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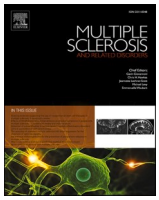

Review article

Insights into the role of B cells in the cortical pathology of Multiple sclerosis: evidence from animal models and patients

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A B S T R A C T

Multiple sclerosis (MS) is a chronic, immune-mediated disease of the central nervous system (CNS) that affects both white and gray matter. Although it has been traditionally considered as a T cell mediated disease, the role of B cell in MS pathology has become a topic of great research interest. Cortical lesions, key feature of the progressive forms of MS, are involved in cognitive impairment and worsening of the patients' outcome. These lesions present pathognomonic hallmarks, such as: absence of blood-brain barrier (BBB) disruption, limited inflammatory events, reactive microglia, neurodegeneration, demyelination and meningeal inflammation. B cells located in the meninges, either as part of diffuse inflammation or as part of follicle-like structures, are strongly associated with cortical damage. The function of CD20-expressing B cells in MS is further highlighted by the success of specific therapies using anti-CD20 antibodies. The possible roles of B cells in pathology go beyond their ability to produce antibodies, as they also present antigens to T cells, secrete cytokines (both pathogenic and protective) within the CNS to modulate T and myeloid cell functions, and are involved in meningeal inflammation. Here, we will review the contributions of B cells to the pathogenesis of meningeal inflammation and cortical lesions in MS patients as well as in preclinical animal models.

1. Introduction

Multiple sclerosis (MS) is a chronic immune-mediated disease of the central nervous system (CNS) that affects both white and gray matter of unknown aetiology. The anatomopathology of the disease is characterized by neuroinflammation, demyelination and axonal degeneration, leading to loss of functions in the CNS, which depends on the location of the lesions (Lassmann, 2013; Miller et al., 2008). It is a heterogeneous disease that displays different clinical phenotypes, including episodes with periods of relapse and remission of symptoms known as "Relapsing Remitting MS" (RRMS), which can progress to a secondary progressive form, "Secondary Progressive MS" (SPMS) (Lublin et al., 2014). However, some other patients experience a persistent progression of symptoms from the disease onset, called "Primary Progressive MS" (PPMS). Cortical lesions are key features of both PPMS and SPMS and they also were found in early MS (Calabrese and Castellaro, 2017; Lucchinetti et al., 2011). These lesions present absence of BBB disruption, limited inflammatory events, reactive microglia, neurodegeneration, demyelination and meningeal inflammation (Silva and Ferrari, 2018). Cortical lesions are clinically pertinent because they are considered responsible for cognitive impairment and worsening of disease outcome (Calabrese et al., 2012a; Nelson et al., 2011; Roosendaal et al., 2009).

Although MS was previously considered to be a T cell-mediated disease, the role of B cells has recently become a central issue in its pathogenesis. B cells are found in the meninges either as part of diffuse inflammation or forming part of follicle-like structures, which are strongly associated with cortical damage (Magliozzi et al., 2010). Additionally, Magliozzi et al., 2007 described the presence of Epstein Barr Virus (EBV) in brain-infiltrating B cells and plasma cells in the majority of MS forms, including cortical pathology, demonstrating that EBV contributes to B-cell dysregulation in cortical MS (Bar-Or et al., 2020; Magliozzi et al., 2013). Therefore, B cells are being pointed out as suitable targets for the development of new therapeutic agents (Rahmanzadeh et al., 2018; Sellebjerg et al., 2020).

This review will provide an overview of B cell function in both MS patients and animal models of the disease. We will explore the contribution of B cells to the pathogenesis of cortical lesions, including their role in antigen presentation, antibody production and cytokine secretion.

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2. Role of B cells in MS

The recent success of specific therapies targeting B cells has brought attention to their role in MS pathology. Besides to their classical role in antibody production, B cells present other functions relevant for MS: they activate T cells via antigen presentation, they secrete pathogenic and/or protective cytokines that modulate both T and myeloid cells in the CNS and they act as a reservoir for EBV infection.

B cells are very efficient antigen-presenting cells (APCs) as they can present protein antigens at low concentrations due to the high affinity of neuroantigens for B cell receptors (BCR). B cells are able to recognize and internalize soluble antigens and conformational epitopes, presenting them on their surface alongside the major histocompatibility complex class II (MHCII), ready to be recognized by T cells (Harp et al., 2008). Peripheral B cells can present myelin antigens, such as myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP), to T cells, leading to their activation. Activated T cells, in turn, migrate into the CNS inducing the destruction of myelin sheaths, as observed in MS patients (Jelcic et al., 2018; Michel et al., 2015). The APCs promote T cell proliferation, differentiation into T helper 1 and 17 cells (Th1 and Th17) and cytokine production (Gharibi et al., 2019; Weber et al., 2010).

During the antigen presentation process, co-stimulatory molecules in the surface of both B and T cells are necessary to induce activation, expansion and differentiation of CNS-specific pathogenic T cells. In fact, B cells from MS patients present a higher expression of co-stimulatory molecules CD86 and CD80 compared to healthy controls (Fraussen et al., 2016; Genc et al., 1997). The interaction between co-stimulatory molecules CD40-CD40L enhances B-T cell collaboration by upregulating MHCII and cell adhesion molecule expression, which leads the activation of NF-κB pathways, thus activating B cell proliferation and, subsequently, antibody and cytokine production. Memory B cells from RRMS patients increase their proliferation in response to CD40 binding, compared to those from healthy donors (Ireland et al., 2012). Moreover, elevated levels of phosphorylated NF-κB (p-P65) after CD40 stimulation were described in RRMS and SPMS patients compared to healthy controls (Chen et al., 2016).

B cells also have the capacity to differentiate into antibody secreting plasm cells. In MS, these cells are responsible for the production of intrathecal G and M immunoglobulins (IgG and IgM, respectively), which constitute the oligoclonal bands (OCB) in patients. The IgG OCBs have been reported in the cerebrospinal fluid (CSF) of 90% of MS patients, regardless of the disease phenotype. The presence of IgG OCBs is associated with a more active inflammatory disease in PPMS patients (Villar et al., 2014). On the other hand, IgM OCBs are found in 30–40% of the cases. Intrathecal synthesis of lipid-specific IgM OCBs predicts a more aggressive disease course in RRMS patients. In addition, the presence of IgM OCBs in the CSF of patients predicts earlier second relapse, more relapses and increased disability (Villar et al., 2005).

Besides OCBs, other antibodies have also been implicated in MS pathogenesis, among them, a group of myelin fragment-binding antibodies found within tissue phagocytes (Genain et al., 1999; Prineas and Graham, 1981). The most common demyelinating lesion pattern (Pattern II) is characterized by prominent deposition of these antibodies as well as complement (Lucchinetti et al., 2000). Even though glycolipids, axonal and glial proteins and viruses have been suggested as targets of these antibodies, the specific antigens recognized by them are still unknown (Blauth et al., 2015; Krumbholz et al., 2012).

Interestingly, B cells have a dual role in MS progression. In fact, based on their cytokine profile, B cells are capable of both enhancing or downregulating the immune response in MS. They can exert a pro-inflammatory role by secreting interleukin (IL)-6, IL-2, IL-15, tumor necrosis factor (TNF)-α, granulocyte-monocyte colony stimulating factor (GM-CSF) and lymphotoxin (LT)-α. IL-6-expressing B cells are involved in the differentiation of Th17 responses, which contribute to disease progression in both MS and experimental autoimmune encephalomyelitis (EAE) (Barr et al., 2012). B cells from mice with EAE secreted high levels of IL-6 and mice with IL-6 deficiency exhibited an attenuated version of the disease (Barr et al., 2012). IL-6 secreted by B cells enhance both T cell proliferation and Th17 cell responses *in vitro* (Barr et al., 2010; Lampropoulou et al., 2008). Conversely, B cells can also secrete anti-inflammatory cytokines, such as IL-10 and IL-35 (Li et al., 2015a; Li et al., 2015b). Notably, pro-inflammatory IL-6 induces the differentiation of IL-10-secreting regulatory B cells, probably as a self-regulatory mechanism to prevent excessive inflammation (Rosser et al., 2014).

IL-1β, TNF-α, and GM-CSF expression by B cell lines derived from MS patients significantly correlate with disability, according to the expanded disability status scale (EDSS). Moreover, B cells from patients with the progressive forms of MS produce more TNF-α and LTα than those from RRMS patients (Stein et al., 2018). In addition, GM-CSF expressed by B cells activates myeloid cells and, in turn, regulates the generation of regulatory IL-10-expressing B cells *in vitro* (Li et al., 2015b). Moreover, GM-CSF knockout mice are resistant to EAE induction, demonstrating that GM-CSF is required for inducing chronic tissue damage (Duncker et al., 2018; McQualter et al., 2001). In humans, the subset of B cells that secrete GM-CSF also express high levels of TNF-α and IL-6 (Li et al., 2015b). All the aforementioned cytokines, chemokines and LTs expressed by B cells have been involved in the formation follicle-like meningeal structures of MS patients (Corsiero et al., 2016).

The regulatory function of B cells is associated to the expression of anti-inflammatory cytokines, such as IL-10, IL-35, TGF-β. In a model of chronic EAE, the regulatory and protective functions of B cells were mediated through IL-10 production (Thi Cuc et al., 2019). Moreover, MOG-immunized knockout mice selectively lacking IL-10-producing B cells, failed to recover from EAE (Fillatreau et al., 2002). IL-35, an anti-inflammatory cytokine of the IL-12 family, produced by B cells, plays an important role in the recovery from the EAE, probably either by inhibiting the immune response or, through the induction of IL-10 by regulatory B cells with concomitant suppression of Th17 response (Kang et al., 2020; Wang et al., 2014) (summarized in Fig. 1). EAE transgenic mice with TGF-β1-deficient B cells demonstrated an earlier onset of the disease and exacerbation of the susceptibility to the disease caused by downregulating encephalitogenic Th1/17 response (Bjarnadottir et al., 2016).

