Current Clinical Neurology
Series Editor: Daniel Tarsy

Henry J. Kaminski
Linda L. Kusner
Editors

Myasthenia Gravis and Related Disorders

Third Edition

Humana Press

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Current Clinical Neurology
ISBN 978-3-319-73584-9 ISBN 978-3-319-73585-6 (eBook)
https://doi.org/10.1007/978-3-319-73585-6

Library of Congress Control Number: 2018934837

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Printed on acid-free paper

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The registered company is Springer International Publishing AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

Series Editor's Introduction

This third edition of *Myasthenia Gravis and Related Disorders* continues to provide a very useful update to the two previous editions edited by Dr. Henry Kaminski which were published in the Current Clinical Neurology series in 2003 and 2009. As stated in their preface, Drs. Kaminski and Kusner indicate that this volume marks a recent period of great achievement in the clinical, translational, and basic sciences of the neuromuscular transmission disorders. As befitting a new edition, many chapters have been extensively updated. In addition, recent developments in understanding the basic mechanisms of myasthenia gravis and other disorders of neuromuscular transmission have been expanded by Dr. Kusner. At the same time new chapters reviewing emerging therapeutics and clinical trial design in myasthenia gravis, coauthored by Dr. Kaminski, enhance what is currently known concerning the clinical treatment and management of this disorder which will serve to move the therapeutics of myasthenia gravis from expert opinion in the direction of evidence based medicine.

Boston, MA, USA — Daniel Tarsy, MD

Preface

The period from the second to the third edition of *Myasthenia Gravis and Related Disorders* marks a time of great achievements in the clinical, translational, and basic sciences of neuromuscular transmission disorders. When the second edition was undergoing development, some chapter authors questioned a need for an update. This was not the case for the newest edition. The work required the addition of a second editor, Dr. Linda L. Kusner, to provide greater depth to oversight of chapters dedicated to the basic science of myasthenia gravis (MG). Dr. Kusner and colleagues have added summaries of preclinical research standards for autoimmune MG. Along with Dr. Gary Cutter and Radwa Aly, I have written a broad summary of MG clinical trial performance. All other chapters have been updated extensively. We now have greater understanding of the clinical presentation of MuSK-related MG and identification of potential new autoantigens, including LRP-4. Clinical care has been enhanced with the development of treatment guidelines by groups in Japan, the United Kingdom, Germany, and an international consortium. New authors provide updates of neuromuscular transmission, autoimmune pathology, and genetics of MG.

Challenges exist for neuromuscular transmission disorders. Their rarity, variability in clinical course, and pathophysiological heterogeneity, i.e., thymoma-associated myasthenia gravis, age-related differences, and clinical phenotypes (ocular vs. bulbar vs. generalized), make clinical trials and biological studies of mechanism difficult. Therefore, clinical trials still are few and a robust evidence base is lacking, making expert opinion the bulwark of treatment recommendations for the most challenging of patients. The ultimate goal of personalized medicine for MG patients has an unclear timeline.

We thank all the contributing authors, Diane Lamsback, and Springer Publishing for making the book a reality. Appreciation is extended to the Department of Veterans Affairs, National Eye Institute, the National Institute of Neurological Disorders and Stroke, the Muscular Dystrophy Association, the Myasthenia Gravis Foundation of Illinois, and the Myasthenia Gravis Foundation of America that have supported the editors' research as well as the work of many of the contributors. To all patients with MG, we are with you in your challenges and commit ourselves as scientists and clinicians to a cure. The editors dedicate this book to the love of our life, Adam Kaminski.

Washington, DC, USA — Henry J. Kaminski
Washington, DC, USA — Linda L. Kusner

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1 Neuromuscular Junction Physiology and Pathophysiology

Jaap J. Plomp

Introduction

The crucial role of the neuromuscular junction (NMJ) is to reliably transmit action potentials from the motor neuron to the skeletal muscle fiber so that sustained muscle contraction is guaranteed. To be able to understand the NMJ dysfunction in myasthenia gravis (MG) and related disorders, it is important to know how this synapse is structurally organized and the way it achieves successful transmission The goal of this chapter is to provide information about the structure and electrophysiological function of the NMJ and to explain the synaptopathic functional deficits which lead to disturbed muscle contraction in MG and other disorders of the NMJ.

Structural Composition of the Neuromuscular Junction

The Presynaptic Compartment

The NMJ, or endplate, is a tiny structure on skeletal muscle fibers. Depending on the species and muscle type, its length ranges from tens to a few hundreds of microns (Fig. 1.1), while the muscle fiber itself can be as long as several tens of centimeters. At this synaptic connection, the pre- and postsynaptic membranes and their adjacent cytoplasmatic environments are structurally highly specialized and harbor elaborate molecular machineries which govern the transmission of action potentials from motor neuron to muscle fiber. Muscles are innervated by a peripheral nerve which contains the bundled axons of motor and sensory neurons. Within the muscle, a motor axon forms multiple branches, each of which connects to a single skeletal muscle fiber, typically in their central region (see Fig. 1.1a). The most distal portion of the branch becomes devoid of myelin and forms a presynaptic nerve terminal that exactly opposes the specialized postsynaptic area. A small distance of ~50 nm is kept between the pre- and postsynaptic membrane, forming the synaptic cleft (Fig. 1.2). Human NMJs are relatively small (usually <100 μm^2) as compared to the most often studied rodent NMJ (which is often >250 μm^2) [1]. Furthermore, their geometry differs in that the human nerve terminal forms several interconnected spotlike boutons instead of a much more continuous "pretzel" shape in rodents (see Fig. 1.1b). NMJs are covered by a few (~3–5) perisynaptic Schwann cells which play (as yet poorly defined) structural and trophic roles in maturation and maintenance of the NMJ and in aiding regeneration of the motor nerve terminal after damage [2, 3].

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H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*, Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_1

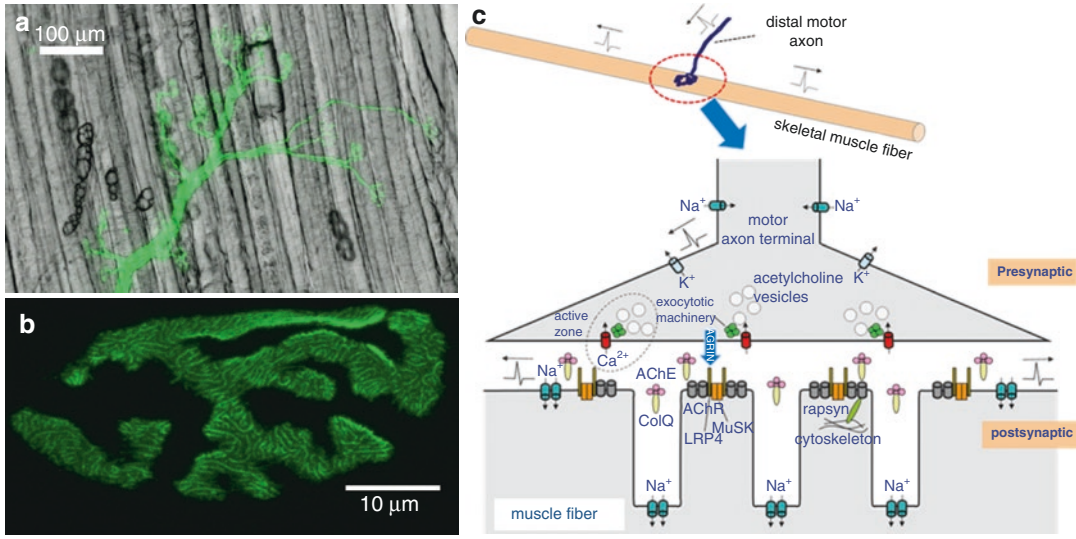


Fig. 1.1 Morphology of the neuromuscular junction. (**a**) Branching of distal motor axons in levator auris longus muscle of the mouse, forming nerve terminals on muscle fibers. Transgenic expression of YFP (green) in motor axons, shown with fluorescence microscopy. Muscle fibers visualized with bright-field microscopy. (**b**) Laser-scanning confocal fluorescence microscopical image of a single, pretzel-shaped mouse diaphragm neuromuscular junction. Staining for acetylcholine receptors with green-fluorescently labeled α-bungarotoxin reveals a fingerprint-like geometric pattern formed by the high density of receptors on the tops of the postsynaptic folds. Courtesy of Lizette van der Pijl. (**c**) Top: schematic illustration of synaptic transmission of action potentials from motor neuron to skeletal muscle fiber at the neuromuscular junction. Bottom: schematic representation of the pre- and postsynaptic machinery underlying synaptic transmission at the neuromuscular junction. *AChE* acetylcholinesterase, *AChR* acetylcholine receptor, *ColQ* collagen-Q, *LRP4* low-density lipoprotein receptor-related protein 4, *MuSK* muscle-specific kinase

