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Myasthenia Gravis: Clinical and Immunological Aspects

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<http://dx.doi.org/10.5772/67684>

Abstract

Autoimmune diseases such as myasthenia gravis (MG) result from an altered balance between the processes of activation and regulation of immune response. MG is the most common autoimmune disorder characterized by failure of transmission at the neuromuscular junction (NMJ). Autoantibodies in MG target the acetylcholine receptors (AChRs) as well as non-AChR components like muscle-specific tyrosine kinase (MuSK). Autoantibodies against AChRs are produced by B cells in the germinal centres (GCs), formed in the medulla of MG thymus and circulated to the post-synaptic side of the neuromuscular junction (NMJ) leading to complement-mediated destruction of the post-synaptic folds of NMJ and internalization of AChRs. The incidence and prevalence of MG have increased particularly in elderly, but clinical presentations vary substantially and recognition depends on classic disease phenotype. This chapter focuses on clinical and immunological aspects of MG and its subgroups based on its characterization of the antigenic targets.

Keywords: autoimmune disorders, autoantibodies, myasthenia gravis, acetylcholine receptors, B-cell receptors, cell-based assays, seronegative myasthenia gravis

1. History of myasthenia gravis

Myasthenia gravis (MG) is an autoimmune syndrome caused by the failure of neuromuscular transmission, which results from the binding of autoantibodies to proteins involved in signalling at the neuromuscular junction. Neurobiology of myasthenia gravis states that it is an antibody-mediated autoimmune disorder where antibodies to the acetylcholine receptors (AChR) cause complement-mediated destruction of the post-synaptic folds of the neuromuscular junction (NMJ) and internalisation of AChRs. This results in reduced muscle-nerve synaptic transmission and fatigable muscle weakness, one of the characteristic clinical features, in MG patients. It mainly affects the voluntary muscles including muscles of the neck, eyelids,

limb and diaphragm. Antibodies to AChRs and their effects on AChR number and function have been recognized since 45 years. However, their characterization and increasing recognition of antigenic targets added to understand different forms of MG.

Other subgroups of MG includes early-onset MG, late-onset MG, MuSK MG, seronegative MG (SNMG), neonatal MG, depends on the presentation and proteins involved in the disease. Some MG patients do not have detectable antibodies against AChRs and are termed as SNMG. In SNMG, antibodies are directed against the extracellular domain of Musk and inhibit agrin-induced AChR clustering in muscle myotubes [1]. Immunoglobulin G antibodies against Musk have been described in [2] and IgM alters AChR in *in vitro* assays [1].

The incidence and prevalence has increased particularly in older individuals [3, 4]. The yearly incidence has also risen in all studies [5] due to significant increase among older males as well as females [6].

The role of the antibodies that cause myasthenia gravis was clearly established in the 1970s but characterization of antigenic targets evolved till now. Confirmation that antibodies to AChR alone could cause myasthenia gravis, came from immunisation against purified AChRs [7], and the fact that monoclonal antibodies to AChR can produce similar effects in laboratory animals [8]. However they also tested that plasma exchange, removes circulating antibodies, which leads to a substantial but transient improvement in muscle function lasting up to 2 months [9].

2. Diagnostic and clinical classification of MG

Autoantibodies against AChR were present in 85% sera of MG patient [10]. After few years, antibodies against MuSK were reported in 70% of patients with generalized seronegative MG [11]. Approximately 15% of patients with generalized MG do not have AChR antibodies, previously defined as 'seronegative' MG and about 40% of them have antibodies against MuSK; about two-third of the remaining 60% has low-affinity antibodies against AChR undetectable by conventional assays [12, 13]. In MG, 90% of all cases are associated with MuSK and AChR antibodies and are convincingly pathogenic. Besides, some of the antibodies can be associated with special clinical phenotypes. The clinical hallmark of MG is fatigable weakness, involving susceptible muscle groups in the body. It is the most characteristic feature of MG which becomes more evident on exertion and improves with rest. The course of MG is variable. Many patients experience intermittent worsening of symptoms triggered by infections, emotional stress, surgeries or medications, particularly during the first year of the disease.

3. Autoantibodies in MG

Early-onset myasthenia gravis is defined as presenting before age 40 years and is more common in women as per MG Foundation of America. Most are positive for AChR antibodies, and the thymus gland is enlarged. These patients have antibodies to other muscle antigens,

but might have other organ-specific autoantibodies [14–16]. Recently, it has been found that, tested serum from EOMG patients, females produced higher amounts of antibodies against clustered AChRs than males. On titration, no significant decrease in level of antibodies was observed. All these observations are summarized in **Table 1** [17].

