Restoring immune tolerance in neuromyelitis optica

Part II

ABSTRACT

Neuromyelitis optica spectrum disorder (NMO/SD) and its clinical variants have at their core the loss of immune tolerance to aquaporin-4 and perhaps other autoantigens. The characteristic phenotype is disruption of astrocyte function and demyelination of spinal cord, optic nerves, and particular brain regions. In this second of a 2-part article, we present further perspectives regarding the pathogenesis of NMO/SD and how this disease might be amenable to emerging technologies aimed at restoring immune tolerance to disease-implicated self-antigens. NMO/SD appears to be particularly well-suited for these strategies since aquaporin-4 has already been identified as the dominant autoantigen. The recent technical advances in reintroducing immune tolerance in experimental models of disease as well as in humans should encourage quantum leaps in this area that may prove productive for novel therapy. In this part of the article series, the potential for regulatory T and B cells is brought into focus, as are new approaches to oral tolerization. Finally, a roadmap is provided to help identify potential issues in clinical development and guide applications in tolerization therapy to solving NMO/SD through the use of emerging technologies. Each of these perspectives is intended to shine new light on potential cures for NMO/SD and other autoimmune diseases, while sparing normal host defense mechanisms. Neurology.org/nn © 2016 American Academy of Neurology

GLOSSARY

AChR = acetylcholine receptor; AQP4 = aquaporin-4; BcR = B cell receptor; Breg = regulatory B cell; EAE = experimental autoimmune encephalomyelitis; GVHD = graft-vs-host disease; IBD = inflammatory bowel disease; IFN = interferon; IgG = immunoglobulin G; IL = interleukin; MS = multiple sclerosis; NMO = neuromyelitis optica; SD = spectrum disease; T1D = type 1 diabetes; TGFβ = transforming growth factor β; Treg = regulatory T cell.

Neuromyelitis optica spectrum disorder (NMO/SD) remains a vexing neuroinflammatory and demyelinating disease that most frequently involves the spinal cord and optic nerve(s).1 The pathogenesis of NMO/SD stems from reactivity to aquaporin-4 (AQP4).2 Complement-fixing antibodies directed against AQP4 can be detected in serum and/or CSF in a majority of individuals diagnosed with NMO/SD. While existing agents may appear clinically beneficial, none has proven effectiveness or received regulatory approval for NMO/SD.3 Nonspecific immunosuppressant therapies often have adverse effects that may be amplified over chronic exposure. These concerns compelled the Guthy-Jackson Charitable Foundation to facilitate...
development of strategies for retolerization to AQP4 as potentially curative solutions for NMO/SD. Several experts in NMO/SD and immunology have contributed to this second of a 2-part series. Here, the state of the art in restoring immune tolerance is examined, and a roadmap is presented for developing antigen-specific approaches to NMO/SD. This discussion addresses current understanding of B and T cell immunobiology, distinct strategies for tolerization and developmental milestones for clinical application. The increasing awareness and understanding of NMO/SD, and the disruption of immune function that it represents, should provide opportunities for benefiting patients with the disease.

**POTENTIAL STRATEGIES FOR RESTORING IMMUNE TOLERANCE IN NMO/SD**

Enhancing regulatory T cell function. Thymus-derived CD4+CD25+Foxp3+ regulatory T cells (Tregs) have several roles in maintaining immune self-tolerance.1-4 These cells undergo thymic selection, then migrate to the periphery where they perform their regulatory functions. Most Tregs constitutively express CD25, the interleukin (IL)-2 receptor α-chain,5,6 and Foxp3,8 a transcription factor involved in Treg function.9-11

