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A REVIEW ARTICLE ON MYASTHENIA GRAVIS

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Abstract

Myasthenia gravis (MG) is an autoimmune neurological disorder characterized by defective transmission at the neuromuscular junction. The incidence of the disease is 4.1 to 30 cases per million person-years, and the prevalence rate ranges from 150 to 200 cases per million. MG is considered a classic example of antibody-mediated autoimmune disease. Most patients with MG have autoantibodies against the acetylcholine receptors (AChRs). Less commonly identified autoantibodies include those targeted to muscle-specific kinase (MuSK), lowdensity lipoprotein receptor-related protein 4 (Lrp4), and agrin. These autoantibodies disrupt cholinergic transmission between nerve terminals and muscle fibers by causing downregulation, destruction, functional blocking of AChRs, or disrupting the clustering of AChRs in the postsynaptic membrane. The core clinical manifestation of MG is fatigable muscle weakness, which may affect ocular, bulbar, respiratory and limb muscles. Clinical manifestations vary according to the type of autoantibody, and whether a thymoma is present.

Keywords: Myasthenia gravis, Acetylcholine receptor, Pathophysiology, Treatment of MG

Introduction

Myasthenia gravis (MG) is the most common autoimmune disorder that affects the neuromuscular junction. MG is largely a treatable disease but can result in significant morbidity and even mortality. This can usually be prevented with a timely diagnosis and appropriate treatment of the disease. MG is a heterogeneous disease from a phenotypic and pathogenesis standpoint. The spectrum of symptoms ranges from a purely ocular form to severe weakness of the limb, bulbar and respiratory muscles. The age of onset is variable from childhood to late adulthood with disease peaks in younger adult women and older men.

MG is considered a classic example of antibody-mediated autoimmune disease. It can also be viewed as an example of a class II hypersensitivity reaction, as IgG autoantibodies react with intra or extracellular antigens, leading to end-organ damage. Most patients with MG have autoantibodies against the acetylcholine receptors (AChRs) [2,3], and a minority are seropositive for antibodies directed to muscle-specific kinase (MuSK) [4,5],

low-density lipoprotein receptor-related protein 4 (Lrp4) [6,7] or agrin [8,9]. These antibodies also provide the basis for defining disease subgroups and help delineate phenotypic variants. In a subgroup of MG patients, striational antibodies have also been identified, which include antibodies against titin, ryanodine receptor, and the alpha subunit of the voltage-gated K+ channel (Kv1.4). These antibodies mostly serve as biomarkers of disease severity and are often detected in patients with late-onset MG or with thymoma, and some of them have concomitant myositis and/or myocarditis.

Although MG is mediated by autoantibodies, different subtypes of T cells and their cytokines also play important roles in the pathogenesis. In this review, we briefly discuss the epidemiology, clinical manifestations, and genetic predisposing factors of adult MG, then provide an overview of pediatric MG, followed by an update on MG pathophysiology.

Epidemiology

MG is a rare neurological disease and pediatric MG is even more uncommon. Both incidence and prevalence have significant geographical variations, but it is believed that MG incidence has increased worldwide over the past seven decades. The prevalence of MG was estimated at
1 in 200,000 from 1915 to 1934, increased to 1 per 20,000 after the introduction of anticholinesterase drugs in 1934, and rose to 1 per 17,000 population after the discovery of AChR antibodies in 1969 [12]. Prevalence rates range from 150 to 200 cases per million, and they have steadily increased over the past 50 years, at least partly due to improvements in recognition, diagnosis, treatment, and an overall increase in life expectancy [13]. More recent studies addressing incidence rates have been conducted in Europe and show a wide range from
4.1 to 30 cases per million person-years [14,15].

The annual rate is lower in studies coming from North America and Japan, with the incidence ranging from 3 to 9.1 cases per million [1]. Lower incidence and prevalence rates have been reported in a large study from China at 0.155–0.366 per million, and 2.19–11.07 per 100,000, respectively [16]. Two population-based studies from Korea showed a prevalence of 9.67– 10.42 per 100,000 people in 2010, which increased to 12.99 per 100,000 in 2014 [17,18]. On the other hand, a smaller study using records of a hospital-based Health Maintenance.
Organization estimated an incidence of MG at 38.8 per 1,000,000 person-years for the Argentinian population [19]. Different study methodologies, including diagnostic criteria and other sources of bias, such as the small size of the study population and the underestimation of patients with milder disease, likely play a factor in the significant variability of incidence ratesover time and across different geographical regions.

