Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Muscle Nerve. Author manuscript; available in PMC 2019 February 01.

Published in final edited form as:

Muscle Nerve. 2018 February ; 57(2): 172–184. doi:10.1002/mus.25973.

B cells in the pathophysiology of myasthenia gravis

John S. Yi, PhD[1], **Jeffrey T. Guptill, MD**[2], **Panos Stathopoulos, MD, PhD**[3], **Richard J. Nowak, MD**[3], and **Kevin C. O'Connor, PhD**[3]

[1]Division of Surgical Sciences, Department of Surgery, Duke University Medical Center, Durham, NC, 27710 USA

[2]Department of Neurology, Neuromuscular Section, Duke University Medical Center, Durham, NC, 27710 USA

[3]Department of Neurology, Yale School of Medicine, New Haven, CT, 06511 USA

Abstract

Myasthenia gravis (MG) is an archetypal autoimmune disease. The pathology is characterized by autoantibodies to the acetylcholine receptor (AChR) in most patients, or to muscle-specific tyrosine kinase (MuSK) in others, and to a growing number of other postsynaptic proteins in smaller subsets. A decrease in the number of functional acetylcholine receptors (AChR), or functional interruption of the AChR, within the muscle end plate of the neuromuscular junction is caused by pathogenic autoantibodies. Although the molecular immunology underpinning the pathology is well understood, much remains to be learned about the cellular immunology contributing to the production of autoantibodies. This review will document research concerning the immunopathology of MG, bringing together evidence principally from human studies with an emphasis on the role of adaptive immunity and B cells in particular. Proposed mechanisms for autoimmunity, which take into account that different types of MG may incorporate divergent immunopathology, are offered.

Keywords

Myasthenia Gravis; B cells; B lymphocytes; Autoimmunity; Immunopathology; Autoantibodies; AChR; MuSK

Introduction

Patients with myasthenia gravis (MG) experience skeletal muscle weakness, worsened by activity [1,2]. MG is a multifactorial disease that includes immune dysregulation, predisposing genetics, and environmental factors. The disease is rare; the estimated annual incidence is 1–

Corresponding author: Kevin C. O'Connor, PhD., Yale School of Medicine, Department of Neurology-Room 353J, 300 George Street, New Haven, CT 06511, ph (203) 737-3321; fax (203) 737-2704; kevin.oconnor@yale.edu.

Conflict of interests/financial disclosures: KCO has received personal compensation in the past year from Genentech for educational activities and from Proclara Biosciences and Editas Medicine for consulting services. JTG has received personal compensation in the past year from Jacobus Pharmaceuticals, argenx, and UCB Pharma for consulting services and from Grifols for educational activities. RJN reports support through an investigator-initiated trial agreement from Genentech for placebo/drug for the currently underway clinical trial (www.clincial trials.gov, NCT02110706).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

2 per 100,000, and the prevalence ranges from approximately 7–20 per 100,000 based on regional studies performed since 1990 [3–5]. Recent epidemiological investigations indicate that, like other autoimmune diseases, the incidence of MG is rising considerably [6]. Such increases can be partly attributed to improved diagnostic precision and rising longevity of the populace, but a genuine rise in incidence may point toward the important role of environmental contributions.

MG is an archetype for B cell-mediated autoimmune disorders. The molecular immunopathology (Figure 1) is attributed to the presence of autoantibodies specifically targeting components of the acetylcholine receptor (AChR). The specific disease mechanism is defined by these autoantibodies and their recognition of a number of molecular elements of the AChR, which impairs neuromuscular transmission in the postsynaptic membrane. The specific end-plate abnormalities mediated by the autoantibodies include disruption of receptor signaling and complement-directed tissue damage. Unlike many autoimmune diseases, MG autoantibodies are demonstrably pathogenic [7–12]. This has been substantiated through numerous *in-vitro* approaches and perhaps demonstrated most convincingly through passive transfer of patient-derived serum or immunoglobulin, which reproduces features of the disease in experimental animals [13]. Further evidence is provided by documented examples of maternal-fetal autoantibody transmission [14,15] and neonatal transfer [16,17], both of which can generate disease symptoms.

Genetic factors partly contribute to MG susceptibility [18]. Although families in whom more than one member has MG are rare, limited MG twin-pair studies suggest rough approximations on MG concordance to be near 35% in monozygotic twins, and near 5% in dizygotic twins [19]. These values, which are similar to a number of other autoimmune diseases, re-emphasize that varying degrees of both genetic and environmental factors contribute to the development of the disease [20]. Nearly all of the MG-associated genes identified to date are involved in the immune response; a pattern common to nearly all autoimmune diseases [21]. The human leukocyte antigen (HLA) locus remains the most strongly associated risk factor for the disease, especially HLA-DQA1 [22]. Examples of other genes encoding molecules that are involved in immune modulation include CTLA4, PTPN22, TNFRSF11A (RANK), [22] and TNFAIP3 interacting protein 1 (TNIP1) [23], all of which participate in cell-signaling pathways.

The incidence of MG with AChR autoantibodies has been observed to distribute in a bimodal pattern. Cases of early-onset MG, defined as patients in whom symptoms occur before approximately age 40, are predominantly women. Conversely, the incidence of late-onset disease is higher in men than in women. MG with muscle-specific tyrosine kinase (MuSK) autoantibodies is predominantly found in women and has a peak incidence of less than 40 years of age [24]. Clinical classifications of MG include a number of subgroups [25,26]. Ocular MG, which is restricted to isolated ptosis, diplopia, or both, with no signs or symptoms of weakness elsewhere, is often the first manifestation of the disease. In 40–50% of ocular MG cases, autoantibodies are not detected [27]. This, however, does not exclude the possibility that they are present and causal. During this early stage of the disease they may be below the level of detection of commonly used assays and/or may be enriched at the

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

neuromuscular junction (NMJ), the site of disease pathology, and thus not measurable in the serum.

Generalized MG usually includes symptoms associated with ocular disease, as well as weakness in extremity, bulbar, and/or respiratory muscles. Autoantibody status is used to classify the disease and has treatment implications in some cases. AChR, MuSK, and low-density lipoprotein (LDL) receptor-related protein 4 (LRP4) autoantibody positive and autoantibody seronegative represent additional major subsets. Within the AChR positive population, further subdivision categorizes early and late onset, early onset MG (EOMG) and late onset MG (LOMG) respectively. EOMG is often characterized by thymic hyperplasia, while cases of LOMG have thymic abnormalities less frequently. Concomitant autoimmune diseases in AChR MG patients are not uncommon, being reported in 13–22% [28]. The most frequently associated autoimmune disease is thyroid disease, followed by systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).

The production of autoantibodies clearly implicates a principal role for B cells in the disease pathogenesis. Dysregulation in immune cell types extending beyond B lineages have been documented, indicating that a combination of factors contributes to disease manifestation; this theme is largely consistent throughout human autoimmunity. The contributions of B cells to the production of MG autoantibodies have been the focus of investigative interests, especially with the successful introduction of biological therapeutics that target these cells. In this review, we focus on the contributions that B cells make to the immunopathology of AChR, MuSK, and other subtypes of MG, although studies outside of AChR MG remain limited in number. We highlight T cell subsets that affect B cell responses, tissue compartments harboring B cells, B cell and antibody secreting subsets, and therapeutic intervention. Thymoma-associated MG and animal models are not included.

B cell-mediated MG immunopathology influenced by T cells

The autoantibodies in AChR MG are class switched, somatically mutated, and primarily of the IgG1 subclass. The initiation of the process that produces this affinity-driven maturation is dependent on T cell help. Accordingly, CD4 T cells are the main drivers in the immunopathogenesis of MG disease [29–33]. They play a multi-faceted role in immunity, from promoting inflammation to inducing immune tolerance and supporting B cell function. In MG, each of these roles has been demonstrated to be dysregulated, resulting in the production of autoantibodies and secretion of pro-inflammatory cytokines. Specifically, AChR-specific CD4 T cells produce IFN-γ and IL-17 in response to AChR peptide stimulation, supporting the role of Th1 and Th17 cells in the pathogenesis of MG [34,35]. Further evidence demonstrating Th1 and Th17 cells act as mediators of MG pathogenesis comes from experimental autoimmune MG (EAMG) studies used to evaluate the susceptibility of MG in the absence of Th1 and Th17 responses. Mice deficient for the IL-17 or T-bet gene, a Th1 transcription factor, were less susceptible to EAMG as demonstrated by decreases in clinical score, cytokine production, and AChR antibody production [36,37]. The only two studies profiling T cells in MuSK MG mirror the observations in AChR MG, in which CD4 T cells from MuSK MG patients exhibited enhanced frequencies of Th1 and

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Th17 cytokines [38,39]. Highlighting the infancy of study in this field is the fact that the identification of antigen-specific T cells in MuSK remains to be accomplished.

Regulatory T cells

The detection of AChR-specific Th1 and Th17 cells also coincides with the generation of defective regulatory T cell (Treg) responses in MG patients. Qualitatively, thymic, and peripheral Tregs from MG patients are impaired in their ability to suppress T cell responses [40,41]. This impairment of Tregs is further complicated by data demonstrating that the T cells in MG become resistant to suppression when cultured with functional Treg [42]. Quantitatively, reports on the frequency of Tregs in MG patients have been mixed, with some groups showing a decrease in the frequency of Tregs in MG [43–46], while others show no such alteration [40,47,48,41]. The differences could be attributed to the markers that were used to identify Tregs. Before FOXP3 was identified as the transcription factor for Tregs, Tregs were identified only by the expression of CD25. Collectively, the functional assays for Tregs provide the best support for their role in the disease, because they show that Tregs in MG patients are defective in regulating immune responses compared to those in healthy subjects.

T-follicular helper (Tfh) & T-follicular regulatory (Tfr) cells

AChR MG is a CD4 dependent B cell mediated disease; therefore, the interaction of Tfh, Tfr, and B cells are critical in dictating the development of MG disease. In 1988, before Tfh cells were recognized as a distinct CD4 T cell subset, it was demonstrated that CD4 T cells were critical in the production of anti-AChR autoantibodies by B cells [30–33,49]. Now it is well understood that CD4 T cells differentiate into Tfh cells upon expression of the master transcription factor Bcl6 and surface marker CXCR5, which allows migration into the germinal center where they support differentiation of B cells into memory B cells and antibody-secreting subsets (plasmablasts and plasma cells) [50,51]. In the thymus of MG patients, the frequency of Tfh cells and B cells increased along with the expression of the Tfh associated markers Bcl6, IL-21, PD-1, and ICOS on thymocytes [52,53]. CXCR5+ CD4 T cells are also found in the periphery, and it is suspected that they are memory Tfh cells that have migrated out of the germinal center [54]. Circulating Tfh cells, IL-21, and CXCL13 were found at a higher frequency in MG patients and correlated with disease severity [55,56]. To demonstrate the pathogenic role of Tfh cells in EAMG, knockdown of the Tfh transcription factor Bcl6 decreased the frequency of Tfh cells and IL-21 production, and importantly, diminished disease severity [57].

