Autoimmune mechanisms in myasthenia gravis

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Purpose of review
This article reviews recent findings on factors and mechanisms implicated in the pathogenesis of myasthenia gravis and briefly summarizes data on therapies acting at various stages of the autoimmune process.

Recent findings
Data published over the last year promise to improve understanding of pathogenic mechanisms underlying myasthenia gravis. Animal studies have at last shown that antimuscle-specific kinase (MuSK) autoantibodies, like antiacetylcholine receptor (AChR) autoantibodies, are myasthenogenic. A new autoantigen, the low-density lipoprotein receptor-related protein 4 (LRP4), has been identified in variable proportions of otherwise seronegative patients. Anti-LRP4 antibodies may define a new myasthenia gravis subtype, supporting the concept that myasthenia gravis is not a single disease entity, and that different subtypes can differ in aetiology. Genetic and environmental factors are implicated in myasthenia gravis. The finding of persisting viral infection in the thymus of AChR-myasthenia gravis patients, combined with data on chronic inflammation, suggest that pathogens may favour intrathymic AChR-specific autosensitization and maintenance of autoimmunity in genetically susceptible individuals. Defective immunoregulatory mechanisms, involving pathogenic Th17 and regulatory T cells, contribute to tolerance loss and perpetuation of the autoimmune response in myasthenia gravis patients.

Summary
The recent identification of mechanisms initiating and perpetuating autoimmunity in myasthenia gravis may stimulate the development of more effective therapies.

Keywords
autoantibodies, autoimmunity, myasthenia gravis, thymus

INTRODUCTION

The pathogenic mechanisms causing autoimmunity in myasthenia gravis, an antibody-mediated disorder affecting the neuromuscular junction (NMJ), are incompletely understood. Most current treatments for myasthenia gravis do not lead to stable remission, highlighting the need to better understand events initiating and perpetuating the condition, in order to develop more effective therapies.

This review focuses on the following aspects of myasthenia gravis pathogenesis: autoimmune responses and associated clinical phenotypes; autoimmune processes at the NMJ; genetic and environmental triggers; intrathymic and peripheral mechanisms of loss of tolerance; and current and emerging therapies.

MYASTHENIA GRAVIS: A PROTOTYPIC BUT MULTIFACETED AUTOIMMUNE DISEASE

Myasthenia gravis is a prototypic humoural-mediated autoimmune disease. Autoantibodies to the acetylcholine receptor (AChR) are present in approximately 80% of patients [1] and cause the typical muscle weakness and fatigability. Defining criteria for autoimmune diseases [2] are fulfilled; in particular, active immunization of animals with AChR leads to experimental myasthenia gravis (EAMG); IgG and complement are present at the NMJ in which AChR is reduced; and disease symptoms occur in mice on injection of IgG from myasthenia gravis patients [1,3]. However, the clinical myasthenia gravis features vary widely and

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KEY POINTS

- A mechanism by which anti-MuSK autoantibodies exert their pathogenic effect at the NMJ has been described. If confirmed, this will have important implications for MuSK-myasthenia gravis therapy.
- The discovery of anti-LRP4 antibodies in a proportion of seronegative myasthenia gravis patients could lead to the identification of a new myasthenia gravis subtype and stimulate the search for specific therapies effective against this subtype.
- The autoimmune regulator AIRE appears to be involved in intrathymic immunoregulation by influencing the balance between pathogenic Th17 T cells and suppressive T-regulatory cells.
- Persisting viral infection has been identified in the thymus of AChR-positive myasthenia gravis patients, suggesting virus-mediated mechanisms in AChR-myasthenia gravis pathogenesis.

mechanisms leading to the production of anti-AChR antibodies are still unclear.

