

# UCSF

## UC San Francisco Previously Published Works

### Title

Restoring immune tolerance in neuromyelitis optica

### Permalink

<https://escholarship.org/uc/item/9qr1j9ww>

### Journal

Neurology Neuroimmunology & Neuroinflammation, 3(5)

### ISSN

2332-7812

### Authors

Bar-Or, Amit  
Steinman, Larry  
Behne, Jacinta M  
et al.

### Publication Date

2016-10-01

### DOI

10.1212/nxi.0000000000000277

Peer reviewed

# Restoring immune tolerance in neuromyelitis optica

## Part II

OPEN

Amit Bar-Or, MD, PhD  
 Larry Steinman, MD  
 Jacinta M. Behne, MA  
 Daniel Benitez-Ribas, PhD  
 Peter S. Chin, MD  
 Michael Clare-Salzler, MD  
 Donald Healey, PhD  
 James I. Kim, PhD  
 David M. Kranz, PhD  
 Andreas Lutterotti, MD, PhD  
 Roland Martin, MD, PhD  
 Sven Schippling, MD, PhD  
 Pablo Villoslada, MD, PhD  
 Cheng-Hong Wei, PhD  
 Howard L. Weiner, MD  
 Scott S. Zamvil, MD, PhD  
 Terry J. Smith, MD  
 Michael R. Yeaman, PhD  
 On behalf of the Guthy-Jackson Charitable Foundation International Clinical Consortium

### 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. *Neurol Neuroimmunol Neuroinflamm* 2016;3:e277; doi: 10.1212/NXI.0000000000000277

### 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).<sup>1</sup> The pathogenesis of NMO/SD stems from reactivity to aquaporin-4 (AQP4).<sup>2</sup> 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.<sup>3</sup> Nonspecific immunosuppressant therapies often have adverse effects that may be amplified over chronic exposure. These concerns compelled the Guthy-Jackson Charitable Foundation to facilitate

Correspondence to  
 Dr. Smith:  
 terrysmi@med.umich.edu

Supplemental data  
 at [Neurology.org/nn](http://Neurology.org/nn)

From the Neuroimmunology Unit and Experimental Therapeutics Program (A.B.-O.), Montreal Neurological Institute and Hospital, McGill University, Montreal, Canada; Department of Neurology (L.S.), Stanford University School of Medicine, Palo Alto; The Guthy-Jackson Charitable Foundation (J.M.B.), San Diego, CA; Department of Gastroenterology (D.B.-R., P.V.), Hospital Clínic, CIBERehd and Center of Neuroimmunology & Inflammatory Bowel Disease, Institut d'Investigacions Biomèdiques August Pi Sunyer, Barcelona, Spain; Genentech, Inc. (P.S.C.), South San Francisco, CA; Department of Pathology (M.C.-S.), University of Florida School of Medicine, Gainesville; Opexa Therapeutics (D.H.), The Woodlands, TX; Department of Surgery (J.I.K.), Center for Transplantation Sciences, Massachusetts General Hospital, Harvard Medical School, Boston, MA; Department of Biochemistry (D.M.K.), University of Illinois, Urbana; Neuroimmunology and MS Research (A.L., R.M., S.S.), Department of Neurology, University Hospital Zurich, University Zurich, Switzerland; Ann Romney Center for Neurologic Diseases (H.L.W.), Department of Neurology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA; Department of Neurology and Program in Immunology (S.S.Z.), University of California, San Francisco School of Medicine; Department of Ophthalmology and Visual Sciences (T.J.S.), Kellogg Eye Center, and Division of Metabolism, Endocrine and Diabetes, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor; Department of Medicine (M.R.Y.), Divisions of Molecular Medicine & Infectious Diseases, David Geffen School of Medicine at UCLA, Los Angeles; and Harbor-UCLA Medical Center & LABioMed at Harbor-UCLA Medical Center (M.R.Y.), Torrance, CA. The Guthy-Jackson Charitable Foundation International Clinical Consortium coinvestigators are listed at [Neurology.org/nn](http://Neurology.org/nn).

