Myasthenia Gravis

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9.1 Introduction

At the normal NMJ, the motor nerve ending (presynaptic region) and a specialized portion of muscle membrane (postsynaptic region) are juxtaposed, being separated by a ~50 nm width, termed synaptic cleft. This space comprises the basal lamina that has a central role in NMJ formation, securing a stable concentration of synaptic proteins, both nerve derived (as agrin, neuregulin) and muscle derived (as laminin β-2) and of the enzyme acetylcholinesterase (AChE) [1]. AChE is expressed in an asymmetric form composed of tetramers of catalytic subunits attached to a collagen tail ColQ that anchors the enzyme through binding both perlecan and the muscle protein MuSK (muscle-specific tyrosine kinase receptor) [2].

In the nerve terminal, synaptic vesicles accumulate at the active zones where P/Q-type voltage-gated calcium channels (VGCC) are clustered. Each vesicle contains 5000–10,000 molecules of acetylcholine (ACh) and is referred to as a quantum. The postsynaptic membrane is folded into secondary synaptic folds which greatly increase its area. At the crest of the folds, the acetylcholine receptors (AChRs) are assembled at a high density (10,000–20,000/μm^2), anchored to the dystroglycan complex through rapsyn [1]. The AChR clustering and the maintenance of NMJ require MuSK activation by agrin through its coreceptor Lrp4 (low-density lipoprotein receptor protein 4) [3].

When an action potential (AP) depolarizes the nerve terminal, the opening of VGCCs results in a rapid increase of the intra-nerve Ca^{2+} concentration, which triggers the exocytosis of 50–300 quanta. The binding of two ACh molecules leads to a conformational change in the AChR and opens the ion channel; the influx of Na^+ results in a local membrane depolarization, end plate potential (EPP), which is

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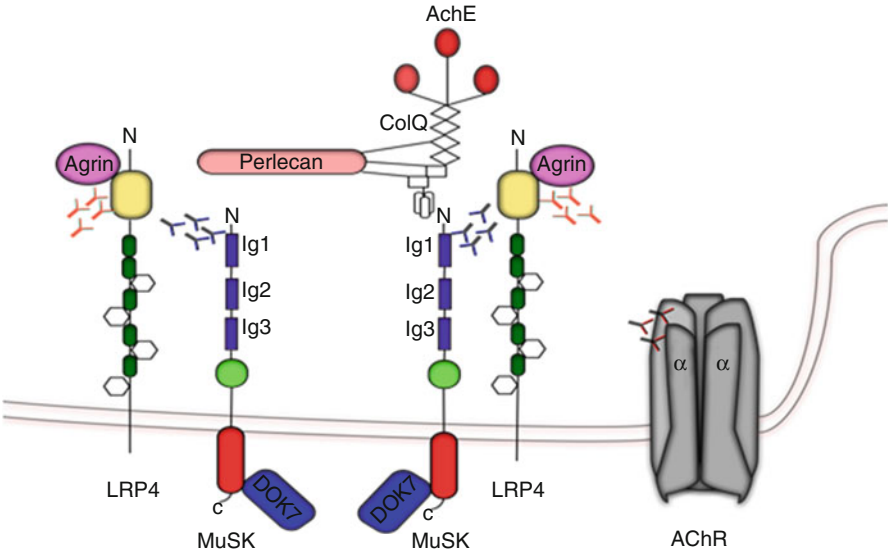

Fig. 9.1 Antibody targets in myasthenia gravis: structure and interactions with related molecules

greatest in the depths of secondary folds where voltage-gated sodium channels (VGSC) are highly concentrated . When the EPP is adequate to open these channels, a muscle AP ensues.

At the normal NMJ, the EPP largely exceeds the threshold for the generation of a propagated muscle AP. This corresponds to the safety factor of neuromuscular transmission (NMT) that depends on presynaptic (amount of quanta released per each nerve depolarization) and postsynaptic (AChR and VGSC density) factors. NMT diseases are characterized by an alteration, generally a reduction, of the safety factor.