Variation in clinical and autoimmune characteristics

The heterogeneity of myasthenia gravis is manifest as follows:

(1) Variable autoantibody status, with some patients positive to antibodies against muscle endplate proteins other than AChR: approximately 5% have antibodies to muscle-specific kinase (MuSK) and approximately 10% are negative for AChR and MuSK (seronegative) [1]; approximately 66% of seronegative cases may have antibodies to clustered AChRs [4].
(2) Variable age of onset, with a female-dominant peak at less than 40 years (early-onset, EOMG) and male-dominant peak at more than 40 years (late-onset, LOMG) [5].
(3) Wide spectrum of symptoms, from ocular to generalized forms, with variation in disease progression [1].
(4) Variable thymic involvement. Although 50–60% of AChR-positive EOMG patients and some seronegative patients have follicular hyperplasia, and 10–15% of usually LOMG patients have thymoma, thymic disorder is absent from most MuSK-myasthenia gravis, and 10–20% of LOMG [6,7].
(5) Variable treatment response, mainly in relation to variable disease severity, but likely also to patient-specific genetic factors [8,9▪].

The clinical variability of myasthenia gravis allows classification into subtypes usually defined by autoantibody status, age at onset and thymic histology (Table 1), and suggests variation in pathogenic mechanisms. Although presence of anti-AChR antibody is useful for diagnosis, disease severity does not correlate with anti-AChR titre [10]. MuSK-myasthenia gravis patients have severe symptoms, with bulbar and respiratory muscle involvement, and resistance to therapy [11], whereas AChR-positive and seronegative patients frequently have generalized weakness [2].

A recent study found that peripheral blood lymphocytes from seronegative myasthenia gravis patients had proliferative responses to AChRα subunit peptides [12▪], suggesting that T-cell proliferation assay can usefully monitor AChR-specific autoimmune response in seronegative patients who have anti-AChR antibodies not detected by routine assay [4]. Most patients with thymoma have antibodies against titin, the ryanodine receptor, and the Kv1.4 α-subunit of voltage-gated potassium channels, as well as AChR. These autoantibodies are normally associated with severe disease [13,14] but a recent series of Kv1.4-positive Caucasian patients, mostly women, had mild LOMG, indicating that anti-Kv1.4 antibodies alone are not markers of severe myasthenia gravis, although they may predict lethal cardiac dysfunction and myocarditis [15▪].

Three recent independent studies [16▪▪–18▪▪] identified a new myasthenia gravis autoantigen, low-density lipoprotein receptor-related protein 4 (LRP4) – an agrin receptor that activates MuSK and participates in AChR clustering [19,20]. Variable proportions of seronegative myasthenia gravis populations from Greece, the US, Germany, and Japan have anti-LRP4 antibodies.

Antibodies to aquaporin 4 – the autoantigen in neuromyelitis optica expressed in the central nervous system (CNS) and at the NMJ – have been identified in myasthenia gravis patients with CNS involvement [21▪,22▪].

Autoimmune processes at the neuromuscular junction

AChR autoantibodies bind various subunits of the receptor, although most are specific for the main immunogenic region of the AChRα subunit [23] that is targeted in acute and chronic EAMG [24▪]. The foetal AChRγ subunit, expressed in extraocular muscles, may also induce an initially ocular autoimmune response progressing to generalized weakness [25]. Anti-AChR antibodies impair neuromuscular transmission mainly via

Table 1. Summary of autoimmune responses and clinical features in myasthenia gravis subtypes

	AChR-MG without thymoma	AChR-MG with thymoma	MuSK-MG	Seronegative-MG
Age at disease onset	Usually <40 years (EOMG); Less often >40 years (LOMG)	Usually >40 years (LOMG)	Usually <40 years (EOMG)	Usually <40 years (EOMG)
Sex	Predominantly women	Male : female ratio= ~1 : 1	Predominantly women	Predominantly women
Autoantigens	AChR in EOMG; AChR, titin, and RyR in LOMG	AChR, titin, RyR, Kv1.4, and other muscle proteins	MuSK	Abs not identified or low-affinity; anti-AChR Abs (~66%) or Abs to LRP4 (variable %)
Autoimmune processes at NMJ	Complement activation, endocytosis of cross-linked AChR molecules or blockage of AChR binding site by anti-AChR Abs	Complement activation, endocytosis of cross-linked AChR molecules or blockage of AChR binding site by anti-AChR Abs	Blocked binding of collagen Q to MuSK, no complement involvement	Unknown; Role of anti-LRP4 Abs unknown
Clinical presentation	Ocular form at onset that frequently progresses to generalized	Generalized form with or without bulbar involvement	Generalized MG with bulbar involvement and frequent crises	Similar to AChR-MG without thymoma
Conventional treatments	Anti-AChE, immunosuppression or thymectomy	Same as AChR-MG (thymectomy necessary)	Same as AChR-MG (no thymectomy)	Same as AChR-MG
Thymic histology	Hyperplasia in most EOMG; Thymic atrophy in most LOMG	Thymoma (hyperplasia common in nonneoplastic thymus)	Thymus resembles that of age-matched controls	Hyperplastic changes in variable proportions of cases