2.1. Role of B cells in cortical MS pathology

2.1.1. Cortical and meningeal MS pathology

Primary demyelinating cortical plaques are specific to MS as they cannot be found in any other chronic neuroinflammatory disorder (Fischer et al., 2013).

The classification of the cortical lesions is based on the distribution of the demyelinating areas along the cortical layers: (a) type I or leukocortical, located in the deeper layers of the gray matter as well as in the adjacent white matter; (b) type II or intracortical, small demyelinating lesions located within the cortex and mostly adjacent to blood vessels; (c) type III or subpial, located on the outermost layer of the cortex, close to the *pia mater*; and (d) type IV or pancortical, distributed throughout the cortex (Dutta and Trapp, 2007; Lucchinetti et al., 2011).

Despite the detailed descriptions available for gray matter pathology, the mechanisms that trigger cortical injury remain unresolved. The first possibility is that the neurodegeneration is primarily originated within the white matter and from there it induces and propagates cortical pathology an anterograde or retrograde manner (Choi et al., 2012; Haider et al., 2016; Lassmann, 2012). The second possibility is that meningeal inflammation is the one responsible of triggering cortical injury.

The relationship between cortical damage and meningeal inflammation has been extensively described and has been linked to cortical pathology and a worse clinical prognosis in the progressive forms of MS (Choi et al., 2012; Howell et al., 2011; James et al., 2020; Magliozzi et al., 2007; Magliozzi et al., 2010). The entrance of peripheral immune

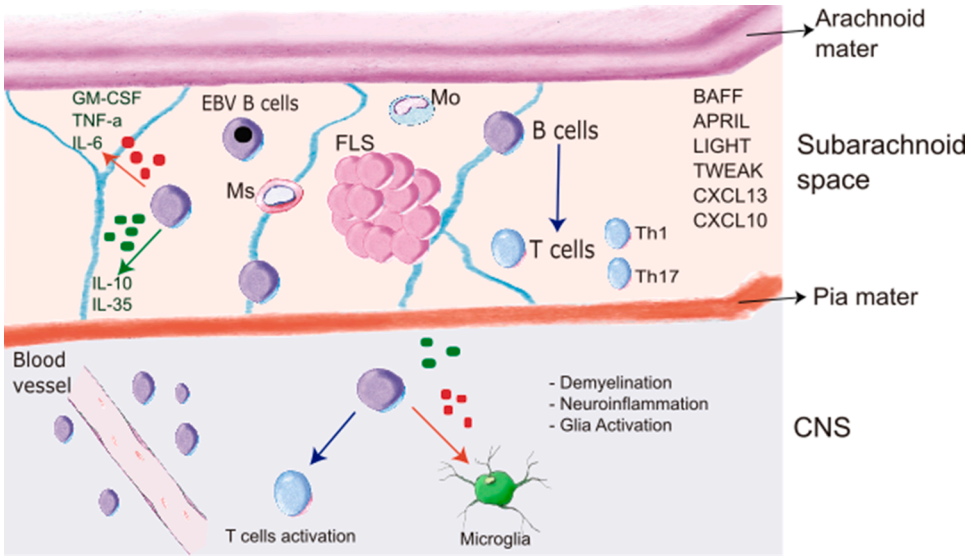

Figure 1. Schematic diagram of the role of B cells in meningeal inflammation and cortical lesions. The subarachnoid space contains the CSF, which include macrophages (Mo) and meningeal cells (Ms), as well as T and B cells. B cells can appear either diffuse or forming follicular-like structures (FLS). B cells can also express pro-inflammatory cytokines (GM-CSF, TNF-α and IL-6) (red dots) and anti-inflammatory cytokines (IL-10 and IL-35) (green dots). In addition, they can express chemokines (CXCL-13 and CXCL-10) and other B cell-related molecules (BAFF, APRIL, LIGHT, TWEAK). Additionally, B cells can activate T cells, leading to Th1 and Th17 responses. T cell re-activation in the meninges promotes the influx of B and T cells into the cortex, which induces loss of local BBB integrity, microglia and astroglia activation, demyelination and neurodegeneration.

cells into the CNS could be explained by the ``two-step'' model, described by Russi and Brown, 2015 (Russi and Brown, 2015). According to this model, the "first wave" includes the immune surveillance of the CNS, where peripheral T cells circulate from the periphery towards the choroid plexus. These cells enter into the ventricles through the blood-CSF barrier of the choroid plexus and eventually reach the subarachnoid space of the meninges. The "second wave" involves the interaction between T cells and APCs loaded with myelin antigens, which triggers T-cell reactivation in the meninges and promotes the influx of peripheral leukocytes into the CNS with the subsequent loss of local BBB integrity. Thus, this second wave of inflammatory cells mediates demyelination and neuronal damage (Axtell and Steinman, 2009; Bartholomaus et al., 2009; Ransohoff, 2009; Reboldi et al., 2009; Wolburg and Paulus, 2010). The progression of cortical pathology associated with meningeal inflammation was demonstrated throughout the stages of MS, with subpial lesions during the earlier stages and deeper lesions in the later stages of the disease, (Magliozzi et al., 2010; Mainero et al., 2015).

In clinical practice, the *in vivo* visualization of cortical lesions remains technically challenging because of the low sensitivity of conventional MRI technique for unequivocal detection of these lesions (Jonkman et al., 2015; Klaver et al., 2015). Distinct MRI techniques have been developed to improve cortical lesion detection, including double inversion recovery, phase-sensitive inversion recovery, magnetization-prepared rapid acquisition with gradient echo sequences and a combination of magnetization-prepared two rapid gradient echo and T2* weighted imaging at 7T (Beck et al., 2018; Favaretto et al., 2015; Nelson et al., 2007; Sethi et al., 2012). Leptomeningeal pathology can also be visualized through brain MRI using post-contrast 3T FLAIR, in which the leakage of gadolinium into the CSF is suggested as an indirect marker of meningeal inflammation in MS (Absinta et al., 2015). The presence of this leptomeningeal contrast enhancement in 3T MRIs has also been associated with cortical atrophy progression, measured over a 5 year period (Zivadinov et al., 2017) and physical disability (Makshakov et al., 2017). However, the presence of this leakage is not specific for MS and is seen in other CNS diseases as well (Absinta et al., 2017). Currently used imaging techniques still require further research in order to improve the current understanding of MS pathology and eventually develop more specific treatments.

2.1.2. Role of B cells in cortico- meningeal MS pathology: evidence from animal models

Since MS is a heterogeneous disease and samples from patients are usually scarce, the development of animal models is crucial for investigating the function of B cells in this pathology. Although animal models are unable to replicate the full complexity of MS pathology, they have contributed to the knowledge of pathological processes of the disease, as well as to the development of therapeutic drugs for its treatment (Bjelobaba et al., 2018; Constantinescu et al., 2011; Kipp et al., 2017; Silva and Ferrari, 2018). Of all the available animal models, EAE has been the one that dominated the field of preclinical research of MS over the last 70 years. EAE can be induced by active immunization using myelin-derived proteins, such as myelin oligodendrocyte glycoprotein (MOG), or peptides in adjuvant or by passive transfer of activated myelin-specific CD4+ T lymphocytes (Robinson et al., 2014). The major concern about MOG-EAE is that it mimics a patients' disease now called "MOG antibody-associated inflammatory demyelinating disease", which differs from MS. EAE is mediated by CD4+ T cells, and the human MS disease is mediated by CD8+ T cells and B cells (Lassmann, 2019). In conclusion, most EAE models are mainly focused on T cell reaction, with no especial focus on B cells.

In order to study the role of B cells, new EAE models based on specific antigen induction, have been created. The first one was based on the immunization of B6 mice with human MOG (hMOG), which induced a severe and chronic course of the disease with CNS meningeal and perivascular inflammatory infiltration with presence of B cells (Boyden et al., 2020; Kuerten and Angelov, 2008; Oliver et al., 2003). Another model was achieved by immunizing C57BL/6 mice with a combination of myelin basic protein (MBP) and three hydrophilic loops of proteolipid protein (PLP), named MP4. These mice presented a large number of infiltrated B cells into both brain and spinal cord, most of which were aggregated forming tertiary lymphoid organs in association with CXCL13+ and CD10+ cells (Kuerten et al., 2008).

Here, we will focus on the data regarding the role of B cells in cortical lesions and the associated meningeal inflammation. The extensive data available related to spinal cord and peripheral response are out of the scope of this manuscript.

The molecules involved in the functional organization of B cell responses within lymphoid follicles were studied in SJL mice immunized with $PLP_{139–151}$ peptides. Magliozzi and colleagues, demonstrated that CXCL13, a chemokine involved in B cell recruitment, and the B cell activating factor of the tumor necrosis factor family (BAFF), essential for proliferation and maturation of B cells, are induced in the CNS of mice with relapsing-remitting and chronic-relapsing EAE. Additionally, the authors demonstrated the presence of follicle-like structures with B cells and CXCL13+ follicular dendritic cells located within the meninges

during the inflammatory process (Magliozzi et al., 2004). LTα1β2 and its receptor were upregulated in mice at the onset of EAE induced by $PLP_{139–151}$. LTα1β2 is member of the TNF-a family expressed on the surface of B and T cells that induces the expression of lymphoid chemokines (among them, CXCL13). Systemic and local blockage of the interaction of LT with its receptor inhibited EAE, impaired the formation of ectopic follicles in the meninges, diminished T and B cell infiltration and prevented the secretion of the chemokines CXCL10 and CXCL13 (Columba-Cabezas et al., 2006).

