B lymphocytes in neuromyelitis optica

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ABSTRACT
Neuromyelitis optica (NMO) is an inflammatory autoimmune disorder of the CNS that predominantly affects the spinal cord and optic nerves. A majority (approximately 75%) of patients with NMO are seropositive for autoantibodies against the astrocyte water channel aquaporin-4 (AQP4). These autoantibodies are predominantly IgG1, and considerable evidence supports their pathogenicity, presumably by binding to AQP4 on CNS astrocytes, resulting in astrocyte injury and inflammation. Convergent clinical and laboratory-based investigations have indicated that B cells play a fundamental role in NMO immunopathology. Multiple mechanisms have been hypothesized: AQP4 autoantibody production, enhanced proinflammatory B cell and plasmablast activity, aberrant B cell tolerance checkpoints, diminished B cell regulatory function, and loss of B cell anergy. Accordingly, many current off-label therapies for NMO deplete B cells or modulate their activity. Understanding the role and mechanisms whereby B cells contribute to initiation, maintenance, and propagation of disease activity is important to advancing our understanding of NMO pathogenesis and developing effective disease-specific therapies.

Neuromyelitis optica (NMO) is a rare demyelinating disorder of the CNS that is diagnosed by a combination of clinical, imaging, and laboratory criteria. The most common manifestations are recurrent optic neuritis and transverse myelitis; however, a broader range of cerebral, diencephalic, and brainstem syndromes are now recognized. Clinical and laboratory-based studies support a prominent role for B cells in disease pathogenesis. Autoantibodies against the aquaporin-4 (AQP4) water channel (AQP4-IgG) are detected in approximately 75% of affected individuals (reviewed in reference 3), and additional neural and non-neural autoantibodies are frequently observed in both seropositive (AQP4-IgG+) and seronegative (AQP4-IgG−) individuals. Both in vivo and in vitro, AQP4-IgG has been shown to reproduce cardinal features of disease pathology, supporting a direct role of this autoantibody in producing CNS injury. Plasmablasts are increased in the peripheral blood (PB) of patients with NMO, and levels of interleukin (IL)-6, a cytokine that supports plasma cell differentiation and survival, are elevated.

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in serum and CSF of both AQP4-IgG+ and AQP4-IgG− patients. In addition, IL-13 and IL-5 also appear to be upregulated in NMO as compared with multiple sclerosis (MS). Together, these observations are consistent with a proinflammatory humoral response in NMO. Moreover, current empiric treatment regimens that reduce the frequency of disease relapses directly deplete B cells (rituximab) or have relatively selective effects on lymphocytes (azathioprine, mycophenolate mofetil, and mitoxantrone). In patients with NMO, disease activity can be reduced without significant reduction in AQP4-IgG titers, suggesting that additional mechanisms, besides those associated with AQP4-IgG, may promote disease activity. In this review, we examine potential mechanisms whereby B cell dysfunction may contribute to NMO pathophysiology: increased proinflammatory B cell activity, diminished B regulatory control, plasmablast expansion and autoantibody production, loss of B cell anergy, and abnormal B cell tolerance. Although many of these mechanisms have yet to be directly implicated in NMO pathology, a critical assessment of each potential mechanism will help inform definitive investigations. Also, while it is understood that many of these mechanisms likely involve complex interactions with other components of the adaptive immune response, the focus of this review on B cells precludes detailed discussion of each of these contributions.

**B CELLS, PLASMA CELLS, PLASMABLASTS, AND ANTIBODIES**

B cells can perform a wide array of normal functions that, when dysregulated, may affect NMO disease activity: antigen presentation, proinflammatory and anti-inflammatory cytokine production, and immunoglobulin production. While the role of B cells in autoimmune disorders may change during different phases of the disease, the apparent ability of B cell depletion to limit new NMO disease activity implies an overall proinflammatory role for B cells in NMO, possibly due to altered numbers or abnormal activity of proinflammatory or regulatory B cell subsets (table 1). Potential mechanisms include expansion of AQP4-specific plasmablast clones, failure to eliminate autoreactive B cell subsets, insufficient antigen-specific regulatory B cells, and/or the loss of anergic maintenance (figure 1).

