Symptomatic treatment of myasthenia gravis and its role in the era of emerging immunomodulatory treatments

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CHAPTER

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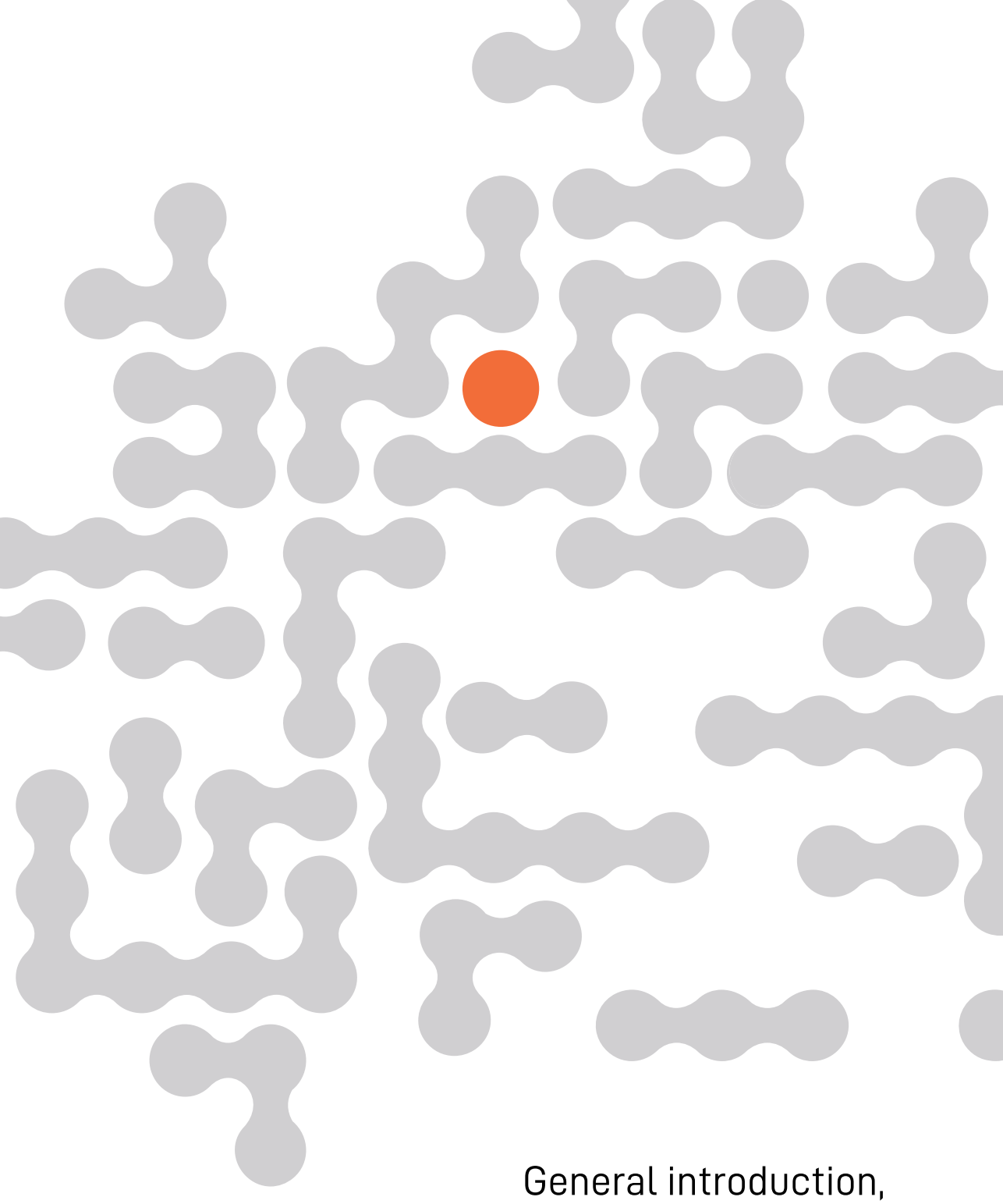