Nerve terminals contain the key elements responsible for the controlled release of the neurotransmitter acetylcholine (ACh) (see Fig. 1.1c). ACh is present in "quanta," which are more or less fixed amounts of ~10,000 molecules, contained in ~50 nm diameter synaptic vesicles (see Fig. 1.2). Each nerve terminal harbors ~150,000–300,000 vesicles. The phospholipid bilayer membrane of these vesicles contains several transmembrane proteins. These include a transporter protein that loads the vesicle with ACh from the nerve terminal's cytoplasm where it is biosynthesized. Furthermore present are the SNARE proteins synaptobrevin, which is involved in neuroexocytosis, and synaptotagmin, a Ca^{2+}-sensing protein. These two molecules, in conjunction with the presynaptic membrane SNARE proteins SNAP-25 and syntaxin, form the core of a complex molecular machinery (modulated by many other proteins) which enables Ca^{2+}-dependent fusion of the vesicle and

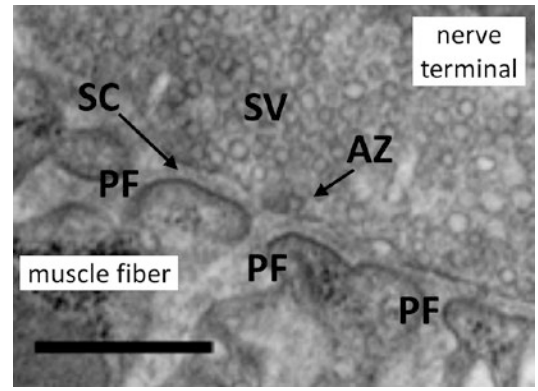


Fig. 1.2 Electron microscopical picture of a detail of a mouse neuromuscular junction. *SV* synaptic vesicles, *SC* synaptic cleft, *AZ* active zone, *PF* postsynaptic folds. Scale bar = 0.5 µm

exocytosis of its ACh quantum from the lumen into the synaptic cleft [4]. This process takes place at specialized areas called active zones, where an array of structural proteins forms a

scaffold, which anchors the functional proteins needed for regulated neuroexocytosis (see Figs. 1.1 and 1.2) [5]. Active zones exist at the cytoplasmatic side of the presynaptic membrane in a density of ~2.5 per μm^2, which means that an adult mouse NMJ has ~800 active zones [6]. A central functional protein at active zones is the voltage-gated Ca^{2+} channel, which at the adult mammalian NMJs is of the $Ca_V2.1$ (or P/Q) type [7]. In developing NMJs or NMJs from mice genetically deficient for $Ca_V2.1$, other types (Ca_V1, $Ca_V2.2$, and/or $Ca_V2.3$) can be present [8]. $Ca_V2.1$ channels are positioned at the active zone through binding with the presynaptic protein bassoon and the synaptic cleft molecule laminin-β2 [9]. In this way they are optimally positioned so that the Ca^{2+} influx resulting from their opening by a motor nerve action potential can evoke quantal ACh release through stimulation of the exocytotic machinery. In the membrane, $Ca_V2.1$ channels may reside in lipid rafts, which are cholesterol-rich micro-domains, which contain signaling molecules, as well as the SNARE proteins SNAP-25 and syntaxin [10, 11]. Furthermore, gangliosides are important components of lipid rafts. These are glycosphingolipids enriched in neuronal membranes, especially at synapses [12].

The Synaptic Cleft

The ~50-nm-wide synaptic cleft (see Fig. 1.2) between the pre- and postsynaptic membrane of the NMJ contains several synapse-specific proteins which are anchored in the extracellular basal lamina matrix and are of importance for synaptic structure and function. A key molecule is acetylcholinesterase (AChE), an enzyme responsible for the hydrolytic degradation of released ACh to terminate its signaling action. AChE is embedded in the extracellular matrix through its collagen-Q tail (see Fig. 1.1c), which also connects to perlecan, and possibly via its catalytic domain to laminin [13, 14]. Another important molecule in the synaptic cleft is laminin-β2, which is produced by the muscle fiber and functions as a presynaptic active zone organizer, a process in which its interaction with presynaptic $Ca_V2.1$ channels is essential [15]. The synaptic cleft also contains matrix metalloprotease 9 (MMP9), of which the role may be to cleave fragments from the extracellular part of some pre- and postsynaptic membrane proteins which then serve as signaling peptides in synaptic homeostasis [16]. In addition, several types of synapse-specific collagens are present with putative roles in maturation and stability of the NMJ [14].

The Postsynaptic Compartment

The motor nerve terminal is positioned in a primary gutter on the muscle fiber membrane. Ultrastructural studies show the nerve terminal perpendicular to this gutter with the postsynaptic membrane forming 50–100-nm-wide secondary folds extending into the sarcoplasm (see Fig. 1.2). Across species the length of these folds is inversely related to the area of the NMJ, the relatively small human NMJs having deep folds [1]. The tops of the synaptic folds contain the ionotropic ACh receptors (AChRs) of the nicotinic type (see below) in high density of ~10,000 ion channels per μm^2. When AChRs are labeled with a fluorescent probe and viewed with high-resolution microscopy, the contours of the tops of the folds appear as a fingerprint-like geometric pattern (see Fig. 1.1b). AChR clusters are in dynamic equilibrium; there is intense turnover with equal extents of degradation and insertion of newly synthesized and recycled AChRs so that a constant density is maintained [17]. This balance between AChR insertion and removal is regulated by antagonistic activity of protein kinases A and C [18]. The ultrastructure of the postsynaptic folds and the specific distribution of ion channels facilitate muscle fiber excitation. The confined cytoplasmatic space in the folds increases the electrical resistance, which produces an extra depolarizing effect of the ion current through the AChRs, when being forced to flow through it [19]. At the bottoms of the folds, voltage-gated Na^+ channels of the $Na_V1.4$ type are present at higher density than in the extrasynaptic sarcolemma. This relatively

high $Na_V1.4$ density lowers the firing threshold and thus facilitates excitability at the postsynaptic membrane (see below) [20].

The postsynaptic folding ultrastructure seems dependent on the large proteins dystrophin and its close homologue utrophin. Dystrophin resides throughout the inside of the sarcolemma where it functions to stabilize muscle fibers by connecting intracellular actin to a complex of intracellular, transmembrane, and extracellular proteins (including syntrophin, dystrobrevin, and dystroglycans), with dystroglycan as a core transmembrane protein. At the NMJ, dystrophin is enriched postsynaptically together with utrophin, where they modulate ultrastructure and function, as demonstrated by altered AChR geometry, reduced ACh sensitivity, and diminished postsynaptic folds in mice deficient for dystrophin alone or both dystrophin and utrophin [21–23].

For the embryonic formation of the postsynaptic membrane specialization, and the stability of the NMJ thereafter, a signaling protein complex exists which consists of the transmembrane receptor tyrosine kinase, muscle-specific kinase (MuSK), the one-pass transmembrane protein, low-density lipoprotein receptor-related protein 4 (LRP4), and an additional number of intracellular postsynaptic proteins (see Fig. 1.1c) [24, 25]. This complex forms a signaling cascade which starts with the binding of neurally released agrin to LRP4. This induces MuSK dimerization, and thereby kinase activation, which in turn stimulates the downstream pathway to finally recruit and cluster AChRs to the tops of the postsynaptic folds. Once expressed and clustered in the membrane, AChRs attract the intracellular protein rapsyn which anchors them to the cytoskeleton [26, 27].

The postsynaptic membrane furthermore contains ErbB receptors which can bind neuregulin-1 released from the motor nerve terminal. Neuregulin-1 (formerly known as AChR-inducing activity, ARIA) was initially thought to be indispensable for expression of AChR subunit genes from subsynaptic nuclei during NMJ formation. However, multiple studies have challenged this view, and it is now believed that neuregulin/ErbB signaling is not essential for AChR gene transcription and formation of AChR clusters in developing NMJs. Rather, neuregulin/ErbB signaling appears to be needed for the maintenance of a normal number of AChRs at the adult NMJ, by inhibiting the removal of AChRs in the recycling process via an effect on α-dystrobrevin1 [28–31].

Normal and Pathological Electrophysiology at the Neuromuscular Junction

The duty of the NMJ is to faithfully transmit each motor neuronal action potential onto the innervated skeletal muscle fiber, where it will spread and stimulate the excitation-contraction system (not discussed here, e.g., for review, see [32]). Although this seems a relatively simple task, the above description of NMJ structure indicates that highly elaborate multi-molecular arrangements are required. Malfunction or deficiency of any of these key factors can influence synaptic function. Although synaptic transmission at the NMJ has a large safety margin (see below), the ultimate consequence of severe defects is block of signal transmission. This will obviously cause muscle weakness because tetanic (i.e., sustained) muscle contraction requires a certain duration of repetitive muscle fiber action potentials at high frequency and that can only be achieved if the NMJ transmission seamlessly follows the trains of high-rate action potential firing of the motor neuron. Defects in key synaptic molecules may arise from genetic mutation, autoimmunity, or intoxication. To understand how these situations lead to block of transmission and consequent muscle weakness, it is important to understand the electrophysiology of the NMJ. Below, the synaptic events at the NMJ are described, as well as their deviations in some prototypical neuromuscular synaptopathies, including MG.