Foxp3 deficiencies in Treg development and function cause abnormal immune tolerance in humans and animal models.12-16 In scurfy mice, Foxp3 deficiency results in Treg depletion, dysfunction, defective maturation, and lethal lymphoproliferative disease.13-14 Affected patients develop polyendocrinopathy and enteropathy through X-linked inheritance,15 via defective Treg suppression of autoreactivity.16 It remains unclear whether similar Treg abnormalities are primarily involved in NMO/SD. Harnessing Tregs as potential therapy might attenuate the overly aggressive effector responses involved in its pathogenesis. Tregs modulate activation and function of T cells, B cells, natural killer cells, dendritic cells, macrophages, and mast cells (reviewed in reference 17). For example, they can inhibit CD4+ and CD8+ T cell proliferation, cytokine production, cytolyis, and other effector cell functions. They can also prevent B cells from generating autoreactive antibodies.18,19 Tregs elaborate soluble factors (e.g., IL-10, transforming growth factor β [TGFβ]), surface molecules (CTLA-4 [cytotoxic T-lymphocyte–associated protein 4]), and generate indoleamine 2,3-dioxygenase, which have been implicated in immune regulation.20 They kill target autoreactive cells through perforin- and granzyme-mediated mechanisms, and inhibit T cell–dendritic cell interactions, rendering T cell activation incomplete thus promoting anergy. Tregs have been shown to have therapeutic benefit against both autoreactive humoral and T cell responses in animal models of type 1 diabetes (T1D), experimental autoimmune encephalomyelitis (EAE), graft-vs-host disease (GVHD),21-23 and in human clinical trials for GVHD and T1D (table).24,25 Antigen-specific Tregs may be more effective in modulating autoimmune responses than their polyclonal counterparts.21,26-28

The generation of therapeutic Tregs for NMO/SD may be achieved using several strategies. While administration of autologous polyclonal Tregs expanded in vitro represents a logical first step for most indications, use of AQP4-restricted Tregs might prove particularly effective in NMO/SD. Creating genetically engineered T cells with chimeric antigen receptors is one feasible approach (see part I; reviewed in references 29 and 30). Because chimeric antigen receptors comprise a single chain variable fragment from a known antibody, this basic strategy circumvents inadvertent or off-target major histocompatibility complex restriction. For example, anti-AQP4 monoclonal antibody could be cloned into a lentiviral vector such as single chain variable fragment, along with an appropriate signaling domain, and transduced into Tregs. Resulting cells should have tight AQP4 specificity and retain antigen-directed immunosuppressive activity.30 Proof of concept for this strategy has been demonstrated in a mouse model of colitis.31 Similarly, antigen-specific human Tregs engineered through transduction of T cell receptor into Foxp3+ Tregs efficiently inhibited factor VIII–specific T effector cells in hemophilia.32 Alternatively, Ag-specific Tregs could be expanded ex vivo by exposure to recombinant AQP4 protein in the presence of IL-2, TGFβ, and/or rapamycin. Thereafter, AQP4–responsive Tregs could be isolated, expanded, and administered to patients. It should be noted that, while AQP4 is the prototypic autoantigen in NMO/SD, others are emerging as candidate participants in this disease, such as myelin oligodendrocyte glycoprotein. If validated, such antigens could be targeted using the strategies outlined herein.

Enhancing regulatory B cell function. Abnormal B cell functions may enhance autoimmune mechanisms, including those associated with NMO/SD. These cells can polarize naïve CD4+ T cells to Th1, Th2, or Th17 phenotypes, present antigens, produce cytokines, and effect costimulation.33-38 Proinflammatory B cells that activate myeloid cells (in turn, activating proinflammatory T cells) have been implicated in human CNS autoimmune disease.39 Counterbalancing the multiple roles of proinflammatory B cells are regulatory B cells (Bregs40). Bregs polarize toward a Th2 bias and modulate immune reactivity by way of IL-10 and TGFβ production, and a M2 macrophage phenotype. However, pathogenic anti-AQP4 antibody is secreted by activated B
plasma cells. Checkpoint dysfunction in plasma cell precursors offsets these anti-inflammatory effects of Th2 polarization and yields AQP4 autoantibody. In turn, this process triggers proinflammatory complement deposition. Thus, targeting autoreactive B cells may be an effective goal for Breg enhancement of immune tolerance in NMO/SD.