General introduction, aims and thesis outline

Background and clinical characteristics

Myasthenia gravis (MG) is a well-characterized autoimmune disease affecting the neuromuscular junction (NMJ).[1] It is a rare disease with an annual incidence of 10-29 cases per one million people, and a prevalence between 100 to 350 cases per one million people.[2] There are two age-related incidence peaks. The first occurs around the age of 30, predominantly affecting women, and is commonly associated with thymic hyperplasia and the HLA haplotype HLA-A1-B8-DR3. The second peak occurs after the age of 50, more frequently affecting men, and is characterized by thymic atrophy and an association with the HLA-DRB1*15:01 allele.[3]

MG is caused by autoantibodies targeting functionally important proteins on the postsynaptic membrane leading to impairment of neuromuscular transmission. Most commonly antibodies are directed against the acetylcholine receptor (AChR), and less commonly against other components of the NMJ such as muscle-specific tyrosine kinase (MuSK), or lipoprotein receptor-related protein 4 (LRP4).[4] Patients without detectable serum antibodies are classified as seronegative MG.

The clinical hallmark of MG is skeletal muscle weakness that typically worsens with repetitive movement.[2] Muscle weakness primarily affects ocular, bulbar, respiratory and proximal trunk and limb muscles. Most patients initially present with ocular symptoms, specifically diplopia and ptosis.[5] Approximately 15% of patients have solely ocular symptoms, while 85% have more generalized symptoms, including weakness of non-ocular muscles.[1] Muscle weakness can fluctuate in severity both throughout the day, with symptoms worsening as the day progresses, and over weeks to months.

Severe weakness may require hospitalization, and involvement of the respiratory muscles can result in myasthenic crisis requiring mechanical ventilation. Around 15% of patients experience myasthenic crisis, predominantly in the early stages of the disease.[2] Continuous progressive deterioration is not observed in MG.[1] MG is often associated with thymic abnormalities, including thymoma and thymic hyperplasia, which contribute to disease pathogenesis.[6] 10-20% of patients with AChR MG have a thymoma, and over 80% of patients with early-onset AChR MG show follicular hyperplasia in the thymus.[1]

Several MG subtypes may be classified based on clinical presentation (ocular or generalized MG) antibody type (AChR, MuSK, LRP4, seronegative), age at disease onset (juvenile-onset, early-onset and late-onset MG), or thymic pathology (thymoma or non-thymoma).[2]

Pathogenesis of MG

Neuromuscular transmission

Figure 1.1 shows key steps in the process of neuromuscular transmission. Acetylcholine is synthesized from acetyl coenzyme A and choline by the enzyme choline acetyltransferase (ChAT), and is then packed into synaptic vesicles via the vesicular acetylcholine transporter (VAChT).[7] These synaptic vesicles accumulate in the presynaptic terminal near specialized release sites, named active zones.[7] A presynaptic action potential depolarizes the nerve terminal, leading to the opening of voltage-gated calcium channels. The resulting calcium influx triggers the fusion of acetylcholine-filled vesicles with the plasma membrane. This process, mediated by the Soluble N-ethylmaleimide-sensitive factor Attachment protein Receptor (SNARE) complex, leads to the release of multiple acetylcholine quanta into the synaptic cleft[7,8].

Acetylcholine then diffuses across the synaptic cleft and binds to densely packed postsynaptic AChRs, inducing ion channel opening and subsequent cation influx. The resulting depolarization propagates across the postsynaptic membrane, ultimately initiating muscle fiber contraction. To restore the presynaptic membrane potential and terminate calcium influx, voltage-gated potassium channels open and the subsequent efflux of potassium ions repolarizes the nerve terminal.[9] In addition, acetylcholinesterase rapidly hydrolyzes acetylcholine in the synaptic cleft, effectively terminating synaptic transmission.[6]

