THE THYMUS IN MYASTHENIA GRAVIS: SITE OF "INNATE AUTOIMMUNITY"?

PAOLA CAVALCANTE, PhD,[1] ROZEN LE PANSE, PhD,[2] SONIA BERRIH-AKNIN, PhD,[2] LORENZO MAGGI, MD,[1] CARLO ANTOZZI, MD,[1] FULVIO BAGGI, PhD,[1] PIA BERNASCONI, PhD,[1] and RENATO MANTEGAZZA, MD[1]

[1] Department of Neurology IV, Neuromuscular Diseases and Neuroimmunology, Fondazione Istituto Neurologico "Carlo Besta," Via Celoria 11, 20133 Milan, Italy
[2] Unité mixte de recherche, CNRS UMR7215/INSERM U974/UPMC/AIM, Thérapie des maladies du muscle strié, Centre Chirurgical Marie Lannelongue, Le Plessis-Robinson, France

ABSTRACT: Myasthenia gravis (MG) is an autoimmune disorder caused, in most cases, by autoantibodies against components of the neuromuscular junction, frequently the acetylcholine receptor (AChR), and less often the muscle-specific kinase receptor. The thymus plays a major role in the pathogenesis of MG with anti-AChR antibodies: it shows marked pathologic alterations (hyperplastic or tumoral) in most AChR-positive patients and contains the elements required to initiate and sustain an autoimmune reaction (AChR autoantigen, AChR-specific T cells, and autoantibody-secreting plasma cells). In this study we review early and more recent findings implicating the thymus as site of AChR autosensitization in MG and briefly discuss the therapeutic role of thymectomy. We also summarize data showing that the MG thymus is in a state of chronic inflammation, and we review emerging evidence of a viral contribution to the onset and maintenance of the thymic autoimmune response.

Muscle Nerve 44: 467–484, 2011

Several lines of evidence support the involvement of the thymus in myasthenia gravis (MG), an autoimmune disease of the neuromuscular junction (NMJ) that causes muscle weakness and fatigability. Over 80% of MG patients have thymic abnormalities, including hyperplasia and thymoma, and thymectomy results in clinically relevant symptom improvement in a high proportion of cases. Autoantibody production and T-cell sensitization against the muscle acetylcholine receptor (AChR), the main target of autoimmunity, have been shown to occur in MG thymus, indicating that the autoimmune response develops, and probably arises, in this organ.

After a general introduction, we review evidence showing that: (a) the thymus is the main locus of the events that give rise to MG with AChR autoantibodies; (b) thymectomy is an effective therapeutic approach in selected patients; and (c) viruses in the thymus contribute to the etiology of the disease.

OVERVIEW OF THE DISEASE

Epidemiological and Clinical Characteristics. Acquired MG is a relatively rare disease, with a prevalence of 70–200 per million and an annual incidence over the last 10 years estimated at between 0.25 and 20 per million.[1] Age at onset shows a bimodal distribution with a female-dominant peak at <40 years (early-onset MG) and a male-dominant peak at >40 years (late-onset MG).[2]

In most patients, MG onset is marked by weakness of the extrinsic ocular muscles, resulting in diplopia and ptosis. The disease progresses to involve other bulbar muscles, and subsequently many other skeletal muscles, to become generalized MG, whose commonest manifestation is fluctuating muscle weakness with easy fatigability on exertion.[3] The cause of myasthenic symptoms is failure of neuromuscular transmission due to the presence of autoantibodies against the NMJ. Over 80% of patients with generalized MG have IgG class antibodies (IgG1 and IgG3) against the AChR on the postsynaptic motor endplate.[3,4] These antibodies impair neuromuscular transmission in three ways: (1) by mediating the binding and activation of complement at the NMJ, resulting in destruction of the muscle membrane[5,6]; (2) by triggering endocytosis of crosslinked AChR molecules and inducing their degradation, a process termed antigenic modulation[7]; and (3) by blocking the acetylcholine binding site.[8] The main mechanism appears to be complement-induced destruction of the muscle membrane.[5,6] The NMJ of MG patients contains activation fragments of complement component 3, the terminal and lytic component 9, and the membrane attack complex (MAC).[5] Evidence

from animals immunized with purified AChR [experimental autoimmune MG (EAMG)] showed that depletion, inhibition, or functional impairment of complement protects against EAMG.[6,9–11]

Variable proportions of AChR antibody–negative patients (70% in Hoch et al.,[12] 47% in Evoli et al.,[13] 37.5% in Sanders et al.,[14] and 41% in McConville et al.[15]), corresponding to approximately 5% of patients with generalized MG, have antibodies (predominantly IgG4) against muscle-specific tyrosine kinase (MuSK), a transmembrane protein of the NMJ involved in AChR clustering. The presence of anti-MuSK antibodies correlates with more severe symptoms and particularly marked bulbar involvement.[14,15] However, it is unclear how these antibodies give rise to the symptoms of MG. Patients negative for both AChR and MuSK antibodies—usually referred to as seronegative—are clinically similar to patients with AChR antibodies.[16] In one study, application of serum from such patients to cultures of TE671 rhabdomyosarcoma cells reduced AChR numbers, suggesting that anti-AChR antibodies were present at low levels in the serum.[17] Consistent with this observation, Leite and co-workers[18] recently reported on a series of seronegative MG patients, 66% of whom had low-affinity antibodies against AChR that were not detected by routine immunoprecipitation assay, suggesting that wider application of more sensitive AChR antibody assays would considerably reduce the number of seronegative patients. Autoantigens to actin, myosin, ryanodine, and titin are also found in MG patients, particularly those with thymoma, although their role in the pathogenesis of MG is unclear.[19–21]

Susceptibility Factors for MG. As with many autoimmune conditions, both genetic and environmental factors contribute to the development of MG. A genetic component is attested by the 40% concordance rate in monozygotic twins.[22] Human leukocyte antigens (HLAs) are the most important genes involved in autoimmune diseases. The association of HLA with MG varies with thymic histology.[23] Several studies have investigated the highly polymorphic class I and class II HLA loci, finding that the HLA-DR and B8 alleles are associated with generalized MG in Caucasian patients with thymic hyperplasia, but not with other thymic conditions.[24,25] The HLA-DR3 and B8 alleles are part of the most conserved major histocompatibility complex (MHC) haplotype—the 8.1 ancestral haplotype—which has been reported to have intricate effects on MG phenotype in patients with hyperplasia, including particularly high serum titers of AChR autoantibodies.[24,25] Although several studies have demonstrated significant associations between MG

and HLA class I and II alleles in patients with thymoma,[23,26,27] the loci identified varied considerably.

Several other loci not linked to HLA have also been implicated in MG, including interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4),[28] all of which are involved in the immune response, and cholinergic receptor nicotinic alpha-polypeptide 1 (CHRNA1), which is of particular interest as it encodes the α subunit of the AChR.[29,30] The CHRNA1 promoter region has been found to contain the single-nucleotide polymorphism (SNP) rs16862847, whose minor allele (G) was found to be associated with early-onset disease in two independent human populations (France and the UK).[30] The G allele was found to reduce binding of the IRF8 transcription factor to the promoter and was associated with lower CHRNA1 expression in ex vivo medullary thymic epithelial cells (mTECs) when compared with the A allele.[30] This suggests that the G variant is a risk factor for MG, based on the hypothesis that lower CHRNA1 expression in thymic epithelium contributes to loss of central tolerance to the protein in MG-susceptible individuals.[30]

With regard to environmental factors predisposing to MG, viral infections are considered major candidates. Earlier hypotheses and emerging evidence regarding the role of viruses in triggering or perpetuating autoimmunity in MG are discussed in the final section of this review.

Treatments. Mortality for MG has decreased from 23% to 30% in the mid-1950s[31] to 1.2% to 2.2% in recent years,[32,33] in relation to advances in disease treatment and management. Current treatments for MG include: immunosuppressive therapy, such as corticosteroids and azathioprine, which suppress immune responses, including activation of autoreactive cells and autoantibody production (azathioprine reduces nucleic acid synthesis, thereby reducing T- and B-cell proliferation)[34]; administration of anticholinesterase inhibitors, which prolong ACh activity at the NMJ to temporarily improve neuromuscular transmission[35]; and broad anti-immune approaches, such as plasma exchange,[36] intravenous immunoglobulins,[37] and immunoadsorption,[38] which deplete pathogenic autoantibodies from serum. More recently B-cell–depletion therapy with rituximab was successfully employed in an uncontrolled study on patients with MG and Lambert–Eaton myasthenic syndrome, although this agent needs validation in randomized trials.[39] Thymectomy is effective in selected patients,[40] as described in what follows.

EVIDENCE FOR THYMIC INVOLVEMENT IN MG

A wealth of data supports thymic involvement in the pathogenesis of MG with AChR autoantibodies

Table 1. Summary of the major thymic hallmarks potentially related to MG development.

	Follicular and diffuse hyperplasia	Thymoma
Thymic epithelial cells (TECs)	• Expression of AChR α, β, and ε subunits, and HLA class II molecules[76] • Enhance *in vitro* production of anti-AChR antibodies by thymocytes[118] • Signs of complement attack[86] • *In vitro* overexpression of AChR subunits by inflammatory stimuli[83] • *In vitro* overexpression of IL-6 by LPS and inflammatory stimuli[136] • *In vitro* production of high levels of B-cell chemoattractant CXCL13[57]	• Preferential expression of AChR ε subunit[89] • Defective expression of HLA class II molecules[72] • Altered expression of a differentiation factor for thymic epithelium[73] • Decreased expression of autoimmune regulator AIRE[92]
Myoid cells	• Expression of whole functional AChR complex[52–54] • *In vitro* overexpression of AChR subunits by inflammatory stimuli[83] • Localization around GCs and cluster with dendritic cells (sign of antigen cross- presentation)[51] • Signs of attack by complement[86]	• Absence of myoid cells[88]
T cells	• Enrichment in AChR-specific CD4$^+$ T cells[77,78] • Increased Fas expression[101] • Decreased mRNA expression of Foxp3 in CD4$^+$CD25$^+$ Tregs[102]	• Enrichment in AChR-specific CD4$^+$ T-cells[78] • Reduced frequency of Tregs and expression of Foxp3[92,104]
B/plasma cells	• Diffuse infiltration of B cells not organized (diffuse hyperplasia) and organized (follicular hyperplasia) into GCs[43,44] • *In vitro* production of anti-AChR antibodies[110–121] • Signs of EBV persistence and reactivation[44]	• Presence of B cells in extra-epithelial perivascular spaces or areas of medullary differentiation[74,75] • In vitro production of anti-AChR antibodies in relation to the histological nature of adjacent tumor tissue[117,118]
GC features	• Resemble lymphoid follicles of peripheral lymphatic organs in terms of immunoglobulin gene diversification, mutation and selection[50] • Signs of somatic hypermutation, antigen-driven clonal expansion, and selection[48] • Formation associated with lymphoangiogenesis and HEV development[55] • High expression of the anti-apoptotic Bcl-2[61] • Expression of latent markers of EBV[44]	• GCs absent or rarely found[74]
Gene signature	• Overexpression of type I and II IFN-induced genes, immunoglobulin genes, and MHC II[83,106,139] • Overexpression of T- and B-cell–attracting chemokines CXCL13,[57] CCL21,[55,106] IP-10,[136] and IP-10 receptor (CXCR3)[136]	• Lack of evidence for inflammatory state

AChR, acetylcholine receptor; AIRE, autoimmune regulator; EBV, Epstein–Barr virus; GC, germinal center; HEV, high endothelial venules; HLA, human leukocyte antigen; IFN, interferon; IP-10, interferon-γ–induced protein 10 kDa; LPS, lipopolysaccharide; MHC, major complex of histocompatibility; Tregs, T-regulatory cells.