The targeted antibodies in most MG cases are against the Ach-gated cation channel $\alpha 1$ AChR [18]. Two isoforms of AChR, foetal and adult, differ in the composition of five subunits: the two $\alpha 1$, one δ and one $\beta 1$ subunits, the foetal receptor contains one γ and replaced by one ϵ subunit in the adult receptor [17]. The main immunogenic region (MIR) is located to the extracellular top of the $\alpha 1$ subunit on the ACh binding site as shown in **Figure 2**. The antibodies against AChRs are mostly complement-fixing IgG1 or IgG3, which recognizes the native conformation easily. The conventional assay to detect AChR antibodies in the sera is radioimmunoprecipitation assay which is based on the mixture of foetal and adult ^{125}I - α -BuTx labelled AChR purified from a human muscle cell line. The sensitivity of the assay is about 80–85% in generalized MG. Alternatively, a non-radioactive cell-based assay (CBA) using cells co-transfected with AChR subunits and rapsyn that clusters AChR at NMJ, detected AChR antibodies in few patients, earlier regarded as ‘seronegative’ with conventional RIPA [13].

Late-onset myasthenia gravis is defined as its first presentation in people older than 40 years. The thymus gland is not enlarged, but there is an HLA association with B7 and DR2 [17]. Thymoma-associated myasthenia gravis is not age specific, but can be presented at any age and the peak onset is during the 4th– 6th decades. There are no clear HLA associations. The patients usually have antibodies to other muscle antigens such as titin and ryanodine receptor [18].

	Generalised myasthenia gravis(MG) anti-AChR–seropositive (RIA)			
	Ocular MG	Early-onset (EOMG)	Late-onset (LOMG)	Thymoma
Onset-age (years)	4–90	10–45	>45	>10
M:F ratio	3:2	1:3	3:2	1:1
% of all MG	20	20	50	10
HLA/other genes	Not clear	DR3/52a -- B8 PTPN22 TNIP1	DR2/ 51 - B7	Not clear
Autoantibodies	AChR MuSK	AChR	AChR	Musk/AChR/ SN
AChR	+ or –	+++ - +	++	++
MuSK	–	–	–	–
Striated muscle/titin	–	–	++ (70%)	++ (>90%)
Thymic histology	Hyperplasia + or –	Hyperplasia (>80%)	Atrophy	Epithelial neoplasia (+adj. hyperplasia)

Age at onset, gender ratio and other factors in this chart describes clinical basis of Classification ‘OF’ MG with Clasification ‘IN’ MG, based on the estimated results on MG serum testing till 2013.

Table 1. Clinical classification of MG.

Ocular myasthenia gravis is restricted to the eye muscles. The titres of antibodies to AChR are lowest in this subgroup, and undetectable in 40–60% of patients. However, electromyography and *in vitro* studies on muscle biopsy samples indicate that the disease is probably present sub-clinically in other muscles [19]. Ocular weakness, presenting as fluctuating ptosis and/or diplopia, is the most common initial presentation of MG, occurring in approximately 85% of patients [20].

MG with thymoma, about 10–15% of patients have thymoma, while 30% of thymomas are associated with MG. Thymoma is equally common in men and women. It can occur at any age, but the peak of onset is around 50 years. Thymoma associated with titin or especially with RyR antibodies may have more severe disease course similar to MuSK-MG, characterized by progressive oropharyngeal weakness. Thymoma is mostly associated with high titer of AChR antibodies. Its symptoms usually persist after thymectomy [21].

3.1. Neonatal MG

Neonatal MG occurs to the babies born to the women having MG regardless of its presentation at the time of pregnancy. It is estimated to be 10% among individuals. It is caused by placental transfer of maternal IgG AChR antibodies. The mothers had very high titres of antibodies specific for the foetal isoform of the AChR, and low concentrations of antibodies directed towards the adult isoform of the AChR (Observations from EOMG patients).