Myasthenia gravis (MG), the most common of these disorders, is caused by antibodies (Abs) to different proteins of the postsynaptic membrane (Fig. 9.1). Abs to the AChR are detected in the great majority of patients, 5–8 % have Abs against MuSK, and a lower proportion of patients harbor Abs to Lrp4 [4]. The autoimmune attack causes morphologic and functional alterations, responsible for NMT impairment, which results in fatigable weakness of voluntary muscles.

9.2 Epidemiology

MG affects all races and can onset at any age, from the first year of life to the nineties. Epidemiological investigations have mostly been focused on the AChR-positive disease (AChR-MG). On the whole, these studies show a broad variability both in incidence, which varies from 4.3 to 18.0 per million, and in prevalence rate, ranging from 70.6 to 163.5 per million [5]. In Western countries, AChR-MG typically shows

a bimodal age of onset, with predominance of women among early-onset cases (between the second and fourth decade) and of men in a more advanced age; childhood MG with purely ocular symptoms is common in Asian populations.

The positivity rate of MuSK Abs in AChR-negative patients varies across populations, with higher rates in Mediterranean countries in Europe and among Afro-American patients in the USA [6]. In two nationwide studies, MuSK-MG prevalence was 1.9 per million in the Netherlands and 2.9 per million in Greece [5]. The disease shows a marked prevalence in women with an average age at onset in the mid-thirties.

The positivity rate of Lrp4 Abs in AChR- and MuSK-negative patients varies in different studies. In a large series of 635 patients, the overall frequency of Lrp4 Abs was 18.7 %, with variations among populations from different countries [7]. Lrp4-MG appears to be prevalent in women (male/female ratio of 1:2) with mean age of onset in the fourth decade.

9.3 Pathogenesis

9.3.1 MG with Abs to AChR

The AChR is a pentameric ion channel with a stoichiometry $2\alpha1\beta1\gamma\delta$ in embryonic/denervated muscle and $2\alpha1\beta1\varepsilon\delta$ in normal adult muscle. Although AChR Abs are polyclonal and can recognize all receptor subunits, epitope mapping studies have shown that a high proportion of patients have serum Abs to the so-called main immunogenic region (MIR) on the extracellular domain of α1 subunits [8]. MIR Abs are highly pathogenic and their serum level was shown to correlate better with disease severity than total AChR Ab titer [8].

AChR Abs mostly belong to IgG1 and IgG3 subclasses and impair NMT through complement-mediated destruction of the postsynaptic membrane, increased AChR degradation by receptor cross-linking, and competition with ACh binding [4]. Their pathogenicity has been fully demonstrated in experimental MG studies, both by active immunization and patients' IgG injection.

AChR-MG is frequently associated with alterations of the thymus, the organ where T cell maturation and establishing of central tolerance occur. Most patients with early-onset MG (age of onset <50 years) have thymic follicular hyperplasia characterized by expansion of the perivascular spaces with prominent B cell and plasma cell infiltration and germinal center formation. The hyperplastic thymus is thought to be the site where the autosensitization against AChR occurs and Ab production is initiated [9]. An intra-thymic inflammatory milieu, possibly induced by infectious agents, together with immune-regulatory defects and a predisposing genetic background concur to the establishing of the autoimmune response [10]. Early-onset AChR-MG is associated with human leukocyte antigens (HLA) B8 and DR3 [10]. A thymoma is present in 10–20 % of AChR-MG patients, with the highest frequency between the fifth and seventh decades of life. Thymomas are tumors of thymic epithelial cells harboring variable proportions of nonneoplastic

lymphocytes. Thymomas associated with MG are prevalently of "cortical" types with a rudimental medulla and retain the capacity to export mature T cells [10]. Tumor tissue does not produce AChR Abs, but through a defective T cell, selection can contribute to MG pathogenesis by the export of autoreactive $CD4^+$ T cell and a reduced production of T regulatory cells [9]. Lastly, in patients with late-onset disease, the thymus parenchyma is mostly replaced by fat, even though some B cell infiltration and occasional germinal centers can be found [9].