(Table 1), whereas, in seronegative patients, and those with MuSK antibodies, the role of the thymus remains unclear. Three main lines of evidence indicate thymic involvement: (1) the thymus has marked pathologic alterations (Figs. 1 and 2) in most AChR-positive cases; (2) the thymus contains all the elements necessary for initiating an AChR-specific autoimmune response (Fig. 2 and Table 1); and (3) high proportions of true remissions occur in selected patients who undergo total thymectomy.[40]

Pathologic Abnormalities in MG Thymus. Pathologic alterations of the thymus are found in >80% of patients with generalized MG positive for AChR antibodies (Fig. 1). Thymic changes comprise follicular hyperplasia (Fig. 1B), diffuse hyperplasia or thymitis (Fig. 1C), thymic involution (Fig. 1D), and thymoma (Fig. 1E and F). Hyperplasia is the most common alteration in early-onset MG, whereas, in the late-onset form, thymoma and involuted thymus (which shows hyperplastic changes) are predominant. MuSK-positive subjects have minimal histological alterations of the thymus, and the organ generally resembles that from age-matched controls.[41,42] Variable proportions of seronegative patients show hyperplastic changes.[41,42]

Follicular and Diffuse Hyperplasia. Follicular hyperplasia is present in 50–60% of AChR-positive patients,[43] and hyperplastic changes are present in a variable proportion (35–75%) of seronegative
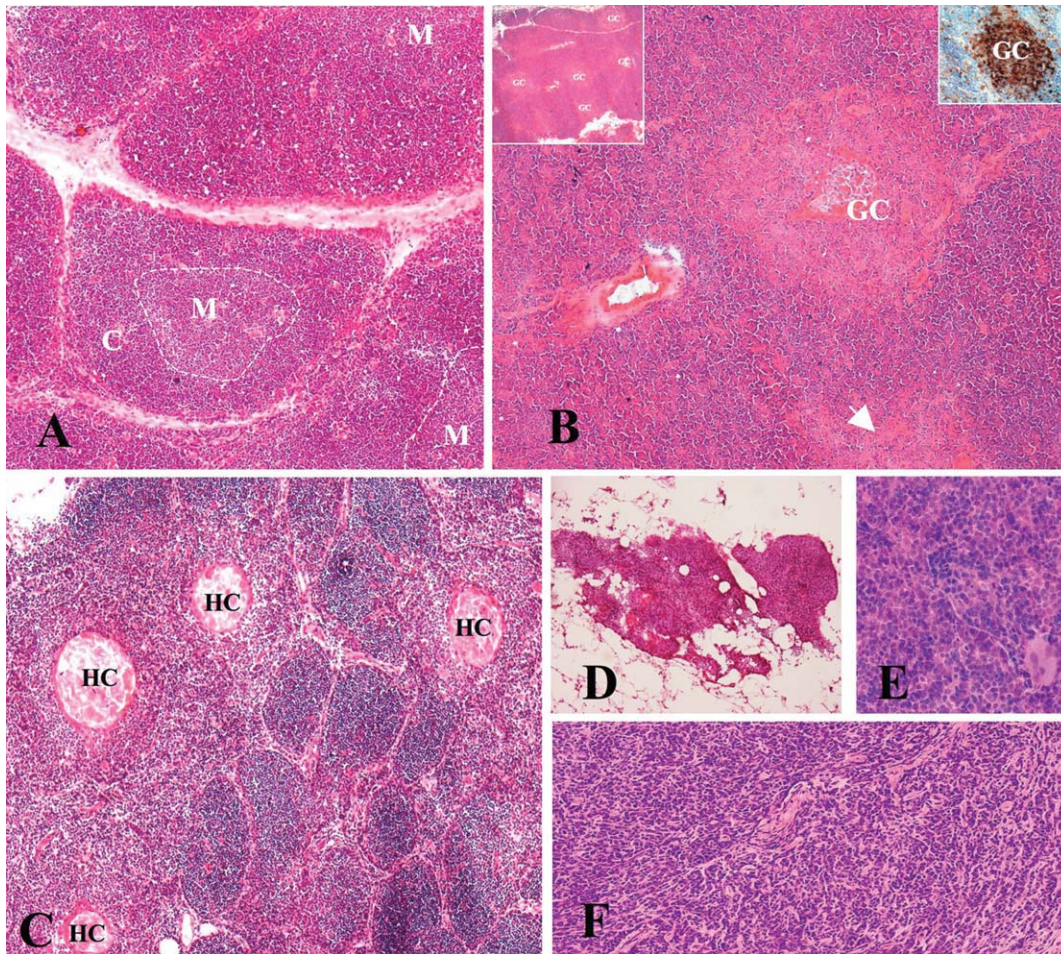
FIGURE 1. Hematoxylin and eosin staining. **(A)** Thymus from a healthy 18-year-old men. The thymic lobules are organized into a darker outer cortex (C) and a lighter inner medulla (M). **(B)** Thymus with follicular hyperplasia from an 18-year-old MG men. The medulla shows diffuse lymphoid infiltrates (arrow) and follicles (lighter areas) containing ectopic germinal centers (GCs). Follicles with GCs are particularly evident in the upper left inset at lower magnification. The upper right inset shows a GC of the same thymus after immunostaining for B-cell marker CD20. **(C)** Thymus with diffuse hyperplasia (thymitis) from a 32-year-old MG women. Large lymphoid infiltrates frequently surrounding Hassall corpuscles (HC) are evident in the medulla. HCs are normally particularly numerous and enlarged in diffuse hyperplasia. **(D)** Involuted thymus from 50-year-old MG women. Much of the parenchyma is substituted by fat. **(E)** World Health Organization (WHO) type B2 thymoma from a 27-year-old MG men. **(F)** WHO type B1/A mixed thymoma from 64-year-old MG men. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

cases.[41,42] Follicular hyperplasia is characterized by the presence of expanded perivascular spaces fused with the thymic medulla that contain B-cell infiltrates, some of which are organized into ectopic B-cell germinal centers (GCs), which, together with other cells (notably follicular dendritic cells), form follicles (Figs. 1B and 2A–I). The B cells not organized into GCs are scattered throughout the thymic medulla and often concentrated around Hassall corpuscles (Fig. 2C and ref. 44).

GCs are present in the affected tissues of several autoimmune diseases,[45] including the multiple sclerosis brain,[45] the rheumatoid arthritis synovium,[46] and the autoimmune thyroiditis thyroid.[47] These GCs contain B cells undergoing somatic hypermutation and antigen-driven selection.[46–49]

In terms of immunoglobulin gene diversification, mutation, and selection, GCs in MG thymus do not differ from those observed in the lymphoid follicles of peripheral lymphatic organs,[50] but uniquely they are often surrounded by plasma cells (Fig. 2E and ref. 44) or muscle-like myoid cells (Fig. 2H and I and ref. 51) expressing AChR.[52–54] GC formation in MG is also associated with lymphoangiogenesis and angioneogenesis. The latter involves the production of specialized post-capillary swellings (high endothelial venules) whose endothelium contains cuboid cells bearing surface chemokines.[55] In particular, these cells overexpress chemokine ligand 21 (CCL21), a chemokine that attracts T and (more strongly) B cells, and plays a role in driving thymic hyperplastic changes in MG.[55]

Diffuse hyperplasia (thymitis) shows histological features similar to those of follicular hyperplasia but lacks GCs (Figs. 1C and 2J–O and ref. 44). Infiltrating B cells are present in the medullary
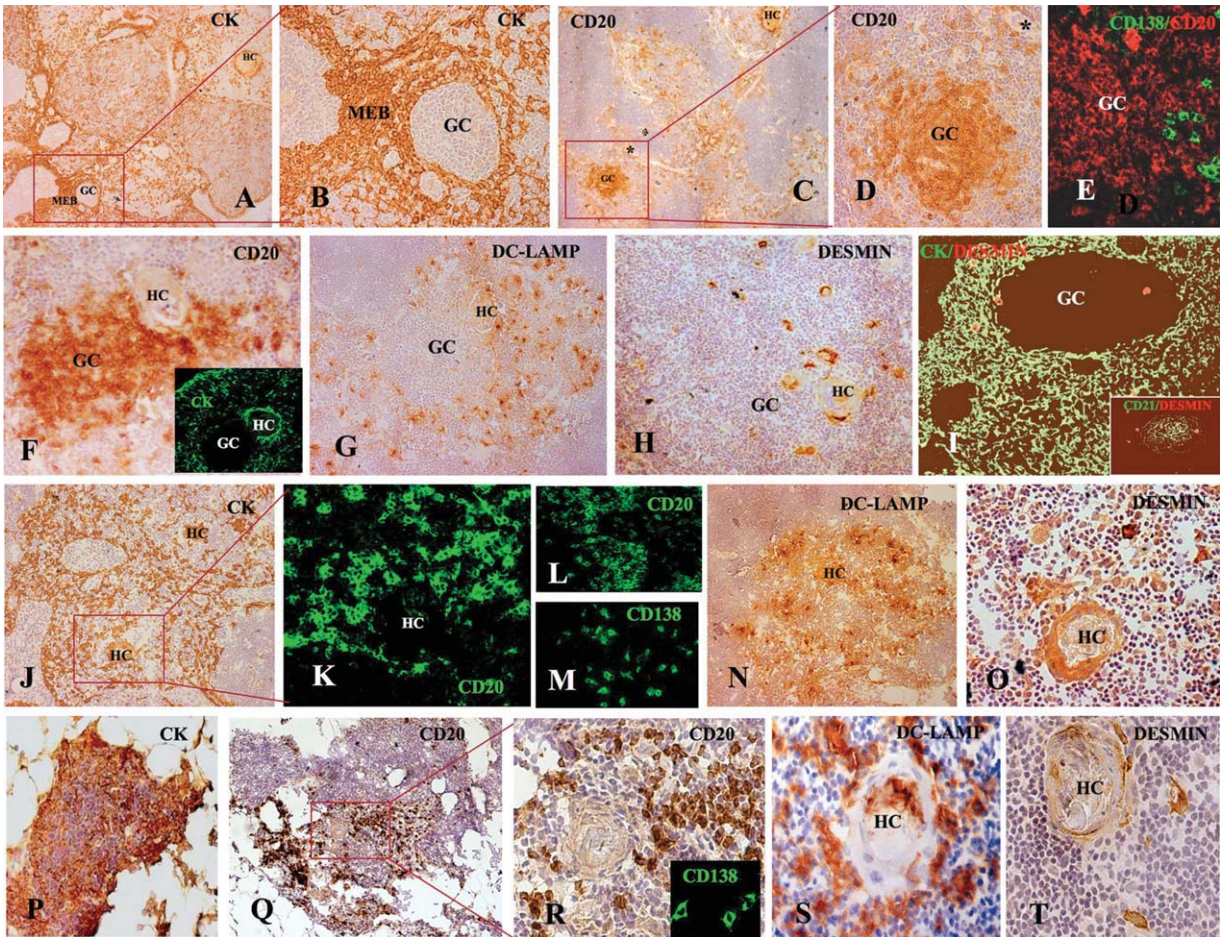
FIGURE 2. Immunohistological features of follicular hyperplasia, thymitis, and thymic involution in MG. **(A–I)** Follicular hyperplasia from an 18-year-old. **(A)** Cytokeratin (CK)-positive medullary TECs intercalated between lymphoid infiltrates; **(C)** abundant infiltrating CD20-positive B cells, many organized into a germinal center (GC). **(B)** and **(D)** are enlarged boxed areas of **(A)** and **(C)** showing GC enclosed by a medullary epithelial band (MEB). High endothelial venules [asterisk in **(C)** and **(D)**] are close to GCs. **(E)** CD138-positive plasma cells at edge of GC, shown in **(A)–(D)**. CD20-positive GCs are often in close contact with Hassall corpuscles (HC), themselves surrounded by CK-positive cells [inset in **(F)**], DC-LAMP–positive dendritic cells **(G)**, and desmin-positive myoid cells **(H)**. Myoid cells may be present inside GCs **(I)**. **(J–O)** Thymitis from a 27-year-old patient. **(J)** TECs are distributed as in follicular hyperplasia. Infiltrating B cells may be located around HCs [**(K)** is an enlargement of boxed area in **(J)**] and widely distributed throughout the medulla **(L)**, where plasma cells **(M)**, myeloid dendritic cells **(N)**, and myoid cells **(O)** are also present. **(P–I)** Involuted thymus from a 50-year-old patient. Thymic parenchyma, prominently substituted by fat **(P)**, is characterized by diffuse B-cell infiltration **(Q, R)**, with plasma cells [inset in **(R)**], myeloid dendritic cells **(S)**, and myoid cells **(T)**. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

parenchyma of follicular and diffuse hyperplasia in greater numbers than typically found in thymuses from age-matched, non-myasthenic individuals.[44,56] This may be because the chemokines, C–X–C motif chemokine 13 (CXCL13) and CCL21, which strongly attract B cells, are expressed at high levels in the thymus of untreated MG patients.[55,57] Levels of these chemokines normalize after corticosteroid treatment produces clinical improvement. The number of GCs also decreases, suggesting that CXCL13 and CCL21 are involved in abnormal peripheral lymphocyte recruitment and GC development.[55,57] Furthermore, expression of the B-cell growth factors, a proliferation-inducing ligand (APRIL) and B-cell activating factor (BAFF), is significantly up-regulated in MG thymus, indicating an environment favorable to B-cell survival.[58] BAFF levels are also significantly higher in the serum of MG patients than in healthy controls, further supporting a role for BAFF in the immunopathogenesis of MG.[59]

Our own recent findings suggest another reason for the B-cell abnormalities in MG thymus.

We found that in MG, but not normal thymuses, B cells of GCs and medullary infiltrates were positive for Epstein–Barr virus (EBV).[44] This virus has a unique ability to stimulate B-cell proliferation, yet it interferes with the normal B-cell differentiation program[60]; therefore, it could be instrumental in stimulating the intrathymic expansion of B cells and inducing them to form GCs.[44]

The high expression levels of the anti-apoptotic Bcl-2 in GC B cells of MG thymus,[61] together with the observation that EBV may induce Bcl-2,[62] strongly suggest a role of EBV as trigger for GC formation and B-cell accumulation in MG thymus. In what follows we summarize the results of a study that suggests an etiological role for EBV in MG.