EAE induced with MOG_{35-55} peptide in mice showed the presence of B and T cells in the meninges of EAE animals compared with saline controls. Leptomeningeal inflammation in EAE-MOG brains was visualized by MRI on a 9.4T scanner with gadolinium as a contrast agent, demonstrating that leptomeningeal enhancement due to inflammation in EAE brains correlated with the presence of CD45+ T cells, CD19+ B cells and a cluster of Iba1+ microglial cells (Pol et al., 2019). The treatment with anti-CD20 demonstrated to delay the neurodegeneration in grey matter, along with improving the severity of the disease in $hMOG_{1-125}$ EAE mice (Pol et al., 2020). Anti-CD20 therapy diminished the proliferation of microglia in the isocortex and depleted CD19-expressing B cells in peripheral blood (Pol et al., 2020).

Even though most of the EAE models have been developed in mice, a model of T cell-driven EAE was developed in the common marmoset. Marmosets immunized with the MOG_{34-56} peptide in incomplete Freund's adjuvant presented CD20+ cells in neocortical lesions (t Hart et al., 2017). Depletion of B cells using a monoclonal antibody against human CD20 suppressed the pathological processes and symptoms of EAE in both cortical and white matter lesions by preventing the activation of pathogenic Th17-producing cytotoxic T cells and by reducing mRNA levels of IL-17, IL-6, IFN-γ and TNF-α in peripheral blood (Jagessar et al., 2012; Kap et al., 2010; Merkler et al., 2006; Pomeroy et al., 2005; Vidaurre et al., 2014). However, when EAE was induced by immunization with recombinant rat MOG in marmoset, the number of B cells in the meninges adjacent to subpial cortical demyelination areas was unaltered compared to control animals. Even though the number of meningeal B cells remained low and unaltered, the meninges of EAE marmosets showed a significant increase of meningeal T cells associated with subpial demyelinated cortex (Kramann et al., 2015).

Another model of progressive MS, Theiler's murine encephalomyelitis virus (TMEV), is characterized by the accumulation of B cells and the production of antibodies in both the CNS and the ectopic lymphoid follicles during CNS inflammation (DiSano et al., 2019b; Gerhauser et al., 2019). These B cells localize in the meninges and the perivascular space with minimal evidence of secondary lymphoid organ formation. During the chronic phase of the disease, the lymphoid structures secrete antibodies, B cell-related cytokines and trophic factors (DiSano et al., 2019a). In addition, CSF of TMEV-infected mice has higher levels of CXCL13, CXCL12, and CCL19 along with an intact BBB, which demonstrates the synthesis of B cell-related chemokines within the CNS. The presence of these molecules cannot be observed in a relapsing model of EAE, suggesting a potentially important role of these chemokines in the chronic progressive phase of demyelinating diseases (DiSano et al., 2019b; Pachner et al., 2011). Interestingly, B-cell depletion in the Theiler's virus model of MS caused exacerbation of the pathology of the TMEV by worsening the early encephalitis and causing a faster progression of disability (Gilli et al., 2015).

Interestingly, CD20 antibody–mediated B cell depletion before EAE induction exacerbated disease symptoms by increasing the T cell influx into the CNS, probably because of the depletion of specific regulatory B10 producing CD1dhiCD5+ cell subset (Matsushita et al., 2008).

Recently, some models of cortical pathology developed in rats described the presence of meningeal B cells. A focal cortical model induced by the long-term expression of IL-1β in the cortex showed lesions with demyelination, neurodegeneration and meningeal inflammation along with presence of follicle-like structures including CD20+ B cells (Gardner et al., 2013; Silva et al., 2018). Other models of subpial demyelination set up in a subclinical EAE model of MOG-immunized dark agouti rats injected with TNF-α and IFN-γ in the subarachnoid space, demonstrated the presence of meningeal inflammation with CD79a+ B cells located in discrete clusters. In this model, B cells were also extended to the cortical lesions (Gardner et al., 2013; James et al., 2020).

2.1.3. Role of B cells in cortico-meningeal MS pathology: evidence from patient's studies

The first study about meningeal infiltrates described the presence of lymphoid follicle-like structures containing CD20+ B cells, CD3+ T cells, CD138+ plasma cells and a network of follicular CD21+/CD35+ dendritic cells producing CXCL13, similar to a germinal center formation, in the meninges of SPMS patients (Serafini et al., 2004). In addition, these follicle-like structures served as sites for intrathecal subarachnoid antibody production and the sequestration and presentation of autoantigens (Magliozzi et al., 2007; Serafini et al., 2004). The presence of these structures indicates that B cell maturation can be sustained locally within the CNS and contribute to the establishment of a compartmentalized humoral immune response.

B cell follicles were detected in the meninges entering the cerebral sulci in SPMS, but not in PPMS (Magliozzi et al., 2007). These cortical and meningeal findings were observed in other SPMS cohorts as well (Haider et al., 2016; Howell et al., 2011). SPMS patients with B cell follicles in the meninges were younger at onset of the disease, had more irreversible disability and an earlier death than SPMS patients without follicles. SPMS cortical lesions associated with B cell follicles in the meninges showed increased demyelination, microglial activation and loss of neurites (Magliozzi et al., 2007). The presence of subpial lesions (type II), suggested a possible pathogenic role for soluble factors diffusing from these follicles. SPMS cortices presenting meningeal follicles exhibited a "surface-in" gradient of neuronal loss in adjacent subpial lesions and a normal-appearing gray matter (Magliozzi et al., 2010). This "surface-in" gradient was observed as a lower density of pyramidal neurons in layers III and V accompanied by glia limitans damage due to astrocyte loss and an opposite gradient of increased density of activated microglia (Magliozzi et al., 2010). Notably, these gradients were not seen in patients without follicles.These findings have raised the hypothesis that soluble factors, potentially released by immune cells in the meninges, diffuse into the CSF and into the superficial brain layers such as the subpial cortical tissue, contributing to the observed gradient of pathological changes (Lisak et al., 2012; Lisak et al., 2017). This hypothesis has been corroborated by *in vitro* experiments. The incubation of a culture of mixed CNS neonatal rat glial cells with the supernatant of CD40-stimulated B cells from the blood of RRMS patients led to oligodendroglial death, together with microglial enlargement and loss of their normal resting bipolar morphology (Lisak et al., 2012). Additionaly, RRMS blood B cells also secrete toxic factors that induced apoptosis in rat and human neurons *in vitro* (Lisak et al., 2017). Furthermore, CSF from MS patients contain soluble mediators that induce axonal damage and apoptosis in cultured rat neurons (Alcazar et al., 2000; Vidaurre et al., 2014).

PPMS patients presented diffuse meningeal inflammation lacking tertiary lymphoid structures, characterized by meningeal and perivascular immune cell infiltration, composed of clusters of CD3+ T cells and CD20+ B cells, but not Ki67+/CD20+ dividing B cells or CD35+ follicular dendritic cells (Choi et al., 2012). These patients had a more severe clinical course and younger age at death, as well as more meningeal inflammation associated with extensive demyelination, microglial activation and neurite loss in the cortical gray matter (Choi et al., 2012).

Significant meningeal inflammation and lymphoid-like structures associated with subpial cortical lesions were found in tissue blocks from MS patients with short disease duration (about 2 years) (Bevan et al., 2018). In concordance with these findings, Lucchinetti and colleagues observed a wide spectrum of cortical lesions associated with meningeal

inflammation in early MS biopsy samples, similar to those seen in samples from patients with chronic MS (Lucchinetti et al., 2011). However, unlike cortical lesion from chronic MS patients, cortical lesions from early MS patients had lymphocytic and macrophagic infiltrates within the lesions (Lucchinetti et al., 2011).

Besides the meninges and the cortex, other compartments of the CNS, such as the subependymal region, present B cell activity in MS. The presence of B cells in the subependymal infiltrate was associated with thalamic lesions in SPMS, in a similar surface-in pattern in the thalamus as previously described for the cerebral cortex (Fadda et al., 2019).

Cortical and deep gray matter lesions can be visualized by MRI in all MS clinical courses, including pediatric and early MS (Brown et al., 2017; Calabrese et al., 2013; Calabrese et al., 2012b; Fadda et al., 2019; Mainero et al., 2015). Specific CSF profiles, in combination with MRI images, have been proposed as a marker of cortico-meningeal compromise, which is considered a prognostic marker of more aggressive and rapid progression for MS (Komori et al., 2015; Magliozzi et al., 2018). These profiles include mainly B cell-related cytokines such as: CXCL13, CXCL10, LTα, IL-6, and IL-10 found in postmortem MS cases and CXCL13, TNF, IFN-γ, CXCL12, IL-6, IL-8, BAFF, APRIL, LIGHT, TWEAK, sTNFR1, sCD163, MMP2, and pentraxin III, found in MS patients at the moment of MS diagnosis (summarized in Fig. 1). In conclusion, the presence of these cytokines in CSF strongly correlates with increased cortical pathology, both at the time of diagnosis and at the time of death, in SPMS patients (Magliozzi et al., 2018).

Regarding the association between CSF and cortical lesions, Magliozzi and colleagues demonstrated, in a longitudinal study, the first association between CSF profile and disease activity in RRMS patients. The presence of B cell-related molecules such as CXCL13, LIGHT and APRIL, in the CSF of RRMS patients at the moment of diagnosis was strongly associated with the risk of disease activity (reflected by the occurrence of new relapses, new or enlarging white matter lesions and disability accumulation) within the next 4 years (Magliozzi et al., 2020). In addition, immunoassay protein and proteomic analysis of CSF from RRMS patients demonstrated the presence of inflammatory mediators linked to B cells, such as CXCL13, CXCL12, CXCL10, BAFF, IL-6, IL-10, GM-CSF, and TNFα (Magliozzi et al., 2019). The correlations between B cell-related molecules and macrophage markers such as CD163 in the CSF could imply that infiltrating B cells might intrathecally interact with macrophages recognizing, internalizing and retaining antigens through the scavenger receptor CD163 (Magliozzi et al., 2019). Elevated CSF levels of CD163, involved in the uptake and metabolism of iron, where found in RRMS patients (Magliozzi et al., 2019). Therefore, CSF analysis might allow the stratification of patients for designing optimal therapeutic approaches (Komori et al., 2015; Magliozzi et al., 2020).