9.3.2 MG with Abs to MuSK

MuSK is a transmembrane protein, made of an extracellular region consisting of three immunoglobulin-like (Ig-like) domains and a cysteine-rich domain, a transmembrane helix, and a cytoplasmic region harboring the kinase activity. MuSK activation by neuronal agrin triggers an intracellular signaling cascade leading to AChR and rapsyn clustering [3]; in addition, its binding to ColQ carboxyl-terminal anchors AChE to the basal lamina [2].

Abs to MuSK are prevalently IgG4 and target mostly the first two Ig-like domains in the extracellular region [11]. Although IgG4 does not activate complement and is relatively inefficient in cross-linking adjacent antigens, IgG4 MuSK Abs were found to correlate with disease severity in patients [12] and induced MG weakness when injected into mice [13]. These Abs were shown to interfere with MuSK-ColQ binding, causing a reduced AChE concentration at the synaptic cleft [14], and to prevent MuSK-Lrp4 binding, thus inhibiting agrin-induced MuSK activation [15]. In addition, immunized animals showed a presynaptic dysfunction as lack of compensatory increase in ACh release, which is a homeostatic response in AChR-MG [13].

The thymus does not seem to be involved in the disease pathogenesis, as pathological examination of specimens from thymectomized patients did not show hyperplastic changes and the association with thymoma has rarely been reported [9]. An association with DR14/DR16 and DQ5 has been observed in these patients [10].

9.3.3 MG with Abs to Lrp4

Lrp4 belongs to the low-density lipoprotein (LDL) receptor family and is expressed in several tissues. At NMJ, Lrp4 acts at both pre- and postsynaptic levels, as it enhances MuSK activation through binding agrin and, in a retrograde manner, stimulates nerve terminal differentiation [16]. Lrp4 is a transmembrane protein consisting of a large extracellular region with multiple LDL repeats, epidermal growth factor (EGF)-like and β-propeller domains, a transmembrane helix, and a short cytoplasmic region. The extracellular region binds both agrin and MuSK [17].

Abs to Lrp4 are mostly IgG1 and were shown to interfere with agrin binding [18]. Immunization with Lrp4 ectodomain induced muscle weakness, AChR cluster fragmentation, and both pre- and postsynaptic NMT dysfunction [19]. A thymoma

has never been found in Lrp4-MG. Though some of these patients were reported to have thymic hyperplasia, there is no convincing evidence of a pathogenic link with the thymus.

In distinct studies, Abs to agrin [20], ColQ [21], and cortactin [22] have been reported in MG patients, often in association with either AChR or MuSK Abs. Their pathogenicity has not been proved, so far, in animal models.

9.4 Clinical Features

The hallmark of MG is fatigable weakness of skeletal muscles. Fatigability is the most consistent feature; weakness is usually present on examination but, in mildly affected cases, may be evident only on exertion. Clinical fluctuations, both daily and over longer periods, are typical. Although all voluntary muscles can be affected, some muscle groups are more commonly involved than others, and clinical presentation is quite characteristic. However, there is a marked variability in weakness extension and severity, from purely ocular symptoms to severe life-threatening disease.

The extrinsic ocular muscles (EOM) are affected in the great majority of patients, and ptosis and diplopia are the most common presenting symptoms. Ptosis is generally asymmetrical (Fig. 9.2, section a) and frequently alternating; it typically fluctuates in severity over short periods. Binocular diplopia can be caused by weakness of a single muscle or of any EOM combination. It is usually intermittent in the early stages of the disease and then tends to become constant. The association of variable diplopia and asymmetrical ptosis is useful in differentiating ocular MG from oculopharyngeal dystrophy, chronic progressive ophthalmoplegia, and thyroid myopathy.