Incidence rates have a bimodal distribution in women, with peaks around age 30 and 50. In men, the incidence increases steadily with age and with the highest rates between age 60 and 89 [20]. Women are more commonly affected before age 40, with a female: male ratio of 3:1 for early-onset MG. In the fifth decade of life, women and men are equally affected, while men have a higher proportion after age 50, with a male: female ratio of 3:2 [21]. Around 10% of cases are pediatric, which is defined as onset before age 18 [13]. MG can affect people of all race and ethnic backgrounds and is slightly more prevalent in patients of African ancestry [22,23,24].

Furthermore, MG phenotype may vary depending on the ethnic background. In a retrospective study from South Africa, black patients were more likely to have treatmentresistant ophthalmoplegia and ptosis than whites, whereas the whites were more likely to develop treatment refractory generalized MG [25]. The age at diagnosis was 17 years higher in Caucasians than non-Caucasians in another cohort of patients with ocular MG [26]. In a US study, Oh et al. found that MG started earlier and had a more severe phenotype in African Americans than in Caucasians [22]. The seronegative African Americans had a higher percent of MuSK seropositivity in that study (50% vs. 17% in the whites). On the other hand, patients of Asian ancestry have higher rates of MuSK antibodies compared to Caucasians and individuals of African ancestry [27,28]. MuSK-associated MG is also more prevalent among those living in latitudes closer to the equator [22,29].

The mortality rate of MG has dramatically declined from the early 20th century after the availability of acetylcholine esterase inhibitors, immunosuppressants, intravenous immunoglobulin and advanced respiratory care. However, the mortality rate from the disease remains at 5–9%, being slightly higher in males than females [12]. Using the US Nationwide Inpatient Sample (NIS) database for the years 2000 to 2005, the overall in-hospital mortality rate was estimated as 2.2%, but higher in those with MG crisis (4.7%), with the main predictorsof death being older age and the presence of respiratory failure [24].

Subtypes of MG and Their Clinical Manifestation

MG ue to Antibodies against AChR (AChR-MG)

Effector Mechanisms

Nicotinic AChR is a heteropentamer consisting of two α-subunits and one each of β-, δsubunit, and γ-subunit (embryonic type) or ε-subunit (adult type), which are organized around a central pore [30]. Antibodies against the AChR are found in approximately 80% of MG patients. At least half of the AChR autoantibodies are directed at the AChR α-subunits [31].

They are believed to be more pathogenic than those directed against the beta subunit [32]. This is likely due to the location of the alpha subunit within the receptor, which leaves it more exposed to antibodies, as well as its role in modulating the receptor sensitivity to ACh binding [33,34]. In addition, there are two alpha subunits per receptor.

AChR antibodies are predominantly of the IgG1 and IgG3 subclasses [35]. IgG2 and IgG4 subclasses are also identified, but in fewer cases [36]. The pathogenic mechanisms and functional spectrum of AChR antibodies are varied, but overall, they impair receptor function by either binding, blocking, or modulating its activity. The predominant mechanism is the binding of the antibody and activation of the complement cascade, leading to the formation of the membrane attack complex (MAC), which causes damage of the postsynaptic membrane and destruction of synaptic folds which contain AChRs and associated proteins, including voltage-gated

sodium channels [35]. Other mechanisms of pathogenicity include: (1) antigenic modulation by the binding and crosslinking of AChRs, leading to increased endocytosis and degradation [37]; and (2) the impairment of AChR function, either by the blocking of ACh binding to the receptor [38] or the prevention of channel opening [39]. Most blocking antibodies appear in association with binding antibodies, and only rarely are they unique. Therefore, this final mechanism is believed to be rare and its clinical implications are not clear [40]. However,the administration of blocking antibodies causes an acute and severe weakness in rodents [41].

Clinical Manifestations

The core clinical manifestation of MG is fatigable muscle weakness, worsened by exertion and improved by rest. The most common presenting symptoms are ocular, with double vision and ptosis. Most patients will develop diplopia and/or ptosis some time during the course of their disease. In addition, up to 80% of patients with ocular onset will go on to develop generalized symptoms, usually within two years of disease onset [42,43]. A recent population-based study conducted by the Mayo Clinic found that 51% of patients presented with ocular onset, and 55% of these developed generalized symptoms [44]. Bulbar muscles are also frequently involved, resulting in flaccid dysarthria, dysphagia, and facial and jaw weakness [45]. Axial weakness canalso be present, with neck flexion weakness usually more common than weakness of neck extension. In a previous retrospective study from our center, about 10% of MG patients developed head-drop some time during the disease course [46]. Head-drop was associated with age >60 and male patients in that study. Limb muscle weakness tends to be symmetric and proximal, and patients commonly complain of difficulty climbing stairs, getting up from chairs, and raising arms above their head. In some instances, distal muscles can be predominantlyaffected, either in a symmetric or asymmetric distribution. For example, some patients complain of weakness in finger and wrist extension and flexion, as well as foot drop. Fina lly, 15–20% of patients with AChR-MG can develop respiratory weakness requiring mechanical ventilation (MG crisis) [21]. Spontaneous remissions for different lengths of time may occur in the course of adult-onset MG. In an earlier study conducted before the widespread useof steroids and other.