A counterpart to Tfh cells, which suppress Tfh and B cell interactions in the germinal center, are follicular regulatory (Tfr) T cells [58–60]. Similar to Tfh cells, Tfr cells express CXCR5, PD-1, ICOS, and Bcl6. But unlike Tfh cells, Tfr cells are derived from natural Treg precursors, and express FOXP3 and BLIMP-1 [61]. Tfr can suppress the production of IL-21 and IL-4 by Tfh cells and inhibit class switching and antibody production by B cells. In MG, the increase in Tfh cells is likely due to a dysregulation in Tfr cells as MG patients exhibit lower ratios of Tfr/Tfh cells and frequency of Tfr cells [62,63]. Moving forward, it is of interest to better understand the dynamics between Tfh, Tfr, and B cells in immune tolerance and disease pathogenesis.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

B cell products: Immunoglobulins that drive MG pathology

Studies conducted many decades ago aimed to identify the circulating agent(s) that caused blocking of neuromuscular transmission in MG [64,65]. Through continued efforts that built upon these, and many other early investigations, it is now well understood that the molecular immunopathology of MG is due to the presence of circulating autoantibodies specifically targeting the AChR, MuSK, or LRP4. Patient-derived AChR, MuSK, and LRP4 autoantibodies in MG are demonstrably pathogenic [7–12,66]. Patients most often harbor only one of these autoantibody specificities. In approximately 75–80% of MG patients, AChR autoantibodies are detectable, MuSK autoantibodies are found in another 5–10% and LRP4 autoantibodies account for up to 20–30% of those without AChR or MuSK autoantibodies [67,68]. This leaves about 5–10% of MG patients without detectable serum autoantibodies to either AChR, MuSK, or LRP4; those individuals are termed seronegative [67,68]. However, the presumption that autoantibodies are absent in such cases is likely incorrect and requires alternative (autoantibody-independent) models to describe the pathology. The introduction of cell-based assays that express MuSK or clustered AChR was instrumental in demonstrating that a considerable portion of MG patients previously characterized as seronegative, did indeed have autoantibodies present in their serum [69,70]. Their autoantibodies were simply not detectable by the commercially available and commonly used radioimmunoassay [71]. Accordingly, it is anticipated that seronegative MG will continue to yield to the influence of higher sensitivity assays and to the discovery of new autoantigen targets [72–74].

Acetylcholine receptor autoantibodies

In the most common form of MG, autoantibodies directed against AChR are predominantly IgG1 and IgG3. These two subclasses effectively activate complement, leading to the formation of membrane attack complex [75–77]. Autoantibody and complement-mediated damage to the NMJ impairs neuromuscular transmission through damage to the postsynaptic neuromuscular junction in the form of simplification of postsynaptic junctional folds, removal of AChR from the membrane, and widening of the synapse [78]. Thus, AChR function is impaired by a number of autoantibody-mediated functions: complement-mediated tissue injury and removal of AChR from the muscle membrane (probably a major factor), cross-linkage of divalent AChR autoantibodies that results in internalization of AChR (by modulating autoantibodies), and rarely, a direct blockade of AChR by the autoantibodies [79–84].

Muscle specific kinase autoantibodies

MuSK is thought to play an important role in clustering AChR at the NMJ to promote efficient neuromuscular transmission. Passive transfer and active immunization studies in animals have shown that MuSK autoantibodies are pathogenic [85,86]. In contrast to AChR MG, MuSK autoantibodies are predominantly IgG4 and do not effectively activate complement [87]. Thus, the pathogenesis of MuSK MG is likely not complement mediated, and disruption of the interaction between MuSK and the postsynaptic protein LRP4 and collagen Q may be the predominant pathologic mechanism of MuSK autoantibodies [88–90]. An interesting feature of secreted IgG4 antibodies is their ability to engage in “Fab-arm

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

exchange" where a monospecific IgG4 protein may swap a heavy and light chain pair with another IgG4 to become bispecific [91]. It has recently been shown that Fab-arm exchanged antibodies are present in MuSK MG and are pathogenic [92].

Other autoantibodies in MG

Recent studies of patients with AChR or MuSK autoantibodies undetectable through using standard assays have revealed additional autoantibodies specific to other NMJ proteins, including LRP4; agrin; collagen Q; cortactin; and the voltage-gated potassium channel, Kv1.4 [93–100]. LRP4 autoantibodies are predominantly IgG1, and studies suggest they are pathogenic [66]. Pathogenicity of the other autoantibodies remains unclear at the moment. Titin autoantibodies, which are known to be present in AChR autoantibody positive patients with thymoma and in LOMG, have recently been observed in patients without identifiable autoantibodies to AChR, MuSK, or LRP4 using a high-sensitivity radioimmunoprecipitation (RIA) assay [101]. These findings suggest that titin autoantibodies may have a role as a diagnostic marker, but independent replication using a large collection of MG patients including those with and without thymoma is needed.

The contributions of B cell subsets to MG immunopathology

B cell tissue compartments in MG

The compartmentalized enrichment of disease-associated B cells can occur in tissue specific autoimmunity [102]. For example, at the synovium, the site of tissue injury in patients with rheumatoid arthritis, B cells producing autoantibodies that are directed toward citrullinated protein/peptide antigens can be observed [103]. Although the site of tissue injury in AChR MG is the muscle end plate, the residence of B cells that express AChR autoantibodies is widely diverse (Figure 1). Over four decades ago, AChR-specific IgG was found in the MG thymus [104]. Shortly afterward, the first identification of AChR autoantibody-producing B cells that occupied the thymus was reported [105]. Thymic abnormality, defined by an AChR specific immune infiltrate, is now recognized as a fundamental characteristic of many (but not all) AChR MG patients.

The thymus in a subset of MG patients includes [106] AChR expression by thymic epithelial cells and myoid cells, the presence of proinflammatory cytokines, and defective regulatory T cells [42]. B cell supporting CD4+ helper T cells are also present [107]. B cells populating the hyperplastic thymus express markers of activation and display functional signs of activation [108]. B cells often organize in the hyperplastic thymus within tertiary lymphoid organs, frequently exemplifying many characteristics of germinal centers. The presence and frequency of these structures positively associate with circulating AChR autoantibodies, reflecting a contributory role in their production. While these characteristics of AChR MG thymus tissue are seminal, they are not applicable to the entire MG population given that the thymus of approximately 30% of AChR MG patients is not hyperplastic and therefore may contain only few, if any, disease-associated B cells [109].

The B cell subset in the thymus tissue that is responsible for the detectable AChR autoantibody has not been precisely defined, but spontaneous production of AChR

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

autoantibodies was demonstrated as most likely due to resident plasma cells [110,111] with possible contributions from plasmablasts. Additional studies demonstrated that AChR autoantibody production by thymic lymphocytes can occur spontaneously or with mitogen stimulation, suggesting that heterogeneous B cell populations make such contributions [112,113]. AChR-specific CD27+ memory B cells are also likely to be present in the hyperplastic MG thymus, although specific identification of such has not been formally demonstrated. B cell repertoire sequencing and B cell immortalization studies have shown that the B cells resident in the MG thymus are broadly, clonally heterogeneous as they lack a dominant clone(s) among the infiltrate [114,115]. They harbor the characteristics of antigen experience, including somatic hypermutation [116–119,114] and biased usage of variable region gene segments, which include over-representation of the VH3 family at the expense of VH4 genes [114]. Of course, among these sequenced B cells are those producing autoantibodies directed toward AChR. However, they represent a minor fraction of the total B cell infiltrate as they are not highly enriched [120,111].

Given the major role of the thymus in MG AChR autoantibody production, thymectomy has been a long-standing treatment strategy. While thymus resection does not extinguish the disease in most cases, a recent, large, placebo-controlled clinical trial has confirmed the long-held belief that the procedure is beneficial [121]. The AChR antibody titer in the majority of MG patients who have had a thymectomy does fall, but invariably does not reach undetectable levels [122,123]. Provided that all of the thymus tissue harboring immune infiltrate is surgically removed, the persistence of both disease and autoantibody is a strong indication that additional locations host autoantibody-producing B cells.

AChR autoantibody-producing B cells can also be found in the circulation [124,30] and lymph nodes [125]. Measurement of AChR-specific B cells present in the circulation are often positively associated with thymic hyperplasia and high serum autoantibody titers [124]. AChR autoantibody-producing B cells have also been identified in the bone marrow [126]. The bone marrow is a well-recognized niche for long-lived plasma cells that directly contribute to the majority of serum immunoglobulin and are accordingly responsible for serological memory. Plasma cells in the bone marrow that produce AChR autoantibodies may contribute to persistently elevated titers after thymectomy [127] and other treatments [128] (discussed below).

Do lymphocytes, including autoantibody-producing B cells, gather at the site of tissue injury, the neuromuscular junction? Lymphocytes that localize to the end-plate region have been observed in the muscle tissue of AChR MG patients [129–131]. Localization in regions that include evidence of tissue damage has also been reported [132] although the specific lineage of such cells was not always elucidated. A subsequent investigation reported macrophages and T cells in MG muscle tissue but overall these infiltrates include few cells, are infrequently found at end-plates and are present in only a subset of patients [76]. Overall, the presence of lymphocytic infiltrates does not appear to associate with the loss of AChR from the end-plates [76]. MG autoantibody-producing B cells, at the end-plate, have not been unambiguously identified to date. Fittingly, it would also be of interest to associate such an infiltrate directly with autoimmune mechanisms to confirm that it is not non-specific inflammation that originates subsequent to the autoimmune-mediated tissue damage. Such

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

investigations would require MG muscle biopsies, which are rarely obtained and represent a recognized constraint in the field.

While clonal B cell enrichment in the hyperplastic thymus tissue is conspicuous, perturbations in periphery are harder to observe. The circulating B cell repertoire was recently characterized through analysis of over 500,000 unique sequences, however only minor, albeit important, deviations from normal controls were evident [133]. This indicates that pathogenic B cells make small changes to the global repertoire that are not readily perceived without very large data sets. Finally, the thymus of patients with MuSK MG presents a picture that is quite different than that of AChR MG patients. Immune cell infiltrates and ectopic germinal centers that are frequently found in the AChR MG thymus tissue are rarely present in MuSK MG [134]. This infers that MuSK autoantibodies may develop and reside in a compartment other than the thymus.

Circulating Memory B cells and Antibody-secreting B cells

Studies directed toward understanding peripheral B cell functional abnormalities in MG are few. The few thorough immunophenotyping studies that have been conducted uncovered some abnormalities in the circulating B cell lineages. Similar to the findings of the repertoire sequencing studies, these perturbations are small and are observed only in B cell subsets. The difference in the frequency of B cell populations between AChR MG and healthy controls is unremarkable and there is no evidence of a general defect in B cell differentiation in MG patients. Double negative ($CD27^{-}IgD^{-}$) B cells, the frequency of which is elevated in some autoimmune diseases [135] and associated with disease-specific autoantibody titer [136], do not appear to be altered in AChR MG [137]. A subpopulation of B cells express CD5+. These cells may be associated with autoantibody production and regulation, although their role in immunobiology is not unambiguously defined. Several autoimmune diseases, such as SLE and Sjogren's syndrome, are associated with enhanced frequencies of CD5(+) B cells. Similarly, there appears to be an increase in CD5+ B cells in a subset of AChR MG patients [138]. Plasmablast frequency, including recently activated subsets ($HLA\text{-}DR^{hi}$) in ocular MG, are elevated in a some AChR MG patients [137]. While such phenotyping studies can identify subset abnormalities that are likely related to autoimmunity, their limitation lies in that identification of autoantibody producers is not possible.

Regulatory B cells

Regulatory B cells (Bregs) are a rare B cell subset with a profound effect in promoting immune tolerance. They inhibit T cell expansion through IL-10, TGF-β, and IL-35 and support the differentiation of T cells into regulatory T cells (Tregs) [139–141]. Unlike Tregs, a specific transcription factor or a surface phenotype that identifies Bregs remains undefined, although several B cell populations including $CD24^{hi}CD27^{+}$ [142], $CD24^{hi}CD38^{hi}$ [143,144], and $CD25^{hi}CD71^{hi}$ [145] B cells have been described to contain an enriched population of Bregs. Recent studies suggest that Bregs are antigen-specific and expand in the presence of an inflammatory event [140,146]. However, the mechanisms driving their generation and maintenance remain undefined.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

One of the better-described subsets of Bregs are B10 cells, which are B cells identified by the production of IL-10, an immunosuppressive cytokine. In humans, B10 cells represent 0.3–0.8% of total B cells and decrease in number with age [142]. Due to their low frequency in circulation, and in attempt to better profile B10 cells, B10 cells are expanded into B10 progenitor cells by a multi-day TLR stimulation with recombinant CD40L, followed up by a re-stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin to detect IL-10 by flow cytometry [142]. In 2014, the first MG study examining B10 cells revealed a decrease in the frequency of B10 cells and a subset of $CD24^{+}CD38^{+}$ B cells in AChR and MuSK MG compared to healthy controls [147]. This decrease in the frequency of B10 cells correlated with disease severity based on MGFA clinical classification assignments. Disease severity also correlated with the functional ability of B10 cells to suppress CD4 T cell proliferation [148]. Additionally, examination of patients undergoing rituximab treatment revealed that the repopulation of B10 cells was associated with the responsiveness to rituximab treatment. Although several EAMG and patient studies have supported the decrease in the frequency of B10 cells in MG, determining the mechanisms that limit B10 differentiation and their function remain an unmet need [149–151]. Moving forward, further research is needed to validate B10 cell frequencies as a biomarker of MG disease severity, and methods to increase B10 cell frequencies may hold promise as a treatment for MG.