No cell markers are known to reliably discriminate between Breg and proinflammatory B cells. Bregs have demonstrated efficacy in attenuating autoimmune reactivity in animal models. For example, B10 cells (CD1d−CD5b) suppress T cell responses in contact hypersensitivity.11 Furthermore, transitional B cells (CD19+CD21hiCD23+CD1d+) ameliorate experimental arthritis, TIM-1+ B cells prolong islet autoreactivity, and systemic lupus erythematosus in experimental mice.54–57 IL-10 deficiency is also associated with multiple sclerosis (MS) severity.59 These apparent paradoxes may reflect the multiplicity of B cell functions in different immune paradigms.42,45,55,58 TGF-β,59,1 IL-4, and interferon (IFN)-γ may differentially influence Breg function, including their expression of major histocompatibility complex class II.31 B cell depletion using anti-CD20 as in MS12–15 depletes B cells exhibiting proinflammatory functions but not autoantibody levels.46–49

Selective depletion of inflammatory B cells targeting AQP4, or enhancement of Bregs targeting AQP4-reactive B or T cells, holds potential therapeutic promise in NMO/SD. A bispecific monoclonal antibody directed against the AQP4-restricted BcR and an anti-idiotype induction in autoimmune diseases.

**Table**

<table>
<thead>
<tr>
<th>Tolerization strategy</th>
<th>Material source</th>
<th>Biological source</th>
<th>Primary target</th>
<th>Animal studies</th>
<th>Example study outcomes</th>
<th>Human studies</th>
<th>Example study outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inverse DNA vaccine</td>
<td>Heterologous</td>
<td>Engineered</td>
<td>APC, Tc, and Bc subsets</td>
<td>Yes52</td>
<td>Induction of tumor suppression</td>
<td>Yes54</td>
<td>Reduction in proinsulin autoantibody-positive CD8+ T cells</td>
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<tr>
<td>Autoreactive Tc vaccine</td>
<td>Autologous</td>
<td>Natural</td>
<td>Autoreactive Tc</td>
<td>Yes55</td>
<td>Mortality reduced from 50% to 0% in SJL/J mice</td>
<td>Yes56</td>
<td>Induction of CD8+ Tc attenuated T1D severity</td>
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<tr>
<td>Dendritic cell vaccine</td>
<td>Autologous</td>
<td>Natural</td>
<td>AQP4 presentation</td>
<td>Yes57,58</td>
<td>Tolerogenic DC vaccine protective in EAE model</td>
<td>Yes59</td>
<td>Mixed outcomes of Ag-specific DC in T1D</td>
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<tr>
<td>Ag-coupled presentation</td>
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<td>Engineered</td>
<td>AQP4 presentation</td>
<td>Yes60</td>
<td>Expansion of Ag-specific CD4+ and CD8+ Tc</td>
<td>Yes61</td>
<td>Treg reactivity to myelin Ag peptide in patients with MS</td>
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<td>Tc receptor engineering</td>
<td>Autologous</td>
<td>Engineered</td>
<td>Autoreactive Tc</td>
<td>Yes62,63</td>
<td>Induction of Treg subset in mouse EAE model</td>
<td>Yes64</td>
<td>Moderate efficacy in human malignancies</td>
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<td>Regulatory Tc induction</td>
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<td>Natural</td>
<td>Proinflammatory cells/pathways</td>
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<td>Treg induction in various preclinical models</td>
<td>Yes36</td>
<td>Expansion of Treg subset attenuates inflammation</td>
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<tr>
<td>Regulatory Bc induction</td>
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<td>Natural</td>
<td>Proinflammatory cells/pathways</td>
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<td>Suppression of Tc autoreactivity; binding immunoglobulin protein induces Breg</td>
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<td>Oral/mucosal tolerization</td>
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<td>Either</td>
<td>APC, Tc, and Bc subsets</td>
<td>Yes14,47</td>
<td>CD4+CD25-Foxp3-LAP Treg induction in various preclinical models</td>
<td>Yes15,16,49,62</td>
<td>Lower TNF-α, higher IL-10 induction by Ag-specific Tc; allergy desensitization</td>
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<td>Adoptive transfer</td>
<td>Autologous or HLA-matched</td>
<td>Conditioned natural</td>
<td>APC, Tc, and Bc subsets</td>
<td>Yes36,37</td>
<td>Modest efficacy in murine arthritis and lupus</td>
<td>Yes34,35</td>
<td>Efficacy in GVHD</td>
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<td>Anti-idiotypic networks</td>
<td>Heterologous</td>
<td>Either</td>
<td>Pathogenic Ab</td>
<td>Yes41</td>
<td>Anti-idiotype induction in NOD mouse model</td>
<td>Yes41,44,45</td>
<td>IVIg efficacy in NMO/SD; anti-idiotype induction in autoimmune diseases</td>
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<td>Passive tolerization</td>
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<td>Pathogenic Ab</td>
<td>Yes50</td>
<td>Passive aquaporinab efficacy in EAE model</td>
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<td>NA</td>
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</table>