Presynaptic Functional Events

The inside of motor neurons and skeletal muscle fibers is negatively charged as compared to the extracellular space. This resting membrane potential of about −80 mV and the existence of several types of ion channels in the membrane of

these cells enable them to generate and transport signals in the form of depolarizations along their membrane. Action potentials are the unitary events (i.e., all-or-none signals of similar magnitude) that are conducted in an active way along the membrane of excitable cells. Their upstroke is caused by influx of Na^+ ions through voltage-gated Na_V channels, which in the skeletal muscle fiber are of the $Na_V1.4$ type. In the motor axon, action conduction is saltatory, i.e., it hops from node of Ranvier to node of Ranvier where the Na_V channel density is relatively high and the lack of a myelin insulation permits ion flux through the membrane. The repolarization phase of action potentials is due to inactivation of Na_V channels in conjunction with opening of voltage-gated rectifying K^+ channels. The latter channels, or members of the protein complex in which they reside, form the antigenic targets of autoantibodies in neuromyotonia/Isaac's syndrome, which is characterized by enhanced ACh release at the NMJ and spontaneous and repetitive motor neuronal firing, most likely generated at the motor nerve terminal [33–36]. These presynaptic K^+ channels are also the target of dendrotoxin, a toxin component of mamba snake venom [37]. Once a motor axonal action potential reaches the synaptic nerve terminal, it causes opening of the $Ca_V2.1$ channels at active zones. This results in Ca^{2+} influx into the motor nerve terminal, driven by the steep concentration gradient between the extracellular space (~2 mM) and the nerve terminal cytoplasm (~200 nM). The local increase of intraterminal Ca^{2+} concentration activates the neuroexocytotic machinery at active zones, leading to synchronous exocytosis of several ACh quanta from multiple active zones. The total number of ACh quanta released from the entire nerve terminal per nerve impulse (i.e., the quantal content) varies between NMJs of species and muscle types, as well as with age, but is roughly correlated with NMJ size and thus with the absolute number of active zones [6]. For instance, mouse diaphragm NMJs (being ~250 μm^2) have a quantal content of ~50, while human NMJs (being only ~100 μm^2) have a lower quantal content of only ~20–30 [23, 38]. From the studies of active zone density and NMJ electrophysiology, it can be deduced that the chance of releasing an ACh quantum from a single active zone in response to an action potential is only ~10% [1]. Most likely this is due to the only brief duration (only ~1 ms) of neural action potentials, which is insufficiently long to activate all $Ca_V2.1$ channels, in combination with the cytoplasmatic increase in the concentration of Ca^{2+} being limited in time and space due to strong cytoplasmatic Ca^{2+} buffering systems [39].

Several, rare disorders are associated with transmission disturbance at the NMJ due to presynaptic malfunction. The prototypical disorder is Lambert-Eaton myasthenic syndrome (LEMS), of which most patients have autoantibodies that target $Ca_V2.1$ channels [40]. Most often these antibodies have a paraneoplastic origin; many LEMS patients suffer from small-cell lung cancer [41]. It is hypothesized that the autoantibodies cross-link and thereby downregulate $Ca_V2.1$ channels, but the exact mechanism is unclear. The result is a reduced presynaptic Ca^{2+} influx, which causes severe reduction of quantal content. This leads to deminished postsynaptic endplate potentials (EPPs), which do not reach the firing threshold (see below) and thus do not trigger a muscle fiber action potential. Prolonged synaptic activity, however, may cause some degree of quantal content increase due to accumulation of presynaptic Ca^{2+} at the active zone, which results in some EPP amplitude increase. At NMJs where the initial EPP is just below the firing threshold, this may lead to temporary recovery of neuromuscular transmission. This explains the LEMS characteristic of short-term improvement in muscle force after maximal voluntary activation [40]. The drug 3,4-diaminopyridine provides symptomatic treatment for LEMS. The drug blocks delayed-rectifier voltage-gated K^+ channels and thereby broadens the presynaptic action potential, which leads to stimulation of more $Ca_V2.1$ channels or opens the available channels for longer duration. The increased Ca^{2+} influx leads to a higher quantal content and, consequently, an increase in EPP amplitude.

Mutations in the gene CACNA1A, encoding the pore-forming subunit of $Ca_V2.1$ channels, are demonstrated in the rare, inherited migraine variant familial hemiplegic migraine (FHM), and also in the clinically overlapping diseases epi-

sodic ataxia type-2 (EA-2) and spinocerebellar type-6 (SCA-6) [42]. Besides the central nervous system symptoms of headache, ataxia, and/or epilepsy in these patients, there are indications of subclinical NMJ malfunction in such patients as well as in *Cacna1a*-mutant mouse strains [8, 43].

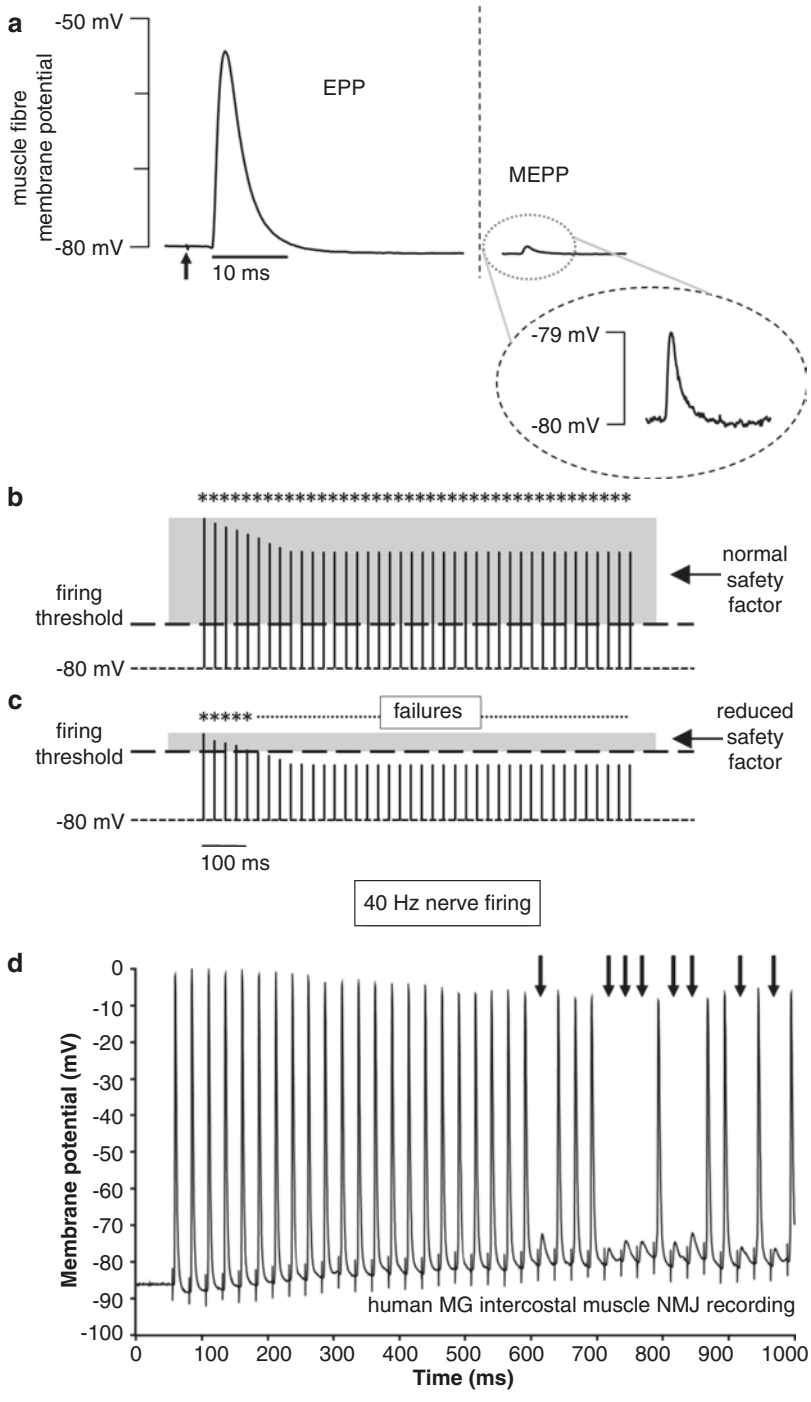
The presynaptic motor nerve terminal at the NMJ is the target of *Clostridial* botulinum neurotoxins, which are the causative agents in the paralytic disorder botulism. The potent toxin binds to gangliosides on the outside of the presynaptic membrane as well as to SV2 and synaptotagmin on the inside of a fusing neuroexocytotic vesicle [44, 45]. The toxin is taken up into the nerve terminal via endocytosis of the synaptic vesicle, and a fragment is released into the cytosol where it cleaves SNARE proteins, thus causing block of ACh release and therewith paralysis. Presynaptic gangliosides may also be the autoimmune target of anti-ganglioside antibodies in the Miller-Fisher syndrome (MFS) variant of the peripheral neuropathy Guillain-Barré syndrome (GBS). Ensuing complement-mediated presynaptic NMJ destruction might contribute to paralytic symptoms of this syndrome [12]. Botulism and MFS/GBS are included in the differential diagnosis of the myasthenic disorders.