Naïve B cell repertoire formation and immunological tolerance

Gene segment recombination that occurs in developing B cells is among the defining features of the adaptive immune system. The process involves stochastically recombined gene segments to generate functional antibodies (B cell receptors) that are expressed on the cell surface. The arbitrary joining is the basis for the vast diversity of the B cell repertoire needed for complete immunity, but this comes at a price; the developing B cell repertoire invariably includes autoreactive antibodies. However, control mechanisms are in place. The majority of autoreactive B cells are eliminated at two separate steps[152,153] during B cell development. First, a central checkpoint in the bone marrow between early immature and immature B cell stages removes the vast majority of developing B cells that express autoreactive antibodies. A second B cell tolerance checkpoint operates in the periphery, and selects against these autoreactive new emigrant B cells before they enter the mature naïve B cell compartment. Patients with autoimmune diseases often exhibit defective B cell tolerance checkpoints; this has been clearly demonstrated in RA, SLE, and type-1 diabetes (T1D) [152,154,155].

B cell tolerance also functions improperly in both AChR and MuSK MG patients [156]. Accordingly, the naïve B cell repertoire in MG is shaped in the context of abnormal counter-selection during B cell development (that is, self-reactive cells are not eliminated). It follows that the manifestation of this abnormality is a naïve repertoire that is shaped differently from those in which B cell tolerance functions normally (Figure 1). Next generation deep sequencing allows for the comprehensive evaluation of the B cell receptor (BCR) repertoire properties in health and disease and provides the depth necessary to adequately depict the circulating peripheral repertoire, which includes up to 10^{11} B cells in humans [157]. Application of this approach to the naïve B cell compartments in AChR and MuSK MG patients revealed repertoire features that were not observed where B cell tolerance

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

functioned properly [133]. This emphasizes the impact of tolerance defects on peripheral autoimmune repertoires. It remains to be determined whether the autoreactive naïve B cell pool is the reservoir from which disease-associated autoantibodies are derived.

Therapeutics

Rituximab and new biologicals that target B cells

Rituximab is a clinically approved B cell directed biologic. It is a chimeric monoclonal antibody that targets the CD20 antigen found on subsets of the B cell lineage. CD20 is a 33-kDa protein expressed by all mature B cells, but not on pre-B or differentiated plasmablasts and plasma cells. Rituximab has been used as part of the standard therapy for non-Hodgkin lymphoma (NHL) and has emerged as a highly effective tool in the management of certain autoimmune diseases [158,159]. Interest in its use for MG began after a patient with both lymphoma and MG responded favorably to rituximab[160]. Several recent studies [128,161–164] have demonstrated the benefits of rituximab treatment in MG. In addition to significant clinical improvement, rituximab also allowed for tapering and subsequent discontinuation of other immunotherapies in both AChR and MuSK MG patients [163]. The beneficial effect, although durable, is not permanent as post-rituximab relapses have been observed [165]. Interestingly, the B cell repertoire that reemerges following CD20-mediated B cell depletion therapy includes evidence of tolerance dysfunction in autoimmune conditions shown to have the checkpoint abnormalities prior to treatment [166]. This indicates that CD20-mediated depletion does not restore checkpoint function and suggests that disease manifestation may reemerge if the abnormal repertoire contributes to pathology.

Given that MG is a B cell mediated disease, the mechanism by which rituximab takes action may seem apparent. That is, B cells are depleted, which results in diminished pathogenic autoantibody production. However, a careful look uncovers that it may be more complicated (Figure 1). Long-lived plasma cells secrete the majority of circulating immunoglobulin. Given the absence of CD20 on their surface, they are not directly affected by rituximab. This is supported by unchanged immunization-generated and plasma cell-dependent antibody titers, such as those for tetanus, following anti-CD20 B cell depletion [128]. As described above, it is apparent that plasma cells and/or plasmablasts produce AChR autoantibodies. In AChR MG the autoantibody titer can remain positive after treatment with rituximab, even during clinical improvement [128]. Thus, it is not exactly clear how the treatment affects improvement. Similarly, NMO autoantibodies directed toward the AQP4 water channel do not fall correspondingly even though remarkable clinical improvement follows rituximab treatment [167]. While these collective data may reflect compartmentalization (in the muscle or CNS) of the respective autoantibodies and/or discordance of serum titer with disease activity, they may also suggest that additional mechanisms, besides those associated with autoantibody, may promote disease activity, at least in the case of NMO. In AChR MG, given the clear dependence of immunopathology on autoantibodies, it is likely that rituximab eliminates autoantibody-producing cells, but the compartment and specific cells remain to be identified. The persistence of a positive autoantibody titer in responding patients may reflect the inability of the assays to discriminate between detection of these autoantibodies and their

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

pathogenic properties, and/or that the serum does not reflect autoantibody status in the central nervous system (CNS) tissue (*in NMO*) or neuromuscular junction (*in MG*).

The situation in MuSK MG may be quite different than that in AChR MG (Figure 1). The markedly diminished MuSK autoantibody titer, as early as three months after rituximab-mediated CD20+ B cell depletion [128], suggests that long-lived plasma cells are not likely to be major contributors to MuSK autoantibody production. Rather, short-lived antibody-secreting cells such as plasmablasts are viable candidates as the primary driver of MuSK autoantibody production. As only a small fraction of these cells expresses CD20 [168], the effectiveness of rituximab in MuSK MG may depend upon depletion of a pool of plasmablast-progenitor CD20+ memory B cells [168,169]. Our group has experimentally tested this model, the results of which suggest that plasmablasts, do indeed, contribute to the production of MuSK-specific autoantibodies [170].

Provided that plasmablasts or plasma cells, the majority of which lack CD20 [168], are a major source of pathogenic AChR autoantibodies, there are existing biologics that can effectively target these cells through their expression of CD19. Inebilizumab (formerly MEDI-551) is an anti-CD19 antibody with enhanced antibody-dependent cell-mediated cytotoxicity against B cell lineages. The drug is currently being evaluated in NMO given the known role of plasmablasts in AQP4 autoantibody production [171,172]. A similar evaluation in AChR MG has not been initiated, but given the similarities in these diseases, testing in rituximab-resistant patients could be considered.

MG induction; a side effect of cancer immunotherapy

The immune system is equipped with checkpoints that enforce immune homeostasis. Our immune system utilizes these checkpoints as a balance to prevent an immune response against self-antigens and thus the onset of autoimmune diseases like MG. Moreover, these checkpoints fine tune the immune response against pathogens to efficiently activate immune cells, and subsequently shut down their effector functions once the pathogen is removed to prevent immunopathogenesis. With an improved understanding of the mechanisms and the key proteins surrounding immune checkpoints, recent developments in cancer immunotherapy have focused on therapies that intervene in the biology of immune checkpoints to promote T cell activation [173].

In the tumor microenvironment, one mechanism of immune evasion by tumor cells is the binding of inhibitory ligands with its corresponding receptor. Notably, T cells in the tumor microenvironment highly express inhibitory receptors and this interaction shuts down the effector function of T cells. Thus, the goal of checkpoint blockade therapy is to tilt this balance towards immune activation by blocking the interaction of inhibitory receptors with its ligand, promoting the generation of efficient tumor-eliminating T cells. Two of the well-studied immune checkpoint targets that have changed the landscape of cancer therapy are CTLA-4 and PD-1 [174,175]. CTLA-4 is upregulated after T cell activation and competes with CD28 for binding to B7-1 and B7-2. This competition favors CTLA-4 because CTLA-4 has a higher affinity to B7-1 and B7-2 than CD28 and subsequently results in immune suppression. PD-1 is an inhibitory receptor that is upregulated following T cell activation and inhibits T cell function.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Based on the mechanisms behind immunotherapy, it is no surprise that the main side effect of unleashing the control of the immune response has been immune-related disease manifesting as dermatologic, endocrine, gastrointestinal, and hepatic events. Although cases of MG after such immunotherapy are rare, the exacerbation of MG is a vital health concern that must be carefully monitored [176]. The first record of ipilimumab-induced (anti-CTLA4) MG was described in two patients with melanoma [177]. The functional burden caused by the development of MG contributed to the death of one patient, while the other patient improved after plasmapheresis. In lung cancer, MG symptoms were reported after combination therapy with either PD-1 or PD-L1 and CTLA-4 inhibitors [178,179]. In these particular cases, the patients developed complications associated with MG exacerbation and did not survive. Interestingly, there are also cases of checkpoint blockade therapy in patients with known history of MG [180,181]. In each of these cases, the patients were given anti-PD-1 therapy to treat melanoma, but developed an exacerbation of their MG disease. Fortunately, their MG symptoms resolved, and although their anti-PD-1 therapy was discontinued, the patients had stable disease or saw a shrinkage of their tumors.

The mechanism by which anti-CTLA-4, anti-PD-1, or anti-PD-L1 therapy induces MG disease is undefined. Available case reports describe a rapid progression to myasthenic crisis; therefore, any neurologic event must be recognized early and immunotherapy must be discontinued immediately, and then likely followed by initiation of high-dose steroids along with IVIg or plasmapheresis. Immunologically, these studies suggest that the MG-specific B and T cells are present in people with no prior clinical evidence of MG pathology. While these autoreactive cells are normally suppressed through immune-tolerance mechanisms, the introduction of checkpoint blockade therapies decreases the ability of T cells to discriminate self and non-self. It is not clear whether the checkpoint-inhibitor induced disease emulates the typical form in terms of immunobiology. Autoantibody specificity and participating cell types first need to be identified so similarities can be defined.

Collectively, with immunotherapy becoming front-line treatment for many cancer types, the number of reports describing exacerbated or emerging MG following the use of checkpoint-inhibitor immunotherapy continues to grow rapidly [182,183184]. Therefore, prior to treatment, patients must be carefully scrutinized for any autoimmune diseases, family history of such, and current physical condition. Knowing a patient’s likelihood of an adverse event following immunotherapy assists clinicians in maximizing the power of immunotherapy while limiting side effects.

Future directions and conclusions

Our understanding of MG immunopathology remains incomplete. It appears that the mechanism used by B cells for autoantibody production in AChR and MuSK MG differ, but details of both are needed to understand the immunopathology that will guide the development of more effective therapies. Clinicians need superior biomarkers that accurately associate with disease activity and severity to help guide management. Directly measuring autoantibody-producing B cells may fulfill this requirement. The B cell response in MG most certainly requires T cell help. While it is understood that pro-inflammatory antigen-

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

specific T cells are at work in AChR MG, very little is known about the role of T cells in MuSK or LRP4 MG.

Translational B cell studies that will have the most impact on the care and treatment of patients are likely to include further investigation of B cell depletion in MG disease subsets, including MuSK. Evaluation of second-generation B cell directed biologics such as ocrelizumab, which appear to have improved efficacy and are better tolerated over the first generation, should be considered [185]. However, highly specific treatment is desired over broad approaches that target an entire immune-cell lineage. Autoantigen-based chimeric immunoreceptors that can direct T cells to kill autoreactive B cells through the specificity of the B cell receptor (BCR) have been developed for the B cell-mediated autoimmune disease, pemphigus [186]. Such an approach may provide an effective strategy for specific targeting of autoreactive B cells in MG, and not only result in clinical improvement but also in an improved safety profile over other less specifically-targeted agents.