Funding information and disclosures are provided at the end of the article. Go to [Neurology.org/nn](http://Neurology.org/nn) for full disclosure forms. The Article Processing Charge was paid by Guthy-Jackson Charitable Foundation.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND), which permits downloading and sharing the work provided it is properly cited. The work cannot be changed in any way or used commercially.

development of strategies for retoleration 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<sup>+</sup>CD25<sup>+</sup>Foxp3<sup>+</sup> regulatory T cells (Tregs) have several roles in maintaining immune self-tolerance.<sup>4,5</sup> 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  $\alpha$ -chain,<sup>6,7</sup> and Foxp3,<sup>8</sup> a transcription factor involved in Treg function.<sup>9–11</sup>

Foxp3 deficiencies in Treg development and function cause abnormal immune tolerance in humans and animal models.<sup>12–16</sup> In scurfy mice, Foxp3 deficiency results in Treg depletion, dysfunction, defective maturation, and lethal lymphoproliferative disease.<sup>12–14</sup> Affected patients develop polyendocrinopathy and enteropathy through X-linked inheritance,<sup>15</sup> via defective Treg suppression of autoreactivity.<sup>16</sup> 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<sup>+</sup> and CD8<sup>+</sup> T cell proliferation, cytokine production, cytolysis, and other effector cell functions. They can also prevent B cells from generating autoreactive antibodies.<sup>18,19</sup> Tregs elaborate soluble factors (e.g., IL-10, transforming growth factor  $\beta$  [TGF $\beta$ ]), surface molecules (CTLA-4 [cytotoxic T-lymphocyte-associated protein 4]), and generate indoleamine 2,3-dioxygenase, which have been implicated in immune regulation.<sup>20</sup> 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),<sup>21–23</sup> and in human clinical trials for GVHD and T1D (table).<sup>24,25</sup> Antigen-specific Tregs may be more effective in modulating autoimmune responses than their polyclonal counterparts.<sup>21,26–28</sup>

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.<sup>5</sup> Proof of concept for this strategy has been demonstrated in a mouse model of colitis.<sup>31</sup> Similarly, antigen-specific human Tregs engineered through transduction of T cell receptor into Foxp3<sup>+</sup> Tregs efficiently inhibited factor VIII–specific T effector cells in hemophilia.<sup>29</sup> Alternatively, Ag-specific Tregs could be expanded *ex vivo*<sup>30,32</sup> by exposure to recombinant AQP4 protein in the presence of IL-2, TGF $\beta$ , 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 naive CD4<sup>+</sup> T cells to Th1, Th2, or Th17 phenotypes, present antigens, produce cytokines, and effect costimulation.<sup>33–38</sup> Proinflammatory B cells that activate myeloid cells (in turn, activating proinflammatory T cells) have been implicated in human CNS autoimmune disease.<sup>39</sup> Counterbalancing the multiple roles of proinflammatory B cells are regulatory B cells (Bregs<sup>40</sup>). Bregs polarize toward a Th2 bias and modulate immune reactivity by way of IL-10 and TGF $\beta$  production, and a M2 macrophage phenotype. However, pathogenic anti-AQP4 antibody is secreted by activated B