immunosuppressants, approximately one fourth of the patients had a complete or near complete spontaneous remission, lasting an average of 4.6 years and up to 17 years [47]. Half of the remissions occurred during the first year after onset. A study of Oosterhuis et al. found that 22% of patients treated with anticholinesterase medications only had spontaneous remission [42]. The remission lasted more than 12 months in duration in half of those patients, with the maximum duration of the remission being 6 years in that study.

AChR MG Subtypes

Ocular MG Most MG patients with ocular symptoms at onset will progress to generalized forms of the disease, usually within two years of onset. Of the remaining, 90% will continue to have ocular manifestations only. Hence, ocular MG is defined by isolated extra-ocular involvement for a period of ≥2 years. Over half of the patients in this group have antibodies against AChRs [21]. There are several explanations as to why extraocular muscles (EOMs) are preferentially affected in MG. EOMs fatigue easily, as they require tonic contractions to sustain gaze in a specific direction, and fibers have a high frequency of synaptic firing, and develop tension faster [48]. In addition, EOMs have a lower density of AChR, thus making them more susceptible to symptoms. It is also theorized that differing epitope expression in EOMs plays a role in their preferential involvement [49].

Generalized AChR Ab Positive MG (AChR-MG): Early vs. Late Onset

Early-onset MG (EOMG) corresponds to patients presenting before age 50. There is a female preponderance, with a female to male ratio of 3:1. Patients in this category have a higher incidence of thymic hyperplasia, and thymectomy has been proven effective in improving clinical outcomes and minimizing the need for immunotherapy [50]. Late-onset MG (LOMG) is defined by onset after the age of 50. There is no female predominance in this group; on the contrary, there can be a slightly higher prevalence among men, especially after age 60.

Thymic hyperplasia is rare and response to thymectomy is poorer [45].

There is a higher prevalence of autoimmune disease among family members and a high correlation with HLA-DR and HLA-B8 haplotypes [51]. There is frequent association of A1B8- DR3 haplotype with early-onset MG [52]. In a study on non-thymomatous AChR Ab + LOMG of Italian ancestry, Spagni et al. found a positive association with HLA-DRB1*07 and HLA- DQB1*02, whereas HLA-DRB1*02, HLA-DRB1*03, HLA-DRB1*11, and HLA-

DQB1*03 were the protective alleles [53]. A study comparing cohorts of patients with EOMG, LOMG and MuSK-MG in a single center in Turkey found a strong association for class I HLA- B/MICA in EOMG patients, specifically HLA-B*08:01. On the other hand, no association was found between LOMG and HLA class I, but it detected an association with HLA-DQA1 and HLA-DRB1 [54]. Another large study on Norwegian MG patients older than 60 showed a strongassociation with HLA-DRB1*15:01 [55].

Genetic variations within other loci have been associated with predisposition to AChR-MG. A large genome-wide association study involving patients from North America and Italy identified different haplotypes across the HLA region, cytotoxic T-lymphocyte-associated protein 4 gene (CTLA4) and tumor necrosis factor receptor 4 superfamily, member 11a, (TNFRSF11A) and NFκB activator genes in early- and late-onset MG cases [56]. Variations in the CTLA4 and HLA-DQA1 loci were associated with both early- and late-onset cases,

whereas genetic variation within the TNFSRF11A locus was a susceptibility factor only in the late-onset cases in that study.

Thymoma-Associated MG

MG is the most common paraneoplastic disorder associated with thymoma. Other thymomarelated disorders with lower association rates include myositis, Morvan syndrome and pure red aplasia. About 50% of patients with a thymoma develop positive AChR antibodies without clinical manifestations, and approximately 30% will develop MG [57]. Conversely, 10– 20% of patients with MG have thymomas [58]. Response to thymectomy is variable, usually worse than in patients with EOMG [50]. Studies on HLA alleles did not reveal a consistent association between HLA and thymomatous MG [59].

