Immunol.* **36**, 339–357 (2018).
4. Busslinger, M. Transcriptional control of early B cell development. *Annu. Rev. Immunol.* **22**, 55–79 (2004).
5. Chung, J. B., Silverman, M. & Monroe, J. G. Transitional B cells: step by step towards immune competence. *Trends Immunol.* **24**, 343–349 (2003).
6. Tierens, A., Delabie, J., Michiels, L., Vandenberghe, P. & De Wolf-Peeters, C. Marginal-zone B cells in the human lymph node and spleen show somatic hypermutations and display clonal expansion. *Blood* **93**, 226–234 (1999).
7. Dono, M. et al. Heterogeneity of tonsillar subepithelial B lymphocytes, the splenic marginal zone equivalents. *J. Immunol.* **164**, 5596–5604 (2000).
8. Pillai, S., Cariappa, A. & Moran, S. T. Marginal zone B cells. *Annu. Rev. Immunol.* **23**, 161–196 (2005).
9. Mayer, C. T. et al. The microanatomic segregation of selection by apoptosis in the germinal center. *Science* **358**, eaao2602 (2017).
10. Zouali, M. B lymphocytes–chief players and therapeutic targets in autoimmune diseases. *Front. Biosci.* **13**, 4852–4861 (2008).
11. Shen, P. et al. IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. *Nature* **507**, 366–370 (2014).
12. Rosser, E. C. & Mauri, C. Regulatory B cells: origin, phenotype, and function. *Immunity* **42**, 607–612 (2015).
13. Hasan, M. M. et al. $CD24^{hi}CD38^{hi}$ and $CD24^{hi}CD27^{+}$ human regulatory B cells display common and distinct functional characteristics. *J. Immunol.* **203**, 2110–2120 (2019).
14. Carter, N. A. et al. Mice lacking endogenous IL-10-producing regulatory B cells develop exacerbated disease and present with an increased frequency of Th1/Th17 but a decrease in regulatory T cells. *J. Immunol.* **186**, 5569–5579 (2011).
15. Matsushita, T., Yanaba, K., Bouaziz, J. D., Fujimoto, M. & Tedder, T. F. Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. *J. Clin. Invest.* **118**, 3420–3430 (2008).
**One of the earliest definitive demonstrations of the importance of $B_{reg}$ cells in experimental inflammatory CNS disease**.
16. Ray, A., Mann, M. K., Basu, S. & Dittel, B. N. A case for regulatory B cells in controlling the severity of autoimmune-mediated inflammation in experimental autoimmune encephalomyelitis and multiple sclerosis. *J. Neuroimmunol.* **230**, 1–9 (2011).
17. Bjarnadottir, K. et al. B cell-derived transforming growth factor-beta1 expression limits the induction phase of autoimmune neuroinflammation. *Sci. Rep.* **6**, 34594 (2016).
18. Duddy, M. et al. Distinct effector cytokine profiles of memory and naive human B cell subsets and implication in multiple sclerosis. *J. Immunol.* **178**, 6092–6099 (2007).
19. Bar-Or, A. et al. Abnormal B-cell cytokine responses a trigger of T-cell-mediated disease in MS? *Ann. Neurol.* **67**, 452–461 (2010).
20. Li, R. et al. Proinflammatory GM-CSF-producing B cells in multiple sclerosis and B cell depletion therapy. *Sci. Transl. Med.* **7**, 310ra166 (2015).
**An important study that demonstrated the existence of a functionally specialized B cell subset that exhibits an inflammatory role mediated via effects on monocyte and macrophage activation**.
21. Li, R., Patterson, K. R. & Bar-Or, A. Reassessing B cell contributions in multiple sclerosis. *Nat. Immunol.* **19**, 696–707 (2018).
22. Lisak, R. P. et al. Secretory products of multiple sclerosis B cells are cytotoxic to oligodendroglia in vitro. *J. Neuroimmunol.* **246**, 85–95 (2012).
23. Lisak, R. P. et al. B cells from patients with multiple sclerosis induce cell death via apoptosis in neurons in vitro. *J. Neuroimmunol.* **309**, 88–99 (2017).
24. Jelcic, I. et al. Memory B cells activate brain-homing, autoreactive $CD4^{+}$ T cells in multiple sclerosis. *Cell* **175**, 85–100.e23 (2018).
**An elegant series of cellular and molecular experiments that demonstrated that B cell–T cell crosstalk is pivotal in the pathophysiology of CNS inflammation in MS**.
25. Okada, Y. et al. Signaling via toll-like receptor 4 and CD40 in B cells plays a regulatory role in the pathogenesis of multiple sclerosis through interleukin-10 production. *J. Autoimmun.* **88**, 103–113 (2018).
26. Knippenberg, S. et al. Reduction in IL-10 producing B cells (Breg) in multiple sclerosis is accompanied by a reduced naive/memory Breg ratio during a relapse but not in remission. *J. Neuroimmunol.* **239**, 80–86 (2011).
27. Kim, Y. et al. Restoration of regulatory B cell deficiency following alemtuzumab therapy in patients with relapsing multiple sclerosis. *J. Neuroinflammation* **15**, 300 (2018).