**Table** Potential strategies for restoring immune tolerance in NMO/SD

Tolerization strategy	Material source	Biological source	Primary target	Animal studies	Example study outcomes	Human studies	Example study outcomes
Inverse DNA vaccine	Heterologous	Engineered	APC, Tc, and Bc subsets	Yes <sup>e53</sup>	Induction of tumor suppression	Yes <sup>e54</sup>	Reduction in proinsulin autoreactive CD8 <sup>+</sup> T cells
Autoreactive Tc vaccine	Autologous	Natural	Autoreactive Tc	Yes <sup>e55</sup>	Mortality reduced from 50% to 0% in SJL/J mice	Yes <sup>e56</sup>	Induction of CD8 <sup>+</sup> Tc attenuated T1D severity
Dendritic cell vaccine	Autologous	Natural	AQP4 presentation	Yes <sup>e57,e58</sup>	Tolerogenic DC vaccine protective in EAE model	Yes <sup>e59</sup>	Mixed outcomes of Ag-specific DC in T1D
Ag-coupled presentation	Autologous	Engineered	AQP4 presentation	Yes <sup>e60</sup>	Expansion of Ag-specific CD4 <sup>+</sup> and CD8 <sup>+</sup> Tc	Yes <sup>e61</sup>	Treg reactivity to myelin Ag peptide in patients with MS
Tc receptor engineering	Autologous	Engineered	Autoreactive Tc	Yes <sup>e62,e63</sup>	Induction of Treg subset in mouse EAE model	Yes <sup>e64</sup>	Moderate efficacy in human malignancies
Regulatory Tc induction	Autologous	Natural	Proinflammatory cells/pathways	Yes <sup>21-23,29,e65</sup>	Treg induction in various preclinical models	Yes <sup>e66</sup>	Expansion of Treg subset attenuates inflammation
Regulatory Bc induction	Autologous	Natural	Proinflammatory cells/pathways	Yes <sup>41-45,49,55,e10-e13,e67</sup>	Suppression of Tc autoreactivity; binding immunoglobulin protein induces Breg	No	NA
Oral/mucosal tolerization	Recombinant	Either	APC, Tc, and Bc subsets	Yes <sup>e14,e17,e27-e31</sup>	CD4 <sup>+</sup> CD25 <sup>+</sup> Foxp3 <sup>+</sup> LAP <sup>+</sup> Treg induction in various preclinical models	Yes <sup>e15,e16,e28,e29</sup>	Lower TNF- $\alpha$ , higher IL-10 induction by Ag-specific Tc; allergy desensitization
Adoptive transfer	Autologous or HLA-matched	Conditioned natural	APC, Tc, and Bc subsets	Yes <sup>e36,e37</sup>	Modest efficacy in murine arthritis and lupus	Yes <sup>e34,e35</sup>	Efficacy in GVHD
Anti-idiotypic networks	Heterologous	Either	Pathogenic Ab	Yes <sup>e41</sup>	Anti-idiotypic induction in NOD mouse model	Yes <sup>e41,e44,e45</sup>	IVIg efficacy in NMO/SD; anti-idiotypic induction in autoimmune diseases
Passive tolerization	Heterologous	Either	Pathogenic Ab	Yes <sup>e50</sup>	Passive aquaporin mAb efficacy in EAE model	No	NA

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-vs-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- $\alpha$  = tumor necrosis factor  $\alpha$ ; Treg = regulatory T cell.

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-CD5<sup>hi</sup>) suppress T cell responses in contact hypersensitivity.<sup>41</sup> Furthermore, transitional B cells (CD19<sup>+</sup>CD21<sup>hi</sup>CD23<sup>hi</sup>CD1d<sup>hi</sup>) ameliorate experimental arthritis, TIM-1<sup>+</sup> B cells prolong islet allograft survival in diabetic mice, and CD1d<sup>hi</sup> B cells abrogate inflammatory bowel disease (IBD).<sup>42-45</sup> Bregs commonly express high levels of IL-10 and cell surface-displayed CD1d.<sup>46</sup> More recently, B cells expressing IL-35 in the absence of IL-10 were also found to have potent anti-inflammatory effects in EAE.<sup>47</sup>

Breg activation typically requires signaling via the B cell receptor (BcR) as well as CD40/CD154 costimulation. In humans, signaling through CD40 or toll-like receptors induces IL-10 expression in naive

(CD27<sup>-</sup>) B cells in the absence of BcR engagement.<sup>48-53</sup> However, profusive IL-10 or IL-35 induction downregulates Breg functions<sup>47,49</sup> and exacerbates autoimmune diseases such as IBD, EAE, arthritis, and systemic lupus erythematosus in experimental mice.<sup>54-57</sup> IL-10 deficiency is also associated with multiple sclerosis (MS) severity.<sup>49</sup> These apparent paradoxes may reflect the multiplicity of B cell functions in different immune paradigms.<sup>42,49,55,58</sup> TGF $\beta$ ,<sup>59,e1</sup> IL-4, and interferon (IFN)- $\gamma$  may differentially influence Breg function, including their expression of major histocompatibility complex class II.<sup>e3</sup> B cell depletion using anti-CD20 as in MS<sup>e2-e5</sup> depletes B cells exhibiting proinflammatory functions but not autoantibody levels.<sup>e6-e9</sup>