Plasma cells in bone marrow (BM) and mucosa-associated lymphoid tissue (MALT) are responsible for the IgG and IgA antibodies that provide long-term humoral immunity. Circulating AQP4-IgG, produced by BM and/or MALT plasma cells, is presumed to initiate CNS injury after gaining access to the CNS compartment through the blood-brain barrier (BBB).

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### Table 1  Circulating human B cell populations of potential relevance in NMO

<table>
<thead>
<tr>
<th>Classification</th>
<th>Cell surface markers</th>
<th>Questions</th>
<th>References</th>
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<tbody>
<tr>
<td>Naive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Newly emigrant</td>
<td>CD19·CD27·CD10·IgM+</td>
<td>BM source of autoreactive B cells?</td>
<td>e-1, e-2</td>
</tr>
<tr>
<td>Mature</td>
<td>CD19·CD27·CD10·IgM+</td>
<td>PB source of autoreactive B cells?</td>
<td>e-1, e-2</td>
</tr>
<tr>
<td>Mature anergic</td>
<td>CD19·IgD·CD21·CD27·IgM−</td>
<td>Pool of silenced autoreactive B cells?</td>
<td>e-3</td>
</tr>
<tr>
<td>Transitional</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>CD19·CD24hi·CD38hi·IgMhi·IgDlo·CD27·</td>
<td>CD27· B10 regulatory population?</td>
<td>e-4</td>
</tr>
<tr>
<td>T2</td>
<td>CD19·CD24hi·CD38hi·IgMhi·IgDlo·CD27·</td>
<td>CD27· B10 regulatory population?</td>
<td>e-4</td>
</tr>
<tr>
<td>T3</td>
<td>CD19·CD24hi·CD38hi·IgMhi·IgDlo·CD27·</td>
<td>Anergic subset?</td>
<td>e-5</td>
</tr>
<tr>
<td>Memory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unswitched</td>
<td>CD19·CD27·IgD+·IgM+</td>
<td>IgD+ autoreactive B cells? Source of CD24+ regulatory B10 subset?</td>
<td>e-6, e-7</td>
</tr>
<tr>
<td>Switched</td>
<td>CD19·CD27·IgD−·IgM−</td>
<td>Source of APCs or proinflammatory B cells?</td>
<td>e-8, e-9</td>
</tr>
<tr>
<td>Double-negative</td>
<td>CD19·CD27·IgD−·IgM+</td>
<td>Source of APCs or proinflammatory B cells?</td>
<td>e-10</td>
</tr>
<tr>
<td>Plasmablasts</td>
<td>CD19·CD20·CD38hi·IgMhi·IgDhi·CD27·</td>
<td>Production of immunoglobulin? Induction of regulatory cells? Induction of proinflammatory B cells?</td>
<td>e-11, e-12</td>
</tr>
</tbody>
</table>

Abbreviations: APC – antigen-presenting cell; BM – bone marrow; NMO – neuromyelitis optica; PB – peripheral blood.