3.2. Anti-Musk MG

Musk is a transmembrane endplate polypeptide involved in a signalling pathway that maintains the normal functional integrity of the NMJ as shown in **Figure 1** [18, 22]. Musk antibodies are mainly IgG4 and are not complement activating, unlike the IgG1 and IgG3 anti-AChR antibodies [26]. Anti-Musk antibodies adversely affect the maintenance of AChR clustering at the muscle endplate, leading to reduced numbers of functional AChRs [20]. Apart from ELISA, a highly sensitive and specific cell-based assay using Musk-transfected HEK cells have been developed and its expression was found very high. Some of the sera of Musk-MG reacted with clustered AChRs as well, showing that low-affinity IgG and IgM antibodies to AChR may be present in a few Musk-MG patients [14]. IgG4 antibodies were previously known in autoimmune disease and thought to occur as a benign phenomenon in conjunction with resolution of allergic reactions. However, they are recognised in other diseases, such as forms of pemphigus [27].

4. Other antibodies

Lrp4 is similarly essential as Musk in the development and function of the adult NMJ, where it performs both anterograde and retrograde signalling roles [24]. These roles highlighted it as a putative antigen of interest, and LRP4 antibodies have been reported in Japanese [28] and European patients. The antibodies were of the complement-activating IgG1 type [28, 29] and impeded agrin-induced clustering of AChRs.

Agrin and collagen Q, antibodies to agrin have been identified in a small number of 'triple negative' MG sera (samples negative for AChR, Musk and LRP4 antibodies) at proportions from 15 to 50%. These antibodies sometimes at low titres were found only with AChR or MuSK antibodies [24]. ColQ tied with MuSK within the synapse, is thought to interact also with Musk. ColQ antibodies were reported in 3–4% of all MG patient sera tested and 1.2–5.5% of the AChR/MuSK/LRP4 negative samples [33].

5. Neuromuscular junction

The NMJ has three basic components, the presynaptic motor nerve terminal, site of acetylcholine synthesis, stored and released. Second is the synaptic space and third is the post-synaptic muscle membrane, which contains the AChRs and the enzyme acetylcholinesterase. Neuromuscular transmission begins with the entry of nerve action potential into the nerve terminal and triggers the release of acetylcholine. Exocytosis of synaptic vesicles containing acetylcholine requires calcium, which enters the depolarised nerve terminal via voltage-gated Ca^{2+} channels. Acetylcholine diffuses across the synaptic cleft and interacts with the AChRs on the post-synaptic side of muscle, leading to depolarisation. The action of acetylcholine