MuSK Antibody-Associated MG (MuSK-MG)

Effector Mechanisms

MuSK is a membrane protein that is critical to the clustering of AChRs in the neuromuscular junction. Agrin, which is secreted from the presynaptic terminal, interacts with Lrp4, resulting in the reorientation of the Lrp4/MuSK complex, which in turn leads to the activation of MuSK through its phosphorylation. Phosphorylated MuSK activates a downstream signaling pathway that leads to the clustering of AChRs (Figure 1) [60].

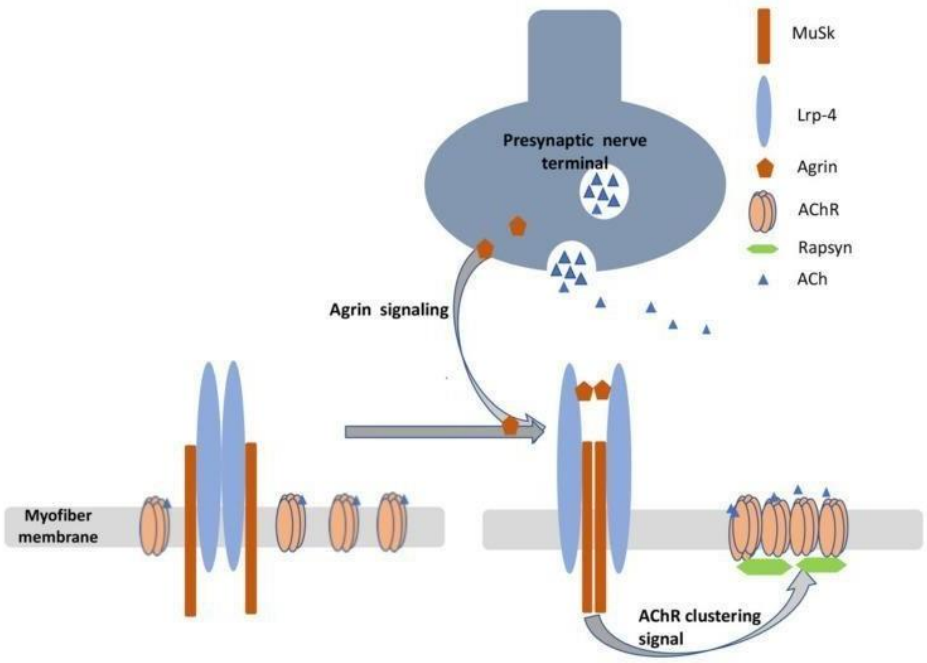

Figure 1
Diagram depicting the secretion of agrin from the presynaptic membrane and its interaction with Lrp4, which results in reorganization and reorientation of MuSK, promoting a signaling pathway that leads to synaptic differentiation, including clustering of AChRs. This involves recruiting rapsyn which links AChRs to the cytoskeleton (not shown).

Antibodies against MuSK are found in approximately 7–10% of all MG patients and up to

40% of patients with generalized MG who are seronegative for AChR Abs. There is a female predominance, with up to 85% of MuSK positive patients being female [61]. Animal studies demonstrated that MuSK antibodies are pathogenic, as they cause severe weakness when administered to healthy mice [62]. In contrast to AChR antibodies, MuSK antibody titers

correlate with disease severity [63]. The concurrence of seropositivity for both AChR and MuSK antibodies has rarely been reported [64], but in general these are considered distinct entities.

MuSK antibodies belong mainly to the IgG4 subclass. They do not fix complement and are not strong activators of cell-mediated cytotoxicity [65]. Given the unique ability of IgG4 to undergo Fab arm exchange, MuSK antibodies are functionally monovalent, and they cannot crosslink antigens of the same class. The mechanism by which MuSK antibodies exert their pathogenic effect on the neuromuscular junction is via binding to the Ig-like domain of the protein, preventing its phosphorylation, and subsequently disrupting the Agrin-Lrp4-MuSKDok-7 signaling pathway. Dok-7 is a muscle-intrinsic activator of MuSK and it is required for synaptogenesis [66]. This ultimately causes a reduction in the density of AChRs in the postsynaptic membrane [67,68].

Clinical Manifestations

MuSK-MG predominantly affects young adults, and is more prevalent among patients of African descent and those living close to the equator in European and Asian nations [45]. This is likely due to a genetic predisposition and not to environmental factors. Muscle weakness preferentially affects cranial and bulbar muscles. Over 40% of patients present with bulbar weakness, usually associated with neck and respiratory involvement [69,70]. Some patients have tongue atrophy. About 30% of patients present with diplopia and/or ptosis. Limb weakness can be uncommon, but when present it tends to be severe and associated with muscle atrophy. Diurnal variations in strength are less common. There is no clear association between MuSK-associated MG and thymic pathology [21]. From the genetic predisposition standpoint, MuSK- associated MG has been associated with DQB1*05 and HLADRB1*14/DRB1*16 [71,72].