This list represents widely accepted minimal marker combinations but is not meant to be exhaustive. References e-1–e-12 are available at Neurology.org/nn.
B cells may play proinflammatory and anti-inflammatory roles in neuromyelitis optica pathogenesis through various mechanisms. Autoreactive B cells may be generated by defective central tolerance (CT; primary checkpoint in bone marrow) or peripheral tolerance (PT; secondary checkpoint in secondary lymphoid tissue). Stimulated B cells leaving germinal centers may differentiate into memory B cells or antibody-producing plasmablasts and plasma cells. In addition to the production of aquaporin-4 (AQP4)-IgG in the bone marrow and CNS, plasma cells and plasmablasts may have additional proinflammatory and anti-inflammatory functions. Plasmablasts may secrete factors such as interleukin (IL)-17, tumor necrosis factor α (TNF-α)/nitrous oxide (NO), and granulocyte-macrophage colony-stimulating factor (GM-CSF), facilitating neutrophil and macrophage CNS infiltration and heightening proinflammatory immune cell activity through modulation of gut microbiota. Alternatively, anti-inflammatory plasma cells (Pregs) may suppress disease activity in part through the production of IL-10 or IL-35. Memory B cells may further promote disease activity by antigen (Ag) presentation, secretion of the proinflammatory cytokines lymphotoxin (LT) and TNF-α, or facilitation of Th17 differentiation (IL-6 production). IL-10-producing B regulatory cells may limit the immune response through antigen-specific or bystander suppression of proinflammatory T cell function. Circulating AQP4-specific anergic B cells may provide a pool of autoreactive disease-relevant B cells that contribute to disease activity. The pool of anergic B cells may be enhanced by deficient B cell tolerance; release of anergic B cells may be enhanced by antigen-complement adducts or decreased levels of IL-6. The location of germinal centers producing AQP4-reactive memory cells and plasmablasts remains unknown (asterisk). Blue arrows: developmental pathways; dashed green arrows and boxes: stimulatory cytokines; dashed red arrows and boxes: inhibitory cytokines. APRIL – a proliferation-inducing ligand; BAFF – B cell-activating factor.

produced AQP4-IgG to the pool of pathogenic CNS antibody remains less clear. Plasmablasts are elevated in the CSF of patients with NMO during relapse, and limited NMO cases have shown AQP4-IgG restricted to the CSF. In contrast, measures of intrathecal IgG synthesis, such as CSF-restricted oligoclonal IgG bands (OCBs), elevated CSF/serum IgG quotient (QlG), or positive AQP4 antibody index (AiAQP4), are observed in only 16.4%, 8%, and 4.3% of patients with NMO, respectively. These discrepancies may arise in part from technical differences; clonally expanded AQP4-specific CSF plasmablasts are detected using flow cytometry and single-cell PCR, whereas QlG, AiAQP4, and OCBs are measured using nephelometric, immunofluorescence, and electrophoretic techniques. These latter methods may be less sensitive in detecting intrathecal immunoglobulin production during active disease when the BBB is dysfunctional. Furthermore, the level of intrathecal AQP4-IgG synthesis could be significantly underestimated due to tissue binding of AQP4-IgG.

Newly generated plasmablasts circulating in the PB of patients with NMO are likely precursors for antibody-producing plasma cells that reside in the BM and CNS. Chihara et al. have identified a
CD19<sup><i>hi</i></sup>CD27<sup><i>hi</i></sup>CD38<sup><i>hi</i></sup>CD180<sup><i>−</i></sup> B cell population that is selectively increased in NMO PB. These cells have phenotypic features of plasmablasts, secrete increased AQP4-IgG following IL-6 stimulation in vitro, and overlap with a similarly defined CSF B cell population. These PB plasmablasts may not only repopulate or replenish BM plasma cell niches but also deliver antibody-secreting cells (ASCs) to the CNS compartment. In addition, subsets of plasma cells may exacerbate or mitigate disease activity through the secretion of proinflammatory or regulatory cytokines (discussed below).

The initial molecular characterization of the humoral immune response in an early AQP4-IgG+ NMO patient revealed a clonally expanded plasmablast population primarily directed against AQP4. In contrast to similar analyses in MS, the repertoire showed significant intraclonal variability and heavy chain variable regions dominated by VH2 rather than VH4 gene segments. These findings provide a molecular signature suggesting that the CNS B cell population in NMO may emanate from recent germinal center activity that is driven by antigenic targets that are distinct from those in MS. Further analyses of the B cell repertoires in AQP4-IgG+ and AQP4-IgG− NMO could help to distinguish ASCs in these two disease subsets and provide an avenue for differentiating NMO from other inflammatory demyelinating disorders. A limited analysis of PB plasmablasts and CSF B cells from the same patient has suggested that circulating plasmablasts may migrate between these compartments during disease activity. Given the results of similar studies in MS, a comprehensive analysis of peripheral and CSF B cell repertoires using high-throughput deep sequencing might clarify the patterns of B cell migration in NMO.