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 apoptosis-promoting surface determinant illustrates an example of this strategy. Alternatively, adoptive transfer of Bregs targeting pathogenic immune cells might also prove effective. This strategy has shown efficacy in attenuating disease severity in models of IBD, MS, arthritis, and systemic lupus erythematosus.<sup>49,55,e10,e11</sup>

Likewise, TGF $\beta$ -producing B cells have been generated *in vitro*, and their adoptive transfer has suppressed experimental T1D by inducing apoptosis of effector T cells.<sup>e12,e13</sup> 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.<sup>e14</sup> While oral tolerization has yet to be successfully translated to human autoimmune diseases, it is effective in human allergy.<sup>e15,e16</sup> In those cases, patients are desensitized to an offending allergen(s) by stepwise exposure to that allergen. Emerging evidence supports the potential for oral tolerization in preventing or treating NMO/SD. For example, NMO-immunoglobulin G (IgG) is predominantly IgG1 and requires T cell endorsement. Thus, antibody production in NMO/SD is largely dependent on antigen-restricted T cells. Furthermore, specific T cell reactivity to AQP4 has been described,<sup>e17–e24</sup> including identification of T cell epitopes.<sup>e25,e26</sup> For example, Varrin-Doye et al.<sup>e17</sup> identified an immunodominant sequence of AQP4 (p61-80) that is recognized by CD4<sup>+</sup> T cells from patients with NMO. Furthermore, these cells exhibit a Th17 phenotype, consistent with the proinflammatory immune profile observed in human NMO/SD, including complement fixation and neutrophil involvement.

Oral tolerization mitigates T cell-mediated disease in animal models of EAE and nonobese diabetes.<sup>e14</sup> Yet, it remains unknown whether orally administered autoantigens can tolerize 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.<sup>e27</sup> In concept, oral tolerization in NMO/SD might be accomplished by feeding AQP4 or its peptides, followed by immunization with AQP4 with 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-Doye et al.<sup>e17</sup> 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 tolerize autoreactive T cells for therapy in human autoimmune disease are being evaluated.<sup>e28</sup> For example, initial results suggest that insulin administered orally may delay T1D, presumably by restoring immune tolerance to insulin-reactive T cells.<sup>e29</sup> 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 e28). 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<sup>+</sup>/TGF $\beta$ <sup>+</sup> regulatory cells.<sup>e30</sup> Similarly, intranasal administration of purified AChR suppressed AChR-directed antibody production and disease manifestations in an experimental rat model of myasthenia gravis.<sup>e31</sup> 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 TGF $\beta$  and IL-10.<sup>e32</sup> Similarly, Bregs can modulate immune responses through the elaboration of IL-10 and TGF $\beta$ .<sup>e33</sup> 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,<sup>e34,e35</sup> yet attempts at adoptive transfer of Tregs in experimental models of collagen-induced arthritis or lupus<sup>e36,e37</sup> 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- $\gamma$ <sup>+</sup> and IL-17<sup>+</sup> phenotypes (i.e., T cell transdifferentiated “*ex-Foxp3*” cells).<sup>e38,e39</sup> Epigenetic modifications or chromatin remodeling are other potential issues in transferred or engrafted regulatory lymphocytes.<sup>e32,e40</sup> 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,<sup>e41</sup> myasthenia gravis,<sup>e42</sup> and neonatal lupus syndrome.<sup>e43</sup> 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.<sup>e44,e45</sup> In T1D, a decline in anti-idiotypic antibodies targeting nonpathogenic, GAD65 autoantibodies precedes disease.<sup>e46</sup> 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<sup>e47</sup> and MS.<sup>e48</sup> 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.<sup>e49</sup> 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.