In around 15 % of patients, MG remains confined to EOM; in the other cases, usually within 2 years from the onset, weakness spreads to other muscle groups [23]. Facial weakness is very common, with inability to close the eyes tightly and to whistle and development of a vertical smile (Fig. 9.2, section b). In limbs, proximal muscles are prevalently involved; weakness of finger extensors is relatively frequent, while ankle dorsiflexion is more rarely affected. Weakness of "bulbar" muscles (masseter, tongue, pharyngeal, and laryngeal muscles) is responsible for

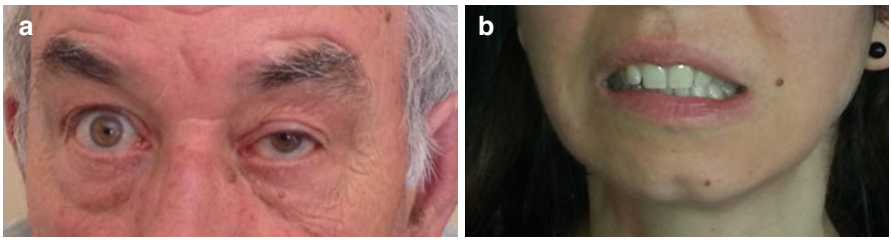

Fig. 9.2 Asymmetrical ptosis in (**a**). Facial weakness with a vertical smile in (**b**)

difficulty in chewing, dysphagia with regurgitation of fluids through the nose, and dysarthria (nasal speech). Among axial muscles, both neck flexors and extensors are involved. Respiratory failure requiring assisted ventilation (the so-called myasthenic crisis) is due to weakness of the diaphragm and intercostal muscles together with upper airway obstruction by bronchial secretions. Crises occur in 15–20 % of patients, and in spite of improvement in MG treatment and critical care, the related mortality rate is still 5 % [24]. Although AChR-MG encompasses the whole clinical spectrum, weakness pattern shows some differences in patient subgroups. Leg muscle involvement is often predominant in younger patients; bulbar and neck weakness is frequent in late-onset disease; early respiratory crises are more common in thymoma-associated MG.

MuSK-MG is nearly always a generalized disease. In most patients, clinical phenotype is characterized by a prevalent involvement of bulbar and axial muscles, with dysarthria, dysphagia, and weakness of the tongue, facial, and neck muscles. Limbs are mildly affected and can be totally spared [25]. Ocular symptoms are common at presentation, but diplopia is generally transient and ptosis is less asymmetrical than in AChR-MG. Myasthenic crises and muscle atrophy are more frequent than in other forms of MG [26]. Muscle atrophy mainly affects facial, tongue, and masseter muscles and can lead to fixed weakness with permanent dysarthria and a myopathic face. Lastly, daily symptom fluctuations are uncommon in these patients, who, however, suffer from frequent MG deteriorations especially in the first years from the onset [26].

The characteristics of Lrp4-MG are not fully defined, but the clinical phenotype in these patients seems to be similar to AChR-MG. In the largest population reported so far, around 22 % of patients had purely ocular symptoms, and those with generalized MG were prevalently affected by mild to moderate weakness [7].

9.5 Diagnosis

Once MG is suspected on clinical grounds, diagnosis confirmation is achieved through serum Ab detection, electrophysiological evidence of a postsynaptic defect of NMT, and clinical response to acetylcholinesterase inhibitors (AChE-I).

9.5.1 Serum Ab Assay

AChR Abs are detected in 85–90 % of patients with generalized MG, in 50 % of those with ocular disease, and in nearly all cases of thymoma-associated MG [27]. Therefore, these are the first Abs to be tested when MG is suspected. All patients with negative results on this assay should be tested for MuSK Abs, taking into account that the latter are very rarely associated with isolated ocular symptoms. AChR and MuSK Abs are very specific [27], and, in practice, their detection in patients with congruent symptoms confirms the diagnosis.