28. Cencioni, M. T., Ali, R., Nicholas, R. & Muraro, P. A. Defective $CD19^{+}CD24^{hi}CD38^{hi}$ transitional B-cell function in patients with relapsing-remitting MS. *Mult. Scler.* https://doi.org/10.1177/1352458520951536 (2020).
29. Thompson, A. J., Reingold, S. C. & Cohen, J. A., International Panel on Diagnosis of Multiple Sclerosis. Applying the 2017 McDonald diagnostic criteria for multiple sclerosis — Authors' reply. *Lancet Neurol.* **17**, 499–500 (2018).
30. Villar, L. M. et al. Intrathecal IgM synthesis predicts the onset of new relapses and a worse disease course in MS. *Neurology* **59**, 555–559 (2002).
31. Villar, L. M. et al. Intrathecal IgM synthesis is a prognostic factor in multiple sclerosis. *Ann. Neurol.* **53**, 222–226 (2003).
32. Obermeier, B. et al. Matching of oligoclonal immunoglobulin transcriptomes and proteomes of cerebrospinal fluid in multiple sclerosis. *Nat. Med.* **14**, 688–693 (2008).
**This study was the first to demonstrate that CSF IgG proteomes match, to a large extent, with the corresponding IgG transcriptome from CSF B cells, confirming that these cells are the source of IgG oligoclonal bands, strongly suggesting their involvement in the pathogenesis of MS**.
33. Esiri, M. M. Immunoglobulin-containing cells in multiple-sclerosis plaques. *Lancet* **2**, 478 (1977).
34. Prineas, J. W. & Wright, R. G. Macrophages, lymphocytes, and plasma cells in the perivascular compartment in chronic multiple sclerosis. *Lab. Invest.* **38**, 409–421 (1978).
35. Esiri, M. M. Multiple sclerosis: a quantitative and qualitative study of immunoglobulin-containing cells in the central nervous system. *Neuropathol. Appl. Neurobiol.* **6**, 9–21 (1980).
36. Serafini, B., Rosicarelli, B., Magliozzi, R., Stigliano, E. & Aloisi, F. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. *Brain Pathol.* **14**, 164–174 (2004).
37. Bevan, R. J. et al. Meningeal inflammation and cortical demyelination in acute multiple sclerosis. *Ann. Neurol.* **84**, 829–842 (2018).
38. Magliozzi, R. et al. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. *Brain* **130**, 1089–1104 (2007).

39. Howell, O. W. et al. Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. *Brain* **134**, 2755–2771 (2011).
40. Choi, S. R. et al. Meningeal inflammation plays a role in the pathology of primary progressive multiple sclerosis. *Brain* **135**, 2925–2937 (2012).
41. Androdias, G. et al. Meningeal T cells associate with diffuse axonal loss in multiple sclerosis spinal cords. *Ann. Neurol.* **68**, 465–476 (2010).
42. Haider, L. et al. The topograpy of demyelination and neurodegeneration in the multiple sclerosis brain. *Brain* **139**, 807–815 (2016).
43. DeLuca, G. C. et al. Casting light on multiple sclerosis heterogeneity: the role of HLA-DRB1 on spinal cord pathology. *Brain* **136**, 1025–1034 (2013).
44. Magliozzi, R. et al. A gradient of neuronal loss and meningeal inflammation in multiple sclerosis. *Ann. Neurol.* **68**, 477–493 (2010).
45. Gardner, C. et al. Cortical grey matter demyelination can be induced by elevated pro-inflammatory cytokines in the subarachnoid space of MOG-immunized rats. *Brain* **136**, 3596–3608 (2013).
46. Magliozzi, R. et al. Inflammatory intrathecal profiles and cortical damage in multiple sclerosis. *Ann. Neurol.* **83**, 739–755 (2018).
47. Lucchinetti, C. F. et al. Inflammatory cortical demyelination in early multiple sclerosis. *N. Engl. J. Med.* **365**, 2188–2197 (2011).
48. Genain, C. P., Cannella, B., Hauser, S. L. & Raine, C. S. Identification of autoantibodies associated with myelin damage in multiple sclerosis. *Nat. Med.* **5**, 170–175 (1999).
49. Lucchinetti, C. et al. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. *Ann. Neurol.* **47**, 707–717 (2000).
50. Barnett, M. H. & Prineas, J. W. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. *Ann. Neurol.* **55**, 458–468 (2004).
51. Sabatino, J. J. Jr., Probstel, A. K. & Zamvil, S. S. B cells in autoimmune and neurodegenerative central nervous system diseases. *Nat. Rev. Neurosci.* **20**, 728–745 (2019).
52. Qin, Y. et al. Clonal expansion and somatic hypermutation of V(H) genes of B cells from cerebrospinal fluid in multiple sclerosis. *J. Clin. Invest.* **102**, 1045–1050 (1998).
53. Baranzini, S. E. et al. B cell repertoire diversity and clonal expansion in multiple sclerosis brain lesions. *J. Immunol.* **163**, 5133–5144 (1999).