Abs, antibodies; AChR, acetylcholine receptor; EOMG, early-onset MG; Kv1.4, α-subunit of voltage-gated potassium channel; LOMG, late-onset MG; LRP4, low-density lipoprotein receptor-related protein 4; MG, myasthenia gravis; MuSK, muscle specific tyrosine kinase; NMJ, neuromuscular junction; RyR, ryanodine receptor.

complement-mediated destruction of the postsynaptic membrane [3].

The pathogenesis of MuSK-myasthenia gravis has begun to emerge recently. Anti-MuSK antibodies were shown to be pathogenic in animal studies that documented both presynaptic and postsynaptic NMJ changes [26▪,27▪]. Noninvolvement of the complement pathway was demonstrated when immunization of C5-deficient mice with MuSK caused severe muscle weakness and NMJ defects [28▪▪]. The likely binding site of anti-MuSK antibodies was identified by in-vitro assays and passive transfer of MuSK-IgG to mice [29▪▪]: autoantibodies blocked collagen Q binding to MuSK compromising agrin-mediated AChR clustering. Anti-MuSK IgG4 are the main antibodies in MuSK-myasthenia gravis. Passive transfer of anti-MuSK IgG4 to mice disrupted postsynaptic membrane ultrastructure and electrophysiological function, indicating that anti-MuSK IgG4 is myasthenogenic on its own [30▪].

A pathogenic role of anti-LRP4 autoantibodies is also likely, as they are mainly of the complement activator IgG1 subclass, inhibit interactions between agrin and LRP4, and reduce AChR clustering in in-vitro systems [16▪▪,17▪▪].

Genetic and environmental triggers

As with other autoimmune diseases, human leukocyte antigen (HLA) loci are implicated in myasthenia gravis susceptibility. The HLA-A1-B8-DR3 (8.1) haplotype has a strong but complex effect on phenotype in EOMG associated with thymic hyperplasia [31]. Some studies point to involvement of HLA-DQ alleles in myasthenia gravis: DQB1*0502 was associated with myasthenia gravis in an Italian population [32] and HLA-DR14-DQ5 may increase susceptibility to MuSK-myasthenia gravis [33]. A high resolution HLA-DR analysis of patients from Texas revealed HLA-DQ associations with EOMG not observed in Europe, suggesting that regional differences in genetic susceptibility may be affected by environmental factors interacting with HLA genes [34▪].

Several non-HLA loci are implicated in myasthenia gravis, including interferon-γ (IFN), cytotoxic lymphocyte-associated protein-4 (CTLA-4), the AChRα subunit (CHRNA1) [35], and protein tyrosine phosphatase nonreceptor-22 (PTPN22), a lymphocyte activation mediator. A large study on Italian myasthenia gravis patients showed that the PTPN22 rs2476601 allele, associated with several autoimmune conditions [36], and known to interfere with autoreactive B-cell removal, and increase T-cell and dendritic-cell responsiveness [37▪,38▪], is not associated with either myasthenia gravis, presence of autoantibodies, thymus abnormality sex, or age of myasthenia gravis onset [39], contrasting with previous findings in a French population [40]. However, the PTPN22 variant rs2488457, that disrupts a promoter binding site for AP-4

transcription factors, correlated with low antibody titres and mild disease, suggesting a role of this variant in myasthenia gravis [39].