Acknowledgments

This work was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health through a Grant to KCO, under award number R01AI114780 and supported, in part, by a pilot research award to KCO from Conquer Myasthenia Gravis. RJN is supported, in part, by the National Institute of Neurological Disorders and Stroke (NINDS) of the National Institutes of Health under award number U01NS084495. JSY is supported by an award from Conquer Myasthenia Gravis. JTG is supported by the National Institute of Neurological Disorders and Stroke of the National Institutes of Health under award number K23NS085049. The authors thank Karen Boss for copy editing and proofreading the manuscript and Charlotte and Eliza Gurley for administrative assistance in its preparation.

List of acronyms and/or abbreviations

MG	Myasthenia gravis
AChR	Acetylcholine receptor
MuSK	Muscle specific kinase
NMO	Neuromyelitis optica
NMJ	Neuromuscular junction
NINDS	Neurological Disorders and Stroke
HLA	Human leukocyte antigen
RANK	TNFRSF11A
TNIP1	TNFAIP3 interacting protein 1
LDL	Low-density lipoprotein
LRP4	Low-density lipoprotein receptor-related protein 4
EAMG	Experimental autoimmune MG
Treg	Regulatory T cell

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Tfr	Follicular regulatory T cell
RIA	Radio-immunoprecipitation assay
BCR	B cell receptor
NHL	Non-Hodgkin's lymphoma
T1D	Type 1 diabetes
BAFF	B cell activating factor
PMA	Phorbol 12 myristate 13 acetate
Tregs	Regulatory T cells
CNS	Central nervous system

References

1. Vincent A. Unravelling the pathogenesis of myasthenia gravis. Nat Rev Immunol. 2002; 2(10):797–804. [PubMed: 12360217]
2. Gilhus NE. Myasthenia Gravis. N Engl J Med. 2016; 375(26):2570–2581. [PubMed: 28029925]
3. Phillips LH 2nd. The epidemiology of myasthenia gravis. Ann N Y Acad Sci. 2003; 998:407–412. [PubMed: 14592908]
4. Santos E, Coutinho E, Moreira I, Silva AM, Lopes D, Costa H, Silveira F, Nadais G, Morais H, Martins J, Branco MC, Veiga A, Silva RS, Ferreira A, Sousa F, Freijo M, Matos I, Andre R, Negrao L, Fraga C, Santos M, Sampaio M, Lopes C, Leite MI, Goncalves G. Epidemiology of myasthenia gravis in Northern Portugal: Frequency estimates and clinical epidemiological distribution of cases. Muscle Nerve. 2016; 54(3):413–421. [PubMed: 26851892]
5. Lefter S, Hardiman O, Ryan AM. A population-based epidemiologic study of adult neuromuscular disease in the Republic of Ireland. Neurology. 2016
6. Cetin H, Fulop G, Zach H, Auff E, Zimprich F. Epidemiology of myasthenia gravis in Austria: rising prevalence in an ageing society. Wien Klin Wochenschr. 2012; 124(21–22):763–768. [PubMed: 23129486]
7. Vincent A, Beeson D, Lang B. Molecular targets for autoimmune and genetic disorders of neuromuscular transmission. Eur J Biochem. 2000; 267(23):6717–6728. [PubMed: 11082182]
8. Koneczny I, Cossins J, Vincent A. The role of muscle-specific tyrosine kinase (MuSK) and mystery of MuSK myasthenia gravis. J Anat. 2013
9. Viegas S, Jacobson L, Waters P, Cossins J, Jacob S, Leite MI, Webster R, Vincent A. Passive and active immunization models of MuSK-Ab positive myasthenia: electrophysiological evidence for pre and postsynaptic defects. Exp Neurol. 2012; 234(2):506–512. [PubMed: 22326541]
10. Lindstrom JM, Engel AG, Seybold ME, Lennon VA, Lambert EH. Pathological mechanisms in experimental autoimmune myasthenia gravis. II. Passive transfer of experimental autoimmune myasthenia gravis in rats with anti-acetylcholine recepotr antibodies. J Exp Med. 1976; 144(3): 739–753. [PubMed: 182897]
11. Oda K, Korenaga S, Ito Y. Myasthenia gravis: passive transfer to mice of antibody to human and mouse acetylcholine receptor. Neurology. 1981; 31(3):282–287. [PubMed: 6259556]
12. Sterz R, Hohlfeld R, Rajki K, Kaul M, Heininger K, Peper K, Toyka KV. Effector mechanisms in myasthenia gravis: end-plate function after passive transfer of IgG, Fab, and F(ab′)2 hybrid molecules. Muscle Nerve. 1986; 9(4):306–312. [PubMed: 2423869]
13. Toyka KV, Brachman DB, Pestronk A, Kao I. Myasthenia gravis: passive transfer from man to mouse. Science. 1975; 190(4212):397–399. [PubMed: 1179220]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

14. Melber D. Maternal-fetal transmission of myasthenia gravis with acetylcholine-receptor antibody. N Engl J Med. 1988; 318(15):996.
15. Vernet-der Garabedian B, Lacokova M, Eymard B, Morel E, Faltin M, Zajac J, Sadovsky O, Dommergues M, Tripon P, Bach JF. Association of neonatal myasthenia gravis with antibodies against the fetal acetylcholine receptor. J Clin Invest. 1994; 94(2):555–559. [PubMed: 8040310]
16. Donaldson JO, Penn AS, Lisak RP, Abramsky O, Brenner T, Schotland DL. Antiacetylcholine receptor antibody in neonatal myasthenia gravis. Am J Dis Child. 1981; 135(3):222–226. [PubMed: 6163352]
17. Keesey J, Lindstrom J, Cokely H. Anti-acetylcholine receptor antibody in neonatal myasthenia gravis. N Engl J Med. 1977; 296(1):55.
18. Avidan N, Le Panse R, Berrih-Aknin S, Miller A. Genetic basis of myasthenia gravis - a comprehensive review. J Autoimmun. 2014; 52:146–153. [PubMed: 24361103]
19. Ramanujam R, Pirskanen R, Ramanujam S, Hammarstrom L. Utilizing twins concordance rates to infer the predisposition to myasthenia gravis. Twin Res Hum Genet. 2011; 14(2):129–136. [PubMed: 21425894]
20. Bogdanos DP, Smyk DS, Rigopoulou EI, Mytilinaiou MG, Heneghan MA, Selmi C, Gershwin ME. Twin studies in autoimmune disease: genetics, gender and environment. J Autoimmun. 2012; 38(2–3):J156–169. [PubMed: 22177232]
21. Uddin M, Sturge M, Rahman P, Woods MO. Autosome-wide copy number variation association analysis for rheumatoid arthritis using the WTCCC high-density SNP genotype data. J Rheumatol. 2011; 38(5):797–801. [PubMed: 21362769]
22. Renton AE, Pliner HA, Provenzano C, Evoli A, Ricciardi R, Nalls MA, Marangi G, Abramzon Y, Arepalli S, Chong S, Hernandez DG, Johnson JO, Bartoccioni E, Scuderi F, Maestri M, Gibbs JR, Errichiello E, Chio A, Restagno G, Sabatelli M, Macek M, Scholz SW, Corse A, Chaudhry V, Benatar M, Barohn RJ, McVey A, Pasnoor M, Dimachkie MM, Rowin J, Kissel J, Freimer M, Kaminski HJ, Sanders DB, Lipscomb B, Massey JM, Chopra M, Howard JF Jr, Koopman WJ, Nicolle MW, Pascuzzi RM, Pestronk A, Wulf C, Florence J, Blackmore D, Soloway A, Siddiqi Z, Muppidi S, Wolfe G, Richman D, Mezei MM, Jiwa T, Oger J, Drachman DB, Traynor BJ. A genome-wide association study of myasthenia gravis. JAMA Neurol. 2015; 72(4):396–404. [PubMed: 25643325]
23. Gregersen PK, Kosoy R, Lee AT, Lamb J, Sussman J, McKee D, Simpfendorfer KR, Pirskanen-Matell R, Piehl F, Pan-Hammarstrom Q, Verschuuren JJ, Titulaer MJ, Niks EH, Marx A, Strobel P, Tackenberg B, Putz M, Maniaol A, Elsais A, Tallaksen C, Harbo HF, Lie BA, Raychaudhuri S, de Bakker PI, Melms A, Garchon HJ, Willcox N, Hammarstrom L, Seldin MF. Risk for myasthenia gravis maps to a (151) Pro-->Ala change in TNIP1 and to human leukocyte antigen-B*08. Ann Neurol. 2012; 72(6):927–935. [PubMed: 23055271]
24. Guptill JT, Sanders DB, Evoli A. Anti-MuSK antibody myasthenia gravis: clinical findings and response to treatment in two large cohorts. Muscle Nerve. 2011; 44(1):36–40. [PubMed: 21674519]
25. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. Lancet Neurol. 2015; 14(10):1023–1036. [PubMed: 26376969]
26. Berrih-Aknin S, Frenkian-Cuvelier M, Eymard B. Diagnostic and clinical classification of autoimmune myasthenia gravis. J Autoimmun. 2014; 48–49:143–148.
27. Jacob S, Viegas S, Leite MI, Webster R, Cossins J, Kennett R, Hilton-Jones D, Morgan BP, Vincent A. Presence and pathogenic relevance of antibodies to clustered acetylcholine receptor in ocular and generalized myasthenia gravis. Arch Neurol. 2012; 69(8):994–1001. [PubMed: 22689047]
28. Nacu A, Andersen JB, Lisnic V, Owe JF, Gilhus NE. Complicating autoimmune diseases in myasthenia gravis: a review. Autoimmunity. 2015; 48(6):362–368. [PubMed: 25915571]
29. Kuchroo VK, Greer JM, Kaul D, Ishioka G, Franco A, Sette A, Sobel RA, Lees MB. A single TCR antagonist peptide inhibits experimental allergic encephalomyelitis mediated by a diverse T cell repertoire. Journal of Immunology. 1994; 153(7):3326–3336.
30. Lisak RP, Laramore C, Zweiman B, Moskovitz A. In vitro synthesis of antibodies to acetylcholine receptor by peripheral blood mononuclear cells of patients with myasthenia gravis. Neurology. 1983; 33(5):604–608. [PubMed: 6682498]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