The efficiency of this system is maintained by a safety factor, ensuring that each nerve impulse generates a suprathreshold response in the muscle fiber. Under physiological conditions, sustained or repetitive contractions, lead to decreasing quantal content.[8] In AChR MG, where fewer AChRs are available at the postsynaptic membrane, the combination of a reduced safety factor and acetylcholine rundown during sustained activity leads to transmission failure, contributing to the hallmark clinical presentation of fluctuating muscle weakness and fatigability.[6]

Effective neuromuscular transmission depends on multiple factors. A critical aspect is the clustering of AChRs on the postsynaptic membrane, directly opposite the active zones of the presynaptic terminal. In healthy neuromuscular junctions the structure is dynamically maintained, through a constant balance exists between mechanisms that promote AChR-clustering and those that are involved with cluster dispersal.[10] Receptor clustering is promoted by the agrin-LRP4-MuSK signaling pathway.[6,7,11] MuSK is located at the postsynaptic membrane and plays a central role in stabilizing postsynaptic AChR clusters.[6] LRP4 interacts with MuSK to form an inactive complex.[7] Agrin, released from the motor nerve terminal, binds to LRP4, inducing a conformational rearrangement that facilitates MuSK dimerization and subsequent autophosphorylation.[11] This activation is further amplified by DOK7

(downstream of tyrosine kinase 7), leading to the clustering of AChRs at the neuromuscular junction via the protein rapsyn. The mechanism by which the signal is transmitted from MuSK to rapsyn remains unclear.[7]

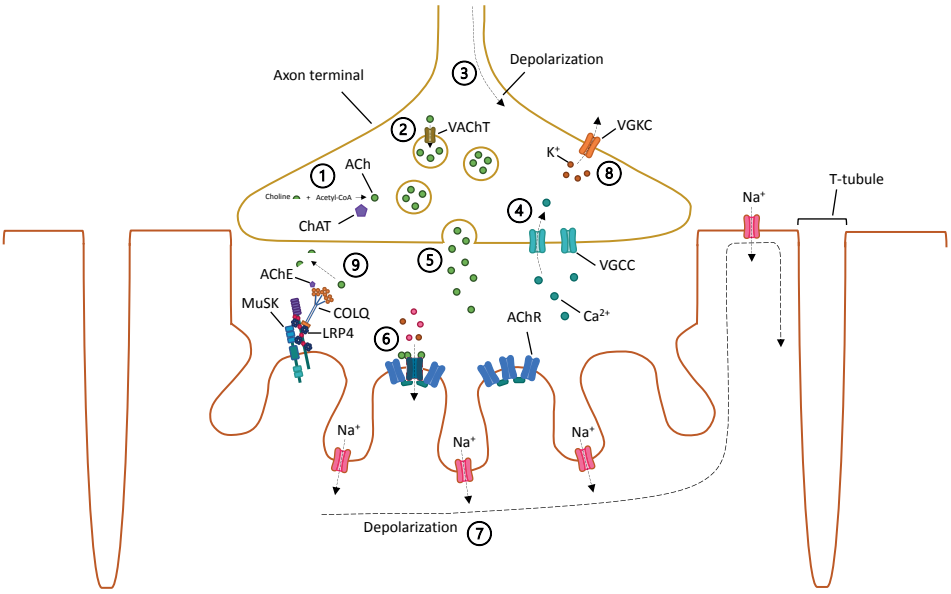

Figure 1.1 Normal neuromuscular transmission
1. Choline acetyltransferase catalyzes the synthesis of acetylcholine from acetyl coenzyme A and choline. 2. Acetylcholine is packed into synaptic vesicles by the vesicular acetylcholine transporter. 3. Depolarization of the nerve terminal by an action potential. 4. Opening of voltage-gated calcium channels, causing calcium influx. 5. Release of acetylcholine quanta into the synaptic cleft. 6. Acetylcholine binds to post-synaptic acetylcholine receptors, triggering cation influx. 7. Postsynaptic depolarization propagates across the muscle membrane. 8. Voltage-gated potassium channels open, repolarizing the presynaptic membrane and stopping calcium influx. 9. Acetylcholine is hydrolyzed, terminating synaptic transmission.
Abbreviations: ACh, acetylcholine; AChE, acetylcholinesterase; AChR, acetylcholine receptor; ChAT, Choline acetyltransferase; VAChT, vesicular acetylcholine transporter; VGCC, voltage-gated calcium channel; VGKC, voltage-gated potassium channel.