Thymoma. Thymoma occurs with variable frequency (up to 30%) in MG.[32,63–65] Patients with thymoma are always positive for AChR antibodies, usually have more severe disease, and are less responsive to treatment than those without thymoma.[21,66–68]

Thymomas are slow-growing, locally invasive epithelial tumors consisting of transformed epithelial cells surrounded by maturing polyclonal T cells.[69] Type B2 thymoma (World Health Organization classification)[70] is most often associated with MG, followed by types AB and B1 (Fig. 1E and F).[21,69,71] In types A and AB, the epithelial cells typically have a medullary or spindle morphology, with focally numerous (AB) or rare (A) thymocytes. Types B1 and B2 consist of polygonal epithelial cells together with numerous developing $CD4^+CD8^+$ T cells, and morphologically resemble thymic cortex. Okumura and co-workers[69] have suggested that the lack of functional medulla in the tumor might result in defective negative selection of T cells, favoring autosensitization against locally expressed autoantigens, such as AChR. Savino and colleagues[72] showed that thymoma epithelial cells are defective in the expression of HLA class II molecules known to play an essential role in intrathymic T-cell differentiation, which might explain thymoma-associated defects in the thymic selection of T cells. Those investigators had previously shown that expression of a differentiation factor for human thymic epithelium—recognized by the anti-p19 monoclonal antibody—is lost in thymomas, suggesting that, during malignant transformation, thymic epithelial cells acquire severe defects likely to give rise to defective T-cell selection.[73]

With regard to B cells in thymomas, a few scattered intratumoral $CD20^+$ B cells are found in non–MG-associated thymomas, but they are numerous in MG-associated tumors (Fig. 3 and refs. 74 and 75). B cells are found mainly in cortical thymomas and well-differentiated thymic carcinomas, where they are present in the extra-epithelial perivascular space and may form lymphoid follicles.[74] B cells also occur frequently in areas of medullary differentiation within the tumor and correspond to the medullary B-cell population of the normal thymus.[74,75]

In about 20% of patients with thymoma, hyperplasia is present in the residual non-neoplastic tissue, whereas the remaining cases have thymic atrophy.[43] It is unclear whether residual hyperplasia is the site of the autoimmune response in patients with thymoma.

Thymic Involution. Ten to 20% of AChR-positive MG cases have atrophic thymus mainly consisting of adipose tissue (Fig. 1D and ref. 43). Atrophy occurs most often in patients >40 years of age and in some cases could be due to treatment with corticosteroids. Although atrophic thymus is very similar to that of age-matched controls in terms of amount of adipose tissue and epithelial space, the residual islands of medullary parenchyma have high numbers of infiltrating B cells that, in some cases, form GCs, whereas plasma cells are scattered throughout the tissue. These are evident signs of hyperplasia and immune activation (Fig. 2P–T and ref. 44).

All Elements Necessary for an Autoimmune Response Are Present in MG Thymus.

The thymus of MG patients with AChR antibodies contains all the components required to initiate and sustain an active autoimmune response: the autoantigen is expressed on muscle-like myoid cells[52–54] and TECs[76]; professional antigen-presenting cells are present[51]; and also present are AChR-specific autoreactive T cells[77,78] and B cells producing anti-AChR antibodies.[79] Transplantation of fragments of MG thymus to immunodeficient mice induces the formation of anti-AChR antibodies and their deposition at skeletal muscle endplates.[80]

Roles of Muscle-like Myoid Cells and TECs in the Autoimmune Process. Because thymic myoid cells express all AChR subunits, possess a functional ACh receptor,[52–54] and have also been reported to express other antigens characteristic of skeletal muscle cells,[81] they have long been considered responsible for sustaining T-cell sensitization and autoantibody production against AChR in thymus.[52–54] These cells mainly express the fetal isoform of AChR (containing the γ subunit), and specific autoantibodies to this isoform are frequently present in MG patients with thymic hyperplasia.[82] Increased expression of AChR components, mainly the α subunit, which contains the main immunogenic region,[82] has been observed in myoid cells in response to proinflammatory cytokines, suggesting that inflammatory conditions can result in enhanced presentation of the autoantigen by these cells as a first step in autosensitization.[83]

Despite the aforementioned characteristics, thymic myoid cells do not express MHC class II molecules and hence are unable to present antigens to T lymphocytes; therefore, it has been suggested that AChR fragments are released from these cells to be taken up by dendritic cells and then presented to supposedly AChR-specific T cells[84] in a process known as cross-presentation.[85] This idea is supported by the observation that, in thymic
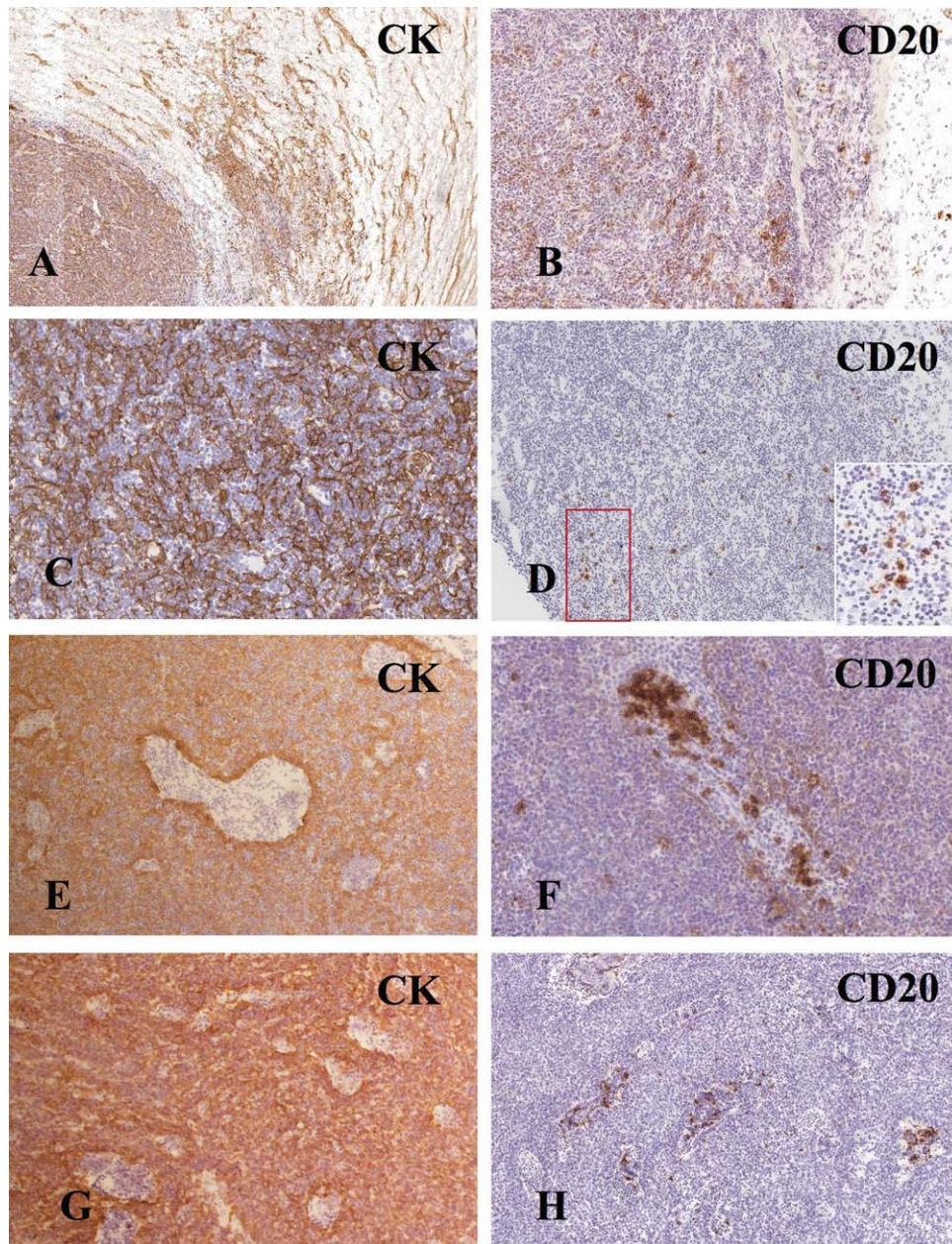
FIGURE 3. Thymoma from MG patients immunostained for cytokeratin (CK) to reveal epithelial cells and CD20 for B cells. **(A**, **B)** Mixed WHO type B1/A thymoma from a 64-year-old patient. Numerous B cells are present in the type B area. **(C, D)** WHO type B1 thymoma from a 54-year-old patient showing B cells scattered within neoplastic tissue **(D)**. The inset, an enlargement of the boxed area, clearly delineates individual B cells. **(E, F)** WHO type B2 thymoma from a 27-year-old patient showing B-cell aggregates. **(G, H)** WHO type B3 thymoma from a 54-year-old patient showing small B-cell aggregates. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

hyperplasia, myoid cells and dendritic cells often cluster together (Fig. 2 and ref. 51). Furthermore, signs of early and persistent complement attack on thymic epithelial and myoid cells in close proximity to GCs in MG thymus have been reported.[86] Persistent autoantibody attack and complement-mediated damage to myoid cells could well be responsible for increasing the levels of autoantigen available to dendritic cells, and for favoring GC formation and antibody diversification—actions

that could be crucial for establishing a self-sustaining AChR-specific autoimmune response. The hypothesis that more antigen is available from myoid cells in MG thymus than normal thymus was originally formulated by Bornemann and Kirchner[87] after observing that myoid cells turned over faster in MG thymus than in control thymus.

Myoid cells are rare or absent from most thymomas,[88] but those present in adjacent non-tumoral thymic tissue have been suggested to be

the source of the α, β, δ, and γ AChR transcripts found in tumor tissue by reverse-transcriptase polymerase chain reaction (PCR).[89] Using quantitative RNase protection assays, MacLennan and colleagues[89] found that mRNA for the ε subunit was present in several MG-associated thymomas (mainly types A and AB), whereas mRNA for the other subunits was not detected. Also, they proposed that preferential expression of the AChR ε subunit might play a distinct role in autosensitization in MG-associated thymomas, particularly of types A and AB.[89] Although thymic myoid cells express high levels of most muscle genes,[81] they express relatively low levels of the AChR ε subunit. This subunit might be expressed by the tumoral epithelial cells of thymomas rather than myoid cells of the non-tumoral component.[89]

With regard to TECs, these do not express the whole AChR, either in normal or MG thymus, but they do express the α, β, and ε subunits of AChR, as well as MHC class II genes.[76] Like myoid cells, they respond to inflammatory stimulation by upregulating AChR subunit transcription and protein production.[83] Based on these findings, TECs are also considered to be involved in the autosensitization against AChR that occurs in the inflamed microenvironment of the MG thymus.[83]

mTECs are essential for the maintenance of central tolerance by expressing tissue-specific antigens and inducing the deletion of self-reactive T cells through direct antigen presentation or cross-presentation by dendritic cells.[90] The main factor responsible for self-antigen expression in mTECs is the autoimmune regulator (AIRE) transcription factor, whose impairment by loss of function mutations causes multiple-organ autoimmune polyendocrinopathy syndrome type 1.[91] AIRE has been shown to modulate CHRNA1 transcriptional levels in human mTECs *ex vivo*, thus suggesting it has a role in setting the autoantigen expression threshold for self-tolerance versus autoimmunity.[30]

In thymomas AIRE is expressed at much lower levels than in normal thymus, and AIRE-positive cells are extremely rare because most thymomas consist mainly of cortical-like epithelial cells that do not express AIRE.[92] Thus, lack of AIRE may facilitate the development of autoreactive T cells, and hence of thymoma-associated autoimmune MG.[92]

Intrathymic Autosensitization of T Cells. AChR-specific CD4^{+} T cells are present in the blood of MG patients,[93] but are particularly abundant in thymus, supporting the idea that T-cell autosensitization in MG takes place in the thymus.[77,78] Treatment with anti-CD4 antibodies has been found to improve MG symptoms, suggesting that CD4^{+} T cells may be involved in initiating the humoral autoimmune response in MG.[94]

Healthy subjects also have circulating T cells that can be activated in the presence of various autoantigens, including AChR, indicating that autoreactive T cells are normally present in the peripheral immune system but are held in check by peripheral tolerance mechanisms.[95,96] Levinson and colleagues[97] observed that animals receiving variants of a thymotrophic gross murine leukemia virus had 4.2 times more CD4^{+} thymic immigrant T cells than animals that received a non-inflammatory control vector, indicating that inflammatory conditions promote migration of peripheral immunocompetent cells to the thymus.

As far back as 1989, Sisely and colleagues[98] showed that peripheral blood T cells of MG patients underwent antigen-induced proliferation when cultured with AChR. Subsequently, numerous antigenic determinants/epitopes for T-cell sensitization were found in each AChR subunit, reflecting the fact that T-cell response is heterogeneous in MG.[99] The strongest response was elicited by α-subunit epitopes, some of which are immunodominant.[99,100] Serial studies on the immune response of individual patients to α-subunit peptides were performed by Ragheb and colleagues,[100] whose results suggest that epitope spreading of the T-cell sensitization to increasingly larger parts of the α subunit occurs as the disease progresses.