Patients who are seropositive for both AChR and MuSK are rare, and it is not certain if they should be categorized as an MG subtype. In a study from southern China, Zhang et al.
demonstrated that the phenotype of double-seropositive patients is more severe than AChRMG and more similar to the MuSK-associated MG [73].

Pathophysiology

Physiology and Organization of the Neuromuscular Junction

The neuromuscular junction is the site of impulse transmission between nerve terminals and muscle fibers. This process requires the release of presynaptic acetylcholine (ACh) and its subsequent binding to a postsynaptic ACh receptor. Synaptic vesicles containing ACh are released from the presynaptic membrane after an action potential activates voltage-gated calcium channels, allowing an influx of calcium into the nerve terminal [97]. The diffusion time of ACh through the synaptic cleft is very short, and it is modulated by the enzyme ACh esterase (AChE), which promotes ACh degradation. The spontaneous release of synaptic vesicles generates the so-called miniature end plate potential (MEPP), while upon nerve fiber stimulation/depolarization, a synchronous release of many synaptic vesicles causes large depolarization of the end plate membrane, generating an evoked endplate potential (EPP) (Figure 2). This, in turn, triggers an action potential in the myofiber, which ultimately leads to its contraction. The amount of ACh released into the synapse is usually higher than that required to generate an action potential, which allows for reliable transmission [97,98]. The binding of ACh to its receptors in the postsynaptic membrane opens the ACh cation-specific channel, leading to localized depolarization and activation of adjacent voltage-gated sodium channels. This allows for the translation of the chemical reaction into an electric signal, which is the muscle fiber action potential. The role of AChE in the hydrolyzation of ACh is therefore crucial, as it prevents a single molecule of ACh from repetitively activating AChRs. The effectiveness of neuromuscular junction transmission is also determined by the amount of ACh released by the nerve terminal, the density of ACh receptors in the postsynaptic membrane, and the density of voltage-gated sodium channels at the endplate. The latter is dependent on the presence of folds in the postsynaptic membrane. These folds determine the density of the voltage-gated sodium channels in the postsynaptic membrane, and therefore increase the efficient coupling of the localized EPP to the myofiber action potential [98].

Neuromuscular junction disorders such as MG disrupt the cascade of events that lead to reliable muscle contraction. In addition, there is a reduction in the number of AChRs and voltage-gated sodium channels as the result of complement-related injury to the postsynaptic membrane in MG.

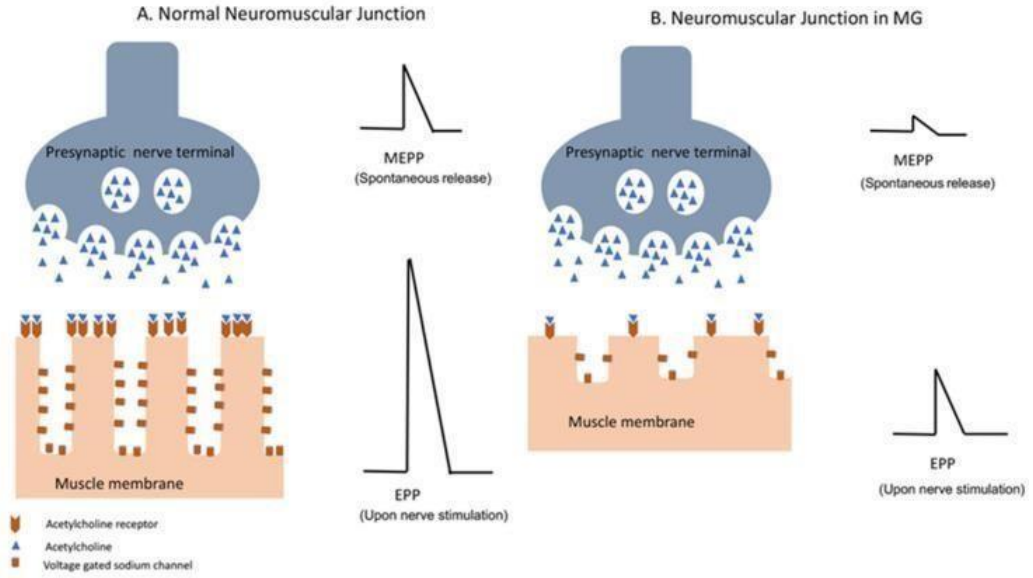

Figure 2
Neuromuscular transmission in normal individuals (**A**) and in patients with MG (**B**). Decreased density of the AChR and complement-mediated damage to the postsynaptic membrane in MG patients result in decrease in miniature end plate potential (MEPP), which occurs with spontaneous release of AChR vesicles, as well as endplate potential (EPP) in response to nerve action potential of the presynaptic membrane. Diminished amplitude of EPP in MG results in impaired neuromuscular transmission.