54. Colombo, M. et al. Accumulation of clonally related B lymphocytes in the cerebrospinal fluid of multiple sclerosis patients. *J. Immunol.* **164**, 2782–2789 (2000).
55. Lovato, L. et al. Related B cell clones populate the meninges and parenchyma of patients with multiple sclerosis. *Brain* **134**, 534–541 (2011).
56. von Budingen, H. C. et al. B cell exchange across the blood-brain barrier in multiple sclerosis. *J. Clin. Invest.* **122**, 4533–4543 (2012).
57. Schafflick, D. et al. Integrated single cell analysis of blood and cerebrospinal fluid leukocytes in multiple sclerosis. *Nat. Commun.* **11**, 247 (2020).
**A study that exploited the latest technological advances in single-cell transcriptomics to simultaneously examine blood and CSF cell composition in patients with MS, which revealed compartmentalized T cell and B cell interactions that experiments in an animal model confirmed as functionally relevant in CNS inflammation**.
58. Ramesh, A. et al. A pathogenic and clonally expanded B cell transcriptome in active multiple sclerosis. *Proc. Natl Acad. Sci. USA* **117**, 22932–22943 (2020).
59. Aloisi, F. & Pujol-Borrell, R. Lymphoid neogenesis in chronic inflammatory diseases. *Nat. Rev. Immunol.* **6**, 205–217 (2006).
60. Drayton, D. L., Liao, S., Mounzer, R. H. & Ruddle, N. H. Lymphoid organ development: from ontogeny to neogenesis. *Nat. Immunol.* **7**, 344–353 (2006).
61. Steri, M. et al. Overexpression of the cytokine BAFF and autoimmunity risk. *N. Engl. J. Med.* **376**, 1615–1626 (2017).
62. Kim, H. J. et al. Epstein-Barr virus-associated lymphoproliferative disorders: review and Update on 2016 WHO classification. *J. Pathol. Transl. Med.* **51**, 352–358 (2017).
63. Munger, K. L. & Ascherio, A. Prevention and treatment of MS: studying the effects of vitamin D. *Mult. Scler.* **17**, 1405–1411 (2011).
64. Almohmeed, Y. H., Avenell, A., Aucott, L. & Vickers, M. A. Systematic review and meta-analysis of the sero-epidemiological association between Epstein Barr virus and multiple sclerosis. *PLoS One* **8**, e61110 (2013).
65. Aloisi, F., Serafini, B., Magliozzi, R., Howell, O. W. & Reynolds, R. Detection of Epstein-Barr virus and B-cell follicles in the multiple sclerosis brain: what you find depends on how and where you look. *Brain* **133**, e157 (2010).
66. Serafini, B. et al. Epstein-Barr virus latent infection and BAFF expression in B cells in the multiple sclerosis brain: implications for viral persistence and intrathecal B-cell activation. *J. Neuropathol. Exp. Neurol.* **69**, 677–693 (2010).
67. Veroni, C., Serafini, B., Rosicarelli, B., Fagnani, C. & Aloisi, F. Transcriptional profile and Epstein-Barr virus infection status of laser-cut immune infiltrates from the brain of patients with progressive multiple sclerosis. *J. Neuroinflammation* **15**, 18 (2018).
68. Tzartos, J. S. et al. Association of innate immune activation with latent Epstein-Barr virus in active MS lesions. *Neurology* **78**, 15–23 (2012).
69. Peferoen, L. A. et al. Epstein Barr virus is not a characteristic feature in the central nervous system in established multiple sclerosis. *Brain* **133**, e137 (2010).
70. Mancuso, R. et al. Detection of viral DNA sequences in the cerebrospinal fluid of patients with multiple sclerosis. *J. Med. Virol.* **82**, 1051–1057 (2010).
71. Willis, S. N. et al. Epstein-Barr virus infection is not a characteristic feature of multiple sclerosis brain. *Brain* **132**, 3318–3328 (2009).
72. Lassmann, H., Niedobitek, G., Aloisi, F. & Middeldorp, J. M., NeuroproMiSe EBV Working Group. Epstein-Barr virus in the multiple sclerosis brain: a controversial issue – report on a focused workshop held in the Centre for Brain Research of the Medical University of Vienna, Austria. *Brain* **134**, 2772–2786 (2011).
73. Cocuzza, C. E. et al. Quantitative detection of Epstein-Barr virus DNA in cerebrospinal fluid and blood samples of patients with relapsing-remitting multiple sclerosis. *PLoS One* **9**, e94497 (2014).
74. Lunemann, J. D. et al. EBNA1-specific T cells from patients with multiple sclerosis cross react with myelin antigens and co-produce IFN-gamma and IL-2. *J. Exp. Med.* **205**, 1763–1773 (2008).
75. Angelini, D. F. et al. Increased $CD8^+$ T cell response to Epstein-Barr virus lytic antigens in the active phase of multiple sclerosis. *PLoS Pathog.* **9**, e1003220 (2013).
76. van Nierop, G. P. et al. Phenotypic and functional characterization of T cells in white matter lesions of multiple sclerosis patients. *Acta Neuropathol.* **134**, 383–401 (2017).