Moulian and co-workers[101] showed that, in AChR-positive MG patients with thymic hyperplasia or involution, thymic T cells were enriched in a subpopulation strongly expressing Fas, a protein involved in apoptosis. They found that all thymocytes proliferated in response to AChR peptides, but that the proliferative response of total MG thymocytes to two different AChR peptides was abolished when the cells strongly expressing Fas were depleted. This suggests that these cells are the autoreactive T cells that induce the autoimmune response in MG thymus.[101]

A severe defect in CD4^{+}CD25^{+} T-regulatory (Treg) cells has been described in MG thymus with hyperplasia, providing an explanation for the intrathymic tolerance loss of AChR-specific T cells.[102] Whether this defect is due to defective expression or regulation of Foxp3—a transcription factor essential for T-cell–regulatory function—is still unclear.[102,103] In MG patients with thymoma, it has also been found that Foxp3 mRNA levels, as well as the intrathymic Treg population, were significantly lower than in non-neoplastic thymuses, a further indication that thymic development of Treg cells is impaired in thymomas.[92,104]

B Cells: Intrathymic Activation and Autoantibody Production. B-cell lymphoid infiltrates, often organized into GCs, are found within chronically inflamed tissues in many autoimmune diseases,[45–47]

including MG thymus with follicular hyperplasia.[43,44] It is unclear whether ectopic GC formation is an early event in the development of the autoimmune disease or the consequence of a disease-related process. However, because thymic alterations and GCs are not present in EAMG animals,[105] B-cell activation and homing to GCs are likely to be key steps in MG initiation rather than secondary events.

A cloning and sequencing study of immunoglobulin genes from GC B cells isolated by laser microdissection from MG thymuses revealed a remarkably heterogeneous population of B cells that were undergoing antigen-driven clonal expansion, somatic hypermutation, and selection, with no single clone dominating the response.[48] Comparison of B-cell clonal sequences from different GCs with known anti-AChR antibodies from other MG patients showed convergent mutations in the complementarity-determining regions, suggesting a common selection process, and in turn indicating that AChR was driving an ongoing response in thymic GCs of MG patients.[48] In a microarray study, a wide range of Ig genes was found to be upregulated in MG thymus,[106] in agreement with other findings indicating B-cell polyclonality in MG patient thymuses.[107] Levinson and collaborators[108] showed that thymic B cells from MG patients were committed to selective IgG secretion rather than IgM secretion as an *in vitro* response to the T-cell–dependent polyclonal activator pokeweed mitogen, whereas blood mononuclear cells from the same patients secreted similar amounts of IgG and IgM, as did blood mononuclear cells from controls. These observations indicate *in vivo* activation of B cells in MG thymus. Evidence for isotype switch in MG thymic B lymphocytes was previously provided by Zweiman and colleagues,[109] who found that thymic B cells from MG patients underwent *in vivo* differentiation, thus presaging the more recent observation that somatic hypermutation and antigen-driven selection characterize GCs of hyperplastic MG thymus.[48]

As far back as 1978, Vincent and colleagues[110,111] provided evidence that cultured thymic tissue from MG patients spontaneously produced antibodies against AChR. The same group showed that autoantibody-producing thymic cell suspensions from MG patients were enriched in plasma cells, which were described as autonomous, long-lived, terminally differentiated, and radio-resistant.[112]

Lisak and colleagues[113] showed that peripheral blood mononuclear cells from MG patients, but not healthy controls, produced anti-AChR antibodies in vitro in the presence of pokeweed mitogen. They further showed that the depletion of CD8^{+} T cells from blood mononuclear cells did not result in autoantibody synthesis in normal subjects, whereas, in MG patients, depletion resulted in increased autoantibody production and increased levels of immunoglobulin cells, indicating that autologous CD8^{+} T cells exert some regulatory control over anti-AChR CD4^{+} T cells in MG.[114,115] Other investigators found that highly enriched B cells from peripheral blood of generalized MG patients could be selectively driven to produce AChR-specific antibodies by coculturing with the AChR-expressing human rhabdomyosarcoma cell line TE671 in the presence of anti-CD40 antibodies and other B-cell growth factors.[116]

In other studies, AChR antibody production by cultured peripheral blood lymphocytes and thymic lymphocytes from MG patients correlated with *in vivo* AChR antibody titers and histological thymic abnormalities, so that cultured lymphocytes from hyperplastic thymuses produced larger quantities of autoantibodies than those from involuted and thymomatous thymuses.[117,118]

Cultured thymic cells from MG patients have been found to produce antibodies specific for influenza A[119] and tetanus toxoid,[120] with the latter being produced by cells from patients booster-immunized to tetanus toxoid before thymectomy. These findings indicate that the B-cell repertoire in MG thymus is heterogeneous and suggest that B cells with different antigen-directed specificities migrate from the periphery to the thymus in MG patients.[120] However, thymic B cells from healthy subjects do not produce AChR antibodies, even in the presence of polyclonal stimulator pokeweed mitogen, whereas MG thymic cells spontaneously produce them; this indicates that, in MG patients, AChR-reactive B cells are an important component of the thymic B-cell repertoire, and that the *in vitro* response of these cells is likely due to prior local activation *in vivo*.[120] Variable effects of pokeweed mitogen on AChR-specific antibody production were observed in thymic B cells from different MG patients, indicating that different functional stages of AChR-reactive B cells are present in MG thymus,[120] probably in relation to quantitative or qualitative differences in the repertoire of receptor-specific T cells between MG patients.

Analyses of purified B cells from hyperplastic thymuses have shown that these cells express activation markers (CD71, 4F2, CD23, B8.7); proliferate in response to two growth factors, B-cell growth factor (BCGF) 12 kDa and recombinant interleukin-2 (rIL-2); and spontaneously secrete anti-AChR antibodies.[121] In hyperplasia, the findings that all proliferating B cells in GCs immunostain strongly for neurotrophic brain-derived growth factor and for its receptor, p75NTR, strongly suggest that this factor is involved in the formation and maintenance of GCs and B-cell proliferation.[122]

Some years ago, Serafini and co-workers[123] found that intrameningeal B-cell follicles from multiple sclerosis patients were enriched in EBV-infected cells, indicating a direct link between EBV infection and B-cell dysregulation in this condition. Our recent findings indicate that active EBV infection of B cells also occurs in MG thymus.[44] We found that GCs and surrounding plasmablasts were the main sites of EBV persistence and reactivation in MG thymuses, suggesting that the virus may play a role in driving abnormal intrathymic B-cell activation, GC formation, and B-cell differentiation into plasma cells.[44]

All these findings point to a situation whereby the intrathymic lymphoid environment maintains the autoimmune reaction by filtering the repertoire of differentiating B cells, selecting high-affinity variants, and generating AChR-specific plasma cells.

Thymectomy in Management of MG. Thymectomy has been shown to be a useful therapeutic procedure for AChR-positive MG, as it results in complete stable remission in a high proportion of cases, especially in patients operated on shortly after diagnosis and those with early-onset MG and thymic hyperplasia.[40,124,125] Its efficacy in these patients is plausibly due to eradication of the site of pathologic autosensitization and autoimmune response maintenance, as indicated by the fact that AChR antibody titers usually fall after thymectomy, and the magnitude of this fall correlates with the quantity of GC B cells in the removed thymus.[126] The severity of follicular hyperplasia and density of lymphoid infiltrates also correlates with improvement after thymectomy.[127] However, it is known that titers do not always fall, suggesting other sites of autoantibody production in some cases.[128]

Because eradication of the main site of autosensitization and response maintenance is the aim, thymectomy should preferably remove all thymic tissue, and several surgical techniques have been developed over the last 50 years to achieve this aim with limited invasiveness.[125] Video-assisted thoracoscopic extended thymectomy (VATET)[129] is one of the least invasive procedures, which, in our own experience, was associated with complete stable remission in 51% of non-thymomatous patients after 6 years of follow-up.[40] Other studies in non-thymomatous patients operated on by the transcervical approach (T-1a,d), transsternal thymectomy (T-3b), combined procedures (T-4), or videoscopic thymectomy (T-2a,c) reported remission rates in the range of 13–81% after 2–7.5 years of follow-up.[125] The recent experience in 26 MG patients who underwent robotic thymectomy was that 82% improved, 18% were unchanged, and none worsened after an average follow-up of 26 months.[130]

Recently, Bachmann and colleagues[131] reported that 42% of patients with generalized MG after thymectomy who were available for follow-up were in complete remission, whereas an additional 39% had improved clinically.

The considerable variation in remission rates after thymectomy was attributed by Sonett and Jaretzki[125] to numerous factors—in addition to surgical technique—including variations in preoperative symptom severity, timing of surgery, use of immunosuppressive agents, and differing postoperative evaluation methods and remission definitions. These investigators[125] emphasized the need for new prospective, randomized studies. So far, no conclusive result of trials comparing conservative versus surgical treatment are available, and selection criteria for thymectomy remain controversial. The task force of the MG Foundation of America recommended development of classification systems and definitions of response to therapy for patients undergoing thymectomy. They also emphasized that prospective, randomized clinical trials were the preferred method for evaluating therapies, and indeed a randomized thymectomy trial (MGTX) is underway. To date, no results are available; however, the task force noted that, when such studies are not feasible, prospective risk-adjusted outcome analyses of non-randomly assigned treatments are also informative.[132]

For AChR-negative MuSK-positive MG, there is consensus that thymectomy is not indicated, as no thymic pathology is evident and the resection produces no benefit.[133] Thymus removal may ameliorate MG symptoms in seronegative patients,[40] particularly those with lymphoid infiltrates and GCs in the thymus.[133] In patients with thymoma, thymectomy is usually necessary to treat the cancer, and the MG symptoms often improve as well. Such improvement has been proposed to occur because self-reactive T cells are no longer produced.[133] Nevertheless, further studies are necessary to clarify the role of the B-cell compartment in thymoma-associated MG and, as discussed previously, to elucidate the contribution of adjacent non-neoplastic pathologic tissue in sustaining the autoimmune response.

INNATE IMMUNITY AND AUTOIMMUNITY IN MG: AN INTRATHYMIC LINK?

Research on the pathogenesis of autoimmune diseases has in general focused on the adaptive immune system. However, it has now become clear that the innate immune system is involved in triggering or enhancing several autoimmune conditions.[134] Innate immune pathways initiate inflammation and orchestrate the acquired immune response to pathogens; however, persistent or

uncontrolled activation of the innate system can lead to chronic inflammation, which in turn can result in autosensitization and autoimmunity.[134] Evidence is now accumulating that tight regulation of innate immune pathways is necessary to maintain tolerance and avoid adaptive autoimmunity. For example, signaling by Toll-like receptors (TLRs) has recently been reported to promote the activation and survival of autoreactive B cells and thereby compromise B-cell tolerance.[135]

In what follows we summarize data on inflammation in the pathogenesis of MG, focusing on new evidence that the innate immune system, possibly activated by viral infection, may trigger the autoimmune condition.

Inflammatory Environment in MG Thymus as Promoter of Autosensitization. Striking evidence of chronic inflammation in MG thymuses comes from a microarray and real-time PCR study[83] showing that transcripts of a large number of IFN-α– and IFN-γ–regulated genes were significantly upregulated in hyperplastic MG thymuses compared with controls. The level of upregulation correlated with the severity of hyperplasia, as measured by the number of GCs in the section. MHC class II genes were also upregulated, possibly due to increased B-cell number. However, MHC class II expression did not correlate with B-cell number and may therefore be related to the inflammatory state of MG thymus.[106] *In vitro* studies showed that application of the cytokines IFN-γ and TNF-α increased AChR transcript levels in myoid cells and TECs, with the AChR α subunit being the most upregulated.[83] It was suggested that, by increasing AChR expression, proinflammatory cytokine activity may contribute to initiating the anti-AChR immune response in hyperplastic MG thymus *in vivo*.[83]

Chronic thymic inflammation could also be directly responsible for the increased expression of the T- and B-cell chemokines CXCL13, CCL21, and interferon-inducible protein-10 (IP-10), shown to occur in myasthenic thymus.[55,57,106,136] By attracting lymphocytes to thymus, these molecules may help maintain the autoimmune response in this organ, giving rise to a vicious circle.

The thymic epithelium plays an important role in promoting the inflammatory response in MG.[137,138] Stimulation of cultured TECs by lipopolysaccharide—a major activator of TLR4 and stimulator of TNF-α and -γ production—has been shown to induce gene expression and production of interleukin-6 (IL-6), which is also a proinflammatory agent and may therefore contribute to the pathologic growth and differentiation of T and B cells in MG thymus.[137] Gene profiling of TEC cultures showed basal overexpression of genes coding

for p38 and extracellular signal–regulated kinases 1 and 2 (ERK1/2) mitogen-activated protein kinases (MAPKs) in hyperplastic MG compared with normal thymus. Components of the signaling pathways of these molecules were also upregulated, including IL-6 and RANTES (regulated-upon-activation normal T-cell expressed and secreted).[138] Production of the latter by thymic epithelium could also support the migration of peripheral lymphocytes to thymus and their survival there, contributing to the pathologic remodeling of the gland, which is typical of MG.[138]

Transcriptional profiling of thymus from untreated and steroid-treated MG patients showed that the inflammatory state was reduced upon treatment.[139] In particular, the expression of type I IFN-induced genes, but not of type II IFN-induced genes or immunoglobulin genes, was normalized, suggesting that inflammatory downmodulation by these drugs occurs through type I IFN pathways.[139] This observation is particularly relevant because type I IFNs play a role during an inflammatory response by lowering the threshold for B-cell induction, thereby promoting fast and polyclonal antibody responses.[139]

A transcriptome analysis of MG thymuses from AChR-positive and seronegative patients showed that some genes were dysregulated in both groups, including MHC class II genes and genes implicated in immune response, transcription, and intracellular signaling.[106] However, seronegative thymuses were characterized by overexpression of antiviral response genes, including ISG12 (neuroprotective against viral infections), DAP12 (involved in natural killer cell–mediated resistance to infections), and SON (repressor of hepatitis B virus activity).[106] This signature is further indication of a possible involvement of viral infection in the pathogenesis of seronegative MG.[106]

Viral Involvement in MG: Old Hypotheses and New Evidence.