The variability of AQP4-IgG titers in treated patients and of CSF AQP4-IgG titers and plasma cells during disease relapse suggest that AQP4-specific ASCs are a labile population that may be modulated at the levels of production, trafficking, and maintenance. IL-5, IL-6, tumor necrosis factor α (TNF-α), B cell-activating factor (BAFF), and a proliferation-inducing ligand (APRIL) are critical for isolated plasma cell survival in vitro (table 2). Elevated CSF levels of IL-6, BAFF, APRIL, and IL-5 likely play a role in facilitating AQP4-specific ASC survival in the CNS of patients with NMO. In BM, eosinophils are the main source of APRIL and IL-6, which are essential for the support of plasma cell niches. Likewise, CNS infiltration of eosinophils may facilitate plasma cell survival and IgG production in NMO lesions. Modulation of eosinophil number and location or direct targeting of IL-6 signaling may have a direct effect on plasma cell survival, autoantibody production, and NMO pathology. For example, the S1P1 receptor agonist fingolimod promotes retention of eosinophils in BM and ASCs in secondary

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Levels in NMO</th>
<th>NMO relevant source(s)</th>
<th>Potential action</th>
<th>References</th>
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<tr>
<td>IL-6</td>
<td>Increased serum</td>
<td>Lymphocytes; monocytes and macrophages; endothelial cells; granulocytes; glial cells</td>
<td>Support plasmablast and plasma cell differentiation and survival; promote Th17</td>
<td>e-13-e-19</td>
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<tr>
<td>IL-5</td>
<td>Unchanged CSF</td>
<td>T cells; eosinophils; mast cells; astrocytes</td>
<td>Maintenance of plasma cell niches and CNS antibody-secreting cells</td>
<td>e-13, e-16, e-18-e-20</td>
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<tr>
<td>BAFF (B)/ APRIL (A)</td>
<td>Increased serum</td>
<td>B cells (A/B); activated T cells (A/B); astrocytes (B); microglia (B); NK cells (B); neutrophils (A/B); monocytes and macrophages (A/B); dendritic cells (A/B); intestinal epithelial cells (B)</td>
<td>Plasmablast and plasma cell survival</td>
<td>e-21-e-24</td>
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<tr>
<td>IL-10</td>
<td>Decreased serum</td>
<td>B10 regulatory cells; plasmablasts; plasma cells</td>
<td>Memory B regulatory cell function; plasma cell regulatory function</td>
<td>e-14, e-18, e-19, e-25, e-29</td>
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<td>IL-35</td>
<td>ND</td>
<td>Plasmablasts; plasma cells</td>
<td>Induction of B10 regulatory cells; facilitate T regulatory cell function</td>
<td>e-25, e-27</td>
</tr>
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<td>TNF-α</td>
<td>+/− Increased serum</td>
<td>B cells; plasma cells; microglia; monocytes</td>
<td>Proinflammatory B cell activity; increase IgA secretion; influence gut microbiota</td>
<td>e-14, e-18, e-19, e-28-e-30</td>
</tr>
<tr>
<td>IL-17</td>
<td>Increased serum</td>
<td>Plasma cells; T cells</td>
<td>Proinflammatory T cell activity; neutrophil recruitment; enhanced B cell survival</td>
<td>e-14, e-18, e-19, e-31-e-33</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Unchanged CSF</td>
<td>Plasmablasts; plasma cells; T cells; dendritic cells</td>
<td>Neutrophil recruitment</td>
<td>e-14, e-19, e-34</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>ND</td>
<td>Plasma cells; microglia</td>
<td>Increase serum IgA/IgA-secreting plasma cells; influence gut microbiota</td>
<td>e-29</td>
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</table>