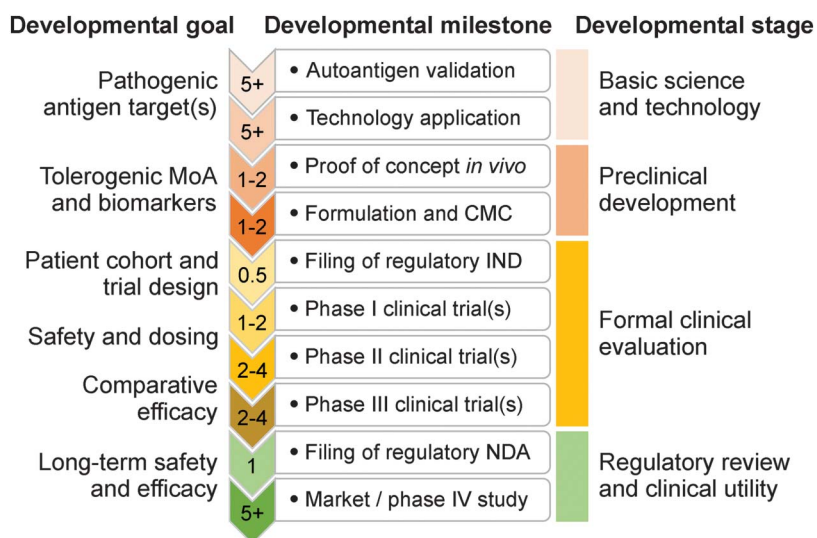
**Target-competitive antibody in tolerization.** Engineering of beneficial antibodies has also been pursued in NMO/SD. A prominent example is aquaporumab, a recombinant monoclonal antibody possessing high affinity for AQP4 but lacking complement activation or antibody-dependent cell cytotoxicity.<sup>e50</sup> 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.

**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 retoleration, 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  $\alpha$ -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.<sup>e51</sup> 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.<sup>e52</sup> 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.

#### ACKNOWLEDGMENT

The authors thank Drs. Gerald Nepom, Philip Birnstein, and William St. Clair for their helpful comments.

#### STUDY FUNDING

This work was supported in part by the Guthy-Jackson Charitable Foundation.

#### DISCLOSURE

A. Bar-Or is on the scientific advisory board for Dionix, Receptos-Celgene, Roche/Genentech, Novartis, GSK, Guthy-Jackson Greater Good Foundation, Immune Tolerance Network, received travel funding and/or speaker honoraria from Receptos-Celgene, Roche/Genentech, Novartis, Sanofi-Genzyme, GSK, served on the editorial board for *Neurology*<sup>®</sup>, *Clinical and Experimental Neuroimmunology*, consulted for Dio-Genix, Receptos-Celgene, Roche/Genentech, Novartis, Sanofi-Genzyme, GSK, received research support from Novartis, Genzyme-Sanofi. L. Steinman served on the scientific advisory board for Novartis,

Receptos, Atreca, Tolerion, Teva, received travel funding and/or speaker honoraria from Biogen, Bayhill, Bayer, Celgene, Receptos, 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, NIAID. D. Healy has a patent pending for the treatment of B cell-mediated autoimmunities with T cell vaccination, has been CSO for Opexa Therapeutics, received research support from and holds stock/stock options in Opexa 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. Martin served on the scientific advisory board for Biogen, Merck & Serono, Teva, Genzyme, Sanofi-Aventis, CellProtect, Neuway, received speaker honoraria from Biogen, Merck & Serono, Novartis, Roche, Genzyme, holds a patent for the therapeutic efficacy of anti-CD25 monoclonal antibody treatment in combination with IFN- $\beta$  in MS, consulted for Myelin Repair Foundation, the Weatherall Institute for Molecular Studies, University of Oxford, the Hertie Foundation, is a member of the Kuratorium of the Jung Foundation for Science, received research support from Novartis, Biogen, Swiss National Science Foundation, European Union Seventh Framework Program, European Research Council. S. 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 II7, received research support from Sanofi-Genzyme, Novartis, University of Zurich, Betty and David Koetser Foundation for Brain Research, Swiss Multiple Sclerosis Society. P. Villoslada 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 neurotrophic compounds for the treatment of brain diseases, Gene signature pattern as a biomarker for MS, Algorithm for quantifying fractal 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 Bionure Inc., Spire Bioventures, Mint-Labs. 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*<sup>®</sup>: *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. Smith received research support from NIH, University of South Denmark, Bell Charitable Foundation, RPB Foundation, is a member of the Guthy-Jackson Charitable Foundation scientific advisory board, and holds patents covering the blockade of insulin-like growth factor receptor-1 in autoimmune diseases. M.R. Yeaman is on the scientific advisory board for Guthy-Jackson Charitable Foundation, served as an associate editor for *PLoS Pathogens*, holds patents for vaccines targeting drug-resistant pathogens, immunotherapies targeting