Pathogens are major environmental candidates for driving/perpetuating autoimmunity. Immunological cross-reactivity between AChR peptides and viral/microbial proteins has been reported [41,42], but evidence for pathogenic molecular mimicry *in vivo* has never been obtained. Early studies on antiviral antibody differences between myasthenia gravis patients and controls produced contrasting results [43,44]. Early attempts to identify/isolate viruses from myasthenia gravis thymuses largely failed too [45,46], although Epstein–Barr virus (EBV) DNA was found in a few myasthenia gravis hyperplastic and thymoma thymuses [47]. More recently, signs of HTLV-I and poliovirus infection were found in myasthenia gravis thymus [48,49]. Myasthenia gravis exacerbations are known to be triggered by infections, whereas influenza vaccines have recently been reported to be beneficial [50▪]. Cases in which myasthenia gravis developed after HIV infection have been reported [51]. Another recent study found that high serum levels of IgG against EBV nuclear antigen 1 were associated with EOMG [52▪] indicating a role of EBV in myasthenia gravis, as in other autoimmune disorders.

Our finding of active EBV infection in hyperplastic myasthenia gravis thymuses led us to suggest EBV involvement in the development/maintenance of the intrathymic autoimmune response [53,54▪]. Two recent studies failed to identify EBV in hyperplastic thymuses [55▪,56▪] but detection sensitivity is likely to be an issue: it would help to reach a consensus on the most sensitive and reliable procedures for analysing EBV in thymus [57▪].

Intrathymic autoimmune mechanisms

A wealth of data implicates the thymus as the main site of autosensitization in AChR-positive myasthenia gravis [58]. About 80% of AChR-positive patients have thymic abnormalities (hyperplasia or thymoma); hyperplastic thymus contains all elements necessary for initiating and maintaining an AChR-specific autoimmune response [autoantigen, expressed on thymic epithelial cells (TECs) and myoid cells; autoreactive T cells, and autoantibody-producing B cells]; and stable remission occurs in high proportions of thymectomized patients.

Thymic hyperplasia is often of the follicular type and characterized by the presence of germinal centres containing B-cell infiltrates. The B cells undergo somatic hypermutation and antigen-driven selection [59] and are surrounded by AChR-expressing myoid cells, suggesting germinal centres involvement in myasthenia gravis pathophysiology [60]. Upregulation of B-cell activating factor (BAFF) and a proliferation-inducing ligand in myasthenia gravis thymus suggests a microenvironment favourable to B-cell maturation/survival [61].

A combination of largely unknown factors likely leads to AChR autosensitization in the thymus of genetically susceptible individuals. Self-tolerance is normally achieved in thymus by negative selection of autoreactive T cells. Medullary TECs (mTECs) express tissue-specific antigens that select and permit deletion of autoreactive T cells [62]. Self-antigen expression is mediated by the autoimmune regulator (AIRE); AIRE loss-of-function mutations cause multiple-organ autoimmune polyglandular syndrome type I (APS-I), whereas AIRE deficiency impairs autoreactive T-cell deletion, resulting in autoimmunity in animals [63]. However, AIRE's role in human autoimmune diseases, including myasthenia gravis, is still unclear. As AIRE modulates CHRNA1 expression in human mTECs *ex vivo*, it has been proposed that, in the presence of specific CHRNA1 variants, AIRE sets the threshold for self-tolerance versus autoimmunity [35]. Although spontaneous myasthenia gravis is not a feature of $AIRE^{-/-}$ mice, nor it is part of the APS-I syndrome in humans, a role of AIRE in the susceptibility of mice to EAMG and a relationship between FoxP3-expressing regulatory T cells (Tregs) and AIRE expression is suggested by recent data showing that $AIRE^{-/-}$ mice have low Tregs and high expression of pathogenic Th17 in thymus [64▪▪]. Defective FoxP3 expression and severely impaired Treg function may also contribute to tolerance loss [65].

Chronic inflammation may also be involved in intrathymic pathogenesis of AChR-myasthenia gravis, by promoting immune-cell activation and increasing AChR expression in mTECs: hyperplastic myasthenia gravis thymus is characterized by high expression of inflammatory cytokines, IFNs, IFN-related genes, MHC class II genes, and lymphocyte-attractant chemokines [54▪,66]. The observation that in-vitro expression of AChR transcripts in myoid cells and TECs is increased by proinflammatory cytokines suggests how inflammation may contribute to intrathymic AChR-specific autosensitization [66].