Autoimmunity and Viral Infections. Several viruses have been implicated in autoimmune conditions by serological studies or by direct localization of viral proteins in affected tissues.[140] EBV, a herpesvirus that infects 90–95% of the world's population, is one of the most frequently implicated agents in B-cell–mediated autoimmune diseases[141] in view of its unique ability to latently infect and immortalize B lymphocytes.[60]

Various other mechanisms have been proposed to explain how viruses, or pathogenic microorganisms in general, might trigger autoimmunity, including general activation of the host immune system, and via viral antigens that mimic self-antigens (molecular mimicry).[140] In the former

process, pathogens may stimulate an innate immune response that in turn favors a self-reactive immune response, mainly by stimulating inflammation and activating the host immune system.[140] The innate immune response is largely mediated by germline-encoded pattern recognition receptors, including TLRs. TLRs are now emerging as important contributors to the onset or progression of inflammatory and autoimmune diseases.[142] TLRs recognize a wide spectrum of molecular patterns specific to bacteria, viruses, and fungi, and activate signaling pathways that eventually result in the destruction of invading pathogens. Some of these pathways lead to the activation of antigen-presenting cells, thereby enhancing their antigen-presenting capacity, and increasing their expression of costimulatory molecules, type I IFNs, other proinflammatory cytokines, and chemokines.[140] These processes initiate and direct the acquired and specific immune response to the pathogen. However, in situations of dysregulated or persistent TLR activation—in susceptible individuals—an aberrant self-specific immune response may be favored, promoting autoimmunity.[140,142] In such situations, pro-inflammatory cytokines are first produced as part of the innate response against the pathogen. Subsequently, their production may be sustained in a non-specific manner as a result of persistent innate immune signaling.[134,143]

In 1974, Abdou and colleagues[144] found, in mixed leukocyte reactions, that thymic cells from MG hyperplastic thymus stimulated autologous peripheral blood lymphocytes, indicating the presence of altered populations of thymic lymphoid cells. In light of current belief that the innate immune system is involved in the development of autoimmune diseases,[134] the altered reactivity of MG thymocytes observed by Abdou and colleagues[144] may be interpreted as an effect of stimulation by the dendritic cells present in MG thymus that have been activated by the innate immune system.

MG and Viral Infections. Viral involvement in MG has long been suspected, but it has not been unequivocally demonstrated. The onset of MG has been documented immediately after proven infection with measles, EBV, or coinfection with human immunodeficiency virus (HIV) and human T-lymphotropic virus (HTLV), strongly suggesting that a virus can trigger onset of the disease, at least in some cases.[145–147]

One approach to identifying microbial agents involved in MG depends on the idea that pathogenic epitopes may mimic epitopes on AChR. In fact, immunological cross-reactivity between AChR peptides and proteins from herpes simplex,[148] *Escherichia coli*, *Proteus vulgaris*, and *Klebsiella pneu-*

moniae has been documented,[149] suggesting that an immune response to these microorganisms can be associated with MG.

Studies to identify antiviral antibodies in MG serum have produced contrasting results (Table 2). In one study, no significant difference was found in antibody titers for influenza A, measles, rubella, cytomegalovirus, herpes zoster, herpes simplex type 1, or mumps between 104 MG patients (with varied clinical features and thymic pathologies) and a group of age-matched healthy controls, suggesting that these common viruses, or the response to them, did not contribute to MG in the patients examined.[150] In another study, however, MG patients not treated with thymectomy or steroids had higher titers of complement-fixing antibodies against cytomegalovirus than healthy controls, whereas thymectomized and steroid-treated patients did not have higher titers.[151] In a small group of juvenile-onset MG patients, EBV antibody levels did not differ from those of age-matched controls.[150]

Several attempts have been made to identify or isolate viruses from homogenates or cell suspensions of MG thymuses, but without success (Table 2).[152,153] However, these early studies used virus detection techniques that would not be considered sufficiently sensitive today, or the methods of storing and treating the thymus tissues may not have been optimal. Nevertheless, McGuire and colleagues[154] found EBV DNA in thymuses of 2 of 4 MG patients with thymic hyperplasia and 2 of 2 of patients with thymoma. More recently, using PCR-based techniques, signs of thymic infection by HTLV-I were found with high frequency in a large group of MG patients.[155]

Poliovirus-Infected Macrophages in MG Thymus. An indication of the possible presence of virus in MG thymuses was obtained from our earlier study, which demonstrated increased expression of TLR4 in some MG thymuses characterized by thymitis and thymic involution.[156] Further examination of the same thymuses for the presence of TLR4 activators (cytomegalovirus, herpes zoster, herpes simplex types 1 and 2, eubacteria, respiratory syncytial virus, and enteroviruses) resulted in the identification of poliovirus (PV) in 14.8% of patients examined and in none of the controls.[157] By PCR we detected plus and minus RNA strands of the PV genome in the thymus in 4 (2 thymitis and 2 thymoma) of the 27 patients examined, and found a linear correlation between levels of the plus and the minus strands, thus indicating persistent PV infection.[157] We confirmed PV infection in all PCR-positive thymuses by immunolocalization of the VP1 capsid protein in the cytoplasm of CD68-positive macrophages expressing high levels of

Table 2. Summary of published findings on viral infection and MG.

Infectious agent	Serological studies	Viral components in MG thymus?
RNA viruses		
CMV, influenza A, measles, rubella, mumps	• No difference between 104 MG patients with variable MG type and thymic pathology and age-matched healthy controls[150] • High titers of antibodies to CMV in MG patients not treated with steroids or thymectomized[151]	• No signs of thymic infection in recent-onset MG patients[152,153] • No signs of CMV infection in thymus of 27 MG patients with variable thymic pathology[157]
HTLV-I	• No antibody against virus detected in serum of 30 MG patients with thymoma[150]	• Detection of HTLV-I tax-rex and pol gene sequences in a high proportion of MG thymuses with hyperplasia and thymoma[155]
Coxsackievirus	• Low incidence of neutralizing antibodies to Coxsackie B1–B6 group viruses in 37 recent-onset MG patients[150]	• No signs of thymic infection in recent-onset MG patients[152,153]
Poliovirus	• No significant difference in antibody titer to poliovirus types 1, 2, and 3 between 27 MG patients and age-matched healthy controls[157]	• No signs of thymic infection in 7 MG patients (recent onset)[152] • Detection of poliovirus type 1 RNA in 4 (2 thymitis and 2 thymoma) of 27 MG thymuses and of VP1 capsid protein in medullary macrophages[157]
DNA viruses		
Herpes simplex virus	• No difference between 104 MG patients and age-matched controls[150]	• No signs of thymic infection in recent-onset MG patients[152,153] • No signs of infection in 27 MG thymuses[157]
Herpes zoster virus	• No difference between 104 MG patients and age-matched controls[150]	• No signs of thymic infection in recent-onset MG patients[152,153] • No signs of infection in 27 MG thymuses[157]
EBV	• IgG antibodies to EBV capsid antigen at expected frequency plus absence of IgM antibodies to EBV in 19 MG patients with onset <20 years[150]	• EBV DNA in thymus of 2 of 4 MG patients with thymic hyperplasia and 2 of 2 with thymoma[154] • Expression of EBV latent (EBERs, EBNA1, LMP1, LMP2A) and lytic (BZLF1, BFRF1, BMFR1, p160, gp350/220) markers and presence of EBV DNA in 17 non-neoplastic MG thymuses[44]

CMV, cytomegalovirus; HTLV-I, human T-cell leukemia virus I; EBV, Epstein–Barr virus; VP1, viral capsid protein 1; EBERs, EBV-encoded small RNAs; EBNA1, EBV nuclear antigen 1; LMP, latent membrane protein.

TLR4. These cells were widely scattered through the thymic medulla. This was the first demonstration of chronic PV infection in MG thymus, strengthening the idea of a link between innate immune response and "adaptive autoimmune response" in MG. Because we detected virus in only a few MG thymuses, "hit-and-run"[140] may be suggested as the mechanism by which viruses may initiate autoimmunity, giving rise to clinical manifestations of MG weeks, months, or years after the pathogen has been cleared from the body. A similar explanation would account for why few studies have detected viral components in pathologic tissues associated with autoimmunity. However, this mechanism does not explain the persistent autoimmune response characteristic of MG.

Active EBV Infection in Pathologic MG Thymus. Another recent study from our laboratory suggested that MG may be yet another EBV-associated autoimmune disease.[44] Following the finding of abnormal accumulation of EBV-harboring B cells and plasma cells in ectopic follicles of multiple sclerosis brains,[123] we decided to look for EBV in MG thymuses. We used *in situ* hybridization to detect EBV-encoded small RNAs, immunohistochemistry

to detect EBV antigens, and PCR to detect viral DNA and mRNA. We found that all MG thymuses examined (6 with follicular hyperplasia, 6 with diffuse hyperplasia, and 5 with thymic involution) were positive for EBV, whereas no sign of infection was found in non-pathologic controls. EBV positivity was found in a high proportion of the B cells and plasma cells forming medullary lymphoid infiltrates and follicles. We found viral DNA and both viral latency and lytic gene mRNAs and proteins, indicating EBV persistence and reactivation, respectively. This suggests that EBV contributes to the chronic B-cell activation characteristic of MG thymus. The presence of viral latency proteins in GCs suggests that these were immunoprivileged niches for EBV persistence, further suggesting that the virus may be responsible for polyclonal B-cell activation as well as expansion of autoreactive B-cell clones in these structures.[44]

Because EBV has the unique ability to disrupt B-cell–regulatory checkpoints by providing activation signals in self-reactive B clones and allowing them to bypass central and peripheral tolerance mechanisms,[60,141] our finding of active EBV infection in MG thymus supports the idea that EBV
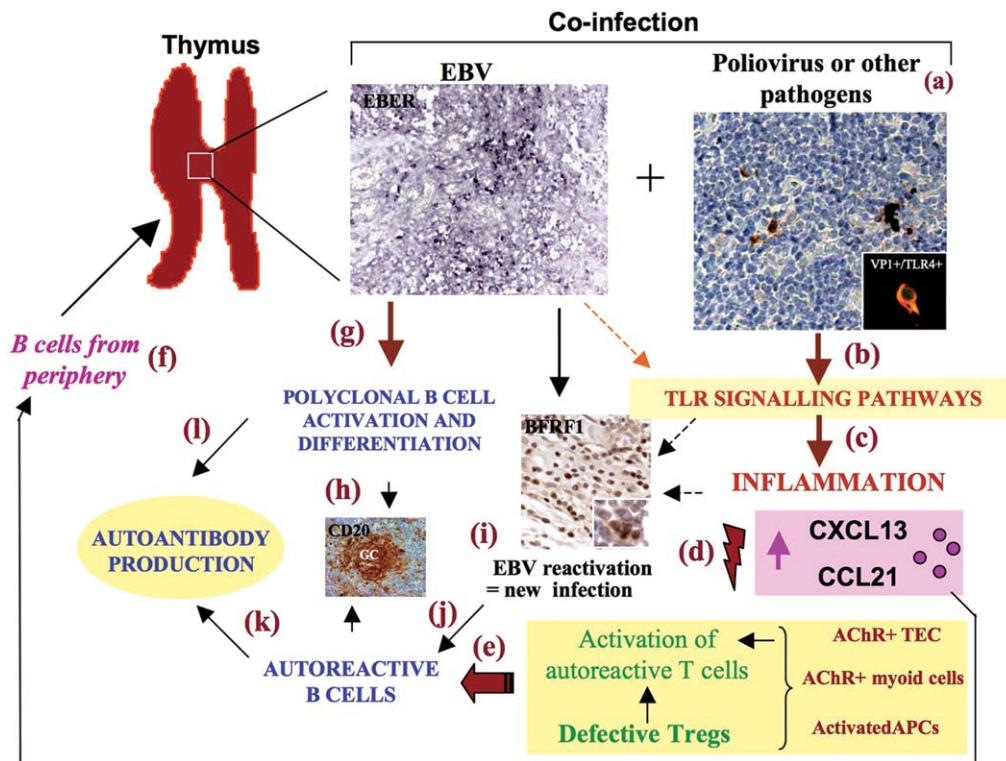
FIGURE 4. Proposed model of virus-induced autoimmunity in MG. A 'danger signal' (e.g., EBV, poliovirus, or other pathogen) **(a)**, stimulates TLR signaling **(b)**, whose dysregulated or persistent activation leads to the chronic inflammation characteristic of MG thymus **(c)**, or (EBV) drives abnormal B-cell activation and differentiation (dashed arrow). Inflammation renders thymus prone to autosensitization **(d)**, by activating professional antigen-presenting cells (APCs), promoting cross-presentation by TECs or myoid cells expressing the autoantigen, and inducing activation of AChR-specific T cells, which in turn promote response by autoreactive B cells **(e)**. Functionally deficient Tregs contribute to lack of tolerance of AChR-specific T cells. Inflammation upregulates production of B-cell attractants CXCL13 and CCL21, which attract circulating B cells to thymus **(f)**, including those harboring EBV. EBV is proposed to drive abnormal polyclonal B-cell activation and proliferation **(g)**, and GC formation **(h)**. EBV reactivation, influenced by the inflammatory state, results in EBV propagation to uninfected cells **(i)** and maintenance of thymic autoimmune response **(j)**. EBV-induced differentiation of B cells to long-lived plasma cells perpetuates autoantibody production **(k)**. EBV-infected B cells could themselves be a source of autoantibodies **(l)**. EBER, EBV-encoded small RNAs; VP1, enteroviral capsid protein; BFRF1, EBV lytic marker; CD20, B-cell marker. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

infection may be a common feature of autoimmune conditions characterized by B-cell dysfunction and lymphoid neogenesis in pathologic tissues.