drug-resistant pathogens, novel anti-infective biological therapeutics, novel anti-infective small molecules, novel biological regulating programmed cell death, consulted for Guthy-Jackson Charitable Foundation, received research support from NovaDigm Therapeutics, Inc., Metacine, Inc., US Department of Defense, NIH, holds stock or stock options for NovaDigm Therapeutics, Inc., Metacine, Inc., receives license fee and royalty payments from NovaDigm Therapeutics. Go to [Neurology.org/nn](http://Neurology.org/nn) for full disclosure forms.

Received February 12, 2016. Accepted in final form July 15, 2016.

## REFERENCES

- Levy M, Wildemann B, Jarius S, et al. Immunopathogenesis of neuromyelitis optica. *Adv Immunol* 2014;121:213–242.
- Lennon VA, Kryzer TJ, Pittock SJ, Verkman AS, Hinson SR. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J Exp Med* 2005;202:473–477.
- Trebst C, Jarius S, Berthele A, et al. Update on the diagnosis and treatment of neuromyelitis optica: recommendations of the Neuromyelitis Optica Study Group (NEMOS). *J Neurol* 2014;261:1–16.
- Sakaguchi S, Setoguchi R, Yang H, Nomura T. Naturally arising Foxp3-expressing CD25+CD4+ regulatory T cells in self-tolerance and autoimmune disease. *Curr Top Microbiol Immunol* 2006;305:51–66.
- Shevach EM. CD4+ CD25+ suppressor T cells: more questions than answers. *Nat Rev Immunol* 2002;2:389–400.
- Thornton AM, Shevach EM. CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. *J Exp Med* 1998;188:287–296.
- Baecher-Allan C, Brown JA, Freeman GJ, Hafler DA. CD4+CD25 high regulatory cells in human peripheral blood. *J Immunol* 2001;167:1245–1253.
- Fontenot JD, Rasmussen JP, Williams LM, Dooley JL, Farr AG, Rudensky AY. Regulatory T cell lineage specification by the forkhead transcription factor Foxp3. *Immunity* 2005;22:329–341.
- Kim JM, Rudensky AY. The role of the transcription factor Foxp3 in the development of regulatory T cells. *Immunol Rev* 2006;212:86–98.
- Gavin MA, Rasmussen JP, Fontenot JD, et al. Foxp3-dependent programme of regulatory T-cell differentiation. *Nature* 2007;445:771–775.
- Williams LM, Rudensky AY. Maintenance of the Foxp3-dependent developmental program in mature regulatory T cells requires continued expression of Foxp3. *Nat Immunol* 2007;8:277–284.
- Brunkow ME, Jeffery EW, Hjerrild KA, et al. Disruption of a new forkhead/winged-helix protein, scurf1, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat Genet* 2001;27:68–73.
- Lahl K, Loddenkemper C, Drouin C, et al. Selective depletion of Foxp3+ regulatory T cells induces a scurfy-like disease. *J Exp Med* 2007;204:57–63.
- Kim JM, Rasmussen JP, Rudensky AY. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. *Nat Immunol* 2007;8:191–197.
- Ziegler SF. FOXP3: of mice and men. *Annu Rev Immunol* 2006;24:209–226.
- Buckner JH. Mechanisms of impaired regulation by CD4(+)CD25(+)FOXP3(+) regulatory T cells in human autoimmune diseases. *Nat Rev Immunol* 2010;10:849–859.