Abbreviations: Ab – antibody; Ag – antigen; APC – antigen-presenting cell; AQP4 – aquaporin-4; Bc – B cell; Breg – regulatory B cell; DC = dendritic cell; EAE = experimental autoimmune encephalomyelitis; GVHD = graft-versus-host disease; HLA = human leukocyte antigen; IL-10 = interleukin 10; IVIg = IV immunoglobulin; MS = multiple sclerosis; NMO/SD = neuromyelitis optica/spectrum disease; NOD = nonobese diabetes; T1D = type 1 diabetes; Tc = T cell; TNF-α = tumor necrosis factor α; Treg = regulatory T cell.
Likewise, TGFβ-producing B cells have been generated in vitro, and their adoptive transfer has suppressed experimental T1D by inducing apoptosis of effector T cells.21,22,23 These and related approaches could be applied to NMO/SD.

**Oral tolerization in NMO/SD.** The gut-associated lymphoid tissue is the largest immune organ and naturally induces tolerance to ingested proteins. Thus, oral tolerance represents a nontoxic and physiologic mechanism by which to induce tolerance in an antigen-specific manner.24 While oral tolerization has yet to be successfully translated to human autoimmune diseases, it is effective in animal models of EAE and nonobese diabetes.25,26 Varrin-Doyer et al.27 suggest that information regarding optimal dosing in initial deletion. Animal studies could provide important insight into how different strategies might affect Treg function.

Oral tolerization mitigates T cell–mediated disease in animal models of EAE and nonobese diabetes.28 Yet, it remains unknown whether orally administered autoantigens can tolerate T cells and attenuate an antibody-mediated neurologic disease. This question appears to have been answered in principle using a rat model of myasthenia gravis, where orally administered acetylcholine receptor (AChR) peptide attenuated the disease.29 In concept, oral tolerization in NMO/SD might be accomplished by feeding AQP4 or its peptides, followed by immunization with AQP4 and an appropriate adjuvant. This would be followed by assaying cell proliferation and antibody production to assess AQP4 tolerogenic responses. Tolerization might be optimal at either low or high dosages of antigen, although the mechanisms involved may differ. For example, at lower dosages, Tregs would be expected to have a prominent role, whereas higher dosages may provoke anergy or cell deletion. Animal studies could provide important information regarding optimal dosing in initial human NMO/SD. Varrin-Doyer et al.30 suggest that administration of either AQP4 protein or its immunodominant peptide(s) may reduce relapse frequency and/or severity in animal models of NMO/SD.