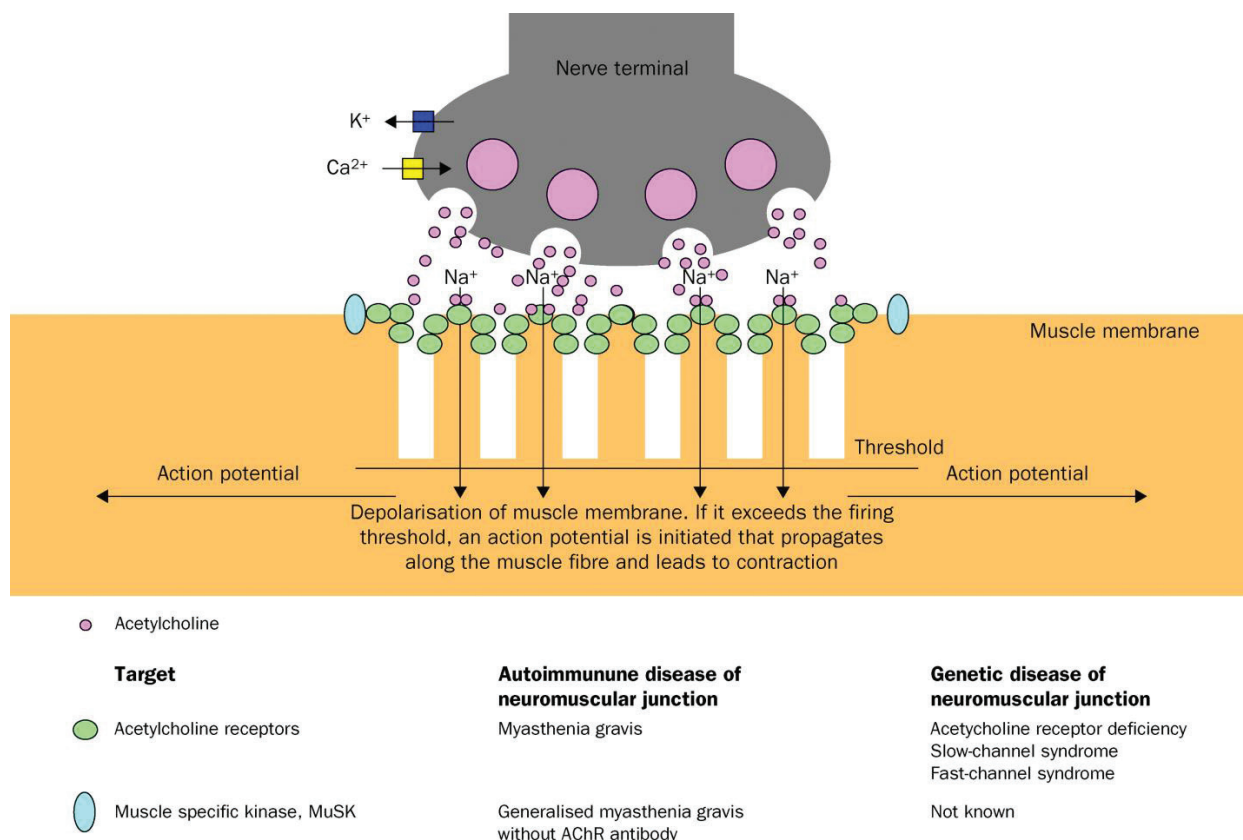


Figure 1. Structure of NMJ, the ion channels involved in neuromuscular transmission and disorders that affect it. The receptor muscle-specific kinase MuSK that now seems to be a major target in AChR seronegative myasthenia gravis is also shown along with other receptors [18].

on the post-synaptic membrane is terminated by acetylcholinesterase. In MG, loss of functional AChRs results in the decrease of threshold required for generation of muscle nerve fibre action potential during repetitive nerve depolarisations, resulting into neuromuscular transmission failure.

6. Structural characterization of AChRs

AChR remains the major antigenic target in MG followed by Musk, LRP4 and agrin. AChR is a pentameric membrane protein consisting of two α , one β , one δ and one ϵ subunit in the adult muscle, whereas, during development on child birth, the γ subunit takes the place of the ϵ . These subunits in respective isoforms are organized around a central cation channel. The two binding sites between α and ϵ or γ and α and δ need to be occupied to be in open state. The main immunogenic region (MIR) is on the extracellular component of each α subunit [13] (Figure 2).

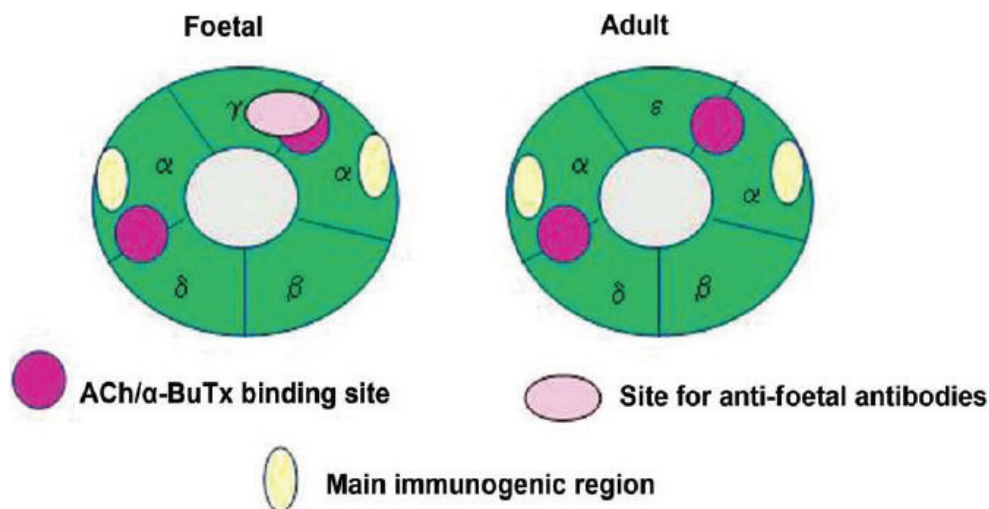


Figure 2. The acetylcholine receptor is a pentameric membrane protein; the membrane consists of five subunits: two α , one β , one δ and either gamma (foetal type) or epsilon (adult type). Acetylcholine and alpha-bungarotoxin bind to sites on the interfaces between the alpha subunit and adjacent subunits. Most of the antibodies in Myasthenia gravis bind to a main immunogenic region on the two α subunits [19].