The ACh-CDK5-calpain pathway is one of the key mechanisms that negatively regulates AChR clustering at the neuromuscular junction.[7] This signaling cascade is initiated by acetylcholine binding to AChRs, which leads to the activation of cyclin-dependent kinase 5 (CDK5). CDK5 subsequently activates the calcium-dependent protease calpain, resulting in the degradation of proteins such as rapsyn. This degradation destabilizes the postsynaptic membrane and promotes the dispersal of AChR clusters.[7] In parallel, a second acetylcholine-driven mechanism acts in opposition to the agrin-LRP4-MuSK signaling pathway.[6] Here, acetylcholine binding to its receptors initiates a modest calcium influx that is amplified

through IP receptor-mediated calcium release.[6] This elevated intracellular calcium triggers caspase-3 activation, which disrupts MuSK signaling integrity and thereby weakens the stabilization of AChR clusters.[6]

Effector mechanisms of autoantibodies

In AChR MG, three primary mechanisms contribute to the reduction in AChR density and impaired receptor function at the postsynaptic membrane (**Figure 1.2**).[6] First, AChR autoantibodies, which are predominantly of the IgG1 and IgG3 subtypes, activate the classical complement pathway, leading to the formation of the membrane attack complex.[6] This disrupts the postsynaptic membrane, reduces AChR density, and results in a loss of voltage-gated sodium channels.[10] Second, due to their bivalent nature, IgG1 and IgG3 autoantibodies can induce AChR crosslinking, triggering receptor internalization and accelerated lysosomal degradation, thereby decreasing receptor density at the NMJ.[6,10] Third, autoantibodies can directly interfere with acetylcholine binding, either by occupying the binding site itself or by binding in close proximity, thereby preventing acetylcholine from accessing its receptor due to spatial constraints.[12] AChR antibodies are polyclonal and can bind to multiple receptor subunits.[13,14] In individual patients, these antibodies may simultaneously mediate different pathogenic mechanisms,[12] and even an individual autoreactive clone can contribute to one or more of these mechanisms.[14]

MuSK antibodies, in contrast to AChR antibodies, are predominantly of the IgG4 subclass. These IgG4 antibodies are anti-inflammatory and do not activate the complement system.[6] IgG4 MuSK antibodies bind to the first immunoglobulin-like domain of MuSK, thereby blocking its interaction with LRP4 and inhibiting agrin-induced MuSK phosphorylation.[15] Furthermore, evidence suggests that the retrograde signaling of LRP4 is impaired in MuSK MG,[16] thereby preventing the upregulation of quantal content, unlike in AChR MG. This is supported by the observation that repetitive nerve stimulation in MuSK MG produces a decremental response pattern similar to that seen in Lambert-Eaton Myasthenic Syndrome (LEMS), consistent with a presynaptic dysfunction.[17] Additionally, MuSK antibodies are thought to interfere with the binding of collagen Q (ColQ) to MuSK.[18] Since acetylcholinesterase is anchored to the synaptic basal lamina via ColQ, this mechanism may underlie the hypersensitivity to acetylcholinesterase inhibitor therapy observed in some patients with MuSK MG.[6]