77. Cencioni, M. T. et al. Programmed death 1 is highly expressed on $CD8^+$ $CD57^+$ T cells in patients with stable multiple sclerosis and inhibits their cytotoxic response to Epstein-Barr virus. *Immunology* **152**, 660–676 (2017).
78. Pittet, C. L., Newcombe, J., Antel, J. P. & Arbour, N. The majority of infiltrating CD8 T lymphocytes in multiple sclerosis lesions is insensitive to enhanced PD-L1 levels on CNS cells. *Glia* **59**, 841–856 (2011).
79. Bar-Or, A. et al. Epstein-Barr virus in multiple sclerosis: theory and emerging immunotherapies. *Trends Mol. Med.* **26**, 296–310 (2020).
80. Krumbholz, M., Derfuss, T., Hohlfeld, R. & Meinl, E. B cells and antibodies in multiple sclerosis pathogenesis and therapy. *Nat. Rev. Neurol.* **8**, 613–623 (2012).
81. Staun-Ram, E. & Miller, A. Effector and regulatory B cells in multiple sclerosis. *Clin. Immunol.* **184**, 11–25 (2017).
82. Beers, S. A., Chan, C. H., French, R. R., Cragg, M. S. & Glennie, M. J. CD20 as a target for therapeutic type I and II monoclonal antibodies. *Semin. Hematol.* **47**, 107–114 (2010).
83. Tipton, T. R. et al. Antigenic modulation limits the effector cell mechanisms employed by type I anti-CD20 monoclonal antibodies. *Blood* **125**, 1901–1909 (2015).
84. Moreno Torres, I. & Garcia-Merino, A. Anti-CD20 monoclonal antibodies in multiple sclerosis. *Expert Rev. Neurother.* **17**, 359–371 (2017).
85. Bar-Or, A. et al. Rituximab in relapsing-remitting multiple sclerosis: a 72-week, open-label, phase I trial. *Ann. Neurol.* **63**, 395–400 (2008).
86. Hauser, S. L. et al. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. *N. Engl. J. Med.* **358**, 676–688 (2008).
**Seminal clinical trial evidence of the efficacy of B cell depleting therapy in MS**.
87. Hawker, K. et al. Rituximab in patients with primary progressive multiple sclerosis: results of a randomized double-blind placebo-controlled multicenter trial. *Ann. Neurol.* **66**, 460–471 (2009).
88. Svenningsson, A. et al. Rapid depletion of B lymphocytes by ultra-low-dose rituximab delivered intrathecally. *Neurol. Neuroimmunol. Neuroinflamm* **2**, e79 (2015).
89. Komori, M. et al. Insufficient disease inhibition by intrathecal rituximab in progressive multiple sclerosis. *Ann. Clin. Transl. Neurol.* **3**, 166–179 (2016).
90. Palanichamy, A. et al. Rituximab efficiently depletes increased CD20-expressing T cells in multiple sclerosis patients. *J. Immunol.* **193**, 580–586 (2014).
91. Martin, F. & Chan, A. C. B cell immunobiology in disease: evolving concepts from the clinic. *Annu. Rev. Immunol.* **24**, 467–496 (2006).
92. Sacco, K. A. & Abraham, R. S. Consequences of B-cell-depleting therapy: hypogammaglobulinemia and impaired B-cell reconstitution. *Immunotherapy* **10**, 713–728 (2018).
93. Myhr, K.-M., Torkildsen, Ø., Lossius, A., Bø, L. & Holmøy, T. B cell depletion in the treatment of multiple sclerosis. *Expert Opin. Biol. Ther.* **19**, 261–271 (2019).
94. van de Veerdonk, F. L. et al. The anti-CD20 antibody rituximab reduces the Th17 cell response. *Arthritis Rheum.* **63**, 1507–1516 (2011).
95. Nissimov, N. et al. B cells reappear less mature and more activated after their anti-CD20-mediated depletion in multiple sclerosis. *Proc. Natl Acad. Sci. USA* **117**, 25690–25699 (2020).
96. Quan, C. et al. The immune balance between memory and regulatory B cells in NMO and the changes of the balance after methylprednisolone or rituximab therapy. *J. Neuroimmunol.* **282**, 45–53 (2015).
97. Maurer, M. A. et al. Rituximab induces sustained reduction of pathogenic B cells in patients with peripheral nervous system autoimmunity. *J. Clin. Invest.* **122**, 1393–1402 (2012).
98. Weber, M. S. et al. B-cell activation influences T-cell polarization and outcome of anti-CD20 B-cell depletion in central nervous system autoimmunity. *Ann. Neurol.* **68**, 369–383 (2010).
99. Lehmann-Horn, K. et al. Anti-CD20 B-cell depletion enhances monocyte reactivity in neuroimmunological disorders. *J. Neuroinflammation* **8**, 146 (2011).
100. Cross, A. H., Stark, J. L., Lauber, J., Ramsbottom, M. J. & Lyons, J. A. Rituximab reduces B cells and T cells in cerebrospinal fluid of multiple sclerosis patients. *J. Neuroimmunol.* **180**, 63–70 (2006).
101. Barr, T. A. et al. B cell depletion therapy ameliorates autoimmune disease through ablation of IL-6-producing B cells. *J. Exp. Med.* **209**, 1001–1010 (2012).