Oral modalities designed to tolerance autoreactive T cells for therapy in human autoimmune disease are being evaluated.31,32 For example, initial results suggest that insulin administered orally may delay T1D, presumably by restoring immune tolerance to insulin-reactive T cells.33 For human NMO/SD, T cell reactivity and AQP4 antibody levels would be monitored pre- and postimmunization. This modality should be well tolerated. Theoretically, oral administration of anti-CD3 monoclonal antibody could induce Tregs to retolerize in NMO/SD. Results using similar strategies have been encouraging in EAE, diabetes mellitus, and lupus (reviewed in reference 34). Other modes of antigen administration have also been explored with promising results. For example, intranasal dosing of myelin basic protein mitigates EAE in rats by activating IL-4+ TGFβ+ regulatory cells.35 Similarly, intranasal administration of purified AChR suppressed AChR-directed antibody production and disease manifestations in an experimental rat model of myasthenia gravis.36 Similarly, other studies have used nasal dosing in autoimmune myocarditis and like conditions.

These approaches would in concept treat NMO/SD regardless of the identities of the pathogenic autoantigen(s) involved. However, the nonspecific expansion of Tregs could result in untoward consequences, including infection or malignancy. Therefore, while promising in principle, therapeutic efficacy of oral tolerization in NMO/SD and other human autoimmune diseases has yet to be proven, and faces challenges.

**OTHER STRATEGIES FOR IMMUNE TOLERIZATION/ IMMUNE DEVIATION**

Adoptive transfer immunotherapy. Immune modulatory functions of Tregs are conferred by expression of one or more cell-associated determinants including CTLA-4, inducible T cell costimulator (CD278), or lymphocyte activation gene 3, as well as secreted factors such as TGFβ and IL-10.37 Similarly, Bregs can modulate immune responses through the elaboration of IL-10 and TGFβ.38 AQP4-restricted Tregs and Bregs could be adoptively transferred into a human leukocyte antigen–matched recipient, where anti-ergotypic lymphocytes could modulate pathogenic effector cells. Safe and effective use of autologous Treg immunotherapy has been demonstrated in human GVHD,39,40 yet attempts at adoptive transfer of Tregs in experimental models of collagen-induced arthritis or lupus41,42 have been only modestly successful. Challenges exist in the use of adoptive transfer modalities. For
example, minority Treg populations can lose Foxp3 expression and become pathogenic, IFN-γ+ and IL-17+ phenotypes (i.e., T cell transdifferentiated “ex-Foxp3” cells). Epigenetic modifications or chromatin remodeling are other potential issues in transferred or engrafted regulatory lymphocytes. Thus, durability and tissue-specific targeting of regulatory lymphocytes are key areas that remain to be optimized for immunotherapy of NMO/SD and other autoimmune diseases.

**Anti-idiotypic networks.** Anti-AQP4 and antibodies directed at alternate antigens have been implicated in most patients with NMO/SD. Less well understood is the potential role for protective or anti-idiotypic antibodies in this disease. Anti-idiotypic antibodies are known to modulate T1D, myasthenia gravis, and neonatal lupus syndrome. Such antibodies target antigen-binding (Fab) domains of pathogenic antibodies, interfering in their pathogenic interactions with cognate targets. This mechanistic principle is a likely basis for efficacy of IVIg therapy in NMO/SD. In T1D, a decline in anti-idiotypic antibodies targeting nonpathogenic, GAD65 autoantibodies precedes disease. While this may be a valid surrogate biomarker, it suggests that protective humoral responses may fend off the onset or progression of certain autoimmune diseases. Beyond passive administration of IVIg, anti-idiotypic antibodies may mediate T cell tolerization strategies targeting lupus and MS. Likewise, deviating B cell response from proinflammatory IgG1 to noninflammatory IgG4 isotype illustrates a potential strategy for antibody response in NMO/SD to be beneficially shifted. It is also noteworthy that presence of anti-idiotypic antibodies can obscure autoantibodies in certain diseases. For example, anti-idiotypic antibodies might limit NMO/SD severity or render assays seronegative for NMO-IgG. Anti-idiotypic antibody therapy may be feasible and is worthy of exploration in NMO/SD.

**Target-competitive antibody in tolerization.** Engineering of beneficial antibodies has also been pursued in NMO/SD. A prominent example is aquaporumab, a recombinant monoclonal antibody possessing high affinity for AQP4 but lacking complement activation or antibody-dependent cell cytotoxicity. Currently in preclinical development, aquaporumab aims to passively tolerize patients by competing with endogenous anti-AQP4, thereby sparing the pathogenic targeting of astrocytes or other CNS targets.