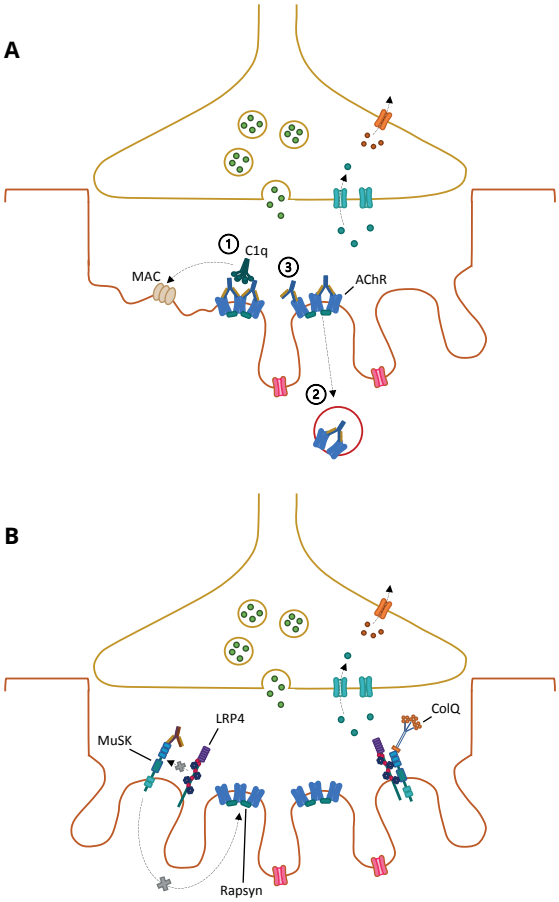

Figure 1.2 Effector mechanisms of AChR and MuSK autoantibodies
A.1. Complement activation. 2. Enhanced internalization of the receptor leading to accelerated lysosomal degradation. 3. Direct antagonism of the receptor. B. MuSK antibodies block LRP4 from activating MuSK leading to reduced acetylcholine receptor clustering.
Abbrevations: AChR, acetylcholine receptor; C1q, complement component 1q; ColQ; collagen Q; LRP4, low density lipoprotein receptor-related protein 4; MAC, membrane attack complex; MuSK, muscle-specific kinase;

Thymic changes

The thymus is the primary site for T cell maturation and central immune tolerance by eliminating self-reactive T cells.[19,20] Under normal conditions, thymic epithelial cells present peripheral tissue proteins, including self-antigens, to developing T cells in the thymus. The main factor responsible for self-antigen expression is the autoimmune regulator (AIRE),[21] a transcription factor that promotes the expression of these proteins in the thymic medulla.[6] T cells that strongly recognize self-antigens undergo apoptosis.[1] Some self-reactive T cells

escape thymic deletion and enter the peripheral circulation. In the periphery, mechanisms like apoptosis, functional inactivation, or suppression by regulatory T cells help maintain tolerance by controlling these self-reactive cells.[1]

In AChR MG, B cells play a central pathogenic role, but their activation and autoantibody production depend on T cell help.[19] Increasing evidence points to T cell dysregulation as a key factor maintaining autoreactive B cells.[19,20] In early-onset AChR MG, thymic regulatory T cells have impaired suppressive function,[19] leading to increased expression of AChR and pro-inflammatory signals by thymic epithelial cells.[19] These changes support the expansion of autoreactive T cells and the recruitment of B cells into the thymus, where ectopic germinal centers can form.[6] Within these structures, B cells undergo affinity maturation and differentiate into long-lived plasma cells producing AChR antibodies.[19] In thymoma-associated MG, the autoimmune regulator AIRE is absent in nearly all thymomas.[6,19] These tumors abnormally express muscle protein fragments, promoting the development of autoantibodies not only against AChR, but also against titin, the ryanodine receptor, and neurofilament proteins.[19]

This facilitates the development of autoreactive T cells within the thymoma, which, only in patients with MG, then migrate to the periphery to support B cells targeting these antigens.[6] In contrast, in MuSK MG thymic abnormalities are typically absent,[6,22] and thymectomy in these patients is ineffective. [23,24]

Current treatment guidelines

Symptomatic treatment

According to national and international guidelines, the first-line treatment for MG is symptomatic therapy, most commonly pyridostigmine.[25-28] Pyridostigmine is a reversible acetylcholinesterase inhibitor that increases the level and duration of action of acetylcholine in the synaptic cleft by inhibiting its breakdown. Other acetylcholinesterase inhibitors that act on the peripheral nervous system include physostigmine, neostigmine, edrophonium, and ambenonium.[5,29] These agents differ in their route of administration (oral or parenteral), and duration of action. Based on years of clinical experience, pyridostigmine is considered to have a more favorable balance between efficacy, duration of action, and side effect profile compared to the other acetylcholinesterase inhibitors. However, data on its efficacy and side effects are limited, and current understanding is based only on (small) observational studies.[30]