102. Topping, J. et al. The effects of intrathecal rituximab on biomarkers in multiple sclerosis. *Mult. Scler. Relat. Disord.* **6**, 49–53 (2016).
103. Sorensen, P. S. & Blinkenberg, M. The potential role for ocrelizumab in the treatment of multiple sclerosis: current evidence and future prospects. *Ther. Adv. Neurol. Disord.* **9**, 44–52 (2016).
104. Klein, C. et al. Epitope interactions of monoclonal antibodies targeting CD20 and their relationship to functional properties. *mAbs* **5**, 22–33 (2013).
105. Hauser, S. L. et al. Ocrelizumab versus interferon beta-1a in relapsing multiple sclerosis. *N. Engl. J. Med.* **376**, 221–234 (2017).
106. Montalban, X. et al. Ocrelizumab versus placebo in primary progressive multiple sclerosis. *N. Engl. J. Med.* **376**, 209–220 (2017).
107. Laurent, S. Effect of ocrelizumab on B and T cell immune repertoires in patients with relapsing multiple sclerosis. *Mult. Scler.* **23**, 85–426 (2017).
108. Gingele, S. et al. Ocrelizumab depletes $CD20^+$ T cells in multiple sclerosis patients. *Cells* **8**, 12 (2018).
109. Gingele, S., Skripuletz, T. & Jacobs, R. Role of $CD20^+$ T cells in multiple sclerosis: implications for treatment with ocrelizumab. *Neural Regen. Res.* **15**, 663–664 (2020).
110. Bar-Or, A. et al. B cells, T cells and inflammatory CSF biomarkers in primary progressive MS and relapsing MS in the OBOE (Ocrelizumab Biomarker Outcome Evaluation) Trial (1635). *Neurology* **94**, 1635 (2020).
111. Derfuss, T. & al. Serum immunoglobulin levels and risk of serious infections in the pivotal Phase III trials of ocrelizumab in multiple sclerosis and their open-label extensions. ECTRIMS Online Library. https://onlinelibrary.ectrims-congress.eu/ectrims/2019/stockholm/279399/tobias.derfuss.serum.immunoglobulin.levels.and.risk.of.serious.infecti (2019).
112. Teeling, J. L. et al. The biological activity of human CD20 monoclonal antibodies is linked to unique epitopes on CD20. *J. Immunol.* **177**, 362–371 (2006).
113. Bleeker, W. K. et al. Estimation of dose requirements for sustained in vivo activity of a therapeutic human

anti-CD20 antibody. *Br. J. Haematol.* **140**, 303–312 (2008).
114. Bar-Or, A. et al. Subcutaneous ofatumumab in patients with relapsing-remitting multiple sclerosis: the MIRROR study. *Neurology* **90**, e1805–e1814 (2018).
115. Hauser, S. L. et al. Ofatumumab versus teriflunomide in multiple sclerosis. *N. Engl. J. Med.* **383**, 546–557 (2020).
116. Hartung, H. P. & Kieseier, B. C. Atacicept: targeting B cells in multiple sclerosis. *Ther. Adv. Neurol. Disord.* **3**, 205–216 (2010).
117. Benson, M. J. et al. Cutting edge: the dependence of plasma cells and independence of memory B cells on BAFF and APRIL. *J. Immunol.* **180**, 3655–3659 (2008).
118. Dillon, S. R., Gross, J. A., Ansell, S. M. & Novak, A. J. An APRIL to remember: novel TNF ligands as therapeutic targets. *Nat. Rev. Drug Discov.* **5**, 235–246 (2006).
119. Gross, J. A. et al. TACI-Ig neutralizes molecules critical for B cell development and autoimmune disease. impaired B cell maturation in mice lacking BLyS. *Immunity* **15**, 289–302 (2001).
120. Tak, P. P. et al. Atacicept in patients with rheumatoid arthritis: results of a multicenter, phase Ib, double-blind, placebo-controlled, dose-escalating, single- and repeated-dose study. *Arthritis Rheum.* **58**, 61–72 (2008).
121. Dall'Era, M. et al. Reduced B lymphocyte and immunoglobulin levels after atacicept treatment in patients with systemic lupus erythematosus: results of a multicenter, phase Ib, double-blind, placebo-controlled, dose-escalating trial. *Arthritis Rheum.* **56**, 4142–4150 (2007).
122. Munafo, A., Priestley, A., Nestorov, I., Visich, J. & Rogge, M. Safety, pharmacokinetics and pharmacodynamics of atacicept in healthy volunteers. *Eur. J. Clin. Pharmacol.* **63**, 647–656 (2007).
123. Rip, J., Van Der Ploeg, E. K., Hendriks, R. W. & Corneth, O. B. J. The role of Bruton's tyrosine kinase in immune cell signaling and systemic autoimmunity. *Crit. Rev. Immunol.* **38**, 17–62 (2018).
124. Montalban, X. et al. Placebo-controlled trial of an oral BTK inhibitor in multiple sclerosis. *N. Engl. J. Med.* **380**, 2406–2417 (2019).