Abbreviations: APRIL = a proliferation-inducing ligand; BAFF = B cell-activating factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; IL = interleukin; ND = not determined; NK = natural killer; NMO = neuromyelitis optica; TNF-α = tumor necrosis factor α. +/− = contradictory results. References e-13-e-34 are available at Neurology.org/nn.
lymphoid tissue.\textsuperscript{25,26} This effect could result in enhanced serum AQP4-IgG titers and CNS ASC migration, resulting in the exacerbation of NMO disease activity reported with fingolimod treatment.\textsuperscript{27} It is interesting that natalizumab, another MS therapy noted to exacerbate NMO disease activity and tissue injury,\textsuperscript{28,29} increases circulating eosinophils\textsuperscript{30,31} that may contribute directly to lesion formation\textsuperscript{32} or support local ASCs. In contrast, inhibition of IL-6 signaling using the anti-IL-6 receptor monoclonal antibody tocilizumab reduces PB plasmablasts and AQP4-IgG titers in some patients and appears to reduce relapse activity in patients with NMO.\textsuperscript{33} These observations are consistent with a role for IL-6 in the induction of plasma cells that produce autoantibodies against AQP4 and other relevant antigens.\textsuperscript{23}

While some studies have reported a correlation between AQP4-IgG titers and clinical relapse, a consistent relationship with disease activity has yet to emerge.\textsuperscript{22,34} A threshold level of AQP4-IgG that triggers clinical relapse, even in a given individual, has not been identified, and serum levels during relapse differ widely within and between individuals. Moreover, failure of rituximab has been documented in some patients with NMO experiencing relapses despite undetectable CD19\textsuperscript{+} cells in PB and high levels of AQP4-IgG serum titers.\textsuperscript{35} In one study, treatment with anti-CD20 antibody (rituximab) appeared to result in a rapid and transient increase in serum AQP4-IgG titers and clinical relapse in tandem with increased circulating BAFF levels;\textsuperscript{36} however, in another study, successful anti-CD20 therapy did not result in reduced levels of AQP4-IgG, BAFF, or APRIL.\textsuperscript{19} Discordance between AQP4-IgG serum titers and disease activity may also be explained by compartmental localization at the site of tissue damage in the CNS. In addition, antibodies may differ with respect to affinity during the course of disease. These changes may be undetectable in current assays. Indeed, in patients with NMO, CSF AQP4-IgG titers have been observed to correlate more closely with clinical activity and neuroinflammation than serum titers.\textsuperscript{57} Alternatively, antibodies targeted against other self-antigens may contribute to disease activity. For instance, autoantibodies against myelin oligodendrocyte glycoprotein (MOG) have been detected in a small fraction of patients diagnosed with NMO who are AQP4-IgG\textsuperscript{−}. There are also clinical differences between the MOG-IgG\textsuperscript{+} and AQP4-IgG\textsuperscript{+} NMO patients, although the findings are based on small numbers of patients and require further study.\textsuperscript{38,39} Unlike anti–AQP4-IgG, anti–MOG-IgG injected intracerebrally into murine brain with human complement does not reproduce NMO pathology.\textsuperscript{40}

**PROINFLAMMATORY B CELLS IN NMO** B cells may facilitate disease activity in NMO by stimulating pathogenic T cell responses through antigen presentation or cytokine secretion (table 2). B cells express major histocompatibility complex (MHC) class II molecules constitutively and serve as potent antigen-presenting cells.\textsuperscript{41} B cell MHC II expression also contributes to the development of T follicular helper cells that participate in B cell differentiation and immunoglobulin isotype switching.\textsuperscript{41,42} Thus, cooperation between AQP4-specific B cells and AQP4-specific T cells may be particularly important in ASC differentiation and the production of NMO-IgG. Moreover, the presence of AQP4-specific B cells may promote T cell responses against AQP4 that contribute to tissue damage. B cells may produce proinflammatory cytokines through antigen-specific or polyclonal stimulation. IL-6 secretion by proinflammatory memory B cells in NMO may aggravate disease activity by promoting pathogenic Th17 differentiation. Indeed, during NMO exacerbations, IL-17A and IL-23 are elevated, indicating enhanced Th17 pathway activity.\textsuperscript{43} A reduction in IL-6 secretion following anti-CD20 B cell depletion in patients with NMO may explain some of the discordance between relapse activity reduction and persistently elevated AQP4-IgG titers. Reduced Th17 pathway activity resulting from reduction in the number of proinflammatory CD20\textsuperscript{+} memory B cells may significantly reduce clinical activity despite a minimal effect on AQP4-IgG production by CD20\textsuperscript{−} tissue-resident plasma cells. In addition, alterations in the cytokine profile of B cells that reconstitute after rituximab treatment may explain some benefits of treatment. In MS, B cells emerging after anti-CD20 depletion secrete lower levels of IL-6.\textsuperscript{44} Also, reconstituting B cells may have a reduced proinflammatory cytokine profile, with lower secretion of lymphotixin or TNF-α upon activation.\textsuperscript{45}