In addition to acetylcholinesterase inhibitors, there are currently only limited alternatives for the symptomatic treatment of MG. Ephedrine may be effective in AChR MG. Although a small series of n-of-1 trials showed a statistically significant reduction of symptoms, the

effect size was smaller than the predefined minimal clinically important difference.[31] Therefore, its clinical use is limited, particularly in light of its restricted availability. Another symptomatic treatment is amifampridine, a potassium channel blocker. Observational studies and a small crossover trial suggest that amifampridine may be effective in patients with MuSK MG.[32-34] Its use in AChR, however, is supported only by anecdotal evidence.

Thymectomy

The first case description of thymectomy in MG dates back to 1911,[35,36] and its efficacy was confirmed in the MGTX trial published in 2016.[37] This trial evaluated the efficacy of extended transsternal thymectomy combined with alternate-day prednisone versus alternate-day prednisone alone in 126 nonthymomatous MG patients aged 18 to 65 years.[37] Over a three-year follow-up, thymectomy resulted in a lower average Quantitative Myasthenia Gravis (QMG) score compared to prednisone monotherapy. Additionally, patients in the thymectomy group required a lower average dose of alternate-day prednisone, fewer needed adjunctive immunosuppression with azathioprine, and hospitalization rates due to disease exacerbations were reduced relative to the prednisone-only group.[37] The 2-year extension study further substantiated the benefits of thymectomy, demonstrating that after 5 years, patients treated with thymectomy plus prednisone had significantly lower mean QMG scores and reduced mean alternate-day prednisone doses compared to those receiving prednisone alone.[38]

Immunosuppressive therapies

For patients with insufficient response to symptomatic treatment, the next treatment step is the initiation of immunosuppressive therapies such as prednisolone and steroid sparing therapies such as azathioprine, mycophenolate mofetil, methotrexate, rituximab, ciclosporin, or tacrolimus.

Corticosteroid treatment is generally accepted as the first line immunosuppressive therapy.[39] It acts via the glucocorticoid receptor to regulate gene transcription and immune responses, including inhibition of dendritic cells and Th1/Th17 cells, and promotion of regulatory T cells.[40] The clinical efficacy of corticosteroids in MG is established through decades of use, expert consensus, and limited trial data.[41] Generally, patients start with high daily doses of prednisolone for several weeks, followed by gradual tapering, over months, to the lowest effective dose.[39] Both short- and long-term use of corticosteroids are associated with significant adverse effects, including osteoporosis, diabetes mellitus, glaucoma, weight gain, hypertension, and increased risk of cardiovascular disease and infections.[42] Consequently, corticosteroids should be tapered as much as possible, and combined with other immunosuppressive (corticosteroid-sparing) therapies at an early stage.

Azathioprine, mycophenolate mofetil, and methotrexate act by inhibiting the proliferation of T- and B-cells, while the calcineurin inhibitors cyclosporine and tacrolimus primarily block T-cell activation.[43]

Due to the limited number of comparative studies, the choice of a specific immunosuppressive agent is currently primarily based on expert opinion, resulting in substantial variability among countries in the use of immunosuppressive agents.[39] Drug choice is often influenced not only by clinician preference but also by factors such as availability and cost, the latter being particularly relevant in countries with limited financial resources.