The resultant inefficient neuromuscular transmission is reflected in decremental response in the amplitude of compound muscle action potential (CMAP) during repetitive nerve stimulation (RNS) and abnormal jitter or blocking in single fiber EMG [98,99].

Immune Dysregulation in MG

Defective B Cell Tolerance

B cell tolerance is mediated by clonal deletion or receptor editing in newly generated B cell clones in the bone marrow when they reach the stage of immature B cells. A second checkpoint occurs on the new emigrant/transitional B cells before they enter the mature naïve B cell compartment. Lee et al. found that the frequency of new emigrant/transitional B cells and mature B cells that express polyreactive and autoreactive B cell receptors (BCRs) is higher in both AChR-MG and MuSK-MG, which would support the concept that patients with MG have defects in both central and peripheral B cell tolerance (Figure 3) [100]. As a result, these patients are also at higher risk of developing other autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and thyroiditis. A break in tolerance is also supported by

data from deep sequencing of BCR repertoire showing distinct gene segment usage biases in both V_H and V_L sequences within the naïve and memory compartments in AChR-MG and MuSK-MG [101].

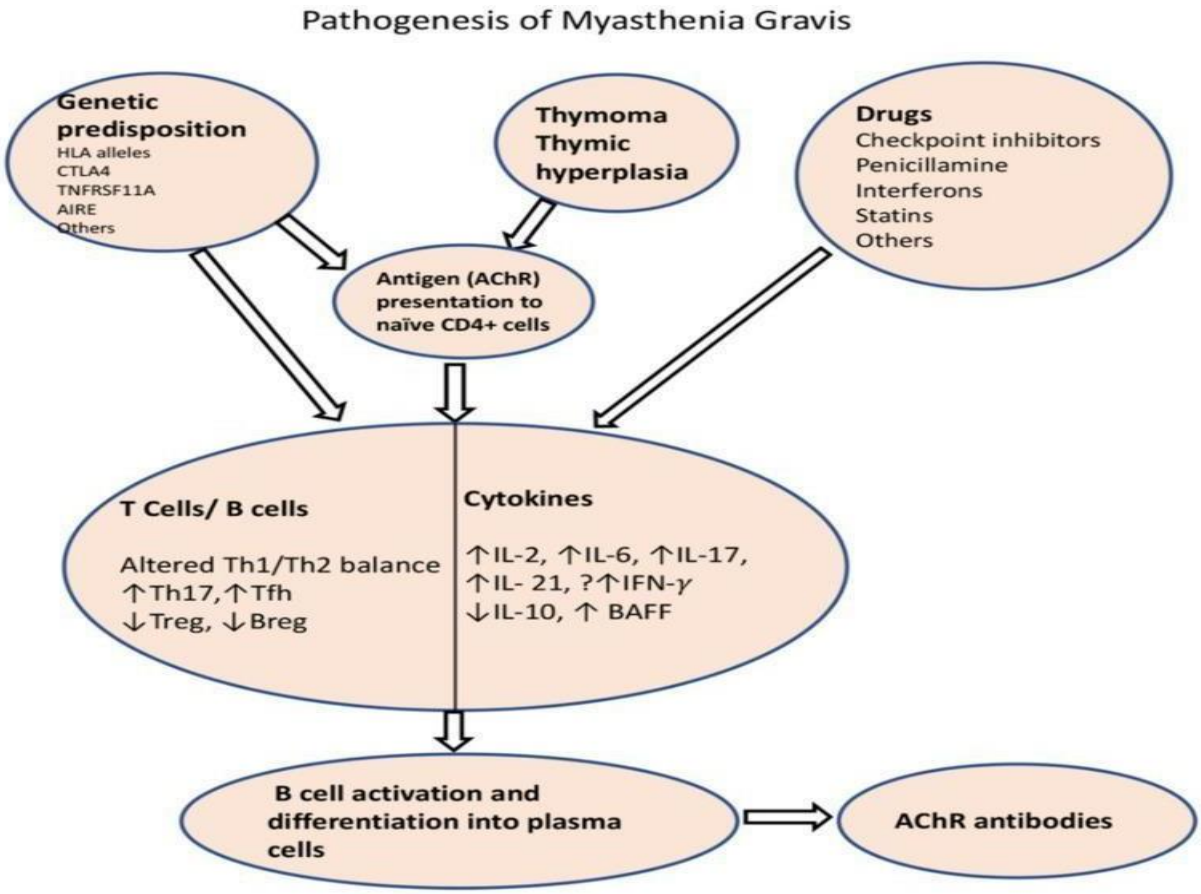

Figure 3

Schematic diagram of pathogenesis of AChR-MG. Impaired tolerance to the AChR is the result of thymoma, thymic dysplasia or due to certain genetic background, which results in presentation of AChR to the naïve T cells by thymic myoid cells or antigen-presenting cells. Among the environmental factors, certain drugs are known to cause de novo MG through alterations of immune homeostasis (drug-induced MG is extensively covered in another paper in this special edition). A number of T cell and B cell subtypes and their cytokines play roles in perturbation of immune homeostasis that results in production of ACR antibodies. HLA: Human Leucocyte antigen; CTLA4: cytotoxic T-lymphocyte-associated protein 4; TNFRSF11A: tumor necrosis factor receptor 4 superfamily, member 11a; AIRE: autoimmune regulator; Th1: T helper 1; Th2: T helper 2; Tfh: T follicular helper; Treg: regulatory T cell; Bregs: regulatory B cells; IL: interleukin; BAFF: B cell activating factor

Treatment of myasthenia gravis

MG treatment is firmly established as the domain of neurologists. Neurologists should be in charge even if the target organ is skeletal muscle, disease mechanisms are systemic, thymus is a target organ for diagnostic, therapeutic and scientific approach, hypoventilation is a lifethreatening symptom, and diplopia often the most troublesome symptom. Ten percent of MG patients have another autoimmune disorder in addition, further supporting the need for complementary medical competence. Close cooperation with other fields of medicine provides knowledge regarding new immunoactive drugs, thus expanding the therapeutic opportunities for MG.

For complicated and rare disorders such as MG, the establishment of medical centres supervising the treatment of the majority of MG patients and of all complicated patients is important to improve treatment quality. Increased treatment experience will optimize present therapy and facilitate the introduction of new and better treatment procedures. Centres with special competence and qualifications in MG treating the majority of patients will further enhance research, including well-controlled and prospective treatment studies.