Bystander activation may also result in the production of B cell cytokines that promote NMO disease activity. Cytokines, such as TNF-α, IL-17, and granulocyte-macrophage colony-stimulating factor (GM-CSF), may be produced by various plasma cell subsets and regulate humoral immunity, alter interactions with commensal microbiota, and modify adaptive and innate immune responses. Nitric oxide and TNF-α secreted by plasma cells in the lamina propria of the small intestine modulate IgA secretion and can alter the composition of the gut flora.\textsuperscript{46} Given that AQP4-specific T cell responses in NMO display cross-reactivity to Clostridium perfringens adenosine triphosphate-binding cassette transporter permease in vitro,\textsuperscript{47} such changes in commensal microbiota may be able to influence downstream T cell reactivity.
During bacterial and parasitic infections, plasma cells are a source of GM-CSF and IL-17. Thus, in the context of NMO, increased levels of GM-CSF and IL-17 may result in increased granulocyte recruitment to the CNS, promoting disease relapse. Future studies are needed to directly determine the quantity, phenotype, and activity of proinflammatory B cells and ASCs in NMO and NMO spectrum diseases.

REGULATORY B CELLS IN NMO  Ongoing advances in the understanding of B cell contributions to autoimmunity include the elucidation of a population of B cells that can negatively regulate cellular immune responses and inflammation, the most studied of which are those secreting IL-10, termed B10 cells (tables 1 and 2). Recently, IL-35, which is essential for the immunosuppressive function of T regulatory cells, was also noted to be necessary for the suppressive function of B cells. Mice lacking IL-35 in their B cells only were unable to recover from experimental autoimmune encephalomyelitis and had higher macrophage and inflammatory T cell activity. IL-35 was also found to be critical for the induction of B regulatory cells. It is interesting that in both infectious and autoimmune models, the major B cell sources of IL-10 and IL-35 transcripts were plasmablasts and plasma cells, suggesting that mature plasma cells expressing IL-35 may be important for the maintenance of B regulatory cell numbers. Additional studies will be needed to confirm plasmablast IL-10 expression, the extent to which such expression may be antigen-restricted, and whether this expression profile is evident during human plasma cell development.

Human B cells with similar features to murine B10 cells have been identified and are expanded in several human autoimmune disorders, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren syndrome, and bullous skin disease. Human B10 regulatory B cells may play an important role in suppressing AQP4-specific and innate immune responses in NMO (figure 1). Quan et al. noted both fewer B10 regulatory cells (CD19^+CD24^hiCD38^hi) in relapsing AQP4+ NMO patients and reduced IL-10 induction following in vitro stimulation of CD19^+ B cells. The study, however, may have underestimated the circulating B10 population, as regulatory B10 cells do not have clearly defined cell differentiation markers, and the expression of CD38^hi may be unreliable for identifying circulating B regulatory cells. It is interesting that a reduction in IL-10 expression by CD19^+ B cells has been observed in patients with MS, and the beneficial effect of parasitic infection in patients with MS has been associated with an increased production of B cell–derived IL-10. Therefore, the possibility that B cells play an important role in suppressing immune activation in NMO warrants further investigation.

B CELL TOLERANCE AND NMO PATHOGENESIS: CENTRAL AND PERIPHERAL TOLERANCE AND B CELL ANERGY  Central and peripheral tolerance.