- Shevach EM. Mechanisms of foxp3+ T regulatory cell-mediated suppression. *Immunity* 2009;30:636–645.
- Fields ML, Seo SJ, Nish SA, Tsai JH, Caton AJ, Erikson J. The regulation and activation potential of autoreactive B cells. *Immunol Res* 2003;27:219–234.
- Fields ML, Hondowicz BD, Wharton GN, et al. The regulation and activation of lupus-associated B cells. *Immunol Rev* 2005;204:165–183.
- Vignali DA, Collison LW, Workman CJ. How regulatory T cells work. *Nat Rev Immunol* 2008;8:523–532.
- Tarbell KV, Yamazaki S, Olson K, Toy P, Steinman RM. CD25+ CD4+ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. *J Exp Med* 2004;199:1467–1477.
- Kohm AP, Carpentier PA, Anger HA, Miller SD. Cutting edge: CD4+CD25+ regulatory T cells suppress antigen-specific autoreactive immune responses and central nervous system inflammation during active experimental autoimmune encephalomyelitis. *J Immunol* 2002;169:4712–4716.
- Ermann J, Hoffmann P, Edinger M, et al. Only the CD62L+ subpopulation of CD4+CD25+ regulatory T cells protects from lethal acute GVHD. *Blood* 2005;105:2220–2226.
- Di Ianni M, Falzetti F, Carotti A, et al. Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. *Blood* 2011;117:3921–3928.
- Marek-Trzonkowska N, Mysliwiec M, Dobyszuk A, et al. Therapy of type 1 diabetes with CD4(+)CD25(high)CD127-regulatory T cells prolongs survival of pancreatic islets: results of one year follow-up. *Clin Immunol* 2014;153:23–30.
- Tang Q, Henriksen KJ, Bi M, et al. In vitro-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J Exp Med* 2004;199:1455–1465.
- Jaekel E, von Boehmer H, Manns MP. Antigen-specific FoxP3-transduced T-cells can control established type 1 diabetes. *Diabetes* 2005;54:306–310.
- Sagoo P, Ali N, Garg G, Nestle FO, Lechler RI, Lombardi G. Human regulatory T cells with alloantigen specificity are more potent inhibitors of alloimmune skin graft damage than polyclonal regulatory T cells. *Sci Transl Med* 2011;3:83ra42.
- Kim YC, Zhang AH, Su Y, et al. Engineered antigen-specific human regulatory T cells: immunosuppression of FVIII-specific T- and B-cell responses. *Blood* 2015;125:1107–1115.
- Jiang S, Camara N, Lombardi G, Lechler RI. Induction of allopeptide-specific human CD4+CD25+ regulatory T cells ex vivo. *Blood* 2003;102:2180–2186.
- Blat D, Zigmund E, Alteber Z, Waks T, Eshhar Z. Suppression of murine colitis and its associated cancer by carcinoembryonic antigen-specific regulatory T cells. *Mol Ther* 2014;22:1018–1028.
- Putnam AL, Safinia N, Medvec A, et al. Clinical grade manufacturing of human alloantigen-reactive regulatory T cells for use in transplantation. *Am J Transplant* 2013;13:3010–3020.
- Bouaziz JD, Yanaba K, Venturi GM, et al. Therapeutic B cell depletion impairs adaptive and autoreactive CD4+ T cell activation in mice. *Proc Natl Acad Sci USA* 2007;104:20878–20883.
- Constant S, Schweitzer N, West J, Ranney P, Bottomly K. B lymphocytes can be competent antigen-presenting cells