Azathioprine is the most commonly used first-line non-steroidal immunosuppressant, and its use is supported by evidence from a randomized clinical trial demonstrating a significant steroid-sparing effect,[44] and expert consensus.[25] Mycophenolate is widely used and expert consensus holds that it is effective for the treatment of MG, despite two negative trials whose outcomes were likely influenced by methodological limitations.[45-47] The effect of methotrexate was assessed in two trials with conflicting results, likely due to differences in patient selection and trial duration.[48,49] Two recent studies have provided further insight into the choice of the initial immunosuppressive therapy.[50,51] A prospective cohort study demonstrated comparable clinical efficacy and improved quality of life with both azathioprine and mycophenolate; however, azathioprine was associated with more adverse events (most frequently hepatotoxicity), while mycophenolate had fewer side effects, but included a higher teratogenic risk.[51] A cross-sectional study further showed that azathioprine was more frequently discontinued due to side effects than mycophenolate or methotrexate, with liver dysfunction, diarrhea, and fatigue being the most common reported side effects for each drug respectively.[50] Over the long-term, skin problems were the primary reason for discontinuing azathioprine after 10 years.[50] In addition, although extensive long-term data for mycophenolate are limited, it appears to carry a lower risk of squamous cell carcinoma compared to azathioprine in transplant recipients.[52] These findings have led to a shift in first-line non-steroidal immunosuppressive therapy in the recently updated Dutch national MG guideline, which now recommends mycophenolate over azathioprine, except for women of childbearing age, for whom azathioprine should be considered as the first-choice treatment.[28]

Immunoglobulin therapy and plasma exchange

Due to the rapid onset of action, both immunoglobulin therapy, either intravenously (IVIg) or subcutaneously (SCIg), and plasma exchange have been used as rescue medication.[25] Immunoglobulin therapy has immunomodulatory effects by saturation of Fc receptors, inhibiting complement activation and neutralizing cytokines and autoantibodies, while plasma exchange reduces the levels of circulating pathogenic factors, including antibodies, complement, cytokines, and adhesion molecules.[1] There are several situations in which treatment with immunoglobulins or plasma exchange as short-term therapies should be considered:[25]

- In patients presenting with life-threatening symptoms, such as respiratory failure or severe dysphagia
- Preoperatively in patients with marked bulbar muscle weakness
- When a rapid clinical improvement is required
- As a preventive measure to avoid exacerbation when initiating corticosteroids

Plasma exchange may provide greater short-term symptom relief in MG compared to IVIg.[53,54] However, IVIg is associated with shorter hospital stays and a lower risk of complications.[53] For that reason, the choice between these treatments should be guided by individual patient and disease characteristics.

In addition to the use of immunoglobulin therapy and plasma exchange in acute indications, both therapies can be considered as maintenance therapy in patients with refractory MG, or in patients in whom immunosuppressive therapies are relatively contraindicated.[25] However, the scientific evidence on this topic is contradictory. A randomized controlled trial showed that IVIg infusions in adult patients with MG did not increase the proportion of patients achieving a ≥50% reduction in corticosteroid dose compared to placebo.[55] A second study found no difference in the primary outcome, although several efficacy parameters showed numerically greater improvements in the IVIg group than in the placebo group. This study was small and may have been underpowered to detect significant differences.[56]

Novel immunomodulatory therapies

Advances in the understanding of the pathophysiology of MG have led to the development of several novel therapeutic options in recent years.

Complement inhibitors

As outlined above, complement plays a crucial role in the pathogenesis of AChR-positive MG, and has therefore emerged as a promising therapeutic target to prevent destruction of the postsynaptic membrane at the neuromuscular junction.

Eculizumab is a humanized monoclonal antibody that binds to the complement protein C5, thereby inhibiting its cleavage into C5a and C5b and preventing the formation of the C5b-induced membrane attack complex. Treatment requires biweekly intravenous administration. Its efficacy has been demonstrated in patients with refractory AChR MG.[57] Although the primary endpoint of the phase 3 REGAIN trial did not reach statistical significance,[57] multiple secondary outcomes and long-term data demonstrated sustained clinical benefit and good tolerability.[57,58]

Ravulizumab is a modified version of eculizumab designed for prolonged complement inhibition. This allows for less frequent dosing, with intravenous administrations once every eight weeks.[59] In the CHAMPION MG trial, ravulizumab demonstrated significant efficacy on MG-ADL and QMG scores, with clinical responses typically observed within 2-4 weeks, and showed a favorable safety profile.[59]