It remains unclear, however, whether active intrathymic EBV infection is a primary event in MG; that is, it could be the consequence of an underlying intrathymic process that results in both attraction of circulating EBV-infected cells and EBV reactivation in MG thymus. The very high prevalence of EBV infection in the population and low incidence of MG suggest that genetic or other environmental factors (or both) must intervene together with EBV to cause MG. It is also possible that a preexisting inflammatory state might be necessary to trigger the recruitment of EBV-infected B cells and other heterogeneously activated B cells, consistent with the polyclonal profile present in MG thymus.[107,139]

Although our study provides the first evidence linking active intrathymic EBV infection with MG, it cannot be considered definitive, because patient numbers were small and other autoimmune disease controls were lacking. Serafini and colleagues[123] used similar techniques to identify meningeal B-cell follicles in multiple sclerosis brains and determine that they were major sites of EBV persistence and reactivation. However, other groups failed not only to detect EBV but also to indentify meningeal B-cell follicles.[158,159] Although we used PCR (as well as the immunohistochemistry and *in situ* hybridization of Serafini et al.[123]) to confirm EBV in MG thymuses, it is clear that our data require confirmation from other groups, even though the storage, selection, and processing of the tissues, as well as the application of the methods used, can vary from group to group and affect the ability to detect EBV.[160,161]

Based on new and established data, we propose a tentative model to explain how viruses may sustain autoimmunity in MG (Fig. 4). In brief, and in the context of a genetic background predisposing to MG, the model proposes that abnormal and

persistent intrathymic activation of TLR-mediated innate immune responses is triggered by endogenous or exogenous (e.g., microbial infection) danger signals giving rise to a cascade of events culminating in the creation of a chronic inflammatory state favorable to the recall of circulating T and B cells to thymus and their subsequent AChR-specific sensitization. The inflammatory state may drive the colonization of thymus by EBV-harboring B cells and a subsequent EBV reactivation, providing a possible explanation for the maintenance and perpetuation of the autoimmune response.

CONCLUSIONS

The thymus has long been considered to be the site of development and maintenance of autoimmunity in MG. The old and new findings reviewed herein show that this is almost certainly the case. The recent discovery of a persistent viral presence in some MG thymuses, together with data showing that components of the innate immune system are activated in thymus (thereby linking infection and intrathymic immune autosensitization), combined with the wealth of data showing that the thymus is in a state of chronic inflammation in most MG patients, make it increasingly plausible that microbial agents are involved in the pathogenetic and etiologic mechanisms of the disease. In particular, the finding of active EBV infection in the intrathymic B-cell component in MG patients suggests how the autoimmune response can be perpetuated, because EBV is potentially able to immortalize B cells that are producing AChR antibodies. Although these recent results raise more questions than answers, they do suggest the direction of further research: for example, identification genetic predisposition and virus–host interactions, that may favor a dysregulated innate immune response and the perpetuation of EBV infection.

If confirmed by further studies, viral involvement in the pathogenesis or progression of MG could have several implications for treatment, first by strengthening the rationale of current approaches, particularly anti-inflammatory drug use and thymectomy to remove the site of infection and autoreactivity. Trials of antiviral agents would also be justified, and the development of new treatments that target innate immune components would be stimulated. Finally, and most interestingly, the question raised by Kaminski and Minarovits,[162] would once again become relevant: Could vaccination be an effective treatment for chronic EBV-related MG?

REFERENCES

1. Carr AS, Cardwell CR, McCarron PO, McConville J. A systematic review of population based epidemiological studies in Myasthenia Gravis. BMC Neurol 2010;10:46.
2. Mantegazza R, Baggi F, Antozzi C, Confalonieri P, Morandi L, Bernasconi P, et al. Myasthenia gravis (MG): epidemiological data and prognostic factors. Ann NY Acad Sci 2003;998:413–423.
3. Vincent A. Unravelling the pathogenesis of myasthenia gravis. Nat Rev Immunol 2002;2:797–804.
4. Lindstrom JM, Seybold ME, Lennon VA, Whittingham S, Duane DD. Antibody to acetylcholine receptor in myasthenia gravis. Prevalence, clinical correlates, and diagnostic value. Neurology 1976;26:1054–1059.
5. Sahashi K, Engel AG, Lambert EH, Howard FM Jr. Ultrastructural localization of the terminal and lytic ninth complement component (C9) at the motor end-plate in myasthenia gravis. J Neuropathol Exp Neurol 1980;39:160–172.
6. Piddlesden SJ, Jiang S, Levin JL, Vincent A, Morgan BP. Soluble complement receptor 1 (sCR1) protects against experimental autoimmune myasthenia gravis. J Neuroimmunol 1996;71:173–177.
7. Drachman DB, Angus CW, Adams RN, Michelson JD, Hoffman GJ. Myasthenia antibodies cross-link acetylcholine receptors to accelerate degradation. N Engl J Med 1978;298:1116–1122.
8. Gomez CM, Richman DP. Anti-acetylcholine receptor antibodies directed against the alpha-bungarotoxin binding site induce a unique form of experimental myasthenia. Proc Natl Acad Sci USA 1983;80:4089–4093.
9. Lennon VA, Seybold ME, Lindstrom JM, Cochrane C, Ulevitch R. Role of complement in the pathogenesis of experimental autoimmune myasthenia gravis. J Exp Med 1978;147:973–983.
10. Biesecker G, Gomez CM. Inhibition of acute passive transfer experimental autoimmune myasthenia gravis with Fab antibody to complement C6. J Immunol 1989;142:2654–2659.
11. Christadoss P. C5 gene influences the development of murine myasthenia gravis. J Immunol 1988;140:2589–2592.
12. Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. Nat Med 2001;7:365–368.
13. Evoli A, Tonali PA, Padua L, Monaco ML, Scuderi F, Batocchi AP, et al. Clinical correlates with anti-MuSK antibodies in generalized seronegative myasthenia gravis. Brain 2003;126:2304–2311.
14. Sanders DB, El-Salem K, Massey JM, McConville J, Vincent A. Clinical aspects of MuSK antibody positive seronegative MG. Neurology 2003;60:1978–1980.
15. McConville J, Farrugia ME, Beeson D, Kishore U, Metcalfe R, Newsom-Davis J, et al. Detection and characterization of MuSK antibodies in seronegative myasthenia gravis. Ann Neurol 2004;55:580–584.
16. Vincent A, McConville J, Farrugia ME, Newsom-Dawis J. Seronegative myasthenia gravis. Semin Neurol 2004;24:125–133.
17. Farrugia ME, Bonifati DM, Clover L, Cossins J, Beeson D, Vincent A. Effect of sera from AChR-antibody negative myasthenia gravis patients on AChR and MuSK in cell cultures. J Neuroimmunol 2007;185:136–144.
18. Leite MI, Jacob S, Viegas S, Cossins J, Clover L, Morgan BP, et al. IgG1 antibodies to acetylcholine receptors in 'seronegative' myasthenia gravis. Brain 2008;131:1940–1952.
19. Gautel M, Lakey A, Barlow DP, Holmes Z, Scales S, Leonard K, et al. Titin antibodies in myasthenia gravis: identification of a major immunogenic region of titin. Neurology 1993;43:1581–1585.
20. Baggi F, Andreetta F, Antozzi C, Simoncini O, Confalonieri P, Labeit S, et al. Anti-titin and antiryanodine receptor antibodies in myasthenia gravis patients with thymoma. Ann NY Acad Sci 1998;841:538–541.
21. Maggi L, Andreetta F, Antozzi C, Baggi F, Bernasconi P, Cavalcante P, et al. Thymoma-associated myasthenia gravis: outcome, clinical and pathological correlations in 197 on a 20-year experience. J Neuroimmunol 2008;15:202:237–244.
22. Murphy J, Murphy SF. Myasthenia gravis in identical twins. Neurology 1986;36:78–80.
23. Machens A, Loliger C, Pichlmeier U, Emskotter T, Busch C, Izbicki JR. Correlation of thymic pathology with HLA in myasthenia gravis. Clin Immunol 1999;91:296–301.
24. Vandiedonck C, Giraud M, Garchon HJ. Genetics of autoimmune myasthenia gravis: the multifaceted contribution of the HLA complex. J Autoimmun 2005;25:6–11.
25. Vandiedonck C, Beaurain G, Giraud M, Hue-Beauvais C, Eymard B, Tranchant C, et al. Pleiotropic effects of the 8.1 HLA haplotype in

patients with autoimmune myasthenia gravis and thymus hyperplasia. Proc Natl Acad Sci USA 2004;101:1564–1569.

26. Vieira ML, Caillat-Zucman S, Gajdos P, Cohen-Kaminsky S, Casteur A, Bach JF. Identification of genomic typing non-DR3 HLA class II genes associated myasthenia gravis. J Neuroimmunol 1993;47: 115–122.

27. Vandiedonck C, Raffoux C, Eymard B, Krumeich CS, Gajdos P, Garchon HJ. Association of HLA-A in autoimmune myasthenia gravis with thymoma. J Neuroimmunol 2009;210:120–123.

28. Giraud M, Vandiedonck C, Garchon HJ. Genetic factors in autoimmune myasthenia gravis. Ann NY Acad Sci 2008;1132:180–192.

29. Garchon HJ, Djabiri F, Viard JP, Gajdos P, Bach JF. Involvement of human muscle acetylcholine receptor alpha-subunit gene (CHRNA) in susceptibility to myasthenia gravis. Proc Natl Acad Sci USA 1994; 91:4668–4672.

30. Giraud M, Taubert R, Vandiedonck C, Ke X, Lévi-Strauss M, Pagani F, et al. An IRF8-binding promoter variant and AIRE control CHRNA1 promiscuous expression in thymus. Nature 2007;448:934–937.

31. Rowland LP, Hoefer PF, Aranow H, Merritt HH. Fatalities in myasthenia gravis: a review of 39 cases with 26 autopsies. Neurology 1958;6:310–326.

32. Mantegazza R, Beghi E, Pareyson D, Antozzi C, Peluchetti D, Sghirlanzoni A, et al. A multicentre follow-up study of 1152 patients with myasthenia gravis in Italy. J Neurol 1990;237:339–344.

33. Alshekhlee A, Miles JD, Katirji B, Preston DC, Kaminski HJ. Incidence and mortality rates of myasthenia gravis and myasthenic crisis in US hospitals. Neurology 2009;72:1548–1554.

34. Sanders DB, Evoli A. Immunosuppressive therapies in myasthenia gravis. Autoimmunity 2010;43:428–435.

35. Punga AR, Stålberg E. Acetylcholinesterase inhibitors in MG: to be or not to be? Muscle Nerve 2009;39:724–728.

36. Antozzi C, Gemma M, Regi B, Berta E, Confalonieri P, Peluchetti D, et al. A short plasma exchange protocol is effective in severe myasthenia gravis. J Neurol 1991;238:103–107.

37. Dalakas M. IVIg in other autoimmune neurological disorders: current status and future prospects. J Neurol 2008;3:12–16.

38. Antozzi C, Berta E, Confalonieri P, Zuffi M, Cornelio F, Mantegazza R. Protein-A immunoadsorption in immunosuppression-resistant myasthenia gravis. Lancet 1994;343:124.

39. Maddison P, McConville J, Farrugia ME, Davies N, Rose M, Norwood F, et al. The use of rituximab in myasthenia gravis and Lambert–Eaton myasthenic syndrome. J Neurol Neurosurg Psychiatry (in press).

40. Mantegazza R, Baggi F, Bernasconi P, Antozzi C, Confalonieri P, Novellino L, et al. Video-assisted thoracoscopic extended thymectomy and extended transsternal thymectomy (T-3b) in non-thymomatous myasthenia gravis patients: remission after 6 years of follow-up. J Neurol Sci 2003;212:31–36.