7. Intra-thymic auto-immune mechanisms in MG

The thymus is an epithelial organ that can be divided into cortex, medulla and cortico-medullary zone. The cortex contains immature lymphocytes alongside epithelial cells and macrophages. The medulla is less cellular containing more mature T lymphocytes, B lymphocytes, epithelial cells, dendritic cells and rare myoid cells. It plays a critical role in self-tolerance with a balance between the generation of T lymphocytes and deletion of auto-reactive T cells, when required [25].

The thymus also has a critical role in AChR Ab+ EOMG patients, having lymphocytic infiltrates in medullary region and germinal centres with distinct areas for B-cell proliferation, differentiation, somatic hypermutation and class switching [25] and thymoma cells do not [23]. Native AChR is also expressed by the myoid cells but are more abundant in hyperplastic thymus [17]. Since a high proportion of patients with MG, demonstrate germinal centre hyperplasia of the thymus or cortical epithelial cell thymoma, the thymus gland was considered a solution to all forms of MG [31]. Whereas, thymectomy is associated with clinical improvement, especially in young patients with thymus hyperplasia and recent disease onset [32]. Normally, the thymus functions in early life to prevent autoimmune disorders by its inherent role in clonal deletion by negative selection of auto-reactive T cells and becomes regulatory T cells in early life [33]. Germinal centres normally arise in primary follicles within the secondary lymphoid organs, namely the spleen, lymph nodes and Peyer's patches; these organs provide the necessary microenvironment for the germinal centre response. This justifies the chances for having an autoimmune disorder was not affected by the age of onset of MG. Hence, indicate that acquired and non-genetic factors are involved in the establishment of the immune tolerance breakdown. The clinical impact of exposure to these acquired factors occurs later in life than the clinical impact of thymic involvement [34].

8. Molecular characterization of thymic B cells

Auto-reactive B cells and antibodies can be detected in a variety of neurological diseases. Their causative roles have been established in some disorders and are found to be involved in the pathogenesis of others. During the immune response against an antigen, B cells bearing antigen-specific receptors stimulate to proliferate and differentiate into antibody-secreting plasma cells within the germinal centres. This requires the presence of follicular dendritic cells (FDCs) and activated CD4 T-helper 1(Th-1) cells, CD40/CD40 ligand interaction, and a cocktail of cytokines to create the microenvironment necessary for a germinal centre reaction and a few B cells bearing an appropriate antigen receptors are stimulated to undergo clonal proliferation in the dark zone of the germinal centre and differentiation to centroblasts, centrocytes, memory B cells and plasma cells. Antibody-secreting plasma cells migrate out of the follicle into the surrounding tissue [35].

Several markers on B cells during its proliferation, differentiation and development, characterize them into subsets. CD 20, CD 19, CD 27 and CD 138 which are found on the surface of B cells and plasmablasts, are currently in research to develop B-cell-targeted immunotherapy to treat MG and other related autoimmune neurological disorders.

9. B-cell-directed immunotherapies

Pyridostigmine and corticosteroids plays a central role in the management of MG [36]. Use of azathioprine and other immunosuppressant drugs have been supported to treat MG. Intravenous Immunoglobulin and plasma exchange were also successful treatments for MG.

Rituximab is a chimeric monoclonal antibody directed against the B-cell surface marker CD20. It reduces circulating B-cell counts, and on the basis of its potential for targeting auto-reactive B-cell clones, have a therapeutic role in antibody-mediated autoimmune diseases [20]. It has been a useful treatment in IgG4-related diseases which eliminates a population of B or plasma cells responsible for the production of IgG4 antibodies, a targeted B-cell immunotherapy [30].

Ofatumumab is a fully monoclonal anti CD20 antibody which inhibits early B-cell development. Ofatumumab induced enormous depletion of peripheral B lymphocytes in rheumatoid arthritis on the retreatment after rituximab [37]. It has been approved for treating chronic lymphocytic leukaemia. Its effect on development of B cells from MG patients' thymus is currently under study [38–40]. There are several emerging therapies for MG, including tacrolimus, rituximab and antigen-specific apheresis, whereas other treatments await clarification of efficacy and their role in MG. In addition, the complement inhibitory therapy has been shown to be effective in experimental MG [41] and might prove promising in myasthenic crisis and particularly in ocular MG, because of the low expression of complement regulators in extraocular muscle [41].

Recent findings that B cells have critical positive and negative roles in autoimmune disease [42] might lead to particularly effective therapeutic strategies that specifically target anti-AChR antibody-producing memory B cells.

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