31. Lisak RP, Laramore C, Levinson AI, Zweiman B, Moskovitz AR, Witte A. In vitro synthesis of antibodies to acetylcholine receptor by peripheral blood cells: role of suppressor T cells in normal subjects. Neurology. 1984; 34(6):802–805. [PubMed: 6233499]
32. Authier FJ, De Grissac N, Degos JD, Gherardi RK. Transient myasthenia gravis during HIV infection. Muscle Nerve. 1995; 18(8):914–916. [PubMed: 7630356]
33. Nath A, Kerman RH, Novak IS, Wolinsky JS. Immune studies in human immunodeficiency virus infection with myasthenia gravis: a case report. Neurology. 1990; 40(4):581–583. [PubMed: 2138717]
34. Cao Y, Amezquita RA, Kleinstein SH, Stathopoulos P, Nowak RJ, O'Connor KC. Autoreactive T Cells from Patients with Myasthenia Gravis Are Characterized by Elevated IL-17, IFN-gamma, and GM-CSF and Diminished IL-10 Production. Journal of immunology. 2016; 196(5):2075–2084.
35. Wang ZY, Okita DK, Howard J Jr, Conti-Fine BM. T-cell recognition of muscle acetylcholine receptor subunits in generalized and ocular myasthenia gravis. Neurology. 1998; 50(4):1045–1054. [PubMed: 9566393]
36. Liu R, Hao J, Dayao CS, Shi FD, Campagnolo DI. T-bet deficiency decreases susceptibility to experimental myasthenia gravis. Exp Neurol. 2009; 220(2):366–373. [PubMed: 19818352]
37. Schaffert H, Pelz A, Saxena A, Losen M, Meisel A, Thiel A, Kohler S. IL-17-producing CD4(+) T cells contribute to the loss of B-cell tolerance in experimental autoimmune myasthenia gravis. European journal of immunology. 2015; 45(5):1339–1347. [PubMed: 25676041]
38. Yi JS, Guidon A, Sparks S, Osborne R, Juel VC, Massey JM, Sanders DB, Weinhold KJ, Guptill JT. Characterization of CD4 and CD8 T cell responses in MuSK myasthenia gravis. J Autoimmun. 2014; 52:130–138. [PubMed: 24378287]
39. Yilmaz V, Oflazer P, Aysal F, Durmus H, Poulas K, Yentur SP, Gulsen-Parman Y, Tzartos S, Marx A, Tuzun E, Deymeer F, Saruhan-Direskeneli G. Differential Cytokine Changes in Patients with Myasthenia Gravis with Antibodies against AChR and MuSK. PLoS One. 2015; 10(4):e0123546. [PubMed: 25893403]
40. Balandina A, Lecart S, Dartevelle P, Saoudi A, Berrih-Aknin S. Functional defect of regulatory CD4(+)CD25+ T cells in the thymus of patients with autoimmune myasthenia gravis. Blood. 2005; 105(2):735–741. [PubMed: 15454488]
41. Thiruppathi M, Rowin J, Ganesh B, Sheng JR, Prabhakar BS, Meriggioli MN. Impaired regulatory function in circulating CD4(+)CD25(high)CD127(low/-) T cells in patients with myasthenia gravis. Clin Immunol. 2012; 145(3):209–223. [PubMed: 23110942]
42. Gradolatto A, Nazzal D, Truffault F, Bismuth J, Fadel E, Foti M, Berrih-Aknin S. Both Treg cells and Tconv cells are defective in the Myasthenia gravis thymus: roles of IL-17 and TNF-alpha. J Autoimmun. 2014; 52:53–63. [PubMed: 24405842]
43. Fattorossi A, Battaglia A, Buzzonetti A, Ciaraffa F, Scambia G, Evoli A. Circulating and thymic CD4 CD25 T regulatory cells in myasthenia gravis: effect of immunosuppressive treatment. Immunology. 2005; 116(1):134–141. [PubMed: 16108825]
44. Li X, Xiao BG, Xi JY, Lu CZ, Lu JH. Decrease of CD4(+)CD25(high)Foxp3(+) regulatory T cells and elevation of CD19(+)BAFF-R(+) B cells and soluble ICAM-1 in myasthenia gravis. Clin Immunol. 2008; 126(2):180–188. [PubMed: 18054287]
45. Masuda M, Matsumoto M, Tanaka S, Nakajima K, Yamada N, Ido N, Ohtsuka T, Nishida M, Hirano T, Utsumi H. Clinical implication of peripheral CD4+CD25+ regulatory T cells and Th17 cells in myasthenia gravis patients. Journal of neuroimmunology. 2010; 225(1–2):123–131. [PubMed: 20472307]
46. Xu WH, Zhang AM, Ren MS, Zhang XD, Wang F, Xu XC, Li Q, Wang J, Din BS, Wu YB, Chen GH. Changes of Treg-associated molecules on CD4+CD25 +Treg cells in myasthenia gravis and effects of immunosuppressants. J Clin Immunol. 2012; 32(5):975–983. [PubMed: 22467037]
47. Battaglia A, Di Schino C, Fattorossi A, Scambia G, Evoli A. Circulating CD4+CD25+ T regulatory and natural killer T cells in patients with myasthenia gravis: a flow cytometry study. J Biol Regul Homeost Agents. 2005; 19(1–2):54–62. [PubMed: 16178275]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

48. Matsui N, Nakane S, Saito F, Ohigashi I, Nakagawa Y, Kurobe H, Takizawa H, Mitsui T, Kondo K, Kitagawa T, Takahama Y, Kaji R. Undiminished regulatory T cells in the thymus of patients with myasthenia gravis. Neurology. 2010; 74(10):816–820. [PubMed: 20211905]
49. Fujii Y, Lindstrom J. Regulation of antibody production by helper T cell clones in experimental autoimmune myasthenia gravis. J Immunol. 1988; 141(10):3361–3369. [PubMed: 2972772]
50. Zhu Y, Zou L, Liu YC. T follicular helper cells, T follicular regulatory cells and autoimmunity. International immunology. 2015
51. Ma CS, Deenick EK. Human T follicular helper (Tfh) cells and disease. Immunology and cell biology. 2014; 92(1):64–71. [PubMed: 24145858]
52. Song Y, Zhou L, Miao F, Chen G, Zhu Y, Gao X, Wang Y, Pang L, Zhao C, Sun X, Chen Z. Increased frequency of thymic T follicular helper cells in myasthenia gravis patients with thymoma. J Thorac Dis. 2016; 8(3):314–322. [PubMed: 27076925]
53. Zhang X, Liu S, Chang T, Xu J, Zhang C, Tian F, Sun Y, Song C, Yi W, Lin H, Li Z, Yang K. Intrathymic Tfh/B Cells Interaction Leads to Ectopic GCs Formation and Anti-AChR Antibody Production: Central Role in Triggering MG Occurrence. Mol Neurobiol. 2016; 53(1):120–131. [PubMed: 25407929]
54. Schmitt N, Bentebibel SE, Ueno H. Phenotype and functions of memory Tfh cells in human blood. Trends in immunology. 2014; 35(9):436–442. [PubMed: 24998903]
55. Luo C, Li Y, Liu W, Feng H, Wang H, Huang X, Qiu L, Ouyang J. Expansion of circulating counterparts of follicular helper T cells in patients with myasthenia gravis. Journal of neuroimmunology. 2013; 256(1–2):55–61. [PubMed: 23305745]
56. Saito R, Onodera H, Tago H, Suzuki Y, Shimizu M, Matsumura Y, Kondo T, Itoyama Y. Altered expression of chemokine receptor CXCR5 on T cells of myasthenia gravis patients. Journal of neuroimmunology. 2005; 170(1–2):172–178. [PubMed: 16214223]
57. Xin N, Fu L, Shao Z, Guo M, Zhang X, Zhang Y, Dou C, Zheng S, Shen X, Yao Y, Wang J, Wang J, Cui G, Liu Y, Geng D, Xiao C, Zhang Z, Dong R. RNA interference targeting Bcl-6 ameliorates experimental autoimmune myasthenia gravis in mice. Mol Cell Neurosci. 2014; 58:85–94. [PubMed: 24361642]
58. Wollenberg I, Agua-Doce A, Hernandez A, Almeida C, Oliveira VG, Faro J, Graca L. Regulation of the germinal center reaction by Foxp3+ follicular regulatory T cells. Journal of immunology. 2011; 187(9):4553–4560.
59. Linterman MA, Pierson W, Lee SK, Kallies A, Kawamoto S, Rayner TF, Srivastava M, Divekar DP, Beaton L, Hogan JJ, Fagarasan S, Liston A, Smith KG, Vinuesa CG. Foxp3+ follicular regulatory T cells control the germinal center response. Nature medicine. 2011; 17(8):975–982.
60. Chung Y, Tanaka S, Chu F, Nurieva RI, Martinez GJ, Rawal S, Wang YH, Lim H, Reynolds JM, Zhou XH, Fan HM, Liu ZM, Neelapu SS, Dong C. Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. Nature medicine. 2011; 17(8):983–988.
61. Sage PT, Sharpe AH. T follicular regulatory cells. Immunological reviews. 2016; 271(1):246–259. [PubMed: 27088919]
62. Zhang CJ, Gong Y, Zhu W, Qi Y, Yang CS, Fu Y, Chang G, Li Y, Shi S, Wood K, Ladha S, Shi FD, Liu Q, Yan Y. Augmentation of Circulating Follicular Helper T Cells and Their Impact on Autoreactive B Cells in Myasthenia Gravis. Journal of immunology. 2016; 197(7):2610–2617.
63. Wen Y, Yang B, Lu J, Zhang J, Yang H, Li J. Imbalance of circulating CD4(+)CXCR5(+)FOXP3(+) Tfr-like cells and CD4(+)CXCR5(+)FOXP3(-) Tfh-like cells in myasthenia gravis. Neuroscience letters. 2016; 630:176–182. [PubMed: 27473945]
64. Nastuk WL, Strauss AJ, Osserman KE. Search for a neuromuscular blocking agent in the blood of patients with myasthenia gravis. Am J Med. 1959; 26(3):394–409. [PubMed: 13626994]
65. Bergh NP. Biologic assays in myasthenia gravis for any agents causing a neuromuscular block. Scand J Clin Lab Invest. 1953; 5(Suppl 5):5–47.
66. Shen C, Lu Y, Zhang B, Figueiredo D, Bean J, Jung J, Wu H, Barik A, Yin DM, Xiong WC, Mei L. Antibodies against low-density lipoprotein receptor-related protein 4 induce myasthenia gravis. J Clin Invest. 2013; 123(12):5190–5202. [PubMed: 24200689]
67. Binks S, Vincent A, Palace J. Myasthenia gravis: a clinical-immunological update. J Neurol. 2016; 263(4):826–834. [PubMed: 26705120]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

68. Gilhus NE, Skeie GO, Romi F, Lazaridis K, Zisimopoulou P, Tzartos S. Myasthenia gravis - autoantibody characteristics and their implications for therapy. Nat Rev Neurol. 2016; 12(5):259–268. [PubMed: 27103470]
69. Huda S, Waters P, Woodhall M, Leite MI, Jacobson L, De Rosa A, Maestri M, Ricciardi R, Heckmann JM, Maniaol A, Evoli A, Cossins J, Hilton-Jones D, Vincent A. IgG-specific cell-based assay detects potentially pathogenic MuSK-Abs in seronegative MG. Neurol Neuroimmunol Neuroinflamm. 2017; 4(4):e357. [PubMed: 28626780]
70. Leite MI, Jacob S, Viegas S, Cossins J, Clover L, Morgan BP, Beeson D, Willcox N, Vincent A. IgG1 antibodies to acetylcholine receptors in 'seronegative' myasthenia gravis. Brain. 2008; 131(Pt 7):1940–1952. [PubMed: 18515870]
71. Devic P, Petiot P, Simonet T, Stojkovic T, Delmont E, Franques J, Magot A, Vial C, Lagrange E, Nicot AS, Risson V, Eymard B, Schaeffer L. Antibodies to clustered acetylcholine receptor: expanding the phenotype. Eur J Neurol. 2014; 21(1):130–134. [PubMed: 24112557]
72. Cortes-Vicente E, Gallardo E, Martinez MA, Diaz-Manera J, Querol L, Rojas-Garcia R, Illa I. Clinical Characteristics of Patients With Double-Seronegative Myasthenia Gravis and Antibodies to Cortactin. JAMA Neurol. 2016; 73(9):1099–1104. [PubMed: 27379450]
73. Kaminski HJ. Seronegative Myasthenia Gravis-A Vanishing Disorder? JAMA Neurol. 2016; 73(9): 1055–1056. [PubMed: 27380019]
74. Hong Y, Zisimopoulou P, Trakas N, Karagiorgou K, Stergiou C, Skeie GO, Hao HJ, Gao X, Owe JF, Zhang X, Yue YX, Romi F, Wang Q, Li HF, Gilhus NE, Tzartos SJ. Multiple antibody detection in 'seronegative' myasthenia gravis patients. Eur J Neurol. 2017; 24(6):844–850. [PubMed: 28470860]
75. Rodgaard A, Nielsen FC, Djurup R, Somnier F, Gammeltoft S. Acetylcholine receptor antibody in myasthenia gravis: predominance of IgG subclasses 1 and 3. Clin Exp Immunol. 1987; 67(1):82–88. [PubMed: 3621677]
76. Nakano S, Engel AG. Myasthenia gravis: quantitative immunocytochemical analysis of inflammatory cells and detection of complement membrane attack complex at the end-plate in 30 patients. Neurology. 1993; 43(6):1167–1172. [PubMed: 8170563]
77. Lefvert AK, Cuenoud S, Fulpius BW. Binding properties and subclass distribution of anti-acetylcholine receptor antibodies in myasthenia gravis. J Neuroimmunol. 1981; 1(1):125–135. [PubMed: 6799544]
78. Engel AG, Sakakibara H, Sahashi K, Lindstrom JM, Lambert EH, Lennon VA. Passively transferred experimental autoimmune myasthenia gravis. Sequential and quantitative study of the motor end-plate fine structure and ultrastructural localization of immune complexes (IgG and C3), and of the acetylcholine receptor. Neurology. 1979; 29(2):179–188. [PubMed: 571062]
79. Engel AG, Lambert EH, Howard FM. Immune complexes (IgG and C3) at the motor end-plate in myasthenia gravis: ultrastructural and light microscopic localization and electrophysiologic correlations. Mayo Clin Proc. 1977; 52(5):267–280. [PubMed: 870771]
80. Hara H, Hayashi K, Ohta K, Itoh N, Nishitani H, Ohta M. Detection and characterization of blocking-type anti-acetylcholine receptor antibodies in sera from patients with myasthenia gravis. Clin Chem. 1993; 39(10):2053–2057. [PubMed: 8403390]
81. Whiting PJ, Vincent A, Newsom-Davis J. Acetylcholine receptor antibody characteristics in myasthenia gravis. Fractionation of alpha-bungarotoxin binding site antibodies and their relationship to IgG subclass. J Neuroimmunol. 1983; 5(1):1–9. [PubMed: 6874919]
82. Almon RR, Andrew CG, Appel SH. Serum globulin in myasthenia gravis: inhibition of alpha-bungarotoxin binding to acetylcholine receptors. Science. 1974; 186(4158):55–57. [PubMed: 4421998]
83. Drachman DB, Angus CW, Adams RN, Michelson JD, Hoffman GJ. Myasthenic antibodies cross-link acetylcholine receptors to accelerate degradation. The New England journal of medicine. 1978; 298(20):1116–1122. [PubMed: 643030]
84. Loutrari H, Kokla A, Tzartos SJ. Passive transfer of experimental myasthenia gravis via antigenic modulation of acetylcholine receptor. European journal of immunology. 1992; 22(9):2449–2452. [PubMed: 1516631]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