Epstein-Barr virus (EBV) was proposed as a possible trigger for the chronification of the compartmentalized immune response of progressive MS because of its special tropism for B cells and its association with the genesis of MS. The presence of EBV markers in B cells and plasma cells has been described in the perivascular compartment of white matter lesions and the meninges of MS brain tissue. The frequency of EBV-harboring cells correlated with brain inflammation and CD8+ activation (Serafini et al., 2007). Furthermore, B cells in white matter lesions, meninges and ectopic B cell follicles were CD27+ and co-expressed latent membrane protein 1 and latent membrane protein 2A, both EBV-encoded proteins that provide survival and maturation signals to B cells. Moreover, BAFF is expressed in EBV-infected B cells and ectopic B-cell follicles in acute MS lesions (Serafini et al., 2010). The presence of EBV in cortical lesions was corroborated by Magliozzi and colleagues, demonstrating cells of the B lineage expressing EBV-encoded small RNA and plasma cells expressing EBV early lytic proteins (BZLF1, BFRF1) in SPMS intracortical immune infiltrates (Magliozzi et al., 2013). In addition, CD8+ cells, granzyme B, perforin and CD107a were also found in those infiltrates, suggesting a cytotoxic activity related to active immune surveillance. These findings indicate that intracortical inflammation during MS progression may be sustained by an EBV-driven immunopathologic response, along with both meningeal and intraparenchymal inflammation, similar to that found in white matter lesions (Magliozzi et al., 2013). Nonetheless, the relationship of other MS-specific pathologies, e.g demyelination, with EBV infection still remains unclear. In spite of the previously described data, some authors were unable to detect the presence of EBV in B cells in the brain and CSF of MS patients (Peferoen et al., 2010; Sargsyan et al., 2010; Torkildsen et al., 2010; van Nierop et al., 2017; Willis et al., 2009). These discrepant results could be based on variations in the preservation and manipulation of human tissue that could affect EBV detection (Cencioni et al., 2017).

2.2. B cell targeting therapies for Multiple Sclerosis

The systemic administration of anti-CD20 monoclonal antibodies induces B cell depletion associated with reduction of clinical and MRI activity and disability worsening (Li et al., 2018; von Essen et al., 2019). All anti-CD20 therapies induce antibody-dependent cellular cytotoxicity (Sellebjerg et al., 2020). Moreover, type 1 anti-CD20 has a complement-mediated mechanism and type 2 induces a strong programmed cell death (Cragg et al., 2003). Rituximab is a type 1 antibody, a chimeric IgG1 anti-CD20 (Cragg et al., 2003), and was the first anti-CD20 monoclonal antibody used for MS treatment. In RRMS patients, Rituximab was demonstrated to reduce the annualized relapse rate and disease activity in phase II clinical trials and observational studies, but still phase III trials have not yet been performed (Bar-Or et al., 2008; Castillo-Trivino et al., 2013; Hauser et al., 2008; Sellebjerg et al., 2020). In addition, in PPMS, phase II/III, a randomized control trial for Rituximab treatment did not reach the primary endpoint of significant reduction in time to confirmed disease progression after 96 weeks (Hawker et al., 2009).

Ocrelizumab is a humanized IgG1 antibody approved by the Food and Drug Administration (FDA) and by the European Medicines Agency in 2018 for both RRMS and PPMS (Sellebjerg et al., 2020). Its efficacy and safety on RRMS patients was demonstrated in two phase III trials, OPERA I and II (Hauser et al., 2017), resulting in a reduction of annualized relapse rate, MRI activity and EDSS (Hauser et al., 2017). In PPMS patients, Ocrelizumab significantly reduced confirmed disability worsening in the ORATORIO phase III study (Montalban et al., 2017).

Ofatumumab, a fully human IgG1 type 1 monoclonal antibody, activates the complement cascade more efficiently than others type 1 antibodies and was approved in 2020 by the FDA for using in the relapsing forms of MS (Sellebjerg et al., 2020). Its efficacy and safety was demonstrated in phase III trials ASCLEPIOS I and II, showing a significant reduction in the annualized relapse rates, disability worsening and MRI activity in RRMS patients (Hauser et al., 2020).

Ublituximab is type 1 chimeric IgG1 antibody which improves antibody-dependent cellular cytotoxicity activity (Klein et al., 2013; Sawas et al., 2017). Even though it is still being tested in an ongoing phase III trial, a phase II study demonstrated that most of the patients were relapse-free at week 48 without disability worsening (Fox et al., 2020).

Finally, Bruton's tyrosine kinase (BTK) enzyme inhibitors (such as Evobrutinib, Fenebrutinib, and Tolebrutinib) represents another interesting group of B cells targeting therapy. BTK is an essential component of different BCR signal pathway triggered after antigen engagement, including PI3K, MAPK and NF-κB pathways, which regulates survival, activation, proliferation, and differentiation of B cells to antibody producing plasma cells (Liang et al., 2018). Some of them have completed phase II and phase III but additional studies are still ongoing in different phenotypes of MS (Carnero Contentti and Correale, 2020).

Concluding remarks

In recent years, the role of B cells has emerged in the field of MS research, as it is noticeable by the increasing number of publications on

the subject. From now on, research should focus on the interaction between B cells, T cells, myeloid cells and microglia. Much of the available data arose from patients, including the presence of B cells in meningeal inflammation and their role on T cell function. Nevertheless, considering the complexity of obtaining human samples and the heterogeneity of the patients, the development of animal models with a focus on B cells will help to further elucidate the mechanisms underlying the role of B cells in MS.

Conflict of Interest

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CCF and EM have no conflicts of interest

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References

Absinta, M., Cortese, I.C., Vuolo, L., Nair, G., de Alwis, M.P., Ohayon, J., Meani, A., Martinelli, V., Scotti, R., Falini, A., Smith, B.R., Nath, A., Jacobson, S., Filippi, M., Reich, D.S., 2017. Leptomeningeal gadolinium enhancement across the spectrum of chronic neuroinflammatory diseases. Neurology 88 (15), 1439–1444.

Absinta, M., Vuolo, L., Rao, A., Nair, G., Sati, P., Cortese, I.C., Ohayon, J., Fenton, K., Reyes-Mantilla, M.I., Maric, D., Calabresi, P.A., Butman, J.A., Pardo, C.A., Reich, D. S., 2015. Gadolinium-based MRI characterization of leptomeningeal inflammation in multiple sclerosis. Neurology 85 (1), 18–28.

Alcazar, A., Regidor, I., Masjuan, J., Salinas, M., Alvarez-Cermeno, J.C., 2000. Axonal damage induced by cerebrospinal fluid from patients with relapsing-remitting multiple sclerosis. J Neuroimmunol 104 (1), 58–67.

Axtell, R.C., Steinman, L., 2009. Gaining entry to an uninflamed brain. Nat Immunol 10 (5), 453–455.

Bar-Or, A., Calabresi, P.A., Arnold, D., Markowitz, C., Shafer, S., Kasper, L.H., Waubant, E., Gazda, S., Fox, R.J., Panzara, M., Sarkar, N., Agarwal, S., Smith, C.H., 2008. Rituximab in relapsing-remitting multiple sclerosis: a 72-week, open-label, phase I trial. Ann Neurol 63 (3), 395–400.

Bar-Or, A., Pender, M.P., Khanna, R., Steinman, L., Hartung, H.P., Maniar, T., Croze, E., Aftab, B.T., Giovannoni, G., Joshi, M.A., 2020. Epstein-Barr Virus in Multiple Sclerosis: Theory and Emerging Immunotherapies. Trends Mol Med 26 (3), 296–310.

Barr, T.A., Brown, S., Mastroeni, P., Gray, D., 2010. TLR and B cell receptor signals to B cells differentially program primary and memory Th1 responses to Salmonella enterica. J Immunol 185 (5), 2783–2789.

Barr, T.A., Shen, P., Brown, S., Lampropoulou, V., Roch, T., Lawrie, S., Fan, B., O'Connor, R.A., Anderton, S.M., Bar-Or, A., Fillatreau, S., Gray, D., 2012. B cell depletion therapy ameliorates autoimmune disease through ablation of IL-6-producing B cells. J Exp Med 209 (5), 1001–1010.

Bartholomaus, I., Kawakami, N., Odoardi, F., Schlager, C., Miljkovic, D., Ellwart, J.W., Klinkert, W.E., Flugel-Koch, C., Issekutz, T.B., Wekerle, H., Flugel, A., 2009. Effector T cell interactions with meningeal vascular structures in nascent autoimmune CNS lesions. Nature 462 (7269), 94–98.

Beck, E.S., Sati, P., Sethi, V., Kober, T., Dewey, B., Bhargava, P., Nair, G., Cortese, I.C., Reich, D.S., 2018. Improved Visualization of Cortical Lesions in Multiple Sclerosis Using 7T MP2RAGE. AJNR Am J Neuroradiol.

Bevan, R.J., Evans, R., Griffiths, L., Watkins, L.M., Rees, M.I., Magliozzi, R., Allen, I., McDonnell, G., Kee, R., Naughton, M., Fitzgerald, D.C., Reynolds, R., Neal, J.W., Howell, O.W., 2018. Meningeal inflammation and cortical demyelination in acute multiple sclerosis. Ann Neurol 84 (6), 829–842.

Bjarnadottir, K., Benkhoucha, M., Merkler, D., Weber, M.S., Payne, N.L., Bernard, C.C.A., Molnarfi, N., Lalive, P.H., 2016. B cell-derived transforming growth factor-beta1 expression limits the induction phase of autoimmune neuroinflammation. Sci Rep 6, 34594.