Ideally treatment recommendations should be based on scientific evidence of high quality, preferentially more than one blinded and controlled prospective study with a sufficient number of well-defined MG patients. There are disappointingly few such studies for MG. Recommendations therefore, rely on studies of lower quality and even sometimes only on case reports, clinical experience, and knowledge from non-MG treatment. It is important for patients as well as doctors to know which treatment is supported by high-quality evidence and which is more tentative and based on clinical experience and circumstantial evidence.

Drug treatments

General

Patients with the diagnosis of MG should always be considered for symptomatic as well as immunoactive drug treatment. Nearly all patients need some treatment, at least in periods where the disease shows clinical activity with permanent or intermittent muscle weakness. Symptomatic drugs have a short-lasting activity both regarding effect and side effects. Dosage can be rapidly changed and the treatment is flexible. Immunoactive drugs have an effect linked to pathogenesis, and the effect usually needs some time before it becomes manifest. Side effects are relevant and should be considered in a long-term perspective. Immunoactive drugs need special attention in children and MG women of childbearing age [6]. Thus, the considerations for patient and doctor are different for symptomatic and immunoactive drug treatment.

Symptomatic Drugs

Acetylcholine esterase inhibition at the neuromuscular junction has a symptomatic effect in myasthenia and especially in autoimmune MG [3, 10, 14–16]. Optimal dosage is adjusted according to effect and side effects. Side effects appear from the nonneuromuscular cholinergic synapses in the autonomic system, which are overstimulated. Alternative ways to increase the amount of acetylcholine at the neuromuscular end plate have been tried, but with less effect than inhibiting the degradation. Acetylcholine esterase inhibitors have a stable and predictable effect, apparently unchanged over years. No scientific comparisons have been undertaken between the various esterase inhibitors. The most commonly used is pyridostigmine and also the faster acting neostigmine. Ambenonium is used in some countries. Some MG patients with anti-MuSK antibodies are hypersensitive to an increase in acetylcholine concentration.

Immunoactive Drugs

Prednisone/prednisolone remains a first-choice drug in MG [3, 14–18]. It has a well-proven positive effect experienced through decades of clinical practice in a high number of patients.

However, there are no formal trials and no scientific comparisons with other drugs. Side effects occur in most patients, and they are usually of clinical significance.

Prednisone/prednisolone is regarded to be safe in pregnancy. To reduce the amount of side effects, dosing the drug every second morning is usually advocated. Most patients keep a sufficient clinical effect on the MG symptoms with this regimen and with markedly less side effects. Patients often continue to do well on a very low every second day dose, but experience an exacerbation if taking this low dose away. We recommend cautiousness regarding MG patients doing well and being stable on prednisone/prednisolone in a low dose; continued long-term treatment may be necessary.