In thymoma, lack of AIRE expression, absence of myoid cells, and failure to generate Tregs may all promote defective self-tolerance [67]. Recently, the expression of FoxP3 and the B-cell chemoattractant CXCL13 were found to vary with thymoma histology and myasthenia gravis subtype (generalized vs. ocular), prompting the proposal that different immunological processes underlie the two myasthenia gravis subtypes [68].

MuSK-positive patients display minimal thymic alterations, suggesting no pathogenic role of thymus in this type of myasthenia gravis.

Intrathymic viral infection and innate immune system activation

Dysregulated Toll-like receptor (TLR) signalling may drive/favour autoimmunity by inducing antigen presenting cell activation and inflammatory cytokine production, thereby priming adaptive immune cells. A recent study [69▪] in multiple sclerosis (MS) patients supported previous findings [70] of latent EBV infection in active brain lesions overexpressing inflammatory IFNα, and found that noncoding EBV RNA induced TLR3-mediated innate responses in cell culture, suggesting that EBV-driven innate immunity contributes to neuroinflammation in MS. TLR stimuli provide signals for B-cell maturation and survival – which significantly impact B-cell tolerance [71]. TLR activation may also influence self-tolerance via an effect on Tregs: in-vitro and in-vivo activation of dendritic cells by TLR7 ligands reduced Treg generation and suppressive function [72▪].

The inflammatory signature of hyperplastic myasthenia gravis thymus is compatible with the hypothesis that persisting infection and TLR-mediated innate immune activation are responsible for the chronic inflammatory state of thymus, the AChR autosensitization, and the ongoing autoimmune response (Fig. 1). Our previous finding that TLR4 is overexpressed in myasthenia gravis thymus [73] and our more recent discovery of TLR4-positive poliovirus-infected macrophages in some myasthenia gravis thymuses [49] suggest that pathogens eliciting innate immune responses might cause immunological alterations in the thymus, rendering the organ prone to autosensitization. As EBV is potentially able to immortalize autoreactive B cells, the presence of active EBV infection in the intrathymic lymphoid component of myasthenia gravis [53,54▪] may provide a means of maintaining the autoimmune process. EBV itself might contribute to innate immune activation [69▪,74], lymphoid neogenesis or inflammation in myasthenia gravis thymus, whereas loss of tolerance of autoreactive cells inside and outside the thymus could be favoured by defective immunosuppression function of Tregs (Fig. 1).

Altered immunoregulatory mechanisms

Various peripheral immunomodulatory mechanisms, including suppression of autoreactive clones by Tregs, normally eliminate autoreactive cells not picked up centrally. Tregs are essential for self-tolerance and defects in Tregs can cause experimental autoimmunity [75]. Imbalance between pathogenic Th17 and protective Tregs characterizes peripheral blood or target tissues in several autoimmune conditions [76–78], suggesting that altered immunoregulation contributes to autoimmunity.

Th17 cells affect autoantibody production by influencing the Th1/Th2-cytokine balance in myasthenia gravis peripheral blood mononuclear cells, which are characterized by reduced Treg number and FoxP3 expression [79]. Administration to EAMG rats of antibodies to interleukin 6 (IL-6) (a cytokine known to switch immune responses from Tregs induction to Th17 cells) suppresses ongoing myasthenia gravis through downregulation of Th17-related genes, and inhibits B cells and autoantibody production [80▪▪]. Administration of naïve $CD4^+CD25^+$ Tregs to AChR-immunized animals prevents EAMG but does not improve ongoing EAMG, suggesting Treg involvement in controlling early events leading to EAMG [81]. Two recent studies reported higher serum IL-17 and IL-32α (proinflammatory cytokines) in myasthenia gravis patients than controls. IL-17 levels correlated with anti-AChR antibody titres, whereas IL-32α tended to decline with clinical improvement in generalized myasthenia gravis patients, suggesting pathogenic roles of these two cytokines [82,83].