Bjelobaba, I., Begovic-Kupresanin, V., Pekovic, S., Lavrnja, I., 2018. Animal models of multiple sclerosis: Focus on experimental autoimmune encephalomyelitis. J Neurosci Res 96 (6), 1021–1042.

Blauth, K., Soltys, J., Matschulat, A., Reiter, C.R., Ritchie, A., Baird, N.L., Bennett, J.L., Owens, G.P., 2015. Antibodies produced by clonally expanded plasma cells in multiple sclerosis cerebrospinal fluid cause demyelination of spinal cord explants. Acta Neuropathol 130 (6), 765–781.

Boyden, A.W., Brate, A.A., Karandikar, N.J., 2020. Novel B cell-dependent multiple sclerosis model using extracellular domains of myelin proteolipid protein. Sci Rep 10 (1), 5011.

Brown, J.W., Pardini, M., Brownlee, W.J., Fernando, K., Samson, R.S., Prados Carrasco, F., Ourselin, S., Gandini Wheeler-Kingshott, C.A., Miller, D.H., Chard, D.T., 2017. An abnormal periventricular magnetization transfer ratio gradient occurs early in multiple sclerosis. Brain 140 (2), 387–398.

Calabrese, M., Castellaro, M., 2017. Cortical Gray Matter MR Imaging in Multiple Sclerosis. Neuroimaging clinics of North America 27 (2), 301–312.

Calabrese, M., Favaretto, A., Martini, V., Gallo, P., 2013. Grey matter lesions in MS: from histology to clinical implications. Prion 7 (1), 20–27.

Calabrese, M., Poretto, V., Favaretto, A., Alessio, S., Bernardi, V., Romualdi, C., Rinaldi, F., Perini, P., Gallo, P., 2012a. Cortical lesion load associates with progression of disability in multiple sclerosis. Brain 135 (Pt 10), 2952–2961.

Calabrese, M., Seppi, D., Romualdi, C., Rinaldi, F., Alessio, S., Perini, P., Gallo, P., 2012b. Gray matter pathology in MS: a 3-year longitudinal study in a pediatric population. AJNR Am J Neuroradiol 33 (8), 1507–1511.

Carnero Contentti, E., Correale, J., 2020. Bruton's tyrosine kinase inhibitors: a promising emerging treatment option for multiple sclerosis. Expert Opin Emerg Drugs 25 (4), 377–381.

Castillo-Trivino, T., Braithwaite, D., Bacchetti, P., Waubant, E., 2013. Rituximab in relapsing and progressive forms of multiple sclerosis: a systematic review. PLoS One 8 (7), e66308.

Cencioni, M.T., Magliozzi, R., Nicholas, R., Ali, R., Malik, O., Reynolds, R., Borsellino, G., Battistini, L., Muraro, P.A., 2017. 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 (4), 660–676.

Columba-Cabezas, S., Griguoli, M., Rosicarelli, B., Magliozzi, R., Ria, F., Serafini, B., Aloisi, F., 2006. Suppression of established experimental autoimmune encephalomyelitis and formation of meningeal lymphoid follicles by lymphotoxin beta receptor-Ig fusion protein. J Neuroimmunol 179 (1-2), 76–86.

Constantinescu, C.S., Farooqi, N., O'Brien, K., Gran, B., 2011. Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). Br J Pharmacol 164 (4), 1079–1106.

Corsiero, E., Nerviani, A., Bombardieri, M., Pitzalis, C., 2016. Ectopic Lymphoid Structures: Powerhouse of. Autoimmunity. Front Immunol 7, 430.

Cragg, M.S., Morgan, S.M., Chan, H.T., Morgan, B.P., Filatov, A.V., Johnson, P.W., French, R.R., Glennie, M.J., 2003. Complement-mediated lysis by anti-CD20 mAb correlates with segregation into lipid rafts. Blood 101 (3), 1045–1052.

Chen, D., Ireland, S.J., Remington, G., Alvarez, E., Racke, M.K., Greenberg, B., Frohman, E.M., Monson, N.L., 2016. CD40-Mediated NF-kappaB Activation in B Cells Is Increased in Multiple Sclerosis and Modulated by Therapeutics. J Immunol 197 (11), 4257–4265.

Choi, S.R., Howell, O.W., Carassiti, D., Magliozzi, R., Gveric, D., Muraro, P.A., Nicholas, R., Roncaroli, F., Reynolds, R., 2012. Meningeal inflammation plays a role in the pathology of primary progressive multiple sclerosis. Brain 135 (Pt 10), 2925–2937.

DiSano, K.D., Linzey, M.R., Royce, D.B., Pachner, A.R., Gilli, F., 2019a. Differential neuro-immune patterns in two clinically relevant murine models of multiple sclerosis. J Neuroinflammation 16 (1), 109.

DiSano, K.D., Royce, D.B., Gilli, F., Pachner, A.R., 2019b. Central Nervous System Inflammatory Aggregates in the Theiler's Virus Model of. Progressive Multiple Sclerosis. Front Immunol 10, 1821.

Duncker, P.C., Stoolman, J.S., Huber, A.K., Segal, B.M., 2018. GM-CSF Promotes Chronic Disability in Experimental Autoimmune Encephalomyelitis by Altering the Composition of Central Nervous System-Infiltrating Cells, but Is Dispensable for Disease Induction. J Immunol 200 (3), 966–973.

Dutta, R., Trapp, B.D., 2007. Pathogenesis of axonal and neuronal damage in multiple sclerosis. Neurology 68 (22 Suppl 3), S22–S31 discussion S43-54.

34 Fadda, G., Brown, R.A., Magliozzi, R., Aubert-Broche, B., O'Mahony, J., Shinohara, R.T., Banwell, B., Marrie, R.A., Yeh, E.A., Collins, D.L., Arnold, D.L., Bar-Or, A., Canadian Pediatric Demyelinating Disease, N., 2019. A surface-in gradient of thalamic damage evolves in pediatric multiple sclerosis. Ann Neurol 85 (3), 340–351.

Favaretto, A., Poggiali, D., Lazzarotto, A., Rolma, G., Causin, F., Gallo, P., 2015. The Parallel Analysis of Phase Sensitive Inversion Recovery (PSIR) and Double Inversion Recovery (DIR) Images Significantly Improves the Detection of Cortical Lesions in Multiple Sclerosis (MS) since Clinical Onset. PLoS One 10 (5), e0127805.

Fillatreau, S., Sweenie, C.H., McGeachy, M.J., Gray, D., Anderton, S.M., 2002. B cells regulate autoimmunity by provision of IL-10. Nat Immunol 3 (10), 944–950.

Fischer, M.T., Wimmer, I., Hoftberger, R., Gerlach, S., Haider, L., Zrzavy, T., Hametner, S., Mahad, D., Binder, C.J., Krumbholz, M., Bauer, J., Bradl, M., Lassmann, H., 2013. Disease-specific molecular events in cortical multiple sclerosis lesions. Brain 136 (Pt 6), 1799–1815.

Fox, E., Lovett-Racke, A.E., Gormley, M., Liu, Y., Petracca, M., Cocozza, S., Shubin, R., Wray, S., Weiss, M.S., Bosco, J.A., Power, S.A., Mok, K., Inglese, M., 2020. A phase 2 multicenter study of ublituximab, a novel glycoengineered anti-CD20 monoclonal antibody, in patients with relapsing forms of multiple sclerosis. Mult Scler, 1352458520918375.

Fraussen, J., Claes, N., Van Wijmeersch, B., van Horssen, J., Stinissen, P., Hupperts, R., Somers, V., 2016. B cells of multiple sclerosis patients induce autoreactive proinflammatory T cell responses. Clin Immunol 173, 124–132.

Gardner, C., Magliozzi, R., Durrenberger, P.F., Howell, O.W., Rundle, J., Reynolds, R., 2013. Cortical grey matter demyelination can be induced by elevated pro-

inflammatory cytokines in the subarachnoid space of MOG-immunized rats. Brain 136 (Pt 12), 3596–3608.
Genain, C.P., Cannella, B., Hauser, S.L., Raine, C.S., 1999. Identification of autoantibodies associated with myelin damage in multiple sclerosis. Nat Med 5 (2), 170–175.
Genc, K., Dona, D.L., Reder, A.T., 1997. Increased CD80(+) B cells in active multiple sclerosis and reversal by interferon beta-1b therapy. J Clin Invest 99 (11), 2664–2671.
Gerhauser, I., Hansmann, F., Ciurkiewicz, M., Loscher, W., Beineke, A., 2019. Facets of Theiler's Murine Encephalomyelitis Virus-Induced Diseases: An Update. International journal of molecular sciences 20 (2).
Gharibi, T., Hosseini, A., Marofi, F., Oraei, M., Jahandideh, S., Abdollahpour-Alitappeh, M., Hashemi, V., Motallebnezhad, M., Babaloo, Z., Baradaran, B., 2019. IL-21 and IL-21-producing T cells are involved in multiple sclerosis severity and progression. Immunol Lett 216, 12–20.
Gilli, F., Li, L., Campbell, S.J., Anthony, D.C., Pachner, A.R., 2015. The effect of B-cell depletion in the Theiler's model of multiple sclerosis. J Neurol Sci 359 (1-2), 40–47.
Haider, L., Zrzavy, T., Hametner, S., Hoftberger, R., Bagnato, F., Grabner, G., Trattnig, S., Pfeifenbring, S., Bruck, W., Lassmann, H., 2016. The topography of demyelination and neurodegeneration in the multiple sclerosis brain. Brain 139 (Pt 3), 807–815.
Harp, C.T., Lovett-Racke, A.E., Racke, M.K., Frohman, E.M., Monson, N.L., 2008. Impact of myelin-specific antigen presenting B cells on T cell activation in multiple sclerosis. Clin Immunol 128 (3), 382–391.
Hauser, S.L., Bar-Or, A., Cohen, J.A., Comi, G., Correale, J., Coyle, P.K., Cross, A.H., de Seze, J., Leppert, D., Montalban, X., Selmaj, K., Wiendl, H., Kerloeguen, C., Willi, R., Li, B., Kakarieka, A., Tomic, D., Goodyear, A., Pingili, R., Haring, D.A., Ramanathan, K., Merschhemke, M., Kappos, L., Asclepios, I., Groups, A.I.T., 2020. Ofatumumab versus Teriflunomide in Multiple Sclerosis. N Engl J Med 383 (6), 546–557.
Hauser, S.L., Bar-Or, A., Comi, G., Giovannoni, G., Hartung, H.P., Hemmer, B., Lublin, F., Montalban, X., Rammohan, K.W., Selmaj, K., Traboulsee, A., Wolinsky, J.S., Arnold, D.L., Klingelschmitt, G., Masterman, D., Fontoura, P., Belachew, S., Chin, P., Mairon, N., Garren, H., Kappos, L., Opera, I., Investigators, O.I.C., 2017. Ocrelizumab versus Interferon Beta-1a in Relapsing Multiple Sclerosis. N Engl J Med 376 (3), 221–234.
Hauser, S.L., Waubant, E., Arnold, D.L., Vollmer, T., Antel, J., Fox, R.J., Bar-Or, A., Panzara, M., Sarkar, N., Agarwal, S., Langer-Gould, A., Smith, C.H., Group, H.T., 2008. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. N Engl J Med 358 (7), 676–688.
Hawker, K., O'Connor, P., Freedman, M.S., Calabresi, P.A., Antel, J., Simon, J., Hauser, S., Waubant, E., Vollmer, T., Panitch, H., Zhang, J., Chin, P., Smith, C.H., group, O.t., 2009. Rituximab in patients with primary progressive multiple sclerosis: results of a randomized double-blind placebo-controlled multicenter trial. Ann Neurol 66 (4), 460–471.
Howell, O.W., Reeves, C.A., Nicholas, R., Carassiti, D., Radotra, B., Gentleman, S.M., Serafini, B., Aloisi, F., Roncaroli, F., Magliozzi, R., Reynolds, R., 2011. Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. Brain 134 (Pt 9), 2755–2771.
Ireland, S.J., Blazek, M., Harp, C.T., Greenberg, B., Frohman, E.M., Davis, L.S., Monson, N.L., 2012. Antibody-independent B cell effector functions in relapsing remitting multiple sclerosis: clues to increased inflammatory and reduced regulatory B cell capacity. Autoimmunity 45 (5), 400–414.
Jagessar, S.A., Heijmans, N., Bauer, J., Blezer, E.L., Laman, J.D., Hellings, N., t Hart, B.A., 2012. B-cell depletion abrogates T cell-mediated demyelination in an antibody-nondependent common marmoset experimental autoimmune encephalomyelitis model. J Neuropathol Exp Neurol 71 (8), 716–728.
James, R.E., Schalks, R., Browne, E., Eleftheriadou, I., Munoz, C.P., Mazarakis, N.D., Reynolds, R., 2020. Persistent elevation of intrathecal pro-inflammatory cytokines leads to multiple sclerosis-like cortical demyelination and neurodegeneration. Acta Neuropathol Commun 8 (1), 66.
Jelcic, I., Al Nimer, F., Wang, J., Lentsch, V., Planas, R., Jelcic, I., Madjovski, A., Ruhrmann, S., Faigle, W., Frauenknecht, K., Pinilla, C., Santos, R., Hammer, C., Ortiz, Y., Opitz, L., Gronlund, H., Rogler, G., Boyman, O., Reynolds, R., Lutterotti, A., Khademi, M., Olsson, T., Piehl, F., Sospedra, M., Martin, R., 2018. Memory B Cells Activate Brain-Homing, Autoreactive CD4(+) T Cells in Multiple Sclerosis. Cell 175 (1), 85-100 e123.
Jonkman, L.E., Fleysher, L., Steenwijk, M.D., Koeleman, J.A., de Snoo, T.P., Barkhof, F., Inglese, M., Geurts, J.J., 2015. Ultra-high field MTR and qR2* differentiates subpial cortical lesions from normal-appearing gray matter in multiple sclerosis. Mult Scler.
Kang, M., Choi, J.K., Jittayasothorn, Y., Egwuagu, C.E., 2020. Interleukin 35-Producing Exosomes Suppress Neuroinflammation and Autoimmune Uveitis. Front Immunol 11, 1051.
Kap, Y.S., van Driel, N., Blezer, E., Parren, P.W., Bleeker, W.K., Laman, J.D., Craigen, J. L., t Hart, B.A., 2010. Late B cell depletion with a human anti-human CD20 IgG1kappa monoclonal antibody halts the development of experimental autoimmune encephalomyelitis in marmosets. J Immunol 185 (7), 3990–4003.
Kipp, M., Nyamoya, S., Hochstrasser, T., Amor, S., 2017. Multiple sclerosis animal models: a clinical and histopathological perspective. Brain Pathol 27 (2), 123–137.
Klaver, R., Popescu, V., Voorn, P., Galis-de Graaf, Y., van der Valk, P., de Vries, H.E., Schenk, G.J., Geurts, J.J., 2015. Neuronal and axonal loss in normal-appearing gray matter and subpial lesions in multiple sclerosis. J Neuropathol Exp Neurol 74 (5), 453–458.
Klein, C., Lammens, A., Schafer, W., Georges, G., Schwaiger, M., Mossner, E., Hopfner, K. P., Umana, P., Niederfellner, G., 2013. Epitope interactions of monoclonal antibodies targeting CD20 and their relationship to functional properties. mAbs 5 (1), 22–33.
Komori, M., Blake, A., Greenwood, M., Lin, Y.C., Kosa, P., Ghazali, D., Winokur, P., Natrajan, M., Wuest, S.C., Romm, E., Panackal, A.A., Williamson, P.R., Wu, T., Bielekova, B., 2015. Cerebrospinal fluid markers reveal intrathecal inflammation in progressive multiple sclerosis. Ann Neurol 78 (1), 3–20.
Kramann, N., Neid, K., Menken, L., Schlumbohm, C., Stadelmann, C., Fuchs, E., Bruck, W., Wegner, C., 2015. Increased Meningeal T and Plasma Cell Infiltration is Associated with Early Subpial Cortical Demyelination in Common Marmosets with Experimental Autoimmune Encephalomyelitis. Brain Pathol 25 (3), 276–286.
Krumbholz, M., Derfuss, T., Hohlfeld, R., Meinl, E., 2012. B cells and antibodies in multiple sclerosis pathogenesis and therapy. Nat Rev Neurol 8 (11), 613–623.
Kuerten, S., Angelov, D.N., 2008. Comparing the CNS morphology and immunobiology of different EAE models in C57BL/6 mice - a step towards understanding the complexity of multiple sclerosis. Annals of anatomy = Anatomischer Anzeiger: official organ of the Anatomische Gesellschaft 190 (1), 1–15.
Kuerten, S., Javeri, S., Tary-Lehmann, M., Lehmann, P.V., Angelov, D.N., 2008. Fundamental differences in the dynamics of CNS lesion development and composition in MP4- and MOG peptide 35-55-induced experimental autoimmune encephalomyelitis. Clin Immunol 129 (2), 256–267.
Lampropoulou, V., Hoehlig, K., Roch, T., Neves, P., Calderon Gomez, E., Sweenie, C.H., Hao, Y., Freitas, A.A., Steinhoff, U., Anderton, S.M., Fillatreau, S., 2008. TLR-activated B cells suppress T cell-mediated autoimmunity. J Immunol 180 (7), 4763–4773.
Lassmann, H., 2012. Cortical lesions in multiple sclerosis: inflammation versus neurodegeneration. Brain 135 (Pt 10), 2904–2905.
Lassmann, H., 2013. Pathology and disease mechanisms in different stages of multiple sclerosis. J Neurol Sci 333 (1-2), 1–4.
Lassmann, H., 2019. The changing concepts in the neuropathology of acquired demyelinating central nervous system disorders. Curr Opin Neurol.
Li, R., Patterson, K.R., Bar-Or, A., 2018. Reassessing B cell contributions in multiple sclerosis. Nat Immunol 19 (7), 696–707.
Li, R., Rezk, A., Healy, L.M., Muirhead, G., Prat, A., Gommerman, J.L., Bar-Or, A., Team, M.C.B.c.i.M., 2015a. Cytokine-Defined B Cell Responses as Therapeutic Targets in Multiple Sclerosis. Front Immunol 6, 626.
Li, R., Rezk, A., Miyazaki, Y., Hilgenberg, E., Touil, H., Shen, P., Moore, C.S., Michel, L., Althekair, F., Rajasekharan, S., Gommerman, J.L., Prat, A., Fillatreau, S., Bar-Or, A., Canadian, B.c.i.M.S.T., 2015b. Proinflammatory GM-CSF-producing B cells in multiple sclerosis and B cell depletion therapy. Science translational medicine 7 (310), 310ra166.
Liang, C., Tian, D., Ren, X., Ding, S., Jia, M., Xin, M., Thareja, S., 2018. The development of Bruton's tyrosine kinase (BTK) inhibitors from 2012 to 2017: A mini-review. European journal of medicinal chemistry 151, 315–326.
Lisak, R.P., Benjamins, J.A., Nedelkoska, L., Barger, J.L., Ragheb, S., Fan, B., Ouamara, N., Johnson, T.A., Rajasekharan, S., Bar-Or, A., 2012. Secretory products of multiple sclerosis B cells are cytotoxic to oligodendroglia in vitro. J Neuroimmunol 246 (1-2), 85–95.
Lisak, R.P., Nedelkoska, L., Benjamins, J.A., Schalk, D., Bealmear, B., Touil, H., Li, R., Muirhead, G., Bar-Or, A., 2017. B cells from patients with multiple sclerosis induce cell death via apoptosis in neurons in vitro. J Neuroimmunol 309, 88–99.
Lublin, F.D., Reingold, S.C., Cohen, J.A., Cutter, G.R., Sorensen, P.S., Thompson, A.J., Wolinsky, J.S., Balcer, L.J., Banwell, B., Barkhof, F., Bebo Jr., B., Calabresi, P.A., Clanet, M., Comi, G., Fox, R.J., Freedman, M.S., Goodman, A.D., Inglese, M., Kappos, L., Kieseier, B.C., Lincoln, J.A., Lubetzki, C., Miller, A.E., Montalban, X., O'Connor, P.W., Petkau, J., Pozzilli, C., Rudick, R.A., Sormani, M.P., Stuve, O., Waubant, E., Polman, C.H., 2014. Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology 83 (3), 278–286.
Lucchinetti, C., Bruck, W., Parisi, J., Scheithauer, B., Rodriguez, M., Lassmann, H., 2000. Heterogeneity of multiple sclerosis lesions: implications for the pathogenesis of demyelination. Ann Neurol 47 (6), 707–717.
Lucchinetti, C.F., Popescu, B.F., Bunyan, R.F., Moll, N.M., Roemer, S.F., Lassmann, H., Bruck, W., Parisi, J.E., Scheithauer, B.W., Giannini, C., Weigand, S.D., Mandrekar, J., Ransohoff, R.M., 2011. Inflammatory cortical demyelination in early multiple sclerosis. N Engl J Med 365 (23), 2188–2197.
Magliozzi, R., Columba-Cabezas, S., Serafini, B., Aloisi, F., 2004. Intracerebral expression of CXCL13 and BAFF is accompanied by formation of lymphoid follicle-like structures in the meninges of mice with relapsing experimental autoimmune encephalomyelitis. J Neuroimmunol 148 (1-2), 11–23.
Magliozzi, R., Hametner, S., Facchiano, F., Marastoni, D., Rossi, S., Castellaro, M., Poli, A., Lattanzi, F., Visconti, A., Nicholas, R., Reynolds, R., Monaco, S., Lassmann, H., Calabrese, M., 2019. Iron homeostasis, complement, and coagulation cascade as CSF signature of cortical lesions in early multiple sclerosis. Ann Clin Transl Neurol 6 (11), 2150–2163.
Magliozzi, R., Howell, O., Vora, A., Serafini, B., Nicholas, R., Puopolo, M., Reynolds, R., Aloisi, F., 2007. Meningeal B-cell follicles in secondary progressive multiple sclerosis associate with early onset of disease and severe cortical pathology. Brain 130 (Pt 4), 1089–1104.
Magliozzi, R., Howell, O.W., Nicholas, R., Cruciani, C., Castellaro, M., Romualdi, C., Rossi, S., Pitteri, M., Benedetti, M.D., Gajofatto, A., Pizzini, F.B., Montemezzi, S., Rasia, S., Capra, R., Bertoldo, A., Facchiano, F., Monaco, S., Reynolds, R., Calabrese, M., 2018. Inflammatory intrathecal profiles and cortical damage in multiple sclerosis. Ann Neurol.
Magliozzi, R., Howell, O.W., Reeves, C., Roncaroli, F., Nicholas, R., Serafini, B., Aloisi, F., Reynolds, R., 2010. A Gradient of neuronal loss and meningeal inflammation in multiple sclerosis. Ann Neurol 68 (4), 477–493.
Magliozzi, R., Scalfari, A., Pisani, A.I., Ziccardi, S., Marastoni, D., Pizzini, F.B., Bajrami, A., Tamanti, A., Guandalini, M., Bonomi, S., Rossi, S., Mazziotti, V., Castellaro, M., Montemezzi, S., Rasia, S., Capra, R., Pitteri, M., Romualdi, C.,

Reynolds, R., Calabrese, M., 2020. The CSF Profile Linked to Cortical Damage Predicts Multiple Sclerosis Activity. Ann Neurol.
Magliozzi, R., Serafini, B., Rosicarelli, B., Chiappetta, G., Veroni, C., Reynolds, R., Aloisi, F., 2013. B-cell enrichment and Epstein-Barr virus infection in inflammatory cortical lesions in secondary progressive multiple sclerosis. J Neuropathol Exp Neurol 72 (1), 29–41.
Mainero, C., Louapre, C., Govindarajan, S.T., Gianni, C., Nielsen, A.S., Cohen-Adad, J., Sloane, J., Kinkel, R.P., 2015. A gradient in cortical pathology in multiple sclerosis by in vivo quantitative 7 T imaging. Brain 138 (Pt 4), 932–945.
Makshakov, G., Magonov, E., Totolyan, N., Nazarov, V., Lapin, S., Mazing, A., Verbitskaya, E., Trofimova, T., Krasnov, V., Shumilina, M., Skoromets, A., Evdoshenko, E., 2017. Leptomeningeal Contrast Enhancement Is Associated with Disability Progression and Grey Matter Atrophy in Multiple Sclerosis. Neurol Res Int 2017, 8652463.
Matsushita, T., Yanaba, K., Bouaziz, J.D., Fujimoto, M., Tedder, T.F., 2008. Regulatory B cells inhibit EAE initiation in mice while other B cells promote disease progression. J Clin Invest 118 (10), 3420–3430.
McQualter, J.L., Darwiche, R., Ewing, C., Onuki, M., Kay, T.W., Hamilton, J.A., Reid, H. H., Bernard, C.C., 2001. Granulocyte macrophage colony-stimulating factor: a new putative therapeutic target in multiple sclerosis. J Exp Med 194 (7), 873–882.
Merkler, D., Ernsting, T., Kerschensteiner, M., Bruck, W., Stadelmann, C., 2006. A new focal EAE model of cortical demyelination: multiple sclerosis-like lesions with rapid resolution of inflammation and extensive remyelination. Brain 129 (Pt 8), 1972–1983.
Michel, L., Touil, H., Pikor, N.B., Gommerman, J.L., Prat, A., Bar-Or, A., 2015. B Cells in the Multiple Sclerosis Central Nervous System: Trafficking and Contribution to CNS-Compartmentalized Inflammation. Front Immunol 6, 636.
Miller, D.H., Weinshenker, B.G., Filippi, M., Banwell, B.L., Cohen, J.A., Freedman, M.S., Galetta, S.L., Hutchinson, M., Johnson, R.T., Kappos, L., Kira, J., Lublin, F.D., McFarland, H.F., Montalban, X., Panitch, H., Richert, J.R., Reingold, S.C., Polman, C. H., 2008. Differential diagnosis of suspected multiple sclerosis: a consensus approach. Mult Scler 14 (9), 1157–1174.
Montalban, X., Hauser, S.L., Kappos, L., Arnold, D.L., Bar-Or, A., Comi, G., de Seze, J., Giovannoni, G., Hartung, H.P., Hemmer, B., Lublin, F., Rammohan, K.W., Selmaj, K., Traboulsee, A., Sauter, A., Masterman, D., Fontoura, P., Belachew, S., Garren, H., Mairon, N., Chin, P., Wolinsky, J.S., Investigators, O.C., 2017. Ocrelizumab versus Placebo in Primary Progressive Multiple Sclerosis. N Engl J Med 376 (3), 209–220.
Nelson, F., Datta, S., Garcia, N., Rozario, N.L., Perez, F., Cutter, G., Narayana, P.A., Wolinsky, J.S., 2011. Intracortical lesions by 3T magnetic resonance imaging and correlation with cognitive impairment in multiple sclerosis. Mult Scler 17 (9), 1122–1129.
Nelson, F., Poonawalla, A.H., Hou, P., Huang, F., Wolinsky, J.S., Narayana, P.A., 2007. Improved identification of intracortical lesions in multiple sclerosis with phase-sensitive inversion recovery in combination with fast double inversion recovery MR imaging. AJNR Am J Neuroradiol 28 (9), 1645–1649.
Oliver, A.R., Lyon, G.M., Ruddle, N.H., 2003. Rat and human myelin oligodendrocyte glycoproteins induce experimental autoimmune encephalomyelitis by different mechanisms in C57BL/6 mice. J Immunol 171 (1), 462–468.
Pachner, A.R., Li, L., Lagunoff, D., 2011. Plasma cells in the central nervous system in the Theiler's virus model of multiple sclerosis. J Neuroimmunol 232 (1-2), 35–40.
Peferoen, L.A., Lamers, F., Lodder, L.N., Gerritsen, W.H., Huitinga, I., Melief, J., Giovannoni, G., Meier, U., Hintzen, R.Q., Verjans, G.M., van Nierop, G.P., Vos, W., Peferoen-Baert, R.M., Middeldorp, J.M., van der Valk, P., Amor, S., 2010. Epstein Barr virus is not a characteristic feature in the central nervous system in established multiple sclerosis. Brain 133 (Pt 5), e137.
Pol, S., Liang, S., Schweser, F., Dhanraj, R., Schubart, A., Preda, M., Sveinsson, M., Ramasamy, D.P., Dwyer, M.G., Weckbecker, G., Zivadinov, R., 2020. Subcutaneous anti-CD20 antibody treatment delays gray matter atrophy in human myelin oligodendrocyte glycoprotein-induced EAE mice. Exp Neurol 335, 113488.
Pol, S., Schweser, F., Bertolino, N., Preda, M., Sveinsson, M., Sudyn, M., Babek, N., Zivadinov, R., 2019. Characterization of leptomeningeal inflammation in rodent experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis. Exp Neurol 314, 82–90.
Pomeroy, I.M., Matthews, P.M., Frank, J.A., Jordan, E.K., Esiri, M.M., 2005. Demyelinated neocortical lesions in marmoset autoimmune encephalomyelitis mimic those in multiple sclerosis. Brain 128 (Pt 11), 2713–2721.
Prineas, J.W., Graham, J.S., 1981. Multiple sclerosis: capping of surface immunoglobulin G on macrophages engaged in myelin breakdown. Ann Neurol 10 (2), 149–158.
Rahmanzadeh, R., Weber, M.S., Bruck, W., Navardi, S., Sahraian, M.A., 2018. B cells in multiple sclerosis therapy-A comprehensive review. Acta Neurol Scand 137 (6), 544–556.
Ransohoff, R.M., 2009. Immunology: In the beginning. Nature 462 (7269), 41–42.
Reboldi, A., Coisne, C., Baumjohann, D., Benvenuto, F., Bottinelli, D., Lira, S., Uccelli, A., Lanzavecchia, A., Engelhardt, B., Sallusto, F., 2009. C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. Nat Immunol 10 (5), 514–523.
Robinson, A.P., Harp, C.T., Noronha, A., Miller, S.D., 2014. The experimental autoimmune encephalomyelitis (EAE) model of MS: utility for understanding disease pathophysiology and treatment. Handb Clin Neurol 122, 173–189.
Roosendaal, S.D., Moraal, B., Pouwels, P.J., Vrenken, H., Castelijns, J.A., Barkhof, F., Geurts, J.J., 2009. Accumulation of cortical lesions in MS: relation with cognitive impairment. Mult Scler 15 (6), 708–714.
Rosser, E.C., Oleinika, K., Tonon, S., Doyle, R., Bosma, A., Carter, N.A., Harris, K.A., Jones, S.A., Klein, N., Mauri, C., 2014. Regulatory B cells are induced by gut microbiota-driven interleukin-1beta and interleukin-6 production. Nat Med 20 (11), 1334–1339.
Russi, A.E., Brown, M.A., 2015. The meninges: new therapeutic targets for multiple sclerosis. Translational research: the journal of laboratory and clinical medicine 165 (2), 255–269.