85. Punga AR, Lin S, Oliveri F, Meinen S, Ruegg MA. Muscle-selective synaptic disassembly and reorganization in MuSK antibody positive MG mice. Experimental neurology. 2011; 230(2):207–217. [PubMed: 21565192]
86. Klooster R, Plomp JJ, Huijbers MG, Niks EH, Straasheijm KR, Detmers FJ, Hermans PW, Sleijpen K, Verrips A, Losen M, Martinez-Martinez P, De Baets MH, van der Maarel SM, Verschuuren JJ. Muscle-specific kinase myasthenia gravis IgG4 autoantibodies cause severe neuromuscular junction dysfunction in mice. Brain. 2012; 135(Pt 4):1081–1101. [PubMed: 22396395]
87. Niks EH, van Leeuwen Y, Leite MI, Dekker FW, Wintzen AR, Wirtz PW, Vincent A, van Tol MJ, Jol-van der Zijde CM, Verschuuren JJ. Clinical fluctuations in MuSK myasthenia gravis are related to antigen-specific IgG4 instead of IgG1. J Neuroimmunol. 2008; 195(1–2):151–156. [PubMed: 18384886]
88. Koneczny I, Cossins J, Waters P, Beeson D, Vincent A. MuSK myasthenia gravis IgG4 disrupts the interaction of LRP4 with MuSK but both IgG4 and IgG1–3 can disperse preformed agrin-independent AChR clusters. PLoS One. 2013; 8(11):e80695. [PubMed: 24244707]
89. Ohno K, Ito M, Kawakami Y, Krejci E, Engel AG. Specific binding of collagen Q to the neuromuscular junction is exploited to cure congenital myasthenia and to explore bases of myasthenia gravis. Chem Biol Interact. 2013; 203(1):335–340. [PubMed: 22981737]
90. Ohno K, Otsuka K, Ito M. Roles of collagen Q in MuSK antibody-positive myasthenia gravis. Chem Biol Interact. 2016; 259(Pt B):266–270. [PubMed: 27119269]
91. van der Neut Kolfschoten M, Schuurman J, Losen M, Bleeker WK, Martinez-Martinez P, Vermeulen E, den Bleker TH, Wiegman L, Vink T, Aarden LA, De Baets MH, van de Winkel JG, Aalberse RC, Parren PW. Anti-inflammatory activity of human IgG4 antibodies by dynamic Fab arm exchange. Science. 2007; 317(5844):1554–1557. [PubMed: 17872445]
92. Koneczny I, Stevens JA, De Rosa A, Huda S, Huijbers MG, Saxena A, Maestri M, Lazaridis K, Zisimopoulou P, Tzartos S, Verschuuren J, van der Maarel SM, van Damme P, De Baets MH, Molenaar PC, Vincent A, Ricciardi R, Martinez-Martinez P, Losen M. IgG4 autoantibodies against muscle-specific kinase undergo Fab-arm exchange in myasthenia gravis patients. Journal of autoimmunity. 2016
93. Romi F, Suzuki S, Suzuki N, Petzold A, Plant GT, Gilhus NE. Anti-voltage-gated potassium channel Kv1. 4 antibodies in myasthenia gravis. J Neurol. 2012; 259(7):1312–1316. [PubMed: 22167224]
94. Higuchi O, Hamuro J, Motomura M, Yamanashi Y. Autoantibodies to low-density lipoprotein receptor-related protein 4 in myasthenia gravis. Ann Neurol. 2011; 69(2):418–422. [PubMed: 21387385]
95. Pevzner A, Schoser B, Peters K, Cosma NC, Karakatsani A, Schalke B, Melms A, Kroger S. Anti-LRP4 autoantibodies in AChR- and MuSK-antibody-negative myasthenia gravis. J Neurol. 2012; 259(3):427–435. [PubMed: 21814823]
96. Zhang B, Tzartos JS, Belimezi M, Ragheb S, Bealmear B, Lewis RA, Xiong WC, Lisak RP, Tzartos SJ, Mei L. Autoantibodies to lipoprotein-related protein 4 in patients with double-seronegative myasthenia gravis. Arch Neurol. 2012; 69(4):445–451. [PubMed: 22158716]
97. Gasperi C, Melms A, Schoser B, Zhang Y, Meltoranta J, Risson V, Schaeffer L, Schalke B, Kroger S. Anti-agrin autoantibodies in myasthenia gravis. Neurology. 2014; 82(22):1976–1983. [PubMed: 24793185]
98. Gallardo E, Martinez-Hernandez E, Titulaer MJ, Huijbers MG, Martinez MA, Ramos A, Querol L, Diaz-Manera J, Rojas-Garcia R, Hayworth CR, Verschuuren JJ, Balice-Gordon R, Dalmau J, Illa I. Cortactin autoantibodies in myasthenia gravis. Autoimmun Rev. 2014; 13(10):1003–1007. [PubMed: 25193850]
99. Zoltowska Katarzyna M, Belaya K, Leite M, Patrick W, Vincent A, Beeson D. Collagen Q--a potential target for autoantibodies in myasthenia gravis. J Neurol Sci. 2015; 348(1–2):241–244. [PubMed: 25577314]
100. Zhang B, Shen C, Bealmear B, Ragheb S, Xiong WC, Lewis RA, Lisak RP, Mei L. Autoantibodies to agrin in myasthenia gravis patients. PLoS One. 2014; 9(3):e91816. [PubMed: 24632822]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

101. Stergiou C, Lazaridis K, Zouvelou V, Tzartos J, Mantegazza R, Antozzi C, Andreetta F, Evoli A, Deymeer F, Saruhan-Direskeneli G, Durmus H, Brenner T, Vaknin A, Berrih-Aknin S, Behin A, Sharshar T, De Baets M, Losen M, Martinez-Martinez P, Kleopa KA, Zamba-Papanicolaou E, Kyriakides T, Kostera-Pruszczyk A, Szczudlik P, Szyluk B, Lavrnic D, Basta I, Peric S, Tallaksen C, Maniaol A, Gilhus NE, Casasnovas Pons C, Pitha J, Jakubikova M, Hanisch F, Bogomolovas J, Labeit D, Labeit S, Tzartos SJ. Titin antibodies in "seronegative" myasthenia gravis--A new role for an old antigen. J Neuroimmunol. 2016; 292:108–115. [PubMed: 26943968]
102. Corsiero E, Nerviani A, Bombardieri M, Pitzalis C. Ectopic Lymphoid Structures: Powerhouse of Autoimmunity. Front Immunol. 2016; 7:430. [PubMed: 27799933]
103. Humby F, Bombardieri M, Manzo A, Kelly S, Blades MC, Kirkham B, Spencer J, Pitzalis C. Ectopic lymphoid structures support ongoing production of class-switched autoantibodies in rheumatoid synovium. PLoS Med. 2009; 6(1):e1.
104. Mittag T, Kornfeld P, Tormay A, Woo C. Detection of anti-acetylcholine receptor factors in serum and thymus from patients with myasthenia gravis. N Engl J Med. 1976; 294(13):691–694. [PubMed: 1250280]
105. Vincent A, Scadding GK, Thomas HC, Newsom-Davis J. In-vitro synthesis of anti-acetylcholine-receptor antibody by thymic lymphocytes in myasthenia gravis. Lancet. 1978; 1(8059):305–307. [PubMed: 75339]
106. Marx A, Pfister F, Schalke B, Saruhan-Direskeneli G, Melms A, Strobel P. The different roles of the thymus in the pathogenesis of the various myasthenia gravis subtypes. Autoimmun Rev. 2013; 12(9):875–884. [PubMed: 23535159]
107. Newsom-Davis J, Willcox N, Calder L. Thymus cells in myasthenia gravis selectively enhance production of anti-acetylcholine-receptor antibody by autologous blood lymphocytes. N Engl J Med. 1981; 305(22):1313–1318. [PubMed: 7290153]
108. Leprince C, Cohen-Kaminsky S, Berrih-Aknin S, Vernet-Der Garabedian B, Treton D, Galanaud P, Richard Y. Thymic B cells from myasthenia gravis patients are activated B cells. Phenotypic and functional analysis J Immunol. 1990; 145(7):2115–2122. [PubMed: 2144544]
109. Berrih-Aknin S, Le Panse R. Myasthenia gravis: a comprehensive review of immune dysregulation and etiological mechanisms. J Autoimmun. 2014; 52:90–100. [PubMed: 24389034]
110. Willcox HN, Newsom-Davis J, Calder LR. Cell types required for anti-acetylcholine receptor antibody synthesis by cultured thymocytes and blood lymphocytes in myasthenia gravis. Clin Exp Immunol. 1984; 58(1):97–106. [PubMed: 6236921]
111. Scadding GK, Vincent A, Newsom-Davis J, Henry K. Acetylcholine receptor antibody synthesis by thymic lymphocytes: correlation with thymic histology. Neurology. 1981; 31(8):935–943. [PubMed: 6973710]
112. Lisak RP, Levinson AI, Zweiman B, Kornstein MJ. Antibodies to acetylcholine receptor and tetanus toxoid: in vitro synthesis by thymic lymphocytes. J Immunol. 1986; 137(4):1221–1225. [PubMed: 3488346]
113. Levinson AI, Zweiman B, Lisak RP, Dziarski A, Moskovitz AR. Thymic B-cell activation in myasthenia gravis. Neurology. 1984; 34(4):462–468. [PubMed: 6608064]
114. Sims GP, Shiono H, Willcox N, Stott DI. Somatic hypermutation and selection of B cells in thymic germinal centers responding to acetylcholine receptor in myasthenia gravis. J Immunol. 2001; 167(4):1935–1944. [PubMed: 11489973]
115. Vrolix K, Fraussen J, Losen M, Stevens J, Lazaridis K, Molenaar PC, Somers V, Bracho MA, Le Panse R, Stinissen P, Berrih-Aknin S, Maessen JG, Van Garsse L, Buurman WA, Tzartos SJ, De Baets MH, Martinez-Martinez P. Clonal heterogeneity of thymic B cells from early-onset myasthenia gravis patients with antibodies against the acetylcholine receptor. J Autoimmun. 2014; 52:101–112. [PubMed: 24439114]
116. Graus YF, de Baets MH, Parren PW, Berrih-Aknin S, Wokke J, van Breda Vriesman PJ, Burton DR. Human anti-nicotinic acetylcholine receptor recombinant Fab fragments isolated from thymus-derived phage display libraries from myasthenia gravis patients reflect predominant specificities in serum and block the action of pathogenic serum antibodies. J Immunol. 1997; 158(4):1919–1929. [PubMed: 9029134]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