41. Leite MI, Strobel P, Jones M, Micklem K, Moritz R, Gold R, et al. Fewer thymic changes in MuSK antibody-positive than in MuSK antibody-negative MG. Ann Neurol 2005;57:444–448.

42. Lauriola L, Ranelletti F, Maggiano N, Guerriero M, Punzi C, Marsili F, et al. Thymus changes in anti-MuSK-positive and -negative myasthenia gravis. Neurology 2005;64:536–538.

43. Müoller-Hermelink HK, Marx A, Geuder KI, Kirchner T. Thymus. In: Damjanov I, Linder J, editors. Anderson's pathology. Vol. I. St. Louis, MO: Mosby; 1996. p 1218–1243.

44. Cavalcante P, Serafini B, Rosicarelli B, Maggi L, Barberis M, Antozzi C, et al. Epstein–Barr virus persistence and reactivation in myasthenia gravis thymus. Ann Neurol 2010;67:726–738.

45. Aloisi F, Pujol-Borrell R. Lymphoid neogenesis in chronic inflammatory diseases. Nat Rev Immunol 2006;6:205–217.

46. Humby F, Bombardieri M, Manzo A, Kelly S, Blades MC, Kirkham B, et al. Ectopic lymphoid structures support ongoing production of class-switched autoantibodies in rheumatoid synovium. PLoS Med 2009;6:e1

47. Armengol MP, Juan M, Lucas-Martín A, Fernández-Figueras MT, Jaraquemada D, Gallart T, et al. Thyroid autoimmune disease: demonstration of thyroid antigen-specific B cells and recombination-activating gene expression in chemokine-containing active intrathyroidal germinal centers. Am J Pathol 2001;159:861–873.

48. 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: 1935–1944.

49. Stott DI, Hiepe F, Hummel M, Steinhauser G, Berek C. Antigen-driven clonal proliferation of B cells within the target tissue of an autoimmune disease. The salivary glands of patients with Sjögren's syndrome. J Clin Invest 1998;102:938–946.

50. Zucherman NS, Howard WA, Bismuth J, Gibson K, Edelman H, Berrih-Aknin S, et al. Ectopic GC in the thymus of myasthenia gravis patients show characteristic of normal GC. Eur J Immunol 2010;40:1150–1161.

51. Roxanis I, Micklem K, McConville J, Newsom-Davis J, Willcox N. Thymic myoid cells and germinal center formation in myasthenia gravis; possible roles in pathogenesis. J Neuroimmunol 2002;125:185–197.

52. Wekerle H, Ketelsen UP. Intrathymic pathogenesis and dual genetic control of myasthenia gravis. Lancet 1977;1:678–680.

53. Schluep M, Willcox N, Vincent A, Dhoot GK, Newson-Davis J. Acetylcholine receptors in human thymic myoid cells in situ: an immunohistological study. Ann Neurol 1987;22:212–222.

54. Wakkach A, Poëa S, Chastre E, Gespach C, Lecerf F, de la Porte S, et al. Establishment of a human thymic myoid cell line: phenotypic and functional characteristics. Am J Pathol 1999;155:1229–1240.

55. Berrih-Aknin S, Ruhlmann N, Bismuth J, Cizeron-Clairac G, Zelman E, Shachar I, et al. CCL21 overexpressed on lymphatic vessels drives thymic hyperplasia in myasthenia. Ann Neurol 2009;66:521–531.

56. Haynes BF, Hale LP. The human thymus. A chimeric organ comprised of central and peripheral lymphoid components. Immunol Res 1998;18:175–192.

57. Meraouna A, Cizeron-Clairac G, Le Panse R, Bismuth J, Truffault F, Tallaksen C, et al. The chemokine CXCL13 is a key molecule in autoimmune myasthenia gravis. Blood 2006;108:432–440.

58. Tangarajh M, Masterman T, Helgeland L, Rot U, Jonsonn MV, Eide GE, et al. The thymus is a source of B-cell-survival factors—APRIL and BAFF—in myasthenia gravis. J Neuroimmunol 2006;178:161–166.

59. Ragheb S, Lisak R, Lewis R, van Stavern G, Gonzales F, Simon K. A potential role for B-cell activating factor in the pathogenesis of autoimmune myasthenia gravis. Arch Neurol 2008;65:1358–1362.

60. Thorley-Lawson D. Epstein–Barr virus: exploiting the immune system. Nat Rev Immunol 2001;1:75–82.

61. Shiono H, Fujii Y, Okumura M, Takeuchi Y, Inoue M, Matsuda H. Failure to down-regulate Bcl-2 protein in thymic germinal center B cells in myasthenia gravis. Eur J Immunol 1997;27:805–809.

62. Henderson S, Rowe M, Gregory C, Croom-Carter D, Wang F, Longnecker R, et al. Induction of Bcl-2 expression by Epstein–Barr virus latent membrane protein 1 protects infected B cells from programmed cell death. Cell 1991;65:1107–1115.

63. Masaoka A, Yamakawa Y, Niwi H, Fukay I, Kondo S, Kobayashi M, et al. Extended thymectomy for myasthenia gravis patients: a 20-year review. Ann Thorac Surg 1996;62:853–859.

64. Chen G, Marx A, Wen-Hu C, Yong J, Puppe B, Stroebel P, et al. New WHO histologic classification predicts prognosis of thymic epithelial tumors: a clinicopathologic study of 200 thymoma cases from China. Cancer 2002;95:420–429.

65. Okumura M, Shiono H, Minami M, Inoue M, Utsumi T, Kadota Y, et al. Clinical and pathological aspects of thymic epithelial tumors. Gen Thorac Cardiovasc Surg 2008;56:10–16.

66. Jaretzki A III, Penn AS, Younger DS. "Maximal" thymectomy for myasthenia gravis. Results. J Thorac Cardiovasc Surg 1988;95: 747–757.

67. Berrih S, Morel E, Gaud C, Raimond F, Le Brigand H, Bach JF. Anti-AChR antibodies, thymic histology, and T cell subsets in myasthenia gravis. Neurology 1984;34:66–71.

68. Evoli A, Minisci C, Di Schino, Marsili F, Punzi C, Batocchi AP, et al. Thymoma in patients with MG: characteristic and long-term outcome. Neurology 2002;59:1844–1850.

69. Okumura M, Fujii Y, Shiono H, Inoue M, Minami M, Utsumi T, et al. Immunological function of thymoma and pathogenesis of paraneoplastic myasthenia gravis. Gen Thorac Cardiovasc Surg 2008; 56:143–150.

70. Rosai J, Sobin LH. Histological typing of tumors of the thymus. In: World Health Organization. International histological classification of tumors, 2nd ed. New York: Springer; 1999. p 9–14.

71. Ströbel P, Bauer A, Puppe B, Ktaushaar T, Krein A, Toyka K, et al. Tumor recurrence and survival in patients treated for thymomas and thymic squamous cell carcinomas: a retrospective analysis. J Clin Oncol 2004;22:1501–1509.

72. Savino W, Manganella G, Verley JM, Wolff A, Berrih S, Levasseur P, et al. Thymoma epithelial cells secrete thymic hormone but do not express class II antigens of the major histocompatibility complex. J Clin Invest 1985;76:1140–1146.

73. Savino W, Berrih S, Dardenne M. Thymic epithelial antigen, acquired during ontogeny and defined by the anti-p19 monoclonal antibody, is lost in thymomas. Lab Invest 1984;51:292–296.

74. Fend F, Kirchner T, Marx A, Müller-Hermelink HK. B-cells in thymic epithelial tumours. An immunohistochemical analysis of intra- and extraepithelial B-cell compartments. Virchows Arch B Cell Pathol Incl Mod Pathol 1993;63:241–247.

75. Alexiev BA, Drachenberg CB, Burke AP. Thymomas: a cytological and immunohistochemical study, with emphasis on lymphoid and neuroendocrine markers. Diagn Pathol 2007;2:13–22.

76. Wakkach A, Guyon T, Bruand C, Tzartos S, Cohen-Kaminsky S, Berrih-Aknin S. Expression of acetylcholine receptor genes in human thymic epithelial cells: implications for myasthenia gravis. J Immunol 1996;157:3752–3760.

77. Melms A, Schalke BCG, Kirchner T, Müller-Hermelink HK, Albert E, Wekerle H. Thymus in myasthenia gravis: isolation of T-

lymphocyte lines specific for the nicotinic acetylcholine receptor from thymuses of myasthenic patients. J Clin Invest 1988;81: 902–908.

78. Sommer N, Willcox N, Harcourt GC, Newsom-Davis J. Myasthenic thymus and thymoma are selectively enriched in acetylcholine receptor specific T cells. Ann Neurol 1990;28:312–319.

79. 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;201:50–56.

80. Schönbeck S, Padberg F, Hohlfeld R, Wekerle H. Transplantation of thymic autoimmune microenvironment to severe combined immunodeficiency mice. A new model of myasthenia gravis. J Clin Invest 1992;90:245–250.

81. Mesnard-Roullier L, Bismuth J, Wakkach A, Poëa-Guyon S, Berrih-Aknin S. Thymic myoid cells express high levels of muscle genes. J Neuroimmunol 2004;148:97–105.

82. Vincent A, Willcox N, Hill M, Curnow J, MacLennan C, Beeson D. Determinant spreading and immune responses to acetylcholine receptors in myasthenia gravis. Immunol Rev 1998;164:157–168.

83. Poëa-Guyon S, Christadoss P, Le Panse R, Guyon T, De Baets M, Wakkach A, et al. Effects of cytokines on acetylcholine receptor expression: implications for myasthenia gravis. J Immunol 2005;174: 5941–5949.

84. Kirchner T, Hoppc F, Schalke B, Müller-Hermelink HK. Microenvironment of thymic myoid cells in myasthenia gravis. Virchows Arch B Cell Pathol 1988;54:295–302.

85. Villadangos JA, Heath WR, Carbone FR. Outside looking in: the inner workings of the crosspresentation pathway within dendritic cells. Trends Immunol 2007;28:45–47.

86. Leite MI, Jones M, Ströbel P, Marx A, Gold R, Niks E, et al. Myasthenia gravis thymus: complement vulnerability of epithelial and myoid cells, complement attack on them and correlations with autoantibody status. Am J Pathol 2007;171:893–905.

87. Bornemann A, Kirchner T. Thymic myoid cell turnover in myasthenia gravis patients and in normal controls. Cell Tissue Res 1996; 284:481–487.

88. Rosai J, Levine GD. Tumors of the thymus. In: Atlas of tumor pathology, series II. Fascicle 13. Washington, DC: Armed Forces Institute of Pathology; 1976. p 34–161.

89. MacLennan CA, Vincent A, Marx A, Willcox N, Gilhus NE, Newsom-Davis J, et al. Preferential expression of AChR ε-subunit in thymomas from patients with myasthenia gravis. J Neuroimmunol 2008;201:28–32.

90. Kyewski B, Klein L. A central role for central tolerance. Annu Rev Immunol 2006;24:571–606.

91. Ruan QG, She JX. Autoimmune polyglandular syndrome type I and the autoimmune regulator. Clin Lab Med 2004;24:305–317.

92. Scarpino S, Di Napoli A, Stoppacciaro A, Antonelli M, Pilozzi E, Chiarle R, et al. Expression of autoimmune regulator gene (AIRE) and T regulatory cells in human thymomas. Clin Exp Immunol 2007;149:504–512.

93. Hohlfeld R, Kalies I, Kohleisen B, Heininger K, Conti-Tronconi BM, Toyka KV. Myasthenia gravis: stimulation of antireceptor autoantibodies by autoreactive T cell lines. Neurology 1986;36:618–621.

94. Ahlberg R, Yi Q, Pirskanen R, Matell G, Swerup C, Rieber EP, et al. Treatment of myasthenia gravis with anti-CD4 antibody: improvement correlates to decreased T-cell autoreactivity. Neurology 1994; 44:1732–1737.

95. Sommer N, Harcourt GC, Willcox N, Beason D, Newsom-Davis J. Acetylcholine receptor-reactive T lymphocytes from healthy subjects and myasthenia gravis patients. Neurology 1991;41:1270–1276.

96. Moiola L, Karachunski P, Protti MP, Howard JF, Conti-Tronconi BM. Epitopes on the beta subunit of human muscle acetylcholine receptor recognized by CD4+ cells of myasthenia gravis patients and healthy subjects. J Clin Invest 1994;93:1020–1028.

97. Levinson AI, Zheng Y, Gaulton G, Moore J, Pletcher CH, Song D, et al. A new model linking intra-thymic acetylcholine receptor expression and the pathogenesis of myasthenia gravis. Ann NY Acad Sci 2003;998:257–265.

98. Sisely A, Lisak RP, Brenner T. Proliferative response of blood cells of patients with myasthenia gravis to purified mammalian acetylcholine receptor. Pathol Immunopathol Res 1989;8:113–117.