During early B cell development, immunoglobulin variable region gene segments are stochastically recombined to generate functional antibodies (B cell receptors [BCRs]) that are expressed on the cell surface. This process is fundamental for the generation of the wide diversity of the immunoglobulin repertoire but also generates autoreactive B cells alongside those that comprise the non-self-reactive naïve repertoire. Elimination of autoreactive B cells is controlled by tolerance mechanisms. The majority of autoreactive B cells are eliminated at 2 separate steps during B cell development (figure 2). A central checkpoint in the BM between early immature and immature B cell stages removes a large population of developing B cells that express autoreactive and polyreactive antibodies. These antibodies have been shown to display low-level reactivity to multiple self-antigens by in vitro approaches. After passing through the first checkpoint, only a small fraction of B cell clones with low levels of self-reactivity migrate to the periphery. The second B cell tolerance checkpoint selects against these autoreactive newly emigrant B cells before they enter the mature naïve B cell compartment.

The mechanisms underlying tolerance defects in autoimmune diseases have not been fully elucidated. Central tolerance checkpoint integrity is thought to be associated with B cell-inherent characteristics. These mechanisms are specifically associated with signaling through the BCR. Conversely, the peripheral tolerance checkpoint is thought to be dependent on T cell/B cell interactions, particularly those involving regulatory T cells, rather than B cell-inherent characteristics. Dysregulation of central or peripheral B cell tolerance may be measured by evaluating the frequency of autoreactive B cells in the naïve repertoire. B cell tolerance defects have been clearly demonstrated in a number of autoimmune diseases. For example, patients with RA, SLE, and type 1 diabetes exhibit defects in both central and peripheral B cell tolerance checkpoints that result in the accumulation of self-reactive mature naïve B cells in their blood. This autoreactive B cell pool is thought to be the reservoir from which disease-associated autoantibodies are derived. NMO presents an opportunity to establish whether this concept is accurate, as B cells that directly produce the autoantibodies have been isolated.

Not all autoimmune diseases follow this pattern of combined central and peripheral tolerance defects. A subset of patients with MS harbor a naïve B cell...
Anergy.

B cell anergy is another mechanism that contributes to silencing humoral autoimmunity. The role of B cell anergy in NMO and human autoimmune disease is relatively unexplored. During disease relapse, anergic escape may increase the frequency of circulating AQP4-IgG plasmablasts and thereby increase AQP4-IgG titers. In combination with central or peripheral deficiencies of B cell tolerance, anergic escape may lead to a rapid relapse of disease activity during B cell reconstitution. Conversely, if only peripheral B cell tolerance is abnormal, as appears to be the case in MS, normal B cell repertoires should be established following depletion and reconstitution. Hence, understanding B cell tolerance in NMO may be critical for determining whether induction or continuous B cell depletion is needed to maintain treatment response.

Understanding the role of both central and peripheral tolerance in the counterselection of AQP4-reactive B cells in NMO may guide administration of B cell–depleting therapies. If central B cell tolerance is impaired in NMO, then the production of a large population of autoreactive B cells in the BM could lead to a rapid relapse of disease activity during B cell reconstitution. Conversely, if only peripheral B cell tolerance is abnormal, as appears to be the case in MS, normal B cell repertoires should be established following depletion and reconstitution. Hence, understanding B cell tolerance in NMO may be critical for determining whether induction or continuous B cell depletion is needed to maintain treatment response.

Recombinant antibodies produced from newly emigrant (CD21lowCD10+IgMhighCD27–) and mature (CD21+CD10−IgM+CD27+) naïve NMO B cells could be tested for reactivity against AQP4 and other self-antigens to determine whether the frequency of autoreactive B cells is greater in seropositive patients and whether diminished central or peripheral tolerance is responsible for both AQP4-specific and other autoreactivities.