117. Cardona A, Pritsch O, Dumas G, Bach JF, Dighiero G. Evidence for an antigen-driven selection process in human autoantibodies against acetylcholine receptor. Mol Immunol. 1995; 32(16): 1215–1223. [PubMed: 8559146]

118. Farrar J, Portolano S, Willcox N, Vincent A, Jacobson L, Newsom-Davis J, Rapoport B, McLachlan SM. Diverse Fab specific for acetylcholine receptor epitopes from a myasthenia gravis thymus combinatorial library. Int Immunol. 1997; 9(9):1311–1318. [PubMed: 9310834]

119. Zuckerman NS, Howard WA, Bismuth J, Gibson K, Edelman H, Berrih-Aknin S, Dunn-Walters D, Mehr R. Ectopic GC in the thymus of myasthenia gravis patients show characteristics of normal GC. Eur J Immunol. 2010; 40(4):1150–1161. [PubMed: 20104489]

120. Hill ME, Shiono H, Newsom-Davis J, Willcox N. The myasthenia gravis thymus: a rare source of human autoantibody-secreting plasma cells for testing potential therapeutics. J Neuroimmunol. 2008; 201–202:50–56.

121. Wolfe GI, Kaminski HJ, Aban IB, Minisman G, Kuo HC, Marx A, Strobel P, Mazia C, Oger J, Cea JG, Heckmann JM, Evoli A, Nix W, Ciafaloni E, Antonini G, Witoonpanich R, King JO, Beydoun SR, Chalk CH, Barboi AC, Amato AA, Shaibani AI, Katirji B, Lecky BR, Buckley C, Vincent A, Dias-Tosta E, Yoshikawa H, Waddington-Cruz M, Pulley MT, Rivner MH, Kostera-Pruszczyk A, Pascuzzi RM, Jackson CE, Garcia Ramos GS, Verschuuren JJ, Massey JM, Kissel JT, Werneck LC, Benatar M, Barohn RJ, Tandan R, Mozaffar T, Conwit R, Odenkirchen J, Sonett JR, Jaretzki A 3rd, Newsom-Davis J, Cutter GR, Group MS. Randomized Trial of Thymectomy in Myasthenia Gravis. N Engl J Med. 2016; 375(6):511–522. [PubMed: 27509100]

122. Vincent A, Newsom-Davis J, Newton P, Beck N. Acetylcholine receptor antibody and clinical response to thymectomy in myasthenia gravis. Neurology. 1983; 33(10):1276–1282. [PubMed: 6684222]

123. Okumura M, Ohta M, Takeuchi Y, Shiono H, Inoue M, Fukuhara K, Kadota Y, Miyoshi S, Fujii Y, Matsuda H. The immunologic role of thymectomy in the treatment of myasthenia gravis: implication of thymus-associated B-lymphocyte subset in reduction of the anti-acetylcholine receptor antibody titer. J Thorac Cardiovasc Surg. 2003; 126(6):1922–1928. [PubMed: 14688707]

124. Newsom-Davis J, Willcox N, Scadding G, Calder L, Vincent A. Anti-acetylcholine receptor antibody synthesis by cultured lymphocytes in myasthenia gravis: thymic and peripheral blood cell interactions. Ann N Y Acad Sci. 1981; 377:393–402. [PubMed: 6978662]

125. Fujii Y, Monden Y, Hashimoto J, Nakahara K, Kawashima Y. Acetylcholine receptor antibody-producing cells in thymus and lymph nodes in myasthenia gravis. Clin Immunol Immunopathol. 1985; 34(1):141–146. [PubMed: 4038384]

126. Fujii Y, Monden Y, Hashimoto J, Nakahara K, Kawashima Y. Acetylcholine receptor antibody production by bone marrow cells in a patient with myasthenia gravis. Neurology. 1985; 35(4): 577–579. [PubMed: 3982648]

127. Fujii Y, Hashimoto J, Monden Y, Ito T, Nakahara K, Kawashima Y. Specific activation of lymphocytes against acetylcholine receptor in the thymus in myasthenia gravis. J Immunol. 1986; 136(3):887–891. [PubMed: 2416838]

128. Diaz-Manera J, Martinez-Hernandez E, Querol L, Klooster R, Rojas-Garcia R, Suarez-Calvet X, Munoz-Blanco JL, Mazia C, Straasheijm KR, Gallardo E, Juarez C, Verschuuren JJ, Illa I. Long-lasting treatment effect of rituximab in MuSK myasthenia. Neurology. 2012; 78(3):189–193. [PubMed: 22218276]

129. Rowland LP, Hoefer PF, Aranow H Jr, Merritt HH. Fatalities in myasthenia gravis; a review of 39 cases with 26 autopsies. Neurology. 1956; 6(5):307–326. [PubMed: 13309613]

130. Russell DS. Histological changes in the striped muscles in myasthenia gravis. J Pathol Bacteriol. 1953; 65(2):279–289. [PubMed: 13062030]

131. Pascuzzi RM, Campa JF. Lymphorrhage localized to the muscle end-plate in myasthenia gravis. Arch Pathol Lab Med. 1988; 112(9):934–937. [PubMed: 3415445]

132. Maselli RA, Richman DP, Wollmann RL. Inflammation at the neuromuscular junction in myasthenia gravis. Neurology. 1991; 41(9):1497–1504. [PubMed: 1891103]

133. Vander Heiden JA, Stathopoulos P, Zhou JQ, Chen L, Gilbert TJ, Bolen CR, Barohn RJ, Dimachkie MM, Ciafaloni E, Broering TJ, Vigneault F, Nowak RJ, Kleinstein SH, O'Connor

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

KC. Dysregulation of B Cell Repertoire Formation in Myasthenia Gravis Patients Revealed through Deep Sequencing. J Immunol. 2017; 198(4):1460–1473. [PubMed: 28087666]

134. Leite MI, Strobel P, Jones M, Micklem K, Moritz R, Gold R, Niks EH, Berrih-Aknin S, Scaravilli F, Canelhas A, Marx A, Newsom-Davis J, Willcox N, Vincent A. Fewer thymic changes in MuSK antibody-positive than in MuSK antibody-negative MG. Ann Neurol. 2005; 57(3):444–448. [PubMed: 15732104]
135. Claes N, Fraussen J, Vanheusden M, Hellings N, Stinissen P, Van Wijmeersch B, Hupperts R, Somers V. Age-Associated B Cells with Proinflammatory Characteristics Are Expanded in a Proportion of Multiple Sclerosis Patients. J Immunol. 2016; 197(12):4576–4583. [PubMed: 27837111]
136. Wei C, Anolik J, Cappione A, Zheng B, Pugh-Bernard A, Brooks J, Lee EH, Milner EC, Sanz I. A new population of cells lacking expression of CD27 represents a notable component of the B cell memory compartment in systemic lupus erythematosus. J Immunol. 2007; 178(10):6624–6633. [PubMed: 17475894]
137. Kohler S, Keil TO, Swierzy M, Hoffmann S, Schaffert H, Ismail M, Ruckert JC, Alexander T, Hiepe F, Gross C, Thiel A, Meisel A. Disturbed B cell subpopulations and increased plasma cells in myasthenia gravis patients. J Neuroimmunol. 2013
138. Ragheb S, Lisak RP. The frequency of CD5+ B lymphocytes in the peripheral blood of patients with myasthenia gravis. Neurology. 1990; 40(7):1120–1124. [PubMed: 1694020]
139. Tedder TF. B10 cells: a functionally defined regulatory B cell subset. Journal of immunology. 2015; 194(4):1395–1401.
140. Rosser EC, Mauri C. Regulatory B cells: origin, phenotype, and function. Immunity. 2015; 42(4): 607–612. [PubMed: 25902480]
141. Lykken JM, Candando KM, Tedder TF. Regulatory B10 cell development and function. International immunology. 2015; 27(10):471–477. [PubMed: 26254185]
142. Iwata Y, Matsushita T, Horikawa M, Dilillo DJ, Yanaba K, Venturi GM, Szabolcs PM, Bernstein SH, Magro CM, Williams AD, Hall RP, St Clair EW, Tedder TF. Characterization of a rare IL-10-competent B-cell subset in humans that parallels mouse regulatory B10 cells. Blood. 2011; 117(2):530–541. [PubMed: 20962324]
143. Das A, Ellis G, Pallant C, Lopes AR, Khanna P, Peppa D, Chen A, Blair P, Dusheiko G, Gill U, Kennedy PT, Brunetto M, Lampertico P, Mauri C, Maini MK. IL-10-producing regulatory B cells in the pathogenesis of chronic hepatitis B virus infection. Journal of immunology. 2012; 189(8): 3925–3935.
144. Flores-Borja F, Bosma A, Ng D, Reddy V, Ehrenstein MR, Isenberg DA, Mauri C. CD19+CD24hiCD38hi B cells maintain regulatory T cells while limiting TH1 and TH17 differentiation. Science translational medicine. 2013; 5(173):173ra123.
145. van de Veen W, Stanic B, Yaman G, Wawrzyniak M, Sollner S, Akdis DG, Ruckert B, Akdis CA, Akdis M. IgG4 production is confined to human IL-10-producing regulatory B cells that suppress antigen-specific immune responses. J Allergy Clin Immunol. 2013; 131(4):1204–1212. [PubMed: 23453135]
146. Matsumoto M, Fujii Y, Baba A, Hikida M, Kurosaki T, Baba Y. The calcium sensors STIM1 and STIM2 control B cell regulatory function through interleukin-10 production. Immunity. 2011; 34(5):703–714. [PubMed: 21530328]
147. Sun F, Ladha SS, Yang L, Liu Q, Shi SX, Su N, Bomprezzi R, Shi FD. Interleukin-10 producing-B cells and their association with responsiveness to rituximab in myasthenia gravis. Muscle Nerve. 2014; 49(4):487–494. [PubMed: 23868194]
148. Yi JS, Russo MA, Massey JM, Juel V, Hobson-Webb LD, Gable K, Raja SM, Balderson K, Weinhold KJ, Guptill JT. B10 Cell Frequencies and Suppressive Capacity in Myasthenia Gravis Are Associated with Disease Severity. Front Neurol. 2017; 8:34. [PubMed: 28239367]
149. Guptill JT, Yi JS, Sanders DB, Guidon AC, Juel VC, Massey JM, Howard JF Jr, Scuderi F, Bartoccioni E, Evoli A, Weinhold KJ. Characterization of B cells in muscle-specific kinase antibody myasthenia gravis. Neurol Neuroimmunol Neuroinflamm. 2015; 2(2):e77. [PubMed: 25745635]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