**TOLERIZATION CLINICAL DEVELOPMENT ROADMAP** Pilot studies evaluating the efficacy of candidate therapies in NMO/SD have yielded ambiguous results. These studies have proven incomplete in informing phase III trial design. This is especially true in pilot studies lacking control arms, where accurate measures of effect magnitude and relative safety are compromised. These estimates are critical in the initial assessment of benefit–risk. Chief barriers to identifying effective and safe antigen-specific treatments include precisely defined target epitopes, optimal dosing regimens, and identification of the most appropriate subject cohorts. A comprehensive strategy for addressing these challenges will be essential for obtaining robust clinical trial outcomes (figure).

The feasibility of tolerization therapy in NMO/SD is predicated on AQP4 or other pathogenic autoantigen(s) having a central role in disease process. One challenge to success is the heterogeneity of the disease. One strategy that may afford a best chance for success would involve a therapeutic target(s) that is common to a majority of NMO/SD cases. In individuals whose disease involves alternate autoantigens or mechanisms, responses to tolerization directed at AQP4 may diverge, potentially jeopardizing study outcomes. Thus, each proposed tolerizing strategy should include a systematic evaluation of all variables that might confound study interpretation. These measures include subject demographics, heterogeneity in disease phenotype, and treatment history before enrollment.

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**Figure Developmental pathway for tolerization therapies addressing neuromyelitis optica/spectrum disorder**

In this illustration, key developmental milestones are sequenced from laboratory discovery (top) to clinical use (bottom). Approximate time (years) typically required to achieve each developmental milestone is shown in the chevron symbols to the left of respective milestones. Developmental goals (left column) and stages (right column) are also listed in relation to respective developmental milestones. CMC = chemistry/manufacturing/control; IND = investigational new drug application; MoA = mechanism of action; NDA = new drug application.
Patient diversity in NMO/SD remains incompletely understood, particularly in those cases in which NMO-IgG cannot be detected. This point illustrates how the stringency of trial inclusion and exclusion criteria must be counterbalanced by the ultimate importance of assessing as broad a subject population as is possible. Assessment of a diverse patient population is essential to the identification of all patients who might benefit from therapy. Thus, early recognition of likely regulatory requirements and intended scope of clinical use is critical to the design of trial programs.

Disease stage may also determine efficacy of tolerization therapy. Early disease might prove particularly amenable to tolerance enhancement aimed at minimizing epitope spreading. NMO/SD cases that progress might exhibit aberrations of immunity differing from those in early, self-limited disease. In designing clinical trials, patients with advanced disease may have accumulated significant disability. This fact can complicate the demonstration of efficacy, as additional dysfunction could escape clinical detection. Establishing a suitable range of disease duration or severity in the inclusion criteria will be required in assessing treatment efficacy.

Previous treatment exposure is likely to influence reestablishment of immune tolerance. In this regard, stratification of study participants with respect to prior drug treatment, temporal remoteness of earlier therapies, and other therapeutic history must be considered carefully. Because tolerization therapy requiring autologous reagents highlights factors unique to each patient, full assessment of immune function in each subject will be necessary before study enrollment.

Attempts at gaining registration for therapies aiming to restore immune tolerance are likely to encounter multiple hurdles, some of which are unique. Several of the tolerance-restoring strategies mentioned have safely navigated phase I clinical trials in other autoimmune conditions. Nonetheless, prospective and rigorous evaluation of safety in larger numbers of patients with NMO/SD will be necessary for any tolerization strategy going forward. For example, the potential for regulatory cells to revert to inflammatory effector phenotypes is a concern. Likewise, strategies that attenuate regulatory mechanisms in concept might promote disease exacerbation if excessive IL-10 or other factors are elaborated. Because of their potential to significantly alter normal immune function, tolerization therapies will likely engender high-level expectations for safety.