Therapies targeting autoimmune mechanisms

Long-term immunosuppression with corticosteroids is the mainstay treatment for autoimmune disorders. Corticosteroids are effective in most myasthenia gravis patients but do not produce permanent remission and have notable side effects, highlighting the need for improved therapies that target specific pathogenetic mechanisms. Other myasthenia gravis treatments are general immunosuppressants, such as azathioprine and cyclosporine, that preferentially inhibit T-cell proliferation and IL-2 production; cholinesterase inhibitors (e.g., pyridostigmine) that improve neuromuscular transmission by prolonging ACh availability at the NMJ; short-term immunomodulatory therapies (plasma exchange or intravenous immunoglobulins) that reduce circulating autoantibodies in patients with disease exacerbation; and thymectomy, which is effective in selected patients with pathologic or atrophic thymus [8,84].

Recently, B-cell depletion with rituximab has been successful in myasthenia gravis, Lambert–Eaton myasthenic syndrome [85,86], and MuSK-myasthenia gravis. In the latter, rituximab produced remission or minimal manifestation status [87▪].

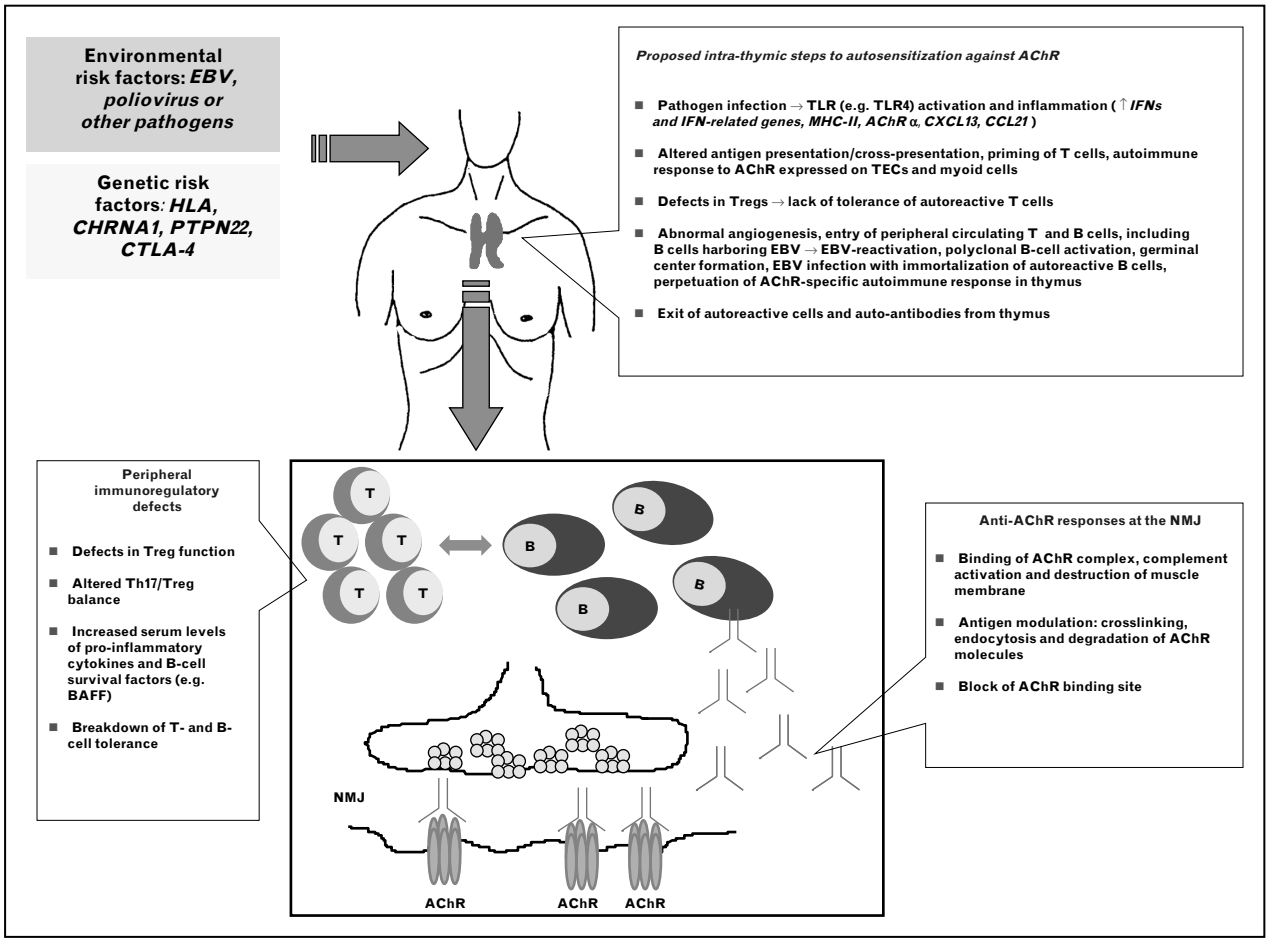

FIGURE 1. Proposed mechanisms for development and maintenance of autoimmune response in AChR-MG associated with nonneoplastic thymic abnormality. Dysregulated or persistent TLR activation by pathogens (particularly viral) or inflammatory triggers leads to the creation of a chronically inflamed thymic microenvironment, that, in the context of a predisposing genetic background, results in autosensitization to AChR, in which professional antigen-presenting cells are activated and antigen cross-presentation by TECs/myoid cells is favoured, resulting in activation of AChR-specific T cells, which in turn promotes an autoimmune response by activated autoreactive B cells. Functionally deficient Tregs contribute to loss of tolerance of AChR-specific T cells. By upregulating the production of T-cell and B-cell chemokines, the inflammatory state favours the recall of circulating T and B cells to thymus and their subsequent AChR-specific sensitization. B cells harbouring EBV are also attracted into the thymus, where EBV reactivation (in the inflamed microenvironment) results in propagation of EBV infection to uninfected cells and maintenance of the thymic autoimmune response, through immortalization of autoreactive B cells. AChR-specific T and B cells, and autoantibodies exit from the thymus to perpetuate