Azathioprine is the other well-established first-choice immunoactive drug used for MG [3, 14– 18]. This drug is often used in combination with prednisone/prednisolone. Formal scientific evidence for its effect in MG is lacking, but a controlled trial showing the superiority of the combination prednisolone—azathioprine over prednisolone alone is much cited [19]. Azathioprine is regarded as safe and with few side effects, also during long-term treatment. It is listed among drugs that should not be used in pregnancy, although formal evidence of teratogenic effects in MG patients is lacking. During the first few months of treatment, the numbers of leucocytes and leucocyte subgroups have to be counted weekly. The clinical effect of azathioprine is slow to appear. Improvement should not be expected to appear until after 3–6 months, and full effect of the drug first occurs after 1-2 years. This is a reason why azathioprine is usually combined with other immunoactive treatments, such as prednisone/prednisolone, and especially in the initial phase. Marked improvement on azathioprine is reported in 70–90% of MG patients in open series.

Mycophenolate mofetil is regarded as an alternative drug for mild MG [3, 14–18]. However, after promising results in open MG patient treatment, randomized and controlled trials failed to confirm a positive effect [20]. The drug has few and mild side effects and is easy to use both for patients and doctors. Despite its limitations, mycophenolate mofetil is still regarded as an alternative drug for mild MG, whereas more severe MG is usually not treated by this drug because of the negative controlled trials**.**

Methotrexate should be used only when first-choice immunosuppressive drugs do not have sufficient effect [3, 14–18]. Methotrexate has a good and proven effect for other autoimmune

disorders, but is not formally tested for MG. Still it should be tried in selected MG patients with a marked functional deficit, partly because it is usually well tolerated.

Cyclosporine *A* is an inhibitor of T cells and has well-documented immunosuppressive effects after organ transplantation. A controlled prospective study with a limited number of included patients proved the effect of

this drug for generalised MG [21]. Due to the danger of side effects, cyclosporine is regarded as a second-choice immunoactive drug for moderate to severe MG not responding to azathioprine and prednisolone [3, 14–18].

Rituximab is a chimaeric monoclonal antibody that targets B lymphocytes through its binding to the CD 20 molecule. MG is a prototype of an antibody-mediated autoimmune disease, and so rituximab and B-cell depletion are a very promising treatment alternative. In a recent review by Benveniste and Hilton-Jones [22], the effect of rituximab in 53 MG patients was recorded, including patients with both AChR and MuSK antibodies. The authors concluded that markedly positive effects were observed with distinct improvement of severe symptoms. Rituximab should be reserved for patients with severe MG, where treatment with prednisolone and at least two other standard immunosuppressive drugs has failed. For milder MG, the risk of progressive multifocal leucoencephalopathy and other potential long-term side effects probably outweigh its therapeutic potential. This drug seems to be particularly useful for anti-MuSK MG.

Tacrolimus (FK506) is a calcineurin inhibitor just as cyclosporine. The drug inhibits the proliferation of activated T lymphocytes, but also acts on ryanodine receptor-mediated calcium release from sarcoplasmic reticulum in muscle cells. The drug has shown a beneficial effect in MG, and it represents an alternative second-choice drug for moderate to severe MG [3, 14–18], perhaps especially for MG patients with ryanodine receptor autoantibodies .

Other drugs, such as cyclophosphamide and several new and selective immunosuppressive drugs, have probably a positive effect on MG, as they have on other autoimmune disorders. However, this effect has not been documented so far or is less well documented than for the above-mentioned drugs. Potential side effects are significant.

Conclusions

MG affects all age groups, with peaks in younger women and older men. There is a great variability in the geographical/regional rates of MG, with the incidence and prevalence rates increasing overall, the latter partly due to better awareness and improvements in the diagnosis of the disease. Juvenile MG is more common in people of Asian and African descent. A subset of AChR-MG is caused by a thymoma or thymic hyperplasia. The rest of AChR-MG as well as all of MuSK, Lrp4, and seronegative-MG cases are primarily autoimmune in nature, though influenced by genetic background and environmental factors that are fully understood. Although primarily an antibody-mediated disorder, different T and B cell subsets, including Th2, Th1, Th17, Tfh, Treg and Breg, and their related cytokines play important roles in MG pathogenesis. A deeper understanding of MG subgroups and their distinct immunopathogenic mechanisms will result in the identification of therapeutic targets and the development of targeted treatmentstrategies.

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