Postsynaptic Functional Events

After presynaptic neuroexocytosis, ACh diffuses into the synaptic cleft. A large part of the transmitter is immediately degraded by the AChE anchored in the basal lamina. The ACh molecules that escape destruction can bind to the postsynaptic AChRs. Afterward, when they come off the AChR, they are degraded by AChE as well.

AChRs are pentameric ligand-gated ion channels of the nicotinic type, consisting of two α, one β, one δ, and one ε subunit and having a single-channel conductance of ~60 pS [46, 47]. To induce opening of the pore, an ACh molecule must occupy each of the two binding sites on the AChR. These are localized at an interface of each of the two α subunits with a non-α subunit [48]. AChRs are rather non-specific cation channels; under physiological conditions they allow influx of Na^+ ions and to a lesser extent K^+ efflux and Ca^{2+} influx. The predominant Na^+ influx through opened AChRs causes a local depolarization at the postsynaptic membrane. The multiple ACh quanta released simultaneously in response to a presynaptic nerve impulse give rise to the EPP, a summed depolarization which stimulates opening of a sufficient number of $Na_V1.4$ channels to form a muscle fiber action potential. This will spread out in two directions over the muscle fiber, away from the NMJ (see Fig. 1.1c), and invade the T-tubuli to switch on the excitation-contraction mechanism that leads to massive Ca^{2+} release from the sarcoplasmatic reticulum which will evoke contraction of the fiber [49]. Depending on species, age, and muscle type, EPPs are ~15–30 mV in amplitude and have a duration of a few milliseconds with a steep rising

Fig. 1.3 Synaptic electrophysiology of the neuromuscular junction. (**a**) A nerve stimulation-evoked endplate potential (EPP) and a spontaneous miniature EPP (MEPP) recorded with an intracellular microelectrode from an ex vivo mouse diaphragm muscle fiber at the neuromuscular junction. The triggering of a muscle fiber action potential is prevented during this measurement by the addition of μ-conotoxin-GIIIB to the experimental bath, which selectively blocks the voltage-gated Na^+ channels on the muscle fiber membrane. The arrow indicates the moment of nerve stimulation. (**b**) Schematic illustration of the concept of firing threshold, safety factor, and physiological rundown of EPP amplitude during high-rate synaptic activity at the neuromuscular junction. Asterisks indicate that an EPP will trigger a muscle fiber action potential. In the depicted normal situation, every EPP will trigger a muscle action potential, and sustained tetanic contraction of the muscle fiber will result. (**c**) Schematic illustration of the situation in myasthenia gravis, where EPPs are reduced in amplitude due to autoimmune-mediated reduction of acetylcholine receptor density at the neuromuscular junction. Due to the reduced safety factor, EPPs will become subthreshold during high-rate synaptic activity, resulting in failures to trigger a muscle fiber action potential. This will lead to muscle contraction fatigue. (**c**) Example of an intracellular microelectrode recording at the neuromuscular junction of an intercostal muscle biopsy from a myasthenia gravis patient. A reduced safety factor in neuromuscular transmission becomes apparent after some time of 40 Hz nerve stimulation, when EPPs become of peri-threshold amplitude and intermittently fail to trigger muscle fiber action potentials (arrows). Obviously, if such pathophysiological events take place at many neuromuscular junctions in a muscle, there will be fatigable muscle weakness


a
-50 mV
muscle fibre membrane potential
-80 mV
EPP
MEPP
10 ms
-79 mV
-80 mV
b
normal safety factor
firing threshold
-80 mV
c
failures
reduced safety factor
firing threshold
-80 mV
100 ms
40 Hz nerve firing
d
Membrane potential (mV)
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
human MG intercostal muscle NMJ recording
0
100
200
300
400
500
600
700
800
900
1000
Time (ms)

phase and an exponential decay phase (Fig. 1.3a). Besides ACh release evoked by nerve activity, a motor nerve terminal spontaneously releases single ACh quanta through exocytosis of single synaptic vesicles. This happens at irregular intervals with an average frequency of ~1–4/s (depending on muscle type) in the adult mouse NMJ and at much lower frequency (~4/min) at the smaller human NMJs. The single ACh quantum released leads to a postsynaptic miniature EPP (MEPP), ranging from ~0.3 to 1.5 mV, depending on species, age, and muscle type and having more or less similar kinetics as the EPP (see Fig. 1.3a). The MEPP is too small to activate many $Na_V1.4$ channels and is thus not capable of inducing a muscle fiber action potential. Presumably, MEPPs form a random spillover from the enormous pool of several hundred thousands of synaptic vesicles present in a motor nerve terminal. While they do not seem to have a functional role, MEPPs are useful in the ex vivo electrophysiological analysis of NMJ function as their mean amplitude forms a measure for the functional AChR density (see below). Furthermore, they can be used to calculate the quantal content at a single NMJ. To this end, the measured EPP amplitude (i.e., the result of simultaneous release of multiple ACh quanta) is divided by the mean measured MEPP amplitude in the same NMJ [50]. First, a correction factor to the EPP amplitude has to be applied, correcting for the nonlinear summation of the depolarizing effect of the multiple ACh quanta [50, 51]. In fruit fly studies a functional role for MEPPs in NMJ maturation has been proposed [52]. The relevance of this finding for the mammalian NMJ remains to be established.

The firing threshold of a muscle fiber is determined by the density and function of the $Na_V1.4$ channels on the muscle fiber membrane. Because $Na_V1.4$ density is higher at the postsynaptic membrane as compared to the extrasynaptic muscle fiber membrane [53, 54], the firing threshold at the NMJ region is lower than elsewhere. This facilitates the triggering of a muscle action potential by the EPP. A substantial safety factor of neuromuscular transmission exists at the NMJ (see Fig. 1.3b). This means that the actual EPP amplitude is much larger than minimally needed to reach the firing threshold. In rat and mouse NMJs, the minimal EPP amplitude to reach the firing threshold is ~10–12 mV, while the actual EPPs are ~20–35 mV [23, 55]. Thus, the safety factor at these rodent NMJs is ~2–3. In human NMJs the safety factor is thought to be at the lower end of this range (~2) [56]. This renders human neuromuscular transmission somewhat more sensitive to conditions that reduce presynaptic ACh release or postsynaptic ACh sensitivity. The safety factor in neuromuscular transmission is needed to ensure sustained successful transmission, even when upon intense use of the NMJ the ACh release shows a physiological rundown. This is due to factors of the neuroexocytotic system becoming limiting (e.g., Cav2.1 channels which inactivate and/or reduction of the pool of available synaptic vesicles). Tetanic muscle contraction is only possible if the NMJ neatly translates the repetitive motor neuronal action potential firing into a similar pattern of action potentials on the muscle fiber. Motor neurons fire trains of action potentials in the frequency range of 20–100 Hz, depending on muscle fiber type [57, 58]. During such tetanic activity, the EPP amplitude in mouse NMJs diminishes by 20–30% to a more or less constant level after about ten impulses (see Fig. 1.3b). At human NMJs, this EPP amplitude rundown is even somewhat more pronounced, by ~40% [59]. Due to the existence of a safety factor in neuromuscular transmission, the NMJ can cope with such EPP rundown during intense activity; EPPs will remain suprathreshold and each will trigger a muscle fiber action potential, causing sustained tetanic muscle fiber contraction (see Fig. 1.3b).

A hallmark of MG is fatigable muscle weakness, which is due to progressive transmission failure at the NMJ during intense synaptic activity. Most MG patients have IgG1 and IgG3 autoantibodies against postsynaptic AChRs at the NMJ. Their antigenic binding has multiple effects: (1) cross-linking of AChRs, causing increased internalization, (2) direct functional block of the AChR, and (3) complement activation, culminating in focal postsynaptic lysis due to membrane attack complex formation [50, 60, 61]. The pri-

mary result of these effects is a reduced postsynaptic ACh sensitivity due to the physical removal and functional block of a proportion of the AChRs. When synaptic signals are electrophysiologically analyzed at NMJ in human MG muscle biopsies or in muscle preparations of MG animal models, this reduced AChR density can be shown to cause a reduction of mean MEPP amplitude [50]. Because MEPPs have no direct physiological role in synaptic transmission the NMJ, this amplitude reduction itself has no acute functional consequences. The extent of reduction, however, forms a good measure for the severity of the autoimmune attack by AChR antibodies. The EPP, caused by the multi-quantal ACh release evoked by a nerve action potential, becomes reduced in amplitude too by the reduced AChR density. This has direct functional consequences for the synaptic function of the NMJ. The safety factor of neuromuscular transmission becomes reduced, and EPPs may be too small to cross the firing threshold of the muscle fiber, especially during intense use of the NMJ when there is EPP amplitude rundown (see Fig. 1.3c). This can lead to a progressive and (intermittent) failure of EPPs to trigger a muscle fiber action potential (see Fig. 1.3c, d) and will result in fatigable muscle weakness. In fact, EPP amplitudes run down more pronouncedly at MG NMJs than at healthy NMJs, presumably due to secondary changes in the presynaptic ACh release mechanism (see below) [59, 62]. This further adds to safety factor reduction in the circumstance of intense synaptic activity.

At severely affected MG NMJs, EPPs may be reduced by such a large extent that the first EPP of a high-frequency train already is subthreshold. Such muscle fibers will not contract at all and will cause an initial/permanent paralysis component of the MG patient, next to the fatigable component caused by the fibers with NMJs that progressively lose transmission during prolonged synaptic activity.

Another factor contributing to the reduced safety factor at MG NMJs is the complement-mediated disruption of the postsynaptic membrane [63]. Besides causing reduction of AChR density, this also leads to concomitant removal of a proportion of the postsynaptic $Na_V1.4$ channels, which are enriched at the bottom of the postsynaptic membrane folds. Because the density of these channels dictates the firing threshold of the muscle fiber at the NMJ, this results in an elevation of the threshold, thereby further reducing the safety factor [64, 65]. Anti-AChR antibodies by themselves do not affect the electrophysiological characteristics of $Na_V1.4$ channels [65]. Furthermore, the complement attack disturbs the geometry of the postsynaptic folds which normally facilitates muscle fiber excitation and thus leads to a less efficient activation of remaining $Na_V1.4$ channels by the ACh-induced ion current through the remaining AChRs, further elevating the firing threshold [65].

The patho-electrophysiology of NMJs in the less frequently occurring MG variants with autoantibodies to postsynaptic antigenic targets other than the AChR, such as MuSK or LRP4, is mostly similar to AChR MG. The precise primary and secondary effects of the non-AChR MG autoantibodies are different from the classical AChR MG autoantibodies [66, 67]. However, the ultimate, secondary effect of these antibodies is dispersal/removal of AChRs, and this leads to reduced EPP amplitude and, consequently, a smaller safety factor in neuromuscular transmission [59, 68–72]. Of note, MuSK MG has two distinctly different features from the classical AChR MG form. First, the MuSK autoantibodies are predominantly of the IgG4, a special IgG subclass, which does not activate the complement cascade. Thus, the observed pathophysiological effects in MuSK MG are most likely complement independent [69]. Second, MuSK MG NMJs seem to lack the phenomenon of compensatory upregulation of ACh release in response to the reduction of postsynaptic AChR density [59, 69, 70, 72, 73]. This homeostatic adaptation of the NMJ in AChR MG is partly compensating for the loss of postsynaptic AChRs and operates at the level of single NMJs via retrograde signaling. Although several post- and presynaptic factors as well as the retrograde signals have been proposed, the mechanism underlying this compensatory response at the MG NMJ is not yet fully understood [74], although it may involve an increase in the size of the pool of releasable ACh vesicles [75]. The homeostatic response might confer some degree of protection

against transmission loss in AChR MG NMJs. However, there is concomitant extra rundown of EPPs due to exaggerated quantal content rundown at AChR MG NMJs. This might partially neutralize such a beneficial effect. Because this compensatory increase in ACh release is lacking in MuSK MG, it might be speculated that MuSK, or its intimate binding partner LRP4, are in some way involved in the homeostatic mechanism, e.g., as postsynaptic sensors or retrograde messengers.

In principle, the patho-electrophysiological mechanisms at the NMJ of intoxications with substances that block NMJ AChRs (e.g., d-tubocurarine-like compounds in plants or α-toxins in the venom of snakes or other animals) will be rather similar to the (non-complement-mediated) effects of AChR autoantibodies in MG [37, 50, 62, 76]. In congenital forms of MG with mutations in genes that encode AChR subunits or MuSK or its downstream signaling factors, the patho-electrophysiological mechanisms might be somewhat different and more complex due to the concomitant structural deficits resulting from developmental aberrations of the NMJ [77, 78]. However, transmission block at NMJs will remain the crucial culprit.

In conclusion, the chapter describes the electrophysiological events at the NMJ in relation to its structural background and has briefly discussed the main features of the patho-electrophysiology in some prototypical NMJ synaptopathies. It is hoped that this chapter has increased the reader's understanding on the physiology of the NMJ and that it will provide a useful background for the reading and interpretation of the following chapters.

Acknowledgments The author gratefully acknowledges support by the Prinses Beatrix Spierfonds, Stichting Spieren voor Spieren, and L'Association Française contre les myopathies.

References

1. Slater CR. Structural factors influencing the efficacy of neuromuscular transmission. Ann N Y Acad Sci. 2008;1132:1–12.
2. Darabid H, Perez-Gonzalez AP, Robitaille R. Neuromuscular synaptogenesis: coordinating partners with multiple functions. Nat Rev Neurosci. 2014;15:703–18.
3. Kang H, Tian L, Mikesh M, Lichtman JW, Thompson WJ. Terminal Schwann cells participate in neuromuscular synapse remodeling during reinnervation following nerve injury. J Neurosci. 2014;34:6323–33.
4. Sudhof TC. Neurotransmitter release: the last millisecond in the life of a synaptic vesicle. Neuron. 2013;80:675–90.
5. Nishimune H. Active zones of mammalian neuromuscular junctions: formation, density, and aging. Ann N Y Acad Sci. 2012;1274:24–32.
6. Chen J, Mizushige T, Nishimune H. Active zone density is conserved during synaptic growth but impaired in aged mice. J Comp Neurol. 2012;520:434–52.
7. Uchitel OD, Protti DA, Sanchez V, Cherksey BD, Sugimori M, Llinas R. P-type voltage-dependent calcium channel mediates presynaptic calcium influx and transmitter release in mammalian synapses. Proc Natl Acad Sci U S A. 1992;89:3330–3.
8. Kaja S, van de Ven RC, van Dijk JG, Verschuuren JJ, Arahata K, Frants RR, et al. Severely impaired neuromuscular synaptic transmission causes muscle weakness in the Cacna1a-mutant mouse rolling Nagoya. Eur J Neurosci. 2007;25:2009–20.
9. Chen J, Billings SE, Nishimune H. Calcium channels link the muscle-derived synapse organizer laminin beta2 to Bassoon and CAST/Erc2 to organize presynaptic active zones. J Neurosci. 2011;31:512–25.
10. Chamberlain LH, Burgoyne RD, Gould GW. SNARE proteins are highly enriched in lipid rafts in PC12 cells: implications for the spatial control of exocytosis. Proc Natl Acad Sci U S A. 2001;98:5619–24.
11. Davies A, Douglas L, Hendrich J, Wratten J, Tran Van MA, Foucault I, et al. The calcium channel alpha2delta-2 subunit partitions with CaV2.1 into lipid rafts in cerebellum: implications for localization and function. J Neurosci. 2006;26:8748–57.
12. Plomp JJ, Willison HJ. Pathophysiological actions of neuropathy-related anti-ganglioside antibodies at the neuromuscular junction. J Physiol. 2009;587:3979–99.
13. Massoulie J, Millard CB. Cholinesterases and the basal lamina at vertebrate neuromuscular junctions. Curr Opin Pharmacol. 2009;9:316–25.
14. Singhal N, Martin PT. Role of extracellular matrix proteins and their receptors in the development of the vertebrate neuromuscular junction. Dev Neurobiol. 2011;71:982–1005.
15. Rogers RS, Nishimune H. The role of laminins in the organization and function of neuromuscular junctions. Matrix Biol. 2017;57–58:86–105.
16. Wu H, Lu Y, Shen C, Patel N, Gan L, Xiong WC, et al. Distinct roles of muscle and motoneuron LRP4 in neuromuscular junction formation. Neuron. 2012;75:94–107.
17. Bruneau E, Sutter D, Hume RI, Akaaboune M. Identification of nicotinic acetylcholine receptor recycling and its role in maintaining receptor density at the neuromuscular junction in vivo. J Neurosci. 2005;25:9949–59.

18. Martinez P, Pires-Oliveira M, Akaaboune M. PKC and PKA regulate AChR dynamics at the neuromuscular junction of living mice. PLoS One. 2013;8:e81311.
19. Vautrin J, Mambrini J. Synaptic current between neuromuscular junction folds. J Theor Biol. 1989;140:479–98.
20. Ruff RL, Lennon VA. End-plate voltage-gated sodium channels are lost in clinical and experimental myasthenia gravis. Ann Neurol. 1998;43:370–9.
21. Grady RM, Teng H, Nichol MC, Cunningham JC, Wilkinson RS, Sanes JR. Skeletal and cardiac myopathies in mice lacking utrophin and dystrophin: a model for Duchenne muscular dystrophy. Cell. 1997;90:729–38.
22. Pilgram GS, Potikanond S, Baines RA, Fradkin LG, Noordermeer JN. The roles of the dystrophin-associated glycoprotein complex at the synapse. Mol Neurobiol. 2010;41:1–21.
23. van der Pijl EM, van Putten M, Niks EH, Verschuuren JJ, Aartsma-Rus A, Plomp JJ. Characterization of neuromuscular synapse function abnormalities in multiple Duchenne muscular dystrophy mouse models. Eur J Neurosci. 2016;43:1623–35.
24. Ghazanfari N, Fernandez KJ, Murata Y, Morsch M, Ngo ST, Reddel SW, et al. Muscle specific kinase: organiser of synaptic membrane domains. Int J Biochem Cell Biol. 2011;43:295–8.
25. Wu H, Xiong WC, Mei L. To build a synapse: signaling pathways in neuromuscular junction assembly. Development. 2010;137:1017–33.
26. Chen PJ, Martinez-Pena y Valenzuela I, Aittaleb M, Akaaboune M. AChRs are essential for the targeting of rapsyn to the postsynaptic membrane of NMJs in living mice. J Neurosci. 2016;36:5680–5.
27. Gautam M, Noakes PG, Mudd J, Nichol M, Chu GC, Sanes JR, et al. Failure of postsynaptic specialization to develop at neuromuscular junctions of rapsyn-deficient mice. Nature. 1995;377:232–6.
28. Escher P, Lacazette E, Courtet M, Blindenbacher A, Landmann L, Bezakova G, et al. Synapses form in skeletal muscles lacking neuregulin receptors. Science. 2005;308:1920–3.
29. Jaworski A, Burden SJ. Neuromuscular synapse formation in mice lacking motor neuron- and skeletal muscle-derived Neuregulin-1. J Neurosci. 2006;26:655–61.
30. Rimer M. Neuregulins at the neuromuscular synapse: past, present, and future. J Neurosci Res. 2007;85:1827–33.
31. Schmidt N, Akaaboune M, Gajendran N, Martinez P, Wakefield S, Thurnheer R, et al. Neuregulin/ErbB regulate neuromuscular junction development by phosphorylation of alpha-dystrobrevin. J Cell Biol. 2011;195:1171–84.
32. Rebbeck RT, Karunasekara Y, Board PG, Beard NA, Casarotto MG, Dulhunty AF. Skeletal muscle excitation-contraction coupling: who are the dancing partners? Int J Biochem Cell Biol. 2014;48:28–38.
33. Lang B, Makuch M, Moloney T, Dettmann I, Mindorf S, Probst C, et al. Intracellular and non-neuronal targets of voltage-gated potassium channel complex antibodies. J Neurol Neurosurg Psychiatry. 2017;88:353–61.
34. Park SB, Lin CS, Krishnan AV, Simon NG, Bostock H, Vincent A, et al. Axonal dysfunction with voltage gated potassium channel complex antibodies. Exp Neurol. 2014;261:337–42.
35. Shillito P, Molenaar PC, Vincent A, Leys K, Zheng W, van den Berg RJ, et al. Acquired neuromyotonia: evidence for autoantibodies directed against K+ channels of peripheral nerves. Ann Neurol. 1995;38:714–22.
36. van SA, Schreurs MW, Wirtz PW, Sillevis Smitt PA, Titulaer MJ. From VGKC to LGI1 and Caspr2 encephalitis: the evolution of a disease entity over time. Autoimmun Rev. 2016;15:970–4.
37. Ranawaka UK, Lalloo DG, de Silva HJ. Neurotoxicity in snakebite—the limits of our knowledge. PLoS Negl Trop Dis. 2013;7:e2302.
38. Plomp JJ, van Kempen GT, De Baets MB, Graus YM, Kuks JB, Molenaar PC. Acetylcholine release in myasthenia gravis: regulation at single end-plate level. Ann Neurol. 1995;37:627–36.
39. Samigullin D, Fatikhov N, Khaziev E, Skorinkin A, Nikolsky E, Bukharaeva E. Estimation of presynaptic calcium currents and endogenous calcium buffers at the frog neuromuscular junction with two different calcium fluorescent dyes. Front Synaptic Neurosci. 2014;6:29.
40. Titulaer MJ, Lang B, Verschuuren JJ. Lambert-Eaton myasthenic syndrome: from clinical characteristics to therapeutic strategies. Lancet Neurol. 2011;10:1098–107.
41. Joubert B, Honnorat J. Autoimmune channelopathies in paraneoplastic neurological syndromes. Biochim Biophys Acta. 2015;1848:2665–76.
42. Plomp JJ, Van den Maagdenberg AM, Molenaar PC, Frants RR, Ferrari MD, Mutant P. Q-type calcium channel electrophysiology and migraine. Curr Opin Investig Drugs. 2001;2:1250–60.
43. Maselli RA, Books W, Dunne V. Effect of inherited abnormalities of calcium regulation on human neuromuscular transmission. Ann N Y Acad Sci. 2003;998:18–28.
44. Bullens RW, O'Hanlon GM, Wagner E, Molenaar PC, Furukawa K, Furukawa K, et al. Complex gangliosides at the neuromuscular junction are membrane receptors for autoantibodies and botulinum neurotoxin but redundant for normal synaptic function. J Neurosci. 2002;22:6876–84.
45. Pirazzini M, Rossetto O, Eleopra R, Montecucco C. Botulinum neurotoxins: biology, pharmacology, and toxicology. Pharmacol Rev. 2017;69:200–35.
46. Milone M, Monaco ML, Evoli A, Servidei S, Tonali P. Ocular myasthenia: diagnostic value of single fibre EMG in the orbicularis oculi muscle. J Neurol Neurosurg Psychiatry. 1993;56:720–1.
47. Newland CF, Beeson D, Vincent A, Newsom-Davis J. Functional and non-functional isoforms of the human muscle acetylcholine receptor. J Physiol. 1995;489:767–78.

48. Sine SM. End-plate acetylcholine receptor: structure, mechanism, pharmacology, and disease. Physiol Rev. 2012;92:1189–234.
49. Bannister RA. Bridging the myoplasmic gap: recent developments in skeletal muscle excitation-contraction coupling. J Muscle Res Cell Motil. 2007;28:275–83.
50. Plomp JJ, Morsch M, Phillips WD, Verschuuren JJ. Electrophysiological analysis of neuromuscular synaptic function in myasthenia gravis patients and animal models. Exp Neurol. 2015;270:41–54.
51. McLachlan EM, Martin AR. Non-linear summation of end-plate potentials in the frog and mouse. J Physiol. 1981;311:307–24.
52. Choi BJ, Imlach WL, Jiao W, Wolfram V, Wu Y, Grbic M, et al. Miniature neurotransmission regulates Drosophila synaptic structural maturation. Neuron. 2014;82:618–34.
53. Flucher BE, Daniels MP. Distribution of Na+ channels and ankyrin in neuromuscular junctions is complementary to that of acetylcholine receptors and the 43 kd protein. Neuron. 1989;3:163–75.
54. Wood SJ, Slater CR. The contribution of postsynaptic folds to the safety factor for neuromuscular transmission in rat fast- and slow-twitch muscles. J Physiol. 1997;500:165–76.
55. Wood SJ, Slater CR. Action potential generation in rat slow- and fast-twitch muscles. J Physiol. 1995;486:401–10.
56. Wood SJ, Slater CR. Safety factor at the neuromuscular junction. Prog Neurobiol. 2001;64:393–429.
57. Eken T. Spontaneous electromyographic activity in adult rat soleus muscle. J Neurophysiol. 1998;80:365–76.
58. Hennig R, Lomo T. Firing patterns of motor units in normal rats. Nature. 1985;314:164–6.
59. Niks EH, Kuks JB, Wokke JH, Veldman H, Bakker E, Verschuuren JJ, et al. Pre- and postsynaptic neuromuscular junction abnormalities in musk myasthenia. Muscle Nerve. 2010;42:283–8.
60. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. Lancet Neurol. 2015;14:1023–36.
61. Phillips WD, Vincent A. Pathogenesis of myasthenia gravis: update on disease types, models, and mechanisms. F1000Res. 2016;5:F1000.
62. Plomp JJ, van Kempen GT, Molenaar PC. Adaptation of quantal content to decreased postsynaptic sensitivity at single endplates in alpha-bungarotoxin-treated rats. J Physiol. 1992;458:487–99.
63. Tuzun E, Christadoss P. Complement associated pathogenic mechanisms in myasthenia gravis. Autoimmun Rev. 2013;12:904–11.
64. Ruff RL, Lennon VA. How myasthenia gravis alters the safety factor for neuromuscular transmission. J Neuroimmunol. 2008;201–202:13–20.
65. Ruff RL. Endplate contributions to the safety factor for neuromuscular transmission. Muscle Nerve. 2011;44:854–61.
66. Huijbers MG, Zhang W, Klooster R, Niks EH, Friese MB, Straasheijm KR, et al. MuSK IgG4 autoantibodies cause myasthenia gravis by inhibiting binding between MuSK and Lrp4. Proc Natl Acad Sci U S A. 2013;110:20783–8.
67. Verschuuren JJ, Huijbers MG, Plomp JJ, Niks EH, Molenaar PC, Martinez-Martinez P, et al. Pathophysiology of myasthenia gravis with antibodies to the acetylcholine receptor, muscle-specific kinase and low-density lipoprotein receptor-related protein 4. Autoimmun Rev. 2013;12:918–23.
68. Cole RN, Reddel SW, Gervasio OL, Phillips WD. Anti-MuSK patient antibodies disrupt the mouse neuromuscular junction. Ann Neurol. 2008;63:782–9.
69. Klooster R, Plomp JJ, Huijbers MG, Niks EH, Straasheijm KR, Detmers FJ, et al. Muscle-specific kinase myasthenia gravis IgG4 autoantibodies cause severe neuromuscular junction dysfunction in mice. Brain. 2012;135:1081–101.
70. Selcen D, Fukuda T, Shen XM, Engel AG. Are MuSK antibodies the primary cause of myasthenic symptoms? Neurology. 2004;62:1945–50.
71. Shen C, Lu Y, Zhang B, Figueiredo D, Bean J, Jung J, et al. Antibodies against low-density lipoprotein receptor-related protein 4 induce myasthenia gravis. J Clin Invest. 2013;123:5190–202.
72. Viegas S, Jacobson L, Waters P, Cossins J, Jacob S, Leite MI, et al. Passive and active immunization models of MuSK-Ab positive myasthenia: electrophysiological evidence for pre and postsynaptic defects. Exp Neurol. 2012;234:506–12.
73. Morsch M, Reddel SW, Ghazanfari N, Toyka KV, Phillips WD. Pyridostigmine but not 3,4-diaminopyridine exacerbates ACh receptor loss and myasthenia induced in mice by muscle-specific kinase autoantibody. J Physiol. 2013;591:2747–62.
74. Plomp JJ. Trans-synaptic homeostasis at the myasthenic neuromuscular junction. Front Biosci (Landmark Ed). 2017;22:1033–51.
75. Wang X, Pinter MJ, Rich MM. Reversible recruitment of a homeostatic reserve pool of synaptic vesicles underlies rapid homeostatic plasticity of quantal content. J Neurosci. 2016;36:828–36.
76. Barber CM, Isbister GK, Hodgson WC. Alpha neurotoxins. Toxicon. 2013;66:47–58.
77. Barisic N, Chaouch A, Muller JS, Lochmuller H. Genetic heterogeneity and pathophysiological mechanisms in congenital myasthenic syndromes. Eur J Paediatr Neurol. 2011;15:189–96.
78. Engel AG, Shen XM, Selcen D, Sine SM. Congenital myasthenic syndromes: pathogenesis, diagnosis, and treatment. Lancet Neurol. 2015;14:420–34.

Acetylcholine Receptor Structure

2

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Introduction

Nicotinic acetylcholine receptors (AChRs) are acetylcholine-gated cation channels [1]. They play a critical postsynaptic role in transmission between motor nerves and skeletal muscles and in autonomic ganglia [2, 3]. In the central nervous system, they also act presynaptically and extrasynaptically to modulate transmission by facilitating the release of many transmitters [4, 5]. In the skin [6], lung [7], bronchial and vascular epithelia [8, 9], and several types of immune cells including monocytes [10–12], dendritic cells [13], macrophages [14–17], T-cells [18, 19], and B-cells [20, 21], they also mediate intercellular communication.

Abnormalities of AChRs are responsible for several human diseases. Mutations in AChRs are known to cause congenital myasthenic syndromes [22] and the rare autosomal dominant nocturnal frontal lobe form of epilepsy (ADNFLE) [23–26]. Autoimmune responses to AChRs are known to cause myasthenia gravis (MG) [27], certain dysautonomias [28–30], and some forms of pemphigus [31].

Nicotine acting on AChRs in the brain causes addiction to tobacco [32]. This is by far the largest medical problem in which AChRs play a direct role and the largest preventable cause of disease, accounting for 430,000 premature deaths annually in the United States [33].

Nicotine acting through AChRs has many physiological effects, including beneficial ones such as inducing vascularization, neuroprotection, cognitive enhancement, anxiolysis, and antinociception. Thus, nicotinic agents are lead compounds for the development of drugs to treat many diseases including Alzheimer's disease, Parkinson's disease, chronic pain, schizophrenia, and Tourette's syndrome [34–40] as well as for smoking cessation [41, 42].

There are many known and potential subtypes of AChRs, each defined by the subunits which compose them [1, 3]. All AChRs are formed by five homologous subunits organized around a central cation channel. There are 17 known AChR subunits: α1–10, β1–4, γ, δ, and ε. By contrast with the many subtypes of neuronal AChRs, there are only two subtypes of muscle AChRs. These are a fetal subtype with an $(\alpha1)_2$ β1γδ stoichiometry and an adult subtype with an $(\alpha1)_2$ β1εδ stoichiometry.

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H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*, Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_2

AChRs are part of a gene superfamily, which includes the genes for subunits of ionotropic receptors for glycine, γ-amino butyric acid (GABA), and serotonin [1, 43]. The structural homologies of all of these receptors, and the sorts of evolutionary steps which produced this diversity of receptors, have been elegantly illustrated by experiments. One showed that changing only three amino acids in the channel-lining part of an AChR subunit to amino acids found in receptors for GABA or glycine receptors resulted in AChRs with anion-selective channels like those of GABA or glycine receptors [44]. Another experiment showed that a chimera of the extracellular domain of an AChR subunit and the remainder of a serotonin receptor subunit produced an ACh-gated cation channel with the conductance properties of a serotonin receptor [45].

Muscle AChRs are the best characterized members of the AChRs [1]. The presence of a single type of synapse in the skeletal muscle (with the exception of extraocular muscle, Chap. 7) facilitated studies of AChR synthesis, developmental plasticity, and electrophysiological function [46–48]. The presence of large amounts of muscle-like AChR in the electric organs of *Torpedo* species permitted the purification and characterization of AChRs, partial sequencing of their subunit proteins, cloning of the subunit cDNAs, and low-resolution electron crystallographic determination of their three-dimensional structure [43, 48–50]. Low stringency hybridization, starting with cDNAs for muscle AChR subunits, leads to the cloning of subunits for neuronal AChRs [48]. Immunization with purified electric organ AChRs leads to the discovery of experimental autoimmune myasthenia gravis (EAMG), the autoimmune nature of MG, and an immunodiagnostic assay for MG [27, 51]. Monoclonal antibodies (mAbs) initially developed as model autoantibodies not only lead to the discovery of the main immunogenic region (MIR) on α1 subunits and the molecular basis of the autoimmune impairment of neuromuscular transmission in MG [27, 52, 53], but also lead to the immunoaffinity purification of neuronal nicotinic AChRs. mAbs have continued to provide useful tools for characterizing AChRs [3].

This chapter reviews the basic structures of muscle and neuronal AChRs. It will describe the antigenic structure of muscle AChRs and consider how this accounts for the pathological mechanisms by which neuromuscular transmission is impaired in MG. This will be briefly contrasted with the antigenic structure of a neuronal AChR involved in autoimmune dysautonomia. This chapter also considers the optimized functional structure of muscle AChRs and how mutations impair AChR function in congenital myasthenic syndromes. The many AChR mutations identified in all of the muscle AChR subunits in myasthenic syndromes are contrasted with the few disease-causing mutations discovered, thus far, in neuronal AChR subunits.

Size and Shape of AChRs

Electron crystallography of two-dimensional helical crystalline arrays of AChRs in fragments of *Torpedo* electric organ membranes has revealed the basic size and shape of this muscle-type AChR to a resolution of 4 Å, as shown in Fig. 2.1 [50, 55]. Viewed from the side, a *Torpedo* AChR is roughly cylindrical, about 140 Å long and 80 Å wide. About 65 Å extends on the extracellular surface, 40 Å crosses the lipid bilayer, and 35 Å extends below. Viewed from the top, the extracellular vestibule is a pentagonal tube with walls about 25 Å thick and a central pore about 20 Å in diameter. The channel across the membrane narrows to a close. Other evidence suggests that the open lumen of the channel becomes narrow (perhaps 7 Å across), sufficient only for rapid flow of hydrated cations like Na^+ or K^+. The five subunits are rodlike, oriented-like barrel staves at a 10° angle around the central channel. Movements of *Torpedo* AChR subunits associated with activation by ACh have been captured by electron microscopy of crystalline arrays of AChRs in membrane fragments sprayed with ACh and fast frozen [56]. Crystal structures of related receptors have been determined in several conformations, and progress is being rapidly made on relating receptor structure to functional state. For example, glycine receptors have been

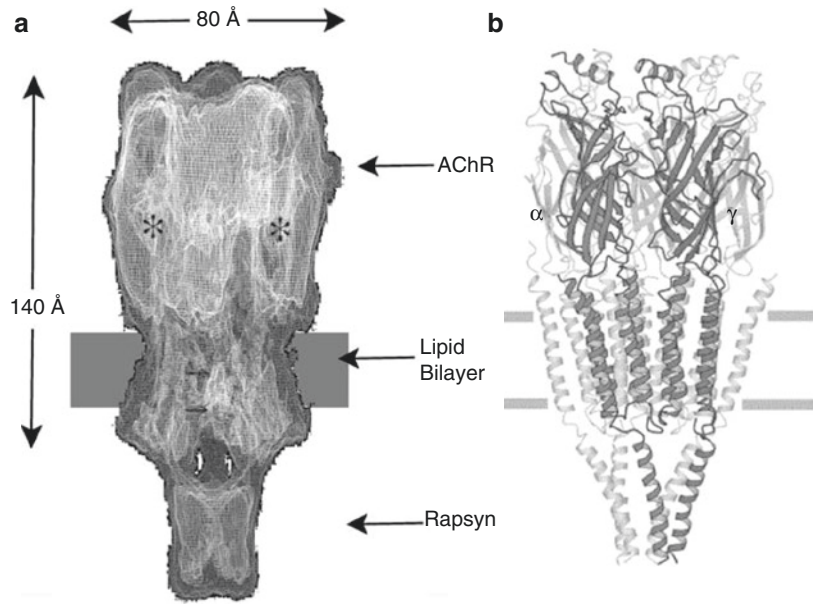


Fig. 2.1 *Torpedo* electric organ AChR structure determined by electron crystallography. (**a**) The large extracellular domain contrasts with the smaller domain on the cytoplasmic surface. Rapsyn is a 43,000 Da peripheral membrane protein through which muscle AChRs are linked to actin in the cytoskeleton to concentrate them at the tips of folds in the postsynaptic membrane adjacent to active zones in the presynaptic membrane at which ACh is released [54]. (**b**) Here the polypeptide chains are shown as ribbon structures, highlighting the α1 and δ-subunits, at whose interface one of the two ACh-binding sites in this (α1)2β1γδ AChR is formed. Reprinted from Journal of Molecular Biology, 346(4), Nigel Unwin, Refined Structure of the Nicotinic Acetylcholine Receptor at 4Å Resolution, 967–989, Copyright 2005, with permission from Elsevier

crystallized in a closed state bound by an antagonist and in an open-channel state bound by glycine [57]. Human neuronal α4β2 AChRs in a desensitized state bound to nicotine have been crystallized with the activation gate in the middle of the cation channel open and a desensitization gate at the cytoplasmic end of the channel closed [58].

X-ray crystallography of a molluscan glial ACh-binding protein (AChBP) has revealed the structure of the extracellular domain of an AChR-like protein at atomic resolution [59–62] (Fig. 2.2). Snail glia were found to release a water-soluble protein which modulated transmission by binding ACh. The cloned protein showed 24% sequence identity with the extracellular domain of human α7. It provides a good model for the basic structure of the extracellular domains of AChRs and other receptors in their superfamily. This is proven by the demonstration that the AChBP, with slight modification to match their interface, can form a chimera with the transmembrane portion of the $5HT_3$ receptor to form an ACh-gated cation channel [65]. Figure 2.2 shows that five AChBP subunits assemble as the extracellular domains of AChR subunits would around the vestibule of the channel. A buffer component was initially found to occupy what was expected to be the ACh-binding site, which is formed at the interface between subunits halfway up the side of the assembled protein. All of the contact amino acids for this site corresponded to ones in AChRs, which had been identified by affinity labeling or mutagenesis studies [43, 49, 66]. Subsequently, AChBP was crystallized in combinations with both agonists, such as nicotine [60], and antagonists, such as snake venom toxins [61]. Basically, most recognizable features were found about where they were expected to be from studies of native AChRs, providing confidence that the structure of the AChBP is relevant to that of AChRs.

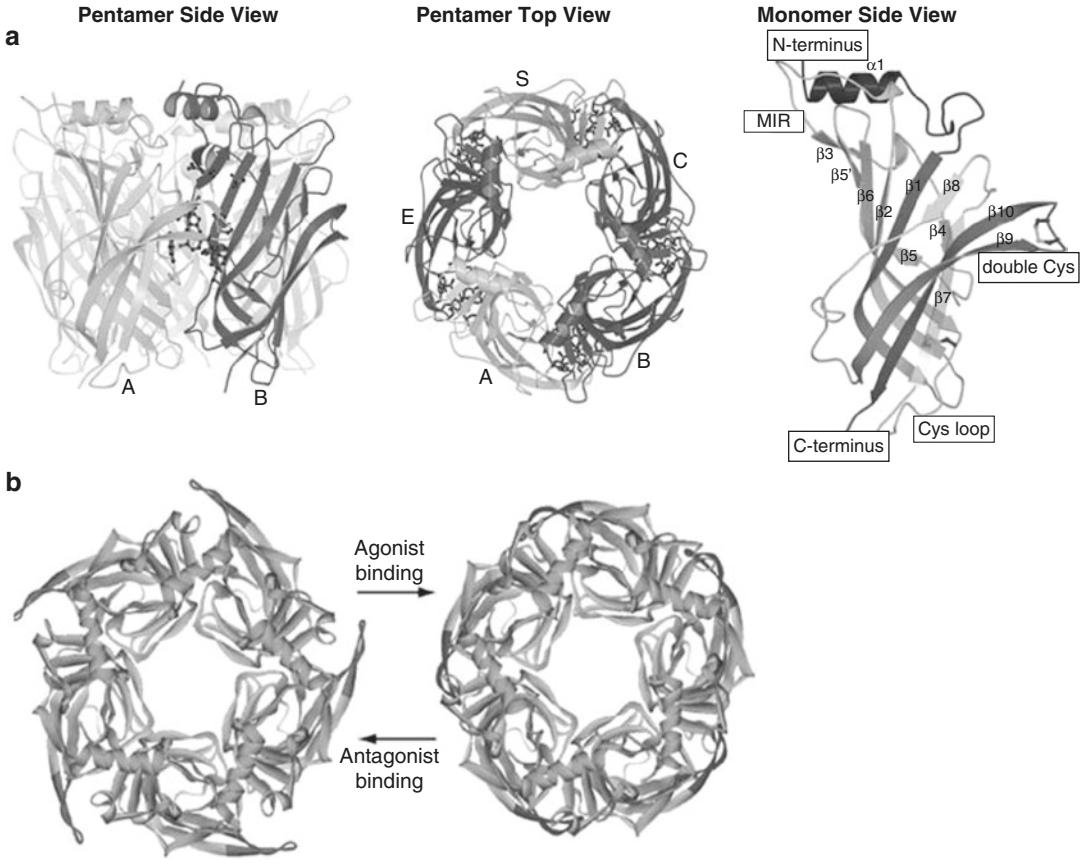


Fig. 2.2 Mollusk AChBP structure determined by X-ray crystallography. (**a**) Atomic-resolution structure of the AChBP reveals the basic structure of AChR extracellular domains. It is a 62-Å-high cylinder that is 80 Å in diameter with an 18 Å diameter central hole. The structure is a homopentamer-like α7 AChRs. There are five ACh-binding sites at subunit interfaces, rather than the two ACh-binding sites expected in a muscle AChR heteropentamer at interfaces between α1 and δ-, γ- or ε-subunits. