B cells contribute to MS pathogenesis through antibody-dependent and antibody-independent mechanisms

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Abstract: For many years, central dogma defined multiple sclerosis (MS) as a T cell-driven autoimmune disorder; however, over the past decade there has been a burgeoning recognition that B cells contribute to the pathogenesis of certain MS disease subtypes. B cells may contribute to MS pathogenesis through production of autoantibodies (or antibodies directed at foreign bodies, which unfortunately cross-react with self-antigens), through promotion of T cell activation via antigen presentation, or through production of cytokines. This review highlights evidence for antibody-dependent and antibody-independent B cell involvement in MS pathogenesis.

Keywords: autoantibodies, antibody targets, clinically isolated MS, primary progressive MS, secondary progressive MS, relapsing and remitting MS, T cells, T regulatory cells

Introduction

Multiple sclerosis (MS) is a common and progressive neurological disease that affects over 1 million people worldwide, with the Canadian Prairies showing among the highest incidence rates in the world.1 This demyelinating autoimmune disease usually presents in the prime of life and is associated with marked physical and cognitive disabilities and a shortened life span.2 Classically described as a neuroinflammatory autoimmune disease that targets the myelin in the brain and spinal cord, this complicated disease has an unknown etiology and no known cure. It presents with varying symptoms such as muscle fatigue, paralysis, loss of sensation/numbness, and pain, as well as emotional impairments such as depression and other mood disorders. The disease has diverse phenotypes.3 The majority of MS patients initially present with subacute attacks, with symptoms and signs referable to the central nervous system (CNS) – defined as a clinically isolated syndrome (CIS).4 When the attack is followed by a complete or partial remission which is then followed by another attack(s), often focused in a different location in the CNS and possibly of higher intensity, the disease course is defined as relapsing and remitting MS (RRMS).4 Patients who present with a gradually progressive course without a well-defined initial attack are presenting with primary progressive MS (PPMS).4 Secondary progressive MS (SPMS) is characterized by CIS or RRMS followed by progressive clinical worsening over time, generally 3 years or more after the onset of disease.4

The pathology of MS includes penetration of leukocytes across the blood–brain barrier (BBB), intrathecal production of antibodies, and neuroinflammation, which leads to demyelination and astrocytic and/or neuronal/axonal injury.2,5 In a recent study, Lucchinetti et al used immunohistochemistry to characterize demyelinating activity,
inflammatory infiltrates, and the presence of meningeal inflammation in cortical lesions from a cohort of patients with early-stage MS. They observed that cortical demyelination was common in the early stages of MS, that the majority of cortical lesions studied were positive for CD3+ T cells, and that a subset were positive for CD20+ B cells. Further, there was a strong topographic association between cortical demyelination and meningeal inflammation suggesting a direct relationship between inflammation and demyelination. The authors speculate that the reason why inflammatory cortical demyelination is not typically observed in chronic, progressive MS may relate to efficient clearance of cortical inflammation over time and thus does not preclude the possibility that inflammation may contribute to demyelination at its onset. Recent work highlighting how B cells contribute to inflammation and pathogenesis of certain MS disease subtypes are explored in this review.

**Evidence that intrathecal B cells contribute to MS pathogenesis**

In the majority of MS patients, B cell numbers are elevated in the CNS. In an extensive histopathological study on actively demyelinating lesions obtained from MS patient biopsies and autopsies, four distinct lesion patterns were observed. Pattern II lesions, but not lesions following pattern I, II, or IV, were positive for B cells and they had prominent antibody deposition and complement components at sites of active myelin destruction. In other studies, immunohistochemical analysis of brain and spinal cord sections revealed lymphoid follicle-like structures containing T cells, B cells, and plasma cells in the cerebral meninges in patients with SPMS, but not in patients with RRMS or PPMS. These results suggest de novo formation and maintenance of ectopic lymphoid structures that contribute to increased B cell production in patients with active SPMS. Meningeal B cell follicles were found in close proximity to large subpial gray matter lesions and diffuse meningeal inflammation, which suggests that the lymphoid-like follicles or products produced by them negatively impacted the integrity of the cortical structures and contributed to gray matter cortical demyelination. In a recent study, Lee-Chang et al determined that patients with CIS and RRMS had reduced transitional B cell numbers in the peripheral blood compared to control patients, but of the transitional B cells present, these cells had upregulated surface expression of integrins (α4 and β1) likely assist these cells to cross the blood–CSF barrier. Overall, these studies suggest that MS patients have increased intrathecal B cells which may contribute to MS pathogenesis through antibody-dependent or antibody-independent mechanisms.

**Antigen-independent mechanisms through which B cells may contribute to MS pathogenesis**

Treatment with rituximab (anti-CD20 antibody, Rituxan®) – a humanized mouse anti-CD20 antibody which depletes CD20+ cells (ie, pre-B cells, immature B cells, mature B cells, and memory B cells, but not stem cells or plasmablasts) – has made it possible to discern whether B cells themselves or their antibody products contribute to MS pathogenesis. In various studies, RRMS patients receiving rituximab showed substantially reduced B cell numbers in their CSF and serum, reduced levels of emerging inflammatory brain lesions, and reduced frequency of clinical attack despite evidence that antibody levels in the CSF were not immediately decreased, suggesting that B cells contribute to pathology via an antibody-independent mechanism. Further, a large scale clinical trial wherein patients diagnosed with RRMS were treated with ocrelizumab (a fully humanized anti-CD20 monoclonal antibody with decreased antibody-dependent, cell-mediated cytotoxic effects compared to rituximab) showed that these patients had reduced numbers of yearly relapses, decreased neuroinflammation, and decreased peripheral B cell levels compared with placebo control patients. Therefore, at least a subset of MS patients treated with anti-CD20 therapy showed improvement of disease, suggesting that B cells can promote pathology through antibody-independent mechanisms.

Beyond their role as producers of antibodies, B cells contribute to the induction, maintenance, and reactivation of CD4+ T cells, they act as antigen-presenting cells, they are required for maintenance and reactivation of memory cells, and they modulate T regulatory (Tregs) cell function. Recently, it was reported that the B cell CXC chemokine ligand 13 was elevated in serum in RRMS patients with active MS. In data obtained from clinical trials, the majority of RRMS patients treated with rituximab responded with a proportional decrease in expression of CXC chemokine ligand 13 in the CSF, decreased B cells in CSF and periphery, and reduced T cells in the CSF. When B cell effector cytokine responses were compared between MS patients and matched controls, activated B cells derived from MS patients exhibited decreased production of the downregulatory cytokine interleukin-10, a cytokine largely...
produced by naive B cells. Bar-Or et al showed that activated B cells derived from MS patients exhibited increased expression of the proinflammatory cytokines lymphotoxin T and tumor necrosis factor α, two cytokines largely produced by memory B cells. When peripheral blood mononuclear cells were subjected to ex vivo B cell depletion, there was reduced T cell proliferation from the cells obtained from MS patients compared with those from healthy controls. The authors proposed that the abnormal expression of B cell-derived cytokines mediate “bystander activation” of proinflammatory T cells which may precipitate new relapsing MS disease activity. If true, these findings offer a potential alternative to antibody-dependent mechanism through which rituximab improves MS symptoms in a subset of patients.

T<sub>reg</sub> cells are negative regulators of immune responses to self- and foreign antigens and they play a critical role in maintaining immune tolerance by suppressing pathologic immune responses. Compared to MS patients with SPMS or healthy controls, patients with RRMS have reduced numbers of T<sub>reg</sub> cells in their peripheral blood but increased numbers of T<sub>reg</sub> cells in the CSF, possibly in an attempt to downregulate local inflammation in the CNS. B cells have also been shown to influence T<sub>reg</sub> cell development, proliferation, and survival in culture. Thus, B cells may promote effector T cell activation while paradoxically they may dampen the adaptive immune response through induction of T<sub>reg</sub> cells. It may be, therefore, that B cells contribute to MS pathogenesis by inappropriately upregulating effector T cells or inappropriately decreasing T<sub>reg</sub> cells, required to maintain immune tolerance.

**Evidence that antibodies contribute to MS pathogenesis**

Immune components and soluble proteins such as serum antibodies pass through the BBB very poorly, if at all. However, sera from MS patients in exacerbation were shown to have significantly reduced expression of the proteins occludin and vascular endothelial-cadherin compared to MS patients not in exacerbation or compared to normal controls. These proteins are major components of the tight-junctions which help create the BBB and their decreased expression in MS patients may result in a more permeable BBB. An in vitro model of BBB serum from SPMS patients showed decreased transendothelial electrical resistance suggesting that serum from SPMS patients affects the permeability of this BBB model. With increased permeability, the brain may be exposed to a multitude of lymphocytes, blood proteins, and antibodies from which they are usually isolated. Because the BBB may be transiently semipermeable in at least some MS clinical disease subtypes, it is conceivable that circulating antibodies may enter the CNS and, if they share affinity for antigens found in the brain, contribute to pathology. Indeed, serum levels in a patient with RRMS showed higher serum myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP) antibodies in times of relapse relative to times of remission further indicating that the BBB in patients with MS may be transiently semipermeable.

The majority of patients with MS present with elevated intrathecal antibody titers. When CSF obtained from patients with MS has been subjected to isoelectric focusing, a technique used to separate proteins by their electrical charge, a pattern of oligoclonal bands becomes evident. Because they have limited heterogeneity, intrathecal B cells undergoing clonal expansion and somatic hypermutation of the expressed antibody gene rearrangement are visualized as oligoclonal bands. In contrast, serum-derived antibodies are produced by a myriad of heterogeneous B cells and thus show a pattern of polyclonal banding upon isoelectric focusing. The majority of oligoclonal bands are complement-activating immunoglobulin (Ig) G1 isotype. Histopathology performed on pattern II demyelinating lesions obtained from MS patient biopsies and autopsies showed prominent antibody deposition and complement components at sites of active myelin destruction. Other studies showed that patients with pattern II histopathologic lesions responded well to plasma exchange. Through magnetic resonance imaging and examination of CSF from patients in the early phases of MS, it was determined that an association between intrathecal antibody synthesis and cortical lesions was highly predictive of an earlier CIS conversion to MS and of higher disease activity. Further, in contrast with patients diagnosed with RRMS or PPMS, patients diagnosed with SPMS who responded positively to treatment with rituximab showed a decline in intrathecal antibody production as well as decreased B cell numbers.

There is precedence that autoantibodies contribute to neurological pathology and disease. Although recently defined as pathologically distinct from MS, many clinicians still consider neuromyelitis optica (NMO; optic-spinal MS) as a part of the MS disease spectrum. NMO-associated IgG antibodies are present in the serum of 70% of patients with NMO. Patients with NMO respond positively to plasma exchange, which suggests that autoantibodies contribute to the pathogenesis of this autoimmune disease. Through a series of elegant experiments, researchers at the Mayo...
Clinic showed that NMO-associated antibodies precipitated Aquaporin 4 from astrocyte cell membranes and definitively established that Aquaporin 4 is the target. Hence, because a pathogenic autoantibody contributes to the neuroinflammatory disorder NMO, it is reasonable to speculate that distinct pathogenic autoantibodies may contribute to other neuroinflammatory disorders such as MS.

Identification antibodies targets which contribute to MS pathogenesis

Foreign antigens

Previous infection with Epstein-Barr virus (EBV) – a virus with lifelong persistence in the host’s B cells – is an established MS risk factor. Whether antibodies against viral proteins contribute to MS pathogenesis by binding to the viral antigen or by binding self-antigens – which share significant morphology to the viral antigen – is currently under investigation. Jaquieri et al assessed EBV-specific humoral and cellular immune responses in the CSF of patients with early MS compared to persons with other inflammatory neurological diseases, noninflammatory neurological diseases, or neurotropic herpesvirus cytomegalovirus (used as a control). They observed enriched intrathecal CD8+ cytotoxic T cells and increased antibody indexes for viral capsid antigen and EBV nuclear antigen 1 (EBNA-1), but not cytomegalovirus antibody indexes, in early MS as compared with other inflammatory neurological diseases and noninflammatory neurological diseases patients. Further, in a survey of 100 subjects with CIS, RRMS, or PPMS over a 5-year period, all of whom had serologic evidence of previous EBV infection, patients with RRMS had significantly higher anti-EBNA-1 titers (a marker of the latent phase of the virus) and gadolinium-enhanced lesions on magnetic resonance images compared with patients with PPMS or CIS. In contrast, Jafari et al – who evaluated anti-EBV antibody response in serum and CSF from a large cohort of patients – determined that there was no evidence for elevated intrathecal anti-EBNA-1 IgG synthesis in MS patients relative to control patients when total IgG content of paired serum and CSF samples were normalized. Further, it was determined that although MS risk tended to be higher in individuals with high titers of neutralizing antibodies against EBV compared to those with low titers, this association was attenuated after adjustment for anti-EBNA-1 IgG antibody titres. Therefore, although there appears to be a strong association between prior EBV exposure and risk of MS, whether antibodies against viral proteins contribute to MS pathogenesis has not yet been definitively established.

Self-antigens

It has proven challenging to definitively identify the antibody targets to which pathogenic autoantibodies bind. While there is evidence that autoantibodies derived from MS patients bind lipids, carbohydrates, and DNA, the vast majority of research has focused on investigating proteins which comprise the myelin sheath such as MBP, MOG, and proteolipid protein as autoantibody targets. Elevated antibody titers against MBP and/or MOG have been reported in serum and CSF derived from MS patients and serum antibodies to MBP and MOG were observed in subgroups of patients with MS, which suggests that autoantibodies specific for myelin-derived proteins may contribute to MS pathogenesis. However, myelin-specific antibodies can also be detected in healthy controls suggesting that these targets are not definitively predictive of disease. Further, although recombinant monoclonal antibodies generated from B cells obtained from CSF from MS patients showed reactivity to sites of degrading myelin and axons, specific reactivity to MOG, MBP, or proteolipid protein could not be confirmed. Thus, pathogenic antibodies which specifically contribute to MS disease remain elusive, and it may be beneficial to expand autoantibody screening beyond myelin-based proteins. CSF and sera from control and MS patients have been screened for autoantibodies using several approaches including phage display libraries, which are constructed using short peptides to mimic epitopes, a human brain complementary DNA expression library, human antigen microarrays, and a cell-based proteomic approach. Such techniques use short, linear amino acid segments to represent antibody binding sites, but these artificial targets fail to identify autoantibodies whose epitopes are comprised of nonadjacent amino acids brought into close proximity through conformational folding of the antigen. Alternatively, they use recombinant antigens which lack posttranslational modification which may be critical for antibody–antigen binding. Studies focused on identifying pathogenic autoantibody targets, which take into account epitopes comprised of nonadjacent amino acids and/or posttranslational modifications, are needed to identify MS biomarkers and therapeutic approaches to prevent or combat MS.

Conclusion

Although the vast majority of MS patients have elevated intrathecal antibody levels, identification of the definitive
antibody targets has remained elusive. Beyond their role in antibody production, intrathecal B cells may contribute to activation/reactivation of effector T cells and the modulation of T<sub>reg</sub> cells, which may contribute to MS pathogenesis. Targeted depletion of pathogenic intrathecal plasma cells/B cells which both eliminate pathogenic antibody production and thwart inappropriate T cell responsiveness may serve as an effective preventative or treatment method in patients with MS.

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