150. Sheng JR, Quan S, Soliven B. IL-10 derived from CD1dhiCD5(+) B cells regulates experimental autoimmune myasthenia gravis. Journal of neuroimmunology. 2015; 289:130–138. [PubMed: 26616882]
151. Sheng JR, Rezania K, Soliven B. Impaired regulatory B cells in myasthenia gravis. Journal of neuroimmunology. 2016; 297:38–45. [PubMed: 27397074]
152. Meffre E, Wardemann H. B-cell tolerance checkpoints in health and autoimmunity. Curr Opin Immunol. 2008; 20(6):632–638. [PubMed: 18848883]
153. Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. Predominant autoantibody production by early human B cell precursors. Science. 2003; 301(5638):1374–1377. [PubMed: 12920303]
154. Samuels J, Ng YS, Coupillaud C, Paget D, Meffre E. Impaired early B cell tolerance in patients with rheumatoid arthritis. J Exp Med. 2005; 201(10):1659–1667. [PubMed: 15897279]
155. Yurasov S, Wardemann H, Hammersen J, Tsuiji M, Meffre E, Pascual V, Nussenzweig MC. Defective B cell tolerance checkpoints in systemic lupus erythematosus. J Exp Med. 2005; 201(5):703–711. [PubMed: 15738055]
156. Lee J-Y, Stathopoulos P, Gupta S, Bannock JM, Barohn RJ, Cotzomi E, Dimachkie MM, Jacobson L, Lee CS, Morbach H, Querol L, Shan J-L, Vander Heiden JA, Waters P, Vincent A, Nowak RJ, O'Connor KC. Compromised fidelity of B-cell tolerance checkpoints in AChR and MuSK myasthenia gravis. Annals of Clinical and Translational Neurology. 2016; 3(6):443–454. [PubMed: 27547772]
157. Glanville J, Zhai W, Berka J, Telman D, Huerta G, Mehta GR, Ni I, Mei L, Sundar PD, Day GMR, Cox D, Rajpal A, Pons J. Precise determination of the diversity of a combinatorial antibody library gives insight into the human immunoglobulin repertoire. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:20216–20221. [PubMed: 19875695]
158. Boye J, Elter T, Engert A. An overview of the current clinical use of the anti-CD20 monoclonal antibody rituximab. Ann Oncol. 2003; 14(4):520–535. [PubMed: 12649096]
159. Dorner T, Isenberg D, Jayne D, Wiendl H, Zillikens D, Burmester G. International Roundtable on BcaTTfI. Current status on B-cell depletion therapy in autoimmune diseases other than rheumatoid arthritis. Autoimmun Rev. 2009; 9(2):82–89. [PubMed: 19716441]
160. Gajra A, Vajpayee N, Grethlein SJ. Response of myasthenia gravis to rituximab in a patient with non-Hodgkin lymphoma. Am J Hematol. 2004; 77(2):196–197. [PubMed: 15389902]
161. Lebrun C, Bourg V, Tieulie N, Thomas P. Successful treatment of refractory generalized myasthenia gravis with rituximab. Eur J Neurol. 2009; 16(2):246–250. [PubMed: 19146644]
162. Illa I, Diaz-Manera J, Rojas-Garcia R, Pradas J, Rey A, Blesa R, Juarez C, Gallardo E. Sustained response to Rituximab in anti-AChR and anti-MuSK positive Myasthenia Gravis patients. J Neuroimmunol. 2008; 201–202:90–94.
163. Nowak RJ, Dicapua DB, Zebardast N, Goldstein JM. Response of patients with refractory myasthenia gravis to rituximab: a retrospective study. Ther Adv Neurol Disord. 2011; 4(5):259–266. [PubMed: 22010039]
164. Zebardast N, Patwa HS, Novella SP, Goldstein JM. Rituximab in the management of refractory myasthenia gravis. Muscle Nerve. 2010; 41(3):375–378. [PubMed: 19852027]
165. Robeson KR, Kumar A, Keung B, DiCapua DB, Grodinsky E, Patwa HS, Stathopoulos PA, Goldstein JM, O'Connor KC, Nowak RJ. Durability of the Rituximab Response in Acetylcholine Receptor Autoantibody-Positive Myasthenia Gravis. JAMA Neurol. 2016
166. Chamberlain N, Massad C, Oe T, Cantaert T, Herold KC, Meffre E. Rituximab does not reset defective early B cell tolerance checkpoints. J Clin Invest. 2016; 126(1):282–287. [PubMed: 26642366]
167. Pellkofer HL, Krumbholz M, Berthele A, Hemmer B, Gerdes LA, Havla J, Bittner R, Canis M, Meinl E, Hohlfeld R, Kuempfel T. Long-term follow-up of patients with neuromyelitis optica after repeated therapy with rituximab. Neurology. 2011; 76(15):1310–1315. [PubMed: 21482945]
168. Quach TD, Rodriguez-Zhurbenko N, Hopkins TJ, Guo X, Hernandez AM, Li W, Rothstein TL. Distinctions among Circulating Antibody-Secreting Cell Populations, Including B-1 Cells, in Human Adult Peripheral Blood. J Immunol. 2016; 196(3):1060–1069. [PubMed: 26740107]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

169. Lanzillotta M, Della-Torre E, Stone JH. Roles of Plasmablasts and B Cells in IgG4-Related Disease: Implications for Therapy and Early Treatment Outcomes. Curr Top Microbiol Immunol. 2017

170. Stathopoulos P, Kumar A, Nowak RJ, O'Connor KC. Post B cell depletion autoantibody-producing plasmablasts identified in muscle-specific kinase myasthenia gravis. J Clin Invest Insight. 2017 in press.

171. Chihara N, Aranami T, Oki S, Matsuoka T, Nakamura M, Kishida H, Yokoyama K, Kuroiwa Y, Hattori N, Okamoto T, Murata M, Toda T, Miyake S, Yamamura T. Plasmablasts as migratory IgG-producing cells in the pathogenesis of neuromyelitis optica. PLoS One. 2013; 8(12):e83036. [PubMed: 24340077]

172. Chihara N, Aranami T, Sato W, Miyazaki Y, Miyake S, Okamoto T, Ogawa M, Toda T, Yamamura T. Interleukin 6 signaling promotes anti-aquaporin 4 autoantibody production from plasmablasts in neuromyelitis optica. Proc Natl Acad Sci U S A. 2011; 108(9):3701–3706. [PubMed: 21321193]

173. Hottinger AF. Neurologic complications of immune checkpoint inhibitors. Curr Opin Neurol. 2016; 29(6):806–812. [PubMed: 27653290]

174. Sharpe AH, Freeman GJ. The B7-CD28 superfamily. Nat Rev Immunol. 2002; 2(2):116–126. [PubMed: 11910893]

175. Zou W, Wolchok JD, Chen L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: Mechanisms, response biomarkers, and combinations. Sci Transl Med. 2016; 8(328):328rv324.

176. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, Pitot HC, Hamid O, Bhatia S, Martins R, Eaton K, Chen S, Salay TM, Alaparthy S, Grosso JF, Korman AJ, Parker SM, Agrawal S, Goldberg SM, Pardoll DM, Gupta A, Wigginton JM. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. The New England journal of medicine. 2012; 366(26):2455–2465. [PubMed: 22658128]

177. Johnson DB, Saranga-Perry V, Lavin PJ, Burnette WB, Clark SW, Uskavitch DR, Wallace DE, Dickson MA, Kudchadkar RR, Sosman JA. Myasthenia Gravis Induced by Ipilimumab in Patients With Metastatic Melanoma. J Clin Oncol. 2015; 33(33):e122–124. [PubMed: 24778401]

178. Antonia S, Goldberg SB, Balmanoukian A, Chaft JE, Sanborn RE, Gupta A, Narwal R, Steele K, Gu Y, Karakunnel JJ, Rizvi NA. Safety and antitumour activity of durvalumab plus tremelimumab in non-small cell lung cancer: a multicentre, phase 1b study. Lancet Oncol. 2016; 17(3):299–308. [PubMed: 26858122]

179. Loochtan AI, Nickolich MS, Hobson-Webb LD. Myasthenia gravis associated with ipilimumab and nivolumab in the treatment of small cell lung cancer. Muscle & nerve. 2015; 52(2):307–308. [PubMed: 25759003]

180. Lau KH, Kumar A, Yang IH, Nowak RJ. Exacerbation of myasthenia gravis in a patient with melanoma treated with pembrolizumab. Muscle & nerve. 2016; 54(1):157–161. [PubMed: 27065302]

181. Maeda O, Yokota K, Atsuta N, Katsuno M, Akiyama M, Ando Y. Nivolumab for the treatment of malignant melanoma in a patient with pre-existing myasthenia gravis. Nagoya J Med Sci. 2016; 78(1):119–122. [PubMed: 27019533]

182. Gonzalez NL, Puwanant A, Lu A, Marks SM, Zivkovic SA. Myasthenia triggered by immune checkpoint inhibitors: New case and literature review. Neuromuscul Disord. 2017; 27(3):266–268. [PubMed: 28109638]

183. Cooper DS, Meriggioli MN, Bonomi PD, Malik R. Severe Exacerbation of Myasthenia Gravis Associated with Checkpoint Inhibitor Immunotherapy. J Neuromuscul Dis. 2017; 4(2):169–173. [PubMed: 28505981]

184. Makarious D, Horwood K, Coward JIG. Myasthenia gravis: An emerging toxicity of immune checkpoint inhibitors. Eur J Cancer. 2017; 82:128–136. [PubMed: 28666240]

185. Hauser SL, Bar-Or A, Comi G, Giovannoni G, Hartung HP, Hemmer B, Lublin F, Montalban X, Rammohan KW, Selmaj K, Traboulsee A, Wolinsky JS, Arnold DL, Klingelschmitt G, Masterman D, Fontoura P, Belachew S, Chin P, Mairon N, Garren H, Kappos L, Opera I. Investigators OIC. Ocrelizumab versus Interferon Beta-1a in Relapsing Multiple Sclerosis. N Engl J Med. 2017; 376(3):221–234. [PubMed: 28002679]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

186. Ellebrecht CT, Bhoj VG, Nace A, Choi EJ, Mao X, Cho MJ, Di Zenzo G, Lanzavecchia A, Seykora JT, Cotsarelis G, Milone MC, Payne AS. Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. Science. 2016; 353(6295):179–184. [PubMed: 27365313]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

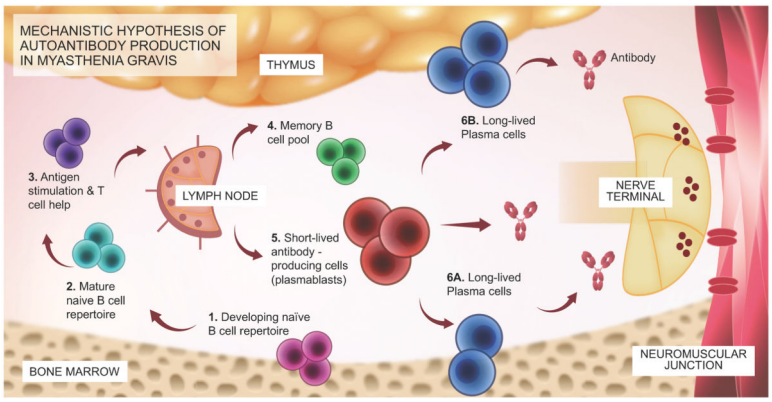

Figure 1.

Schematic diagram outlining the mechanistic hypothesis for the production of AChR or MuSK MG autoantibodies.

The proposed mechanistic path to autoantibody production in MG begins with naïve B cells (Steps 1 and 2), which likely encounter antigen(s) and receive T cell help in the lymph node (3). They then differentiate into memory B cells (4), antibody-secreting plasmablasts (5), and antibody-secreting long-lived plasma cells, which reside in the bone marrow (6A) and may also be present in the thymus (6B) of some patients with AChR MG. Plasmablasts and plasma cells may contribute to MG autoantibody production. B cell depletion therapy eliminates CD20+ memory and naïve B cells but does not directly eliminate plasmablasts or plasma cells, which are CD20-negative. After CD20-targeted depletion, MG serum autoantibody titers markedly diminish (especially in MuSK MG), suggesting that plasma cells are unlikely candidates for autoantibody production. Rather, short-lived plasmablasts are more viable candidates. As only a small fraction of these cells express CD20, the effectiveness of B cell depletion therapy may depend upon depletion of a pool of plasmablast-progenitor CD20+ memory B cells. Conversely, autoantibody titers that remain elevated following CD20-targeted depletion may be the product of long-lived plasma cells.