Sargsyan, S.A., Shearer, A.J., Ritchie, A.M., Burgoon, M.P., Anderson, S., Hemmer, B., Stadelmann, C., Gattenlohner, S., Owens, G.P., Gilden, D., Bennett, J.L., 2010. Absence of Epstein-Barr virus in the brain and CSF of patients with multiple sclerosis. Neurology 74 (14), 1127–1135.
Sawas, A., Farber, C.M., Schreeder, M.T., Khalil, M.Y., Mahadevan, D., Deng, C., Amengual, J.E., Nikolinakos, P.G., Kolesar, J.M., Kuhn, J.G., Sportelli, P., Miskin, H. P., O'Connor, O.A., 2017. A phase 1/2 trial of ublituximab, a novel anti-CD20 monoclonal antibody, in patients with B-cell non-Hodgkin lymphoma or chronic lymphocytic leukaemia previously exposed to rituximab. British journal of haematology 177 (2), 243–253.
Sellebjerg, F., Blinkenberg, M., Sorensen, P.S., 2020. Anti-CD20 Monoclonal Antibodies for Relapsing and Progressive Multiple Sclerosis. CNS Drugs 34 (3), 269–280.
Serafini, B., Rosicarelli, B., Franciotta, D., Magliozzi, R., Reynolds, R., Cinque, P., Andreoni, L., Trivedi, P., Salvetti, M., Faggioni, A., Aloisi, F., 2007. Dysregulated Epstein-Barr virus infection in the multiple sclerosis brain. J Exp Med 204 (12), 2899–2912.
Serafini, B., Rosicarelli, B., Magliozzi, R., Stigliano, E., Aloisi, F., 2004. Detection of ectopic B-cell follicles with germinal centers in the meninges of patients with secondary progressive multiple sclerosis. Brain Pathol 14 (2), 164–174.
Serafini, B., Severa, M., Columba-Cabezas, S., Rosicarelli, B., Veroni, C., Chiappetta, G., Magliozzi, R., Reynolds, R., Coccia, E.M., Aloisi, F., 2010. 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 (7), 677–693.
Sethi, V., Yousry, T.A., Muhlert, N., Ron, M., Golay, X., Wheeler-Kingshott, C., Miller, D. H., Chard, D.T., 2012. Improved detection of cortical MS lesions with phase-sensitive inversion recovery MRI. J Neurol Neurosurg Psychiatry 83 (9), 877–882.
Silva, B.A., Ferrari, C.C., 2018. Cortical and meningeal pathology in progressive multiple sclerosis: a new therapeutic target? Reviews in the neurosciences.
Silva, B.A., Leal, M.C., Farias, M.I., Avalos, J.C., Besada, C.H., Pitossi, F.J., Ferrari, C.C., 2018. A new focal model resembling features of cortical pathology of the progressive forms of multiple sclerosis: Influence of innate immunity. Brain Behav Immun.
Stein, J., Xu, Q., Jackson, K.C., Romm, E., Wuest, S.C., Kosa, P., Wu, T., Bielekova, B., 2018. Intrathecal B Cells in MS Have Significantly Greater Lymphangiogenic Potential Compared to B Cells Derived From Non-MS Subjects. Frontiers in neurology 9, 554.
t Hart, B.A., Dunham, J., Faber, B.W., Laman, J.D., van Horssen, J., Bauer, J., Kap, Y.S., 2017. A B Cell-Driven Autoimmune Pathway Leading to Pathological Hallmarks of Progressive Multiple Sclerosis in the Marmoset Experimental Autoimmune Encephalomyelitis Model. Front Immunol 8, 804.
Thi Cuc, B., Pohar, J., Fillatreau, S., 2019. Understanding regulatory B cells in autoimmune diseases: the case of multiple sclerosis. Current opinion in immunology 61, 26–32.
Torkildsen, O., Stansberg, C., Angelskar, S.M., Kooi, E.J., Geurts, J.J., van der Valk, P., Myhr, K.M., Steen, V.M., Bo, L., 2010. Upregulation of immunoglobulin-related genes in cortical sections from multiple sclerosis patients. Brain Pathol 20 (4), 720–729.
van Nierop, G.P., van Luijn, M.M., Michels, S.S., Melief, M.J., Janssen, M., Langerak, A. W., Ouwendijk, W.J.D., Hintzen, R.Q., Verjans, G., 2017. Phenotypic and functional characterization of T cells in white matter lesions of multiple sclerosis patients. Acta Neuropathol 134 (3), 383–401.
Vidaurre, O.G., Haines, J.D., Katz Sand, I., Adula, K.P., Huynh, J.L., McGraw, C.A., Zhang, F., Varghese, M., Sotirchos, E., Bhargava, P., Bandaru, V.V., Pasinetti, G., Zhang, W., Inglese, M., Calabresi, P.A., Wu, G., Miller, A.E., Haughey, N.J., Lublin, F. D., Casaccia, P., 2014. Cerebrospinal fluid ceramides from patients with multiple sclerosis impair neuronal bioenergetics. Brain 137 (Pt 8), 2271–2286.
Villar, L.M., Casanova, B., Ouamara, N., Comabella, M., Jalili, F., Leppert, D., de Andres, C., Izquierdo, G., Arroyo, R., Avsar, T., Lapin, S.V., Johnson, T., Montalban, X., Fernandez, O., Alvarez-Lafuente, R., Masterman, D., Garcia-Sanchez, M.I., Coret, F., Siva, A., Evdoshenko, E., Alvarez-Cermeno, J.C., Bar-Or, A., 2014. Immunoglobulin M oligoclonal bands: biomarker of targetable inflammation in primary progressive multiple sclerosis. Ann Neurol 76 (2), 231–240.
Villar, L.M., Sadaba, M.C., Roldan, E., Masjuan, J., Gonzalez-Porque, P., Villarrubia, N., Espino, M., Garcia-Trujillo, J.A., Bootello, A., Alvarez-Cermeno, J.C., 2005. Intrathecal synthesis of oligoclonal IgM against myelin lipids predicts an aggressive disease course in MS. J Clin Invest 115 (1), 187–194.
von Essen, M.R., Ammitzboll, C., Hansen, R.H., Petersen, E.R.S., McWilliam, O., Marquart, H.V., Damm, P., Sellebjerg, F., 2019. Proinflammatory CD20+ T cells in the pathogenesis of multiple sclerosis. Brain 142 (1), 120–132.
Wang, R.X., Yu, C.R., Dambuza, I.M., Mahdi, R.M., Dolinska, M.B., Sergeev, Y.V., Wingfield, P.T., Kim, S.H., Egwuagu, C.E., 2014. Interleukin-35 induces regulatory B cells that suppress autoimmune disease. Nat Med 20 (6), 633–641.
Weber, M.S., Prod'homme, T., Patarroyo, J.C., Molnarfi, N., Karnezis, T., Lehmann-Horn, K., Danilenko, D.M., Eastham-Anderson, J., Slavin, A.J., Linington, C., Bernard, C.C., Martin, F., Zamvil, S.S., 2010. B-cell activation influences T-cell polarization and outcome of anti-CD20 B-cell depletion in central nervous system autoimmunity. Ann Neurol 68 (3), 369–383.
Willis, S.N., Stadelmann, C., Rodig, S.J., Caron, T., Gattenloehner, S., Mallozzi, S.S., Roughan, J.E., Almendinger, S.E., Blewett, M.M., Bruck, W., Hafler, D.A.,

O'Connor, K.C., 2009. Epstein-Barr virus infection is not a characteristic feature of multiple sclerosis brain. Brain 132 (Pt 12), 3318–3328.
Wolburg, H., Paulus, W., 2010. Choroid plexus: biology and pathology. Acta Neuropathol 119 (1), 75–88.
Zivadinov, R., Ramasamy, D.P., Vaneckova, M., Gandhi, S., Chandra, A., Hagemeier, J., Bergsland, N., Polak, P., Benedict, R.H., Hojnacki, D., Weinstock-Guttman, B., 2017. Leptomeningeal contrast enhancement is associated with progression of cortical atrophy in MS: A retrospective, pilot, observational longitudinal study. Mult Scler 23 (10), 1336–1345.