A third complement inhibitor is zilucoplan, a small macrocyclic peptide that binds C5 with high affinity and specificity. This binding prevents cleavage into C5a and C5b and also inhibits C6 binding by targeting the region of C5 that forms C5b. Zilucoplan is administered as a once-daily subcutaneous injection, which can be self-administered by the patient. The RAISE study demonstrated a significant improvement in MG-ADL scores at week 12 compared to baseline, and zilucoplan was well tolerated.[60]

FcRn blockers

The neonatal Fc receptor (FcRn) was originally identified in neonatal intestinal epithelial cells, hence its name, but is now known to be widely expressed throughout the body, including in endothelial cells.[61] These cells internalize circulating IgG, which binds to FcRn in acidic endosomes. FcRn protects IgG from degradation by recycling it back to the circulation, thereby prolonging its half-life. FcRn antagonists block this interaction, leading to lysosomal degradation of unbound IgG and a marked decrease in circulating IgG levels.[61] This mechanism is analogous to the effect of plasma exchange, as both therapies reduce serum IgG concentrations.[61]

Efgartigimod is a human IgG1-derived Fc fragment administered intravenously and was the first FcRn-blocker evaluated in a phase 3 trial for generalized MG. In the ADAPT study, efgartigimod demonstrated significant clinical improvement, with 68% of patients classified as MG-ADL responders, compared to 30% in the placebo group.[62] Treatment consisted of weekly infusions over 4 weeks, followed by observation. It was generally well tolerated with the most common adverse events being upper respiratory infections, headache, and urinary tract infections. Long-term efficacy and safety were confirmed in an open-label extension with repeated treatment cycles.[63]

Rozanolixizumab is a humanized IgG4 anti-FcRn monoclonal antibody administered subcutaneously.

In the MycarinG study, rozanolixizumab demonstrated significant improvements in MG-ADL scores compared to placebo in patients with generalized myasthenia gravis.[64] Treatment-emergent adverse events were more common during rozanolixizumab treatment, mainly headache, but serious adverse events were infrequent and comparable across groups.

The efficacy of a third FcRn inhibitor, nipocalimab (a deglycosylated IgG1 anti-FcRn monoclonal antibody) has been demonstrated in the Vivacity-MG3 study.[65] Nipocalimab significantly improved MG-ADL scores over 24 weeks compared to placebo. Adverse event rates were comparable between groups, with infections and headaches being most common.

Aims and outline of this thesis

The aim of this thesis is to provide a comprehensive overview of the therapeutic landscape for patients with MG, with a special emphasis on existing and novel symptomatic treatment strategies (Part I), and on immunomodulatory therapies and their clinical implementation (Part II).

Part I: Symptomatic treatment

Chapter 2 describes a review of the literature on currently available and emerging symptomatic treatment options for MG, highlighting ongoing challenges in their clinical application and the continued need for clinical trials to optimize symptom control. In **Chapter 3** we describe results of a cross-sectional survey in a large cohort of MG patients, quantifying effectiveness, side effects and net benefit of pyridostigmine use in clinical practice. **Chapters 4 and 5** report on the findings of IMPACT-MG, a randomized, double-blind, placebo-controlled crossover study. In **Chapter 4**, we investigated the efficacy and cost-utility of pyridostigmine in AChR-positive MG patients, while **Chapter 5** describes the effect of adding amifampridine in patients where symptom control with pyridostigmine alone was insufficient.

Part II: Immunomodulatory therapies

In **Chapter 6**, an overview of longitudinal treatment patterns of currently available treatment options including pyridostigmine, corticosteroids and immunosuppressive and immunomodulatory therapies, in different subgroups of MG patients is given. **Chapter 7** describes the clinical implementation of efgartigimod, a neonatal Fc receptor blocker, in patients with refractory MG.

Finally, **Chapter 8** provides a summary and general discussion of this thesis, reviewing the results and discussing future perspectives on symptomatic and immunomodulatory therapies in MG.