125. Torke, S. et al. Inhibition of Bruton's tyrosine kinase interferes with pathogenic B-cell development in inflammatory CNS demyelinating disease. *Acta Neuropathol.* **140**, 535–548 (2020).
126. Investigators, C. T. et al. Alemtuzumab vs. interferon beta-1a in early multiple sclerosis. *N. Engl. J. Med.* **359**, 1786–1801 (2008).
127. Cohen, J. A. et al. Alemtuzumab versus interferon beta 1a as first-line treatment for patients with relapsing-remitting multiple sclerosis: a randomised controlled phase 3 trial. *Lancet* **380**, 1819–1828 (2012).
128. Coles, A. J. et al. Alemtuzumab for patients with relapsing multiple sclerosis after disease-modifying therapy: a randomised controlled phase 3 trial. *Lancet* **380**, 1829–1839 (2012).
129. Coles, A. J. et al. Alemtuzumab CARE-MS II 5-year follow-up: efficacy and safety findings. *Neurology* **89**, 1117–1126 (2017).
130. Havrdova, E. et al. Alemtuzumab CARE-MS I 5-year follow-up: durable efficacy in the absence of continuous MS therapy. *Neurology* **89**, 1107–1116 (2017).
131. Jones, J. L. & Coles, A. J. Mode of action and clinical studies with alemtuzumab. *Exp. Neurol.* **262 (Pt A)**, 37–43 (2014).
132. Jones, J. L. et al. Improvement in disability after alemtuzumab treatment of multiple sclerosis is associated with neuroprotective autoimmunity. *Brain* **133**, 2232–2247 (2010).
133. Hu, Y. et al. Investigation of the mechanism of action of alemtuzumab in a human CD52 transgenic mouse model. *Immunology* **128**, 260–270 (2009).
134. Xia, M. Q., Hale, G. & Waldmann, H. Efficient complement-mediated lysis of cells containing the CAMPATH-1 (CDw52) antigen. *Mol. Immunol.* **30**, 1089–1096 (1993).
135. Hill-Cawthorne, G. A. et al. Long term lymphocyte reconstitution after alemtuzumab treatment of multiple sclerosis. *J. Neurol. Neurosurg. Psychiatry* **83**, 298–304 (2012).
136. Thompson, S. A., Jones, J. L., Cox, A. L., Compston, D. A. & Coles, A. J. B-cell reconstitution and BAFF after alemtuzumab (Campath-1H) treatment of multiple sclerosis. *J. Clin. Immunol.* **30**, 99–105 (2010).
137. Cox, A. L. et al. Lymphocyte homeostasis following therapeutic lymphocyte depletion in multiple sclerosis. *Eur. J. Immunol.* **35**, 3332–3342 (2005).
138. Zhang, X. et al. Differential reconstitution of T cell subsets following immunodepleting treatment with alemtuzumab (anti-CD52 monoclonal antibody) in patients with relapsing-remitting multiple sclerosis. *J. Immunol.* **191**, 5867–5874 (2013).
139. von Essen, M. R. et al. Proinflammatory $CD20^+$ T cells in the pathogenesis of multiple sclerosis. *Brain* **142**, 120–132 (2019).
140. Wiendl, H. et al. Lymphocyte pharmacodynamics are not associated with autoimmunity or efficacy after alemtuzumab. *Neurol. Neuroimmunol. Neuroinflamm.* **7**, e635 (2020).
141. Ruck, T. et al. ALAIN01 — Alemtuzumab in autoimmune inflammatory neurodegeneration: mechanisms of action and neuroprotective potential. *BMC Neurol.* **16**, 34 (2016).
142. Giovannoni, G. et al. A placebo-controlled trial of oral cladribine for relapsing multiple sclerosis. *N. Engl. J. Med.* **362**, 416–426 (2010).
143. Comi, G. et al. MRI outcomes with cladribine tablets for multiple sclerosis in the CLARITY study. *J. Neurol.* **260**, 1136–1146 (2013).
144. Giovannoni, G. et al. Sustained disease-activity-free status in patients with relapsing-remitting multiple sclerosis treated with cladribine tablets in the CLARITY study: a post-hoc and subgroup analysis. *Lancet. Neurol.* **10**, 329–337 (2011).
145. De Stefano, N. et al. Reduced brain atrophy rates are associated with lower risk of disability progression in patients with relapsing multiple sclerosis treated with cladribine tablets. *Mult. Scler.* **24**, 222–226 (2018).
146. Leist, T. P. et al. Effect of oral cladribine on time to conversion to clinically definite multiple sclerosis in patients with a first demyelinating event (ORACLE MS): a phase 3 randomised trial. *Lancet Neurol.* **13**, 257–267 (2014).
147. Freedman, M. S. et al. The efficacy of cladribine tablets in CIS patients retrospectively assigned the diagnosis of MS using modern criteria: Results from the ORACLE-MS study. *Mult. Scler. J. Exp. Transl. Clin.* **3**, 2055217317732802 (2017).
148. Giovannoni, G. Cladribine to treat relapsing forms of multiple sclerosis. *Neurotherapeutics* **14**, 874–887 (2017).