Access to an adequate and appropriate subject cohort is essential to establishing the safety, efficacy, and dose-optimization of a candidate agent. Development and validation of new trial outcome measures and diagnostic biomarkers will facilitate trial designs that enhance the likelihood to yield unambiguous results. Optimizing early-phase study design promotes the success of subsequent phase III trials. This experience may be particularly true for strategies attempting immune retolerization, whereby the details a priori of optimal dosing are essentially unknown. Sample size determinations will depend on a number of factors, including the following: (1) stringent and objective definitions of relapse and severity; (2) estimates of relapse frequency; (3) reduction of relapse frequency by an experimental treatment vs a comparator; (4) time to onset of efficacy; (5) error assumptions around the point estimates for control; and (6) choice of power level required to reject the null hypothesis. The choice of appropriate comparator(s) is key to designing any clinical trial. Inclusion of arms receiving pure placebo, unproven currently used therapies, or add-on combinations in NMO/SD has recently been reviewed. Use of an unproven yet widely used drug(s) as a comparator imposes substantial challenges to demonstrating superiority or noninferiority, as the comparator effect would remain undetermined.

Designing robust NMO/SD efficacy and safety trials to support drug registration will require substantial input from the relevant regulatory agencies. Thus, their early guidance on every aspect of the trial design should be sought. Their input regarding safety, dosing, and study endpoints is essential. The data generated from each trial will ultimately inform product labeling and patient access, and will likely shape clinical practice. It should also be noted that 2 adequate and well-controlled confirmatory trials are typically required as evidentiary for approval. In rare circumstances, a single pivotal trial generating unambiguous results may suffice, especially in the orphan disease space. As safety will be a major driver of benefit–risk assessment, early endorsement of the safety monitoring details and plan should be obtained from regulatory authorities.

In summary, meeting the scientific, operational, ethical, and regulatory hurdles for developing novel therapeutics in rare neuroimmunologic diseases such as NMO/SD is challenging. Nonetheless, strategies aimed at restoring immune tolerance may offer favorable long-term safety compared to chronic immunosuppression. No therapies currently used in NMO/SD have been proven effective or safe in controlled, prospective clinical trials. This fact highlights the unmet need and justifies the continued efforts of researchers and clinicians in close collaboration with industry and regulatory partners to facilitate development of tolerizing therapies for NMO/SD.

**CONCLUDING REMARKS** The goal of immune tolerization is to reset the immune system and restore central and peripheral tolerance and in so doing overcome manifestations of autoimmune disease.
This primary goal faces difficult challenges, including disease heterogeneity, the dynamics of adaptive immunity, and inflammatory responses over the course of disease. The strategies discussed in these articles may prove successful in reducing immune reactivity targeting pathogenic autoantigens in NMO/SD. To do so, tolerization therapeutics must delete autoreactive effector cells or attenuate their functions, and prevent their re-emergence. In theory, efficacy may be achieved by modulating inflammatory or enhancing regulatory responses. The value of these approaches will ultimately be determined by their efficacy in reducing or eliminating disease morbidity and mortality. In practice, the combination of traditional and retolerization strategies may be a first step forward, where conditioning of the immune system through one approach affords a permissive groundwork for retolerization. In this respect, a development process in which adaptive trial designs may afford the most efficient advances may be ideal. Obviously, identifying durable curative and preventive measures remains the ultimate goal for these strategies. Despite its challenges, restoring immune tolerance remains a meritorious and promising goal in NMO/SD.

**AUTHOR CONTRIBUTIONS**

Amit Bar-Or: drafting/revising the manuscript. Larry Steinman: drafting/revising the manuscript. Jacinta M. Behne: revising the manuscript. Daniel Benitez-Ribas: drafting/revising the manuscript. Peter S. Chin: drafting/revising the manuscript. Michael Clare-Salzler: drafting/revising the manuscript. Donald Healey: drafting/revising the manuscript. James I. Kim: drafting/revising the manuscript. David M. Kranz: drafting/revising the manuscript. Andreas Lutterotti: drafting/revising the manuscript. Roland Martin: drafting/revising the manuscript. Sven Schippling: drafting/revising the manuscript. Pablo Villoslada: drafting/revising the manuscript. Cheng-Hong Wei: drafting/revising the manuscript. Howard L. Weiner: drafting/revising the manuscript. Scott S. Zamvil: drafting/revising the manuscript. Terry J. Smith: drafting/revising the manuscript. study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research, and will give final approval. Michael R. Yeaman: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research, and will give final approval.