- for priming CD4<sup>+</sup> T cells to protein antigens in vivo. *J Immunol* 1995;155:3734–3741.
35. Harris DP, Haynes L, Sayles PC, et al. Reciprocal regulation of polarized cytokine production by effector B and T cells. *Nat Immunol* 2000;1:475–482.
  36. Linton PJ, Bautista B, Biederman E, et al. Costimulation via OX40L expressed by B cells is sufficient to determine the extent of primary CD4 cell expansion and Th2 cytokine secretion in vivo. *J Exp Med* 2003;197:875–883.
  37. Barr TA, Shen P, Brown S, et al. B cell depletion therapy ameliorates autoimmune disease through ablation of IL-6-producing B cells. *J Exp Med* 2012;209:1001–1010.
  38. Molnarfi N, Schulze-Topphoff U, Weber MS. MHC class II-dependent B cell APC function is required for induction of CNS autoimmunity independent of myelin-specific antibodies. *J Exp Med* 2013;210:2921–2937.
  39. Li R, Rezk A, Miyazaki Y, et al. Proinflammatory GM-CSF producing B cells in multiple sclerosis and B cell depletion therapy. *Sci Transl Med* 2015;7:310ra166.
  40. Carter NA, Vasconcellos R, Rosser EC, et al. Mice lacking endogenous IL-10-producing regulatory B cells develop exacerbated disease and present with an increased frequency of Th1/Th17 but a decrease in regulatory T cells. *J Immunol* 2011;186:5569–5579.
  41. Bouaziz JD, Yanaba K, Tedder TF. Regulatory B cells as inhibitors of immune responses and inflammation. *Immunol Rev* 2008;224:201–214.
  42. Yanaba K, Bouaziz JD, Haas KM, Poe JC, Fujimoto M, Tedder TF. A regulatory B cell subset with a unique CD1dhiCD5<sup>+</sup> phenotype controls T cell-dependent inflammatory responses. *Immunity* 2008;28:639–650.
  43. Ding Q, Yeung M, Camirand G, et al. Regulatory B cells are identified by expression of TIM-1 and can be induced through TIM-1 ligation to promote tolerance in mice. *J Clin Invest* 2011;121:3645–3656.
  44. Lee KM, Kim JI, Stott R, et al. Anti-CD45RB/anti-TIM-1-induced tolerance requires regulatory B cells. *Am J Transpl* 2012;12:2072–2078.
  45. Mizoguchi A, Mizoguchi E, Smith RN, Preffer FI, Bhan AK. Suppressive role of B cells in chronic colitis of T cell receptor alpha mutant mice. *J Exp Med* 1997;186:1749–1756.
  46. Mizoguchi A, Bhan AK. A case for regulatory B cells. *J Immunol* 2006;176:705–710.
  47. Shen P, Roch T, Lampropoulou V, et al. IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. *Nature* 2014;507:366–370.
  48. Duddy ME, Alter A, Bar-Or A. Distinct profiles of human B cell effector cytokines: a role in immune regulation? *J Immunol* 2004;172:3422–3427.
  49. Duddy M, Niino M, Adatia F, et al. Distinct effector cytokine profiles of memory and naïve human B cell subsets and implication in multiple sclerosis. *J Immunol* 2007;178:6092–6099.
  50. Fillatreau S, Sweenie CH, McGeachy MJ, Gray D, Anderton SM. B cells regulate autoimmunity by provision of IL-10. *Nat Immunol* 2002;3:944–950.
  51. Bar-Or A, Fawaz L, Fan B, et al. Abnormal B-cell cytokine responses a trigger of T-cell-mediated disease in MS? *Ann Neurol* 2010;67:452–461.
  52. Lampropoulou V, Hoehlig K, Roch T, et al. TLR-activated B cells suppress T cell-mediated autoimmunity. *J Immunol* 2008;180:4763–4773.
  53. Rieger A, Bar-Or A. B-cell-derived interleukin-10 in autoimmune disease: regulating the regulators. *Nat Rev Immunol* 2008;8:486–487.
  54. Mizoguchi E, Mizoguchi A, Preffer FI, Bhan AK. Regulatory role of mature B cells in a murine model of inflammatory bowel disease. *Int Immunol* 2000;12:597–605.
  55. Mauri C, Gray D, Mushtag N, Londei M. Prevention of arthritis by interleukin 10-producing B cells. *J Exp Med* 2003;197:489–501.
  56. Lenert P, Brummel R, Field EH, Ashman RF. TLR-9 activation of marginal zone B cells in lupus mice regulates immunity through increased IL-10 production. *J Clin Immunol* 2005;25:29–40.
  57. Mangan NE, Fallon RE, Smith P, van Rooijen N, McKenzie AN, Fallon PG. Helminth infection protects mice from anaphylaxis via IL-10-producing B cells. *J Immunol* 2004;173:6346–6356.
  58. Blair PA, Norena LY, Flores-Borja F, et al. CD19(+)CD24(hi)CD38(hi) B cells exhibit regulatory capacity in healthy individuals but are functionally impaired in systemic lupus erythematosus patients. *Immunity* 2010;32:129–140.
  59. Gonnella PA, Waldner HP, Weiner HL. B cell-deficient ( $\mu$  MT) mice have alterations in the cytokine microenvironment of the gut-associated lymphoid tissue (GALT) and a defect in the low dose mechanism of oral tolerance. *J Immunol* 2001;166:4456–4464.