149. Beutler, E. Cladribine (2-chlorodeoxyadenosine). *Lancet* **340**, 952–956 (1992).
150. Comi, G. et al. Effect of cladribine tablets on lymphocyte reduction and repopulation dynamics in patients with relapsing multiple sclerosis. *Mult. Scler. Relat. Disord.* **29**, 168–174 (2019).
151. Stuve, O. et al. Effects of cladribine tablets on lymphocyte subsets in patients with multiple sclerosis: an extended analysis of surface markers. *Ther. Adv. Neurol. Disord.* **12**, 1756286419854986 (2019).
152. Ceronie, B. et al. Cladribine treatment of multiple sclerosis is associated with depletion of memory B cells. *J. Neurol.* **265**, 1199–1209 (2018).
153. Baker, D. et al. Both cladribine and alemtuzumab may effect MS via B-cell depletion. *Neurol. Neuroimmunol. Neuroinflamm* **4**, e360 (2017).
154. Rejdak, K., Stelmasiak, Z. & Grieb, P. Cladribine induces long lasting oligoclonal bands disappearance in relapsing multiple sclerosis patients: 10-year observational study. *Mult. Scler. Relat. Disord.* **27**, 117–120 (2019).
155. Swart, J. F. et al. Haematopoietic stem cell transplantation for autoimmune diseases. *Nat. Rev. Rheumatol.* **13**, 244–256 (2017).
156. Marmont, A. M. Immune ablation followed by allogeneic or autologous bone marrow transplantation: a new treatment for severe autoimmune diseases? *Stem Cell* **12**, 125–135 (1994).
157. Muraro, P. A. et al. Autologous haematopoietic stem cell transplantation for treatment of multiple sclerosis. *Nat. Rev. Neurol.* **13**, 391–405 (2017).
158. Burt, R. K. et al. Association of nonmyeloablative hematopoietic stem cell transplantation with neurological disability in patients with relapsing-remitting multiple sclerosis. *JAMA* **313**, 275–284 (2015).
159. Muraro, P. A. & Abrahamsson, S. V. Resetting autoimmunity in the nervous system: The role of hematopoietic stem cell transplantation. *Curr. Opin. Invest. Drugs* **11**, 1265–1275 (2010).
160. Atkins, H. L. et al. Immunoablation and autologous haemopoietic stem-cell transplantation for aggressive multiple sclerosis: a multicentre single-group phase 2 trial. *Lancet* **388**, 576–585 (2016).
161. Abrahamsson, S., Mattoscio, M., Muraro P. A. in *Multiple Sclerosis Immunology - A Foundation for Current and Future Treatments* (ed Gran B. & Yamamura T.) Ch. 19, 401-431 (Springer-Verlag, 2013).
162. Mancardi, G. & Saccardi, R. Autologous haematopoietic stem-cell transplantation in multiple sclerosis. *Lancet Neurol.* **7**, 626–636 (2008).
163. Saccardi, R. et al. A prospective, randomized, controlled trial of autologous haematopoietic stem cell transplantation for aggressive multiple sclerosis: a position paper. *Mult. Scler.* **18**, 825–834 (2012). **This study provided the key concept and high-level protocol for randomized controlled trials of AHSCT compared with best standard therapy, a design that has been adopted by subsequent trials, including BEAT-MS and STAR-MS**.
164. Saccardi, R. et al. Autologous HSCT for severe progressive multiple sclerosis in a multicenter trial: impact on disease activity and quality of life. *Blood* **105**, 2601–2607 (2005).
165. Mancardi, G. L. et al. Autologous haematopoietic stem cell transplantation with an intermediate intensity conditioning regimen in multiple sclerosis: the Italian multi-centre experience. *Mult. Scler.* **18**, 835–842 (2012).
166. Burt, R. K. et al. Effect of nonmyeloablative hematopoietic stem cell transplantation vs continued disease-modifying therapy on disease progression in patients with relapsing-remitting multiple sclerosis: a randomized clinical trial. *JAMA* **321**, 165–174 (2019). **First proof-of-principle, randomized controlled trial that demonstrated superior clinical efficacy of AHSCT compared with standard disease modifying therapy in patients with MS**.
167. Sormani, M. P. et al. Autologous hematopoietic stem cell transplantation in multiple sclerosis: A meta-analysis. *Neurology* **88**, 2115–2122 (2017).
168. Muraro, P. A. et al. Thymic output generates a new and diverse TCR repertoire after autologous stem cell transplantation in multiple sclerosis patients. *J. Exp. Med.* **201**, 805–816 (2005).
169. Muraro, P. A. et al. T cell repertoire following autologous stem cell transplantation for multiple sclerosis. *J. Clin. Invest.* **124**, 1168–1172 (2014).
170. Harris, K. M. et al. Extensive intrathecal T cell renewal following hematopoietic transplantation for multiple sclerosis. *JCI Insight* **5**, e127655 (2020). **The first high-resolution description of the reconstitution of TCR repertoires in the blood and CSF of patients with MS who underwent AHSCT and the first report of extensive ablation of pre-therapy T cell clones in both blood and CSF, which persisted for the 4-year duration of follow-up**.