99. Protti MP, Manfredi AA, Straub C, Howard JF, Conti-Tronconi BM. Immunodominant regions for T helper-cell sensitization on the human nicotinic receptor alpha subunit in myasthenia gravis. Proc Natl Acad Sci 1990;87:7792–7796.

100. Ragheb S, Mohamed M, Lisak RP. Myasthenia gravis patients, but not healthy subjects, recognize epitopes that are unique to the ε-subunit of the acetylcholine receptor. J Neuroimmunol 2005;159: 137–145.

101. Moulian N, Bidault J, Truffault F, Yamamoto AM, Levasseur P, Berrih-Aknin S. Thymocyte Fas expression is dysregulated in myasthenia gravis patients with anti-acetylcholine receptor antibody. Blood 1997;89:3287–3295.

102. 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; 105:735–741.

103. Matsui N, Nakane S, Saito F, Ohigashi I, Nakagawa Y, Kurobe H, et al. Undiminished regulatory T cells in the thymus of patients with myasthenia gravis. Neurology 2010;74:816–820.

104. Luther C, Poeschel S, Varga M, Melms A, Tolosa E. Decreased frequency of intrathymic regulatory T cells in patients with myasthenia-associated thymoma. J Neuroimmunol 2005;164:124–128.

105. Meinl E, Klinkert WE, Wekerle H. The thymus in myasthenia gravis. Changes typical for the human disease are absent in experimental autoimmune myasthenia gravis of the rat. Am J Pathol 1991;139: 995–1008.

106. Le Panse R, Cizeron-Clairac G, Bismuth J, Berrih-Aknin S. Microarrays reveal distinct gene signatures in the thymus of seropositive and seronegative myasthenia gravis patients and the role of CC chemokine ligand 21 in thymic hyperplasia. J Immunol 2006;177: 7868–7879.

107. Guigou V, Emilie D, Berrih-Aknin S, Fumoux F, Fougereau M, Schiff C. Individual germinal centres of myasthenia gravis human thymuses contain polyclonal activated B cells that express all the VH and VK families. Clin Exp Immunol 1991;83:262–266.

108. Levinson AI, Zweiman B, Lisak RP. Pokeweed mitogen-induced immunoglobulin secretory responses of thymic B cells in myasthenia gravis: selective secretion of IgG versus IgM cannot be explained by helper functions of thymic T cells. Clin Immunol Immunopathol 1990;57:211–217.

109. Zeweiman B, Levinson AI, Lisak RP. Phenotypic characteristics of thymic B lymphocytes in myasthenia gravis. J Clin Immunol 1989;9: 242–247.

110. 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;1:305–307.

111. Scadding GK, Vincent A, Newsom-Davis J, Henry K. Acetylcholine receptor antibody synthesis by thymic lymphocytes: correlation with thymic histology. Neurology 1981;31:935–943.

112. Willcox HN, Newsom-Davis J, Calder LR. Greatly increased autoantibody production in myasthenia gravis by thymocyte suspensions prepared with proteolytic enzymes. Clin Exp Immunol 1983;54: 378–386.

113. 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: 604–608.

114. Lisak RP, Laramore C, Levinson AI, Zewiman 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;34:802–805.

115. Lisak RP, Laramore C, Levinson AI, Zweiman B, Moskovitz AR. Suppressor T cells in myasthenia gravis and antibodies to acetylcholine receptor. Ann Neurol 1986;19:87–89.

116. Padberg F, Matsuda M, Fenk R, Patenge N, Kubuschok B, Hohlfeld R, et al. Myasthenia gravis: selective enrichment of anti-acetylcholine receptor antibody production in untransformed human B cell cultures. Eur J Immunol 1999;29:3538–3548.

117. 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;136:887–891.

118. Safar D, Berrih-Aknin S, Morel E. In vitro anti-acetylcholine receptor antibody synthesis by myasthenia gravis patient lymphocytes: correlations with thymic histology and thymic epithelial cell interaction. J Clin Immunol 1987;7:225–234.

119. 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: 1313–1318.

120. Lisak RP, Levinson AI, Zweiman B, Kornstein MJ. Antibodies to acetylcholine receptor and tetanus toxoid: in vitro synthesis by thymic lymphocytes. J Immunol 1986;137:1221–1225.

121. Leprince C, Cohen-Kaminsky S, Berrih-Aknin S, Vernet-Der Garabedian B, Treton D, Galanaud P, et al. Thymic B cells from myasthenia gravis patients are activated B cells. Phenotypic and functional analysis. J Immunol 1990;145:2115–2122.

122. Berzi A, Ayata CK, Cavalcante P, Falcone C, Candiago E, Motta T, et al. BDNF and its receptors in human myasthenic thymus: implications for cell fate in thymic pathology. J Neuroimmunol 2008; 197:128–139.

123. Serafini B, Rosicarelli B, Franciotta D, Magliozzi R, Reynolds R, Cinque P, et al. Dysregulated Epstein–Barr virus infection in the multiple sclerosis brain. J Exp Med 2007;204:2899–2912.

124. Beghi E, Antozzi C, Batocchi AP, Cornelio F, Cosi V, Evoli A. Prognosis of myasthenia gravis: a multi center follow-up study of 844 patients. J Neurol Sci 1991;106:213–220.

125. Sonett JR, Jaretzki A III. Thymectomy for nonthymomatous myasthenia gravis. A critical analysis. Ann NY Acad Sci 2008;1132: 315–328.
126. Okumura M, Ohta M, Takeuchi Y, Shiono H, Inoue M, Fukuhara K, et al. The immunological 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;126:1922–1928.
127. Mori T, Nomori H, Ikeda K, Kobayashi H, Iwatani K, Kobayashi T. The distribution of parenchyma, follicles, and lymphocyte subsets in thymus of patients with myasthenia gravis, with special reference to remission after thymectomy. J Thorac Cardiovasc Surg 2007;133: 364–368.
128. 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: 141–146.
129. Novellino L, Longoni M, Spinelli L, Andretta M, Cozzi M, Faillace G. 'Extended' thymectomy without sternotomy performed by cervicotomy and thoracoscopic technique in the treatment of myasthenia gravis. Int Surg 1994;79:378–381.
130. Goldstein SD, Yang SC. Assessment of robotic thymectomy using the myasthenia gravis Foundation of America guidelines. Ann Thorac Surg 2010;89:1080–1086.
131. Bachmann K, Burkhardt D, Schreiter I, Kaifi J, Schurr P, Busch C, et al. Thymectomy is more effective than conservative treatment for myasthenia gravis regarding outcome and clinical improvement. Surgery 2009;145:392–398.
132. Jaretzki A III, Barohn RJ, Ernstoff RM, Kaminski HJ, Keesey JC, Penn AS, et al. Myasthenia gravis. Recommendations for clinical research standards. Neurology 2000;55:16–23.
133. Okumura M, Inoue M, Kadota Y, Hayashi A, Tokunaga T, Kusu T, et al. Biological implications of thymectomy for myasthenia gravis. Surg Today 2010;40:102–107.
134. Shi F-D, Ljunggren H-G, Sarvetnick N. Innate immunity and autoimmunity: from self-protection to self-destruction. Trends Immunol 2001;22:97–101.
135. Crampton SP, Voynova E, Bolland S. Innate pathways to B cell activation and tolerance. Ann NY Acad Sci 2010;1183:58–68.
136. Feferman T, Maiti PK, Berrih-Aknin S, Bismuth J, Bidault J, Fuchs S, et al. Overexpression of IFN-induced protein 10 and its receptor CXCR3 in myasthenia gravis. J Immunol 2005;174:5324–5331.
137. Cohen-Kaminsky S, Devergne O, Delattre RM, Klingel-Schmitt I, Emilie D, Galanaud P, et al. Interleukin-6 overproduction by cultured thymic epithelial cells from patients with myasthenia gravis is potentially involved in thymic hyperplasia. Eur Cytokine Netw 1993; 4:121–132.
138. Colombara M, Antonini V, Riviera AP, Mainiero F, Strippoli R, Merola M, et al. Constitutive activation of p38 and ERK1/2 MAPKs in epithelial cells of myasthenic thymus leads to IL-6 and RANTES overexpression: effects on survival and migration of peripheral T and B cells. J Immunol 2005;175:7021–7028.
139. Cizeron-Clairac G, Le Panse R, Frenkian-Cuvelier M, Meraouna A, Truffault F, Bismuth J, et al. Thymus and myasthenia gravis: what can we learn from DNA microarrays?. J Neuroimmunol 2008;201: 57–63.
140. Münz C, Lünemann JD, Getts MT, Miller SD. Antiviral immune responses: triggers of or triggered by autoimmunity? Nat Rev Immunol 2009;9:246–257.
141. Niller HH, Wolf H, Minarovits J. Regulation and dysregulation of Epstein–Barr virus latency: implications for the development of autoimmune diseases. Autoimmunity 2008;41:298–328.
142. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol 2010; 11:373–384.
143. Di Rosa F, Barnaba V. Persisting viruses and chronic inflammation: understanding their relationship to autoimmunity. Immunol Rev 1998;164:17–27.
144. Abdou NI, Lisak RP, Zweiman B, Abrahamsohn I, Penn AS. The thymus in myasthenia gravis. Evidence for altered cell populations. N Engl J Med 1974;291:1271–1275.
145. Korn IL, Abramsky O. Myasthenia gravis following viral infection. Eur Neurol 1981;20:435–439.
146. Bhibhatbhan A, Kline G, Vincent A, Toth C. Anti-MuSK myasthenia gravis presenting with Epstein–Barr virus-associated mononucleosis and immune-mediated diabetes mellitus. Muscle Nerve 2007;36: 264–266.
147. Verma A, Berger J. Myasthenia gravis associated with dual infection of HIV and HTLV-I. Muscle Nerve 1995;18:1355–1356.
148. Schwimmbeck PL, Dyrberg T, Drachman DB, Oldstone MBA. Molecular mimicry and myasthenia gravis. An autoantigenic site of the acetylcholine receptor alpha-subunit that has biologic activity and reacts immunochemically with herpes simplex virus. J Clin Invest 1989;84:1174–1180.
149. Stefansson K, Dieperink ME, Richman DP, Gomez CM, Marton LS. Sharing of antigenic determinants between the nicotinic acetylcholine receptor and proteins in *Escherichia coli*, *Proteus vulgaris*, and *Klebsiella pneumoniae*. Possible role in the pathogenesis of myasthenia gravis. N Engl J Med 1985;312:221–225.
150. Klavinskis LS, Willcox N, Newsom-Davis J. Antivirus antibodies in myasthenia gravis. Neurology 1985;35:1381–1384.
151. Tindall RSA, Cloud R, Lucky J, Rosenberg RN. Serum antibodies to cytomegalovirus in myasthenia gravis: effects of thymectomy and steroids. Neurology 1978;28:273–277.
152. Aoki T, Drachman DB, Asher DM, Gibbs CJ, Bahmanyar S, Wolinsky JS. Attempts to implicate viruses in myasthenia gravis. Neurology 1985;35:185–192.
153. Klavinski LS, Willcox HNA, Richmond JE, Newsom-Davis J. Attempted isolation of viruses from myasthenia gravis thymus. J Neuroimmunol 1986;11:287–299.
154. McGuire LJ, Huang DP, Teoh R, Arnold M, Wong L, Lee JC. Epstein–Barr virus genome in thymoma and thymic lymphoid hyperplasia. Am J Pathol 1988;131:385–390.
155. Manca N, Perandin F, De Simone N, Giannini F, Bonifati D, Angelini C. Detection of HTLV-I tax-rex and pol gene sequences of thymus gland in large group of patients with myasthenia gravis. J Acquir Immune Defic Syndr 2002;29:300–306.
156. Bernasconi P, Barberis M, Baggi F, Passerini L, Cannone M, Arnoldi E, et al. Increased Toll-like receptor 4 expression in thymus of myasthenia gravis patients. Am J Pathol 2005;167:129–139.
157. Cavalcante P, Barberis M, Cannone M, Baggi F, Antozzi C, Maggi L, et al. Detection of poliovirus-infected macrophages in thymus of patients with myasthenia gravis. Neurology 2010;74:1118–1126.
158. Willis SN, Stadelmann C, Rodig SJ, Caron T, Gattenloehner S, Mallozzi SS, et al. Epstein–Barr virus infection is not characteristic feature of multiple sclerosis brain. Brain 2009;132:3318–3328.
159. Peferoen LAN, Lamers F, Lodder LNR, Gerritsen WH, Huitinga I, Melief J, et al. Epstein–Barr virus is not a characteristic feature in the central nervous system in established multiple sclerosis. Brain 2010;133:e137.
160. Sargsyan SA, Shearer AJ, Ritchie AM, Burgoon MP, Anderson S, Hemmer B, et al. Absence of Epstein–Barr virus in the brain and CSF of patients with multiple sclerosis. Neurology 2010;74:1127–1135.
161. Aloisi F, Serafini B, Magliozzi R, Howell OW, Reynolds R. Detection of Epstein–Barr virus and B-cell follicles in the multiple sclerosis brain: what you find depends on how and where you look. Brain 2010;133:e157.
162. Kaminski HJ, Minarovits J. Epstein–Barr virus: trigger for autoimmunity? Ann Neurol 2010;67:697–698.