B cell–depleting therapies, such as rituximab, reduce the relapse rate in NMO (table 3).
References e-35 and e-36 at Neurology.org/nn provide a recent review of NMO treatment. B cell therapeutics in NMO treatment pipeline

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<th>Agent(s)</th>
<th>Target</th>
<th>Potential mechanism(s)</th>
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<tr>
<td>Ritu-ximab, ofatumumab, ocrelizumab</td>
<td>Anti-CD20-mediated B cell depletion</td>
<td>Reduction in proinflammatory B cells; may result in transient BAFF elevation</td>
</tr>
<tr>
<td>Medi-551</td>
<td>Anti-CD19-mediated B cell depletion</td>
<td>Reduction in proinflammatory B cell and APC numbers; may reduce regulatory B cell numbers and activity</td>
</tr>
<tr>
<td>Atacicept</td>
<td>APRIL/TACI scavenger receptor</td>
<td>Reduction of APCs; may reduce regulatory B cell numbers and activity</td>
</tr>
<tr>
<td>Belimumab</td>
<td>Anti-CD22</td>
<td>Reduction in anergic B cells</td>
</tr>
<tr>
<td>Anti-IL-17</td>
<td>Anti-IL-17 antibody</td>
<td>Reduce T cell proinflammatory activity; reduce CNS neutrophil recruitment; reduce B cell survival</td>
</tr>
<tr>
<td>Tocilizumab, SA237</td>
<td>Anti-IL-6 receptor antibody</td>
<td>Reduction in APC survival; autoantibody production; reduction in proinflammatory B cell activity</td>
</tr>
<tr>
<td>Anti-IL-5</td>
<td>Anti-IL-5 antibody</td>
<td>Reduction of APCs; reduction of CNS eosinophil infiltration</td>
</tr>
</tbody>
</table>

Abbreviations: APC = antibody-producing cell; APRIL = a proliferation-inducing ligand; BAFF = B cell-activating factor; IL = interleukin; NMO = neuromyelitis optica; TACI = transmembrane activator and CAML interactor.

Summary and Future Directions

Emerging evidence suggests that B lineage cells may be multipurpose contributors to NMO spectrum disorders. AQP4-IgG is detectable in the serum and CSF of the majority of patients with NMO and reproduces disease-specific pathology. The contribution of B cells to NMO pathogenesis, however, may extend beyond the production of AQP4-IgG to include an imbalance of proinflammatory and anti-inflammatory B cell functions. Accumulating evidence points toward the importance of antigen presentation and cytokine secretion; however, impaired B cell tolerance and aberrant anergic silencing warrant further study. Basic and translational research, closely associated with clinical studies, should be leveraged toward defining the mechanisms of communication between B cells and other immune cells that drive NMO pathogenesis in order to identify novel targets for therapeutic intervention.

Author Contributions

Dr. J.L. Bennett participated in the analysis, interpretation, writing, and critical review of the manuscript for important intellectual content. Dr. K.C. O’Connor participated in the analysis, interpretation, writing, and critical review of the manuscript for important intellectual content. Dr. A. Bar-Or participated in the analysis, interpretation, writing, and critical review of the manuscript for important intellectual content. Dr. H.-C. von Budingen participated in the critical review of the manuscript for important intellectual content. Dr. T.F. Tedder participated in the critical review of the manuscript for important intellectual content. Dr. B. Hemmer participated in the critical review of the manuscript for important intellectual content. Dr. T.J. Smith participated in the critical review of the manuscript for important intellectual content. Dr. K.C. O’Connor participated in the analysis, interpretation, writing, and critical review of the manuscript for important intellectual content.

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DISCLOSURE

J.L. Bennett is on the editorial board for *Journal of Neuro-ophthalmology*, *Multiple Sclerosis Journal*, and *Neurology: Neuroimmunology & Neuroinflammation*; holds patents for compositions and methods for the treatment of neuromyelitis optica and novel blocking monoclonal therapy for neuromyelitis optica; has consulted for EMD-Serono, Questcor Pharmaceuticals, Alnylam Pharmaceuticals, Medimmune, Abbvie, Novartis Pharmaceuticals, Chugai Pharmaceuticals, Genzyme, and Genentech; received research support from Questcor Pharmaceuticals, Novartis Pharmaceuticals, NIH, and Guthy-Jackson Foundation; holds stock in Apsara Therapeutics; and receives license fee and royalty payments for aquaporinmab. K. 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