171. Collins, F., Kazmi, M. & Muraro, P. A. Progress and prospects for the use and the understanding of the mode of action of autologous hematopoietic stem cell transplantation in the treatment of multiple sclerosis. *Expert Rev. Clin. Immunol.* **13**, 611–622 (2017).
172. Farge, D. et al. Analysis of immune reconstitution after autologous bone marrow transplantation in systemic sclerosis. *Arthritis Rheum.* **52**, 1555–1563 (2005).
173. Arruda, L. C. M. et al. Immune rebound associates with a favorable clinical response to autologous HSCT in systemic sclerosis patients. *Blood Adv.* **2**, 126–141 (2018).
174. Alexander, T. et al. Depletion of autoreactive immunologic memory followed by autologous hematopoietic stem cell transplantation in patients with refractory SLE induces long-term remission through de novo generation of a juvenile and tolerant immune system. *Blood* **113**, 214–223 (2009).
175. Karnell, F. G. et al. Reconstitution of immune cell populations in multiple sclerosis patients after autologous stem cell transplantation. *Clin. Exp. Immunol.* **189**, 268–278 (2017).
176. Nash, R. A. et al. High-dose immunosuppressive therapy and autologous hematopoietic cell transplantation for relapsing-remitting multiple sclerosis (HALT-MS): a 3-year interim report. *JAMA Neurol.* **72**, 159–169 (2015).
177. Nash, R. A. et al. High-dose immunosuppressive therapy and autologous peripheral blood stem cell transplantation for severe multiple sclerosis. *Blood* **102**, 2364–2372 (2003).
178. Saiz, A. et al. MRI and CSF oligoclonal bands after autologous hematopoietic stem cell transplantation in MS. *Neurology* **56**, 1084–1089 (2001).
179. Openshaw, H. et al. Peripheral blood stem cell transplantation in multiple sclerosis with busulfan and cyclophosphamide conditioning: report of toxicity and immunological monitoring. *Biol. Blood Marrow Transpl.* **6**, 563–575 (2000).
180. Bowen, J. D. et al. Autologous hematopoietic cell transplantation following high-dose immunosuppressive therapy for advanced multiple sclerosis: long-term results. *Bone Marrow Transpl.* **47**, 946–951 (2012).

181. Nash, R. A. et al. High-dose immunosuppressive therapy and autologous HCT for relapsing-remitting MS. *Neurology* **88**, 842–852 (2017).
182. BEAT-MS. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT04047628 (2021).
183. Villar, L. M. et al. Intrathecal synthesis of oligoclonal IgM against myelin lipids predicts an aggressive disease course in MS. *J. Clin. Invest.* **115**, 187–194 (2005).
184. Villar, L. M. et al. Intrathecal IgM synthesis in neurologic diseases: relationship with disability in MS. *Neurology* **58**, 824–826 (2002).
185. Mehra, V. et al. Epstein-Barr virus and monoclonal gammopathy of clinical significance in autologous stem cell transplantation for multiple sclerosis. *Clin. Infect. Dis.* **69**, 1757–1763 (2019).

**Acknowledgements**
M.T.C. and P.A.M. have received support from the National Institute of Health Research (NIHR-EME Project: 16/126/26 to P.A.M.) and the NIHR Biomedical Research Centre funding scheme to Imperial College London. M.T.C. has received support from the Elena Pecci research project and the Fondazione Careggi Onlus (Firenze, Italy). We thank Rui Li and Diego Espinoza for helpful comments on B cell subsets.

**Author contributions**
M.T.C. and P.A.M. conceptualized the manuscript. M.T.C., M.M., R.M. and P.A.M. wrote the initial draft. A.B.O. critically reviewed the manuscript for important intellectual content and edited the manuscript. P.A.M. supervised, reviewed and revised the manuscript.

**Competing interests**
M.M. discloses travel support and speaker honoraria from Biogen Idec, Genzyme, Merck-Sereno, Novartis, Roche, and Teva and consultation for Celgene, Merck-Serono, Novartis and Roche. A.B.-O. discloses participation as a speaker in meetings sponsored by and receiving consulting fees and/or grant support from Accure, Atara Biotherapeutics, Biogen, BMS/Celgene/Receptos, GlaxoSmithKline, Gossamer, Janssen/Actelion, Medimmune, Merck/EMD Serono, Novartis, Roche/Genentech and Sanofi-Genzyme. P.A.M. discloses travel support and speaker honoraria from unrestricted educational activities organized by Bayer HealthCare, Bayer Pharma, Biogen Idec, Merck-Serono, Novartis and Sanofi Aventis, and consultation for Jasper Therapeutics and Magenta Therapeutics. The other authors declare no competing interests.

**Peer review information**
*Nature Reviews Neurology* thanks J. Laman, M. Weber and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

**Publisher's note**
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Supplementary information**
The online version contains supplementary material available at https://doi.org/10.1038/s41582-021-00498-5.

**RELATED LINKS**
**BEAT-MS:** http://www.beat-ms.org
**STAR-MS:** https://www.sheffield.ac.uk/scharr/research/centres/ctru/starms