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**DISCLOSURE**

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Receptors, is on the editorial board for Multiple Sclerosis Journal, Proceedings of the National Academy of Science, holds patents for antigen-specific tolerance, has a patent pending for cytokines and type 1 interferons, is on the speakers bureau for EMD Serono, received research support from NIH, has stock options and board membership in Tolerion, is on the board of directors for BioAtla. J.M. Behne and D. Benitez-Ribas report no disclosures. P.S. Chin served as the medical director for Genentech and Novartis Pharmaceuticals Corp. M. Clare-Salzler received research support from NIH, NAID. D. Healy has a patent pending for the treatment of B cell-mediated autoimmunities with T cell vaccination, has been CSO for Opera Therapeutics, received research support from and holds stock/stock options in Operaa Therapeutics. J.I. Kim is an associate editor for Journal of Negative Results in Biomedicine, is employed by Unum Therapeutics. D.M. Kranz has patents and patents pending in the areas of yeast display and T cell receptor engineering and receives license fee payments from them, consulted for AbbVie, received research support from NIH, Melanoma Research Alliance. A. Lutterotti served on the scientific advisory board for Bayer, Biogen, Novartis, Genzyme, received travel support from European Charcot Foundation, Fundacio GAEM, holds a patent with the University of Zurich, received research support from Wyss Translational Center, Austrian MS Society. R. 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Schippling served on the scientific advisory board for Bayer Healthcare, Biogen, Merck Serono, Novartis, Sanofi Genzyme, TEVA, received travel funding and/or speaker honoraria from Bayer Healthcare, Biogen, Merck Serono, Novartis, Sanofi-Aventis, TEVA, is an associate editor for Frontiers in Neurology, holds a patent for therapeutic vaccination in PML using VP1 and IFN, received research support from Sanofi-Genzyme, Novartis, University of Zurich, Betty and David Koetsers Foundation for Brain Research, Swiss Multiple Sclerosis Society. P. Villalobos received travel funding and/or speaker honoraria from Novartis, Roche, Genzyme, served as an academic editor for PLoS One, served on the editorial board for Neurology & Therapy, Current Treatment Options in Neurology, Multiple Sclerosis and Demyelinating Disorders, holds a patent for methylthioadenosine for the treatment of MS, Agnostic neuromorphic compounds for the treatment of brain diseases, Gene signature pattern as a biomarker for MS, Algorithm for quantifying fractional dimension in brain MRI, received research support from Novartis, Roche, Genzyme, Instituto de Salud Carlos III, European Commission, National MS Society, Fundacion Maraton TV3, holds stock or stock options in Bionaire Inc., Spire Bioventures, MintLabs. C.-H. Wei reports no disclosures. H.L. Weiner served on the scientific advisory board for the Guthy-Jackson Charitable Foundation, Teva Pharmaceutical Industries, Biogen Idec, Novartis, Sanofi-Aventis, consulted for Therapix, Biogen, Novartis, Serono, Teva, Sanofi, received research support from National Multiple Sclerosis Society. S. Zamvil served on the scientific advisory board for BioMS, Teva Pharmaceuticals, Eli Lilly and Co., Myelin Repair Foundation, is deputy editor for Neurology®: Neuroimmunology & Neuroinflammation, consulted for Biogen Idec, Teva Neuroscience, EMD Serono, Genzyme, Novartis, Roche, is on the speakers bureau for Advanced Health Media, Biogen, received research support from NIH, NMSS. Alexander M. and June L. Maisin Foundation. T.J. 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