The positivity rate of AChR Abs has been further increased by the demonstration that some patients have serum IgG able to bind to AChRs when concentrated on cell

surface, as those at the NMJ. With a cell-based assay (CBA), serum Abs to "clustered" AChR were found in 50–60 % of patients negative on the standard assay, including some ocular MG cases [28].

Abs against Lrp4 have been detected with different techniques at frequencies varying from 3 to 50 % of AChR- and MuSK-negative samples. The recent report of these Abs in high proportion of patients with amyotrophic lateral sclerosis (ALS) casts doubt upon their specificity for MG [29].

The diagnostic value of other Abs is not defined. Moreover, while the standard radioimmunoassay for AChR and MuSK Abs is largely available, the other Abs can be tested in selected laboratories.

AChR-MG is associated with striated muscle (striatonal) Abs that recognize intracellular proteins, as titin and the ryanodine receptor (RyR). These Abs are strongly associated with thymoma (titin Abs are positive in 95 % and RyR Abs in 70 % of thymoma patients) and are present in nearly 50 % late-onset non-thymoma patients, while they are very uncommon in early-onset MG. Striatonal Abs are not diagnostic of MG and presumably not pathogenic, but are markers of thymoma in younger MG patients, and seem to correlate with disease severity [30].

Abs to Kv1.4 that target the muscle voltage-gated potassium channel were found to be associated with severe MG and myocarditis in Japanese patients [31].

9.5.2 Electrophysiological Studies

Repetitive nerve stimulation (RNS) is the most frequently used technique in the electrophysiology of NMT. In MG, low-frequency (2–3 Hz) RNS is typically associated with a decrement, greater than 10 %, of the compound muscle AP (CMAP) amplitude between the first and fourth or fifth stimulus. RNS diagnostic yield depends on testing weak muscles and is related to weakness pattern and severity. The rate of positive results is close to 75 % in patients with generalized MG and less than 50 % in those with isolated ocular symptoms [32]. In MuSK-MG, on account of the predominant bulbar involvement, diagnostic sensitivity is low, unless facial muscles are examined [33]. A decremental response on low-frequency RNS is not specific for MG as it is found in other primary disorders of NMT and in some patients with ASL or radiculopathy [32].

Single fiber electromyography (SF-EMG) records APs from single muscle fibers and measures jitter during voluntary activation or nerve stimulation. In volitional SF-EMG, jitter corresponds to the time interval variations between pairs of APs from two or more muscle fibers belonging to one motor unit. When NMT is impaired as in MG, increased jitter and "impulse blocking" (when EPP does not reach the threshold to generate an AP) occur [32]. SF-EMG is the most sensitive diagnostic test for MG, as, provided that appropriate muscles are examined, positive results are recorded in 98 % of cases, including patients with ocular myasthenia [34] or MuSK-MG [26]. However, an increased jitter is far from specific as, apart from other diseases of NMT, it can be found in neurogenic and myopathic conditions [27].

9.5.3 Pharmacological Test (Response to AChE-Is)

In MG, AChE-Is improve NMT by increasing the lifetime of ACh that can bind repeatedly to AChRs. Short-acting agents, as edrophonium chloride IV and neostigmine IM, are generally used for diagnostic purposes. Response should be evaluated on selected weak muscles and compared with reaction to placebo. With these prerequisites, a definite clinical improvement, although not specific, strongly supports the diagnosis. As edrophonium injection can be associated with bronchoconstriction and severe bradycardia, atropine should always be kept at reach.

In MG the overall rate of positive responses of edrophonium/neostigmine testing is 90 % [35]. However, in MuSK-MG, improvement upon AChE-I injection is much less common (50–70 %); side effects, such as muscle cramps and fasciculations, are frequent; and symptom worsening can be observed [26]. Cholinergic hypersensitivity in MuSK-MG can be ascribed to a relative deficiency of AChE at the synaptic cleft as a result of Ab interference with MuSK-ColQ binding [14].

A positive reaction to edrophonium/neostigmine test is observed in congenital myasthenic syndromes (CMS) and, less frequently, in Lambert-Eaton myasthenic syndrome. “False” responses have been reported in ALS and Guillain-Barrè syndrome [36].

Upon MG confirmation, all patients should undergo a radiological study of the thymus to rule out a thymoma, together with a screening for other autoimmune diseases (especially thyreopathies) and medical conditions that could interfere with treatment.

9.6 Treatment

Treatment decisions are based on weakness extension and severity, pathogenic aspects (associated Abs, thymus pathology), and patient’s characteristics. Current treatment, although largely unspecific, has dramatically reduced mortality and restored lifestyle to normal in many patients.

9.6.1 Symptomatic Treatment

Oral AChE-Is represent the first-line treatment, pyridostigmine bromide (Mestinon) being the agent most commonly used. In general, MG patients respond to AChE-Is, even though a satisfactory control of symptoms can be achieved in a minority of cases. Treatment is usually well tolerated and adverse effects (gastric discomfort, diarrhea, salivation, and cramps) are mild and can be reversed by dose reduction. On the other hand, MuSK-MG patients often show both unresponsiveness to and intolerance of AChE-Is, as – with Mestinon standard doses – they develop signs of cholinergic hypersensitivity [37] that may progress to weakness worsening (due to depolarization block) and respiratory failure [26]. Cholinergic crises are currently very rare in AChR-MG, which are associated with AChE-I overdosage [27].

Both 3,4-diaminopyridine and albuterol proved effective and well tolerated in MuSK-MG animal models [38]. These agents have not been tested in patients. A recent case report suggests that 3,4diaminopyridine may improve MuSK-MG [39].

9.6.2 Thymectomy

Although thymectomy has been in use for many decades, its efficacy has never been ascertained in controlled study (the first randomized trial is ongoing).

Thymectomy is indicated in all thymoma cases. In the absence of a thymoma, it is recommended in patients with generalized MG as an option to increase the probability of remission and improvement [40]. In most centers, it is performed in subjects with early-onset AChR-MG, in whom the removal of a hyperplastic thymus is associated with a high rate of drug-free remission. Patients with late-onset MG show a less satisfactory response, and the indication to surgery in the other disease subtypes is controversial. In particular, in MuSK-MG clinical studies failed to show significant differences in outcome measures between thymectomized and unthymectomized patients [38].

Lastly, it is worth pointing out that thymectomy, even in patients with thymoma, is never to be considered an emergency treatment and should be performed once stable control of the disease has been achieved.

9.6.3 Short-Term Immunomodulation

Plasma exchange (PE) and intravenous immunoglobulin (IVIg) that induce a rapid albeit temporary improvement are mostly used in the treatment of MG exacerbations. In addition, both (in particular IVIg) are used as periodic treatment in selected cases unresponsive to immunosuppression. In two randomized trials, PE and IVIg were shown to have comparable efficacy in an acute setting [41, 42]; there is no evidence for IVIg superiority over steroids in chronic treatment.

PE protocol consists of three to five exchanges performed every other day. Serious complications are mainly related to central venous catheters [43]. Semi-selective immunoadsorption, which does not remove albumin and coagulation factors, can be a safer alternative in patients requiring frequent PE. IVIg is administered at a dose of 400 mg/kg/day for 2–5 days. It is generally well tolerated, although serious complications have occasionally been reported [44].

9.6.4 Immunosuppressive Therapy

Immunosuppressive therapy is performed when symptoms are not adequately controlled with AChE-Is. The initial goal is to improve MG as quickly as possible; thereafter, medications should be reduced to the minimum effective dose to minimize side effects. From these principles, steroids are the first treatment because of their rapid-onset effect; in chronic administration immunosuppressants are associated as steroid-sparing agents.

9.6.4.1 Steroids

Prednisone and prednisolone are the agents mostly used in MG. They are generally administered on a daily basis at the start of treatment, then shifting to an alternate-day regimen with slow dose reduction. In most cases, ocular myasthenia can satisfactorily be managed with low-dose prednisone (25 mg/day as starting dosage), while in patients with generalized MG, higher doses (0.75–1 mg/kg/day) are employed. As steroids may induce a temporary MG deterioration, in patients with generalized disease, treatment should be started in the hospital, and PE or IVIg may be given to reduce symptom severity. The association of high-dose steroids plus PE or IVIg is also the standard treatment for severe bulbar symptoms or respiratory crises.

Steroids are effective in around 80 % of MG patients [45], but symptom relapses are frequent on dose tapering and chronic administration entails the risk of a number of side effects.

9.6.4.2 Immunosuppressants

Several immunosuppressants are used in the treatment of MG and appear to be effective in the great majority of patients, although class I evidence is still limited [45, 46]. All these agents have a long-latency effect; they can be administered in combination with steroids from the beginning and can replace prednisone in long-term treatment. Close monitoring of side effects is recommended, and because of the potential risk of infections and malignancy, the lowest maintenance dose should be determined in each patient [27].

In many countries, the purine analogue azathioprine is the first choice immunosuppressant in MG, at a starting daily dose of 2.5–3 mg/kg and a maintenance dose of 1 mg/kg. Leukopenia and hepatotoxicity are the main adverse effects, which usually subside with dose reduction or withdrawal. As patients with thiopurine methyl transferase (TPMT) deficiency may develop severe bone marrow toxicity, TPMT activity should be measured before treatment.

Mycophenolate mofetil (MMF) inhibits T and B cell proliferation, with higher specificity than azathioprine for activated lymphocytes. At the standard daily dosage of 2–2.5 g, it resulted effective in retrospective analyses and open-label trials [46]. Although these results were not confirmed in two randomized studies [47, 48], MMF, also in view of its favorable toxicity profile, is largely used in patients unresponsive to or intolerant of azathioprine.

Of calcineurin inhibitors, both cyclosporine and tacrolimus were shown to improve MG in small randomized trials [46]. The use of cyclosporine (at an initial dose of 4–6 mg/kg and a maintenance dose $\leq$3–4 mg/kg) is limited by side effects, as nephrotoxicity and hypertension [27]. Tacrolimus seems to be relatively safe at the doses used in MG and can be used as third-line drug [46]. In a recent single-blinded study, methotrexate was found to be effective as steroid-sparing agent, with similar efficacy and tolerability to azathioprine [49]. The use of cyclophosphamide on account of significant toxicity is mostly reserved to patients with severe refractory disease [46].

Immunosuppression in MG as in other autoimmune diseases has been rapidly evolving with the introduction of biologic drugs. In case reports and observational

studies, rituximab, a chimeric monoclonal Ab (mAb) that depletes B cells, was found to be effective and well tolerated in MG, particularly in MuSK-MG [50]. Treatment with eculizumab, a humanized mAb that inhibits terminal complement, was associated with significant AChR-MG improvement in a randomized placebo-controlled trial [51]. New biologics are currently explored as potential therapies in MG. These agents are very promising in view of their specific immune targets. However, lack of controlled studies and safety concerns limit so far their use to MG refractory to conventional treatment.

Highlights

- Myasthenia gravis (MG) is a heterogeneous disease, in which different antibodies affect neuromuscular transmission.
- MG with antibodies to AChR is frequently associated with thymus pathology.
- MuSK antibodies should be tested in all AChR-negative patients.
- In patients without detectable antibodies, other conditions that can mimic MG must be carefully ruled out.
- Treatment strategy should be individualized, taking into account weakness severity, patient's characteristics, and pathogenic aspects.

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