the autoimmune response in the periphery, resulting in antibody attachment to AChR at the NMJ and impaired neuromuscular transmission. Altered peripheral immunoregulatory mechanisms, such as defective Th17/Treg balance, defective immunosuppressive function of Tregs and increased serum levels of proinflammatory cytokines and B-cell growth factors, may contribute to maintaining the AChR-specific autoimmune response. AChR, acetylcholine receptor; B, B cells/plasma cells; EBV, Epstein–Barr virus; IFN, interferon; MG, myasthenia gravis; NMJ, neuromuscular junction; T, T cells; TECs, thymic epithelial cells; TLR, Toll-like receptor.

However, this agent requires validation in randomized trials.

The proteasome inhibitor bortezomib, which depletes plasma cells and autoantibodies [88], and the complement pathway blocker anti-C1q antibody [89] have both been reported effective in EAMG. A recent pilot study using eculizumab, an anti-C5 complement monoclonal antibody, demonstrated a clinical benefit in refractory generalized myasthenia gravis patients [90], and belimumab, a monoclonal antibody inhibiting BAFF, has been proposed for a clinical trial which is not yet started.

Recent findings that EBV infection is a feature of myasthenia gravis thymus [53,54▪] suggest that antiviral agents and treatments targeting the innate

immune system should be investigated. Vaccination (for myasthenia gravis prevention) and antiviral treatment for chronic EBV-related myasthenia gravis might also be worth trying.

CONCLUSION

Recent work has improved our understanding of pathogenic mechanisms in myasthenia gravis, but we are still not at a point where knowledge can drive the development of new treatments. Perhaps discovery of the probable pathogenic mechanism of anti-MuSK antibodies will change this. We believe it is important to focus on the environmental factors that, interacting with the genetic background, generate and maintain autoimmunity. One such factor appears to be active intrathymic EBV infection in AChR-positive myasthenia gravis patients. If confirmed, therapeutic approaches targeting EBV will need testing. This will involve assessing virological, serological, and immunological variables in relation to clinical course and response to therapy; and also performing epidemiologic studies to relate prior EBV exposure to subsequent myasthenia gravis and identify susceptible individuals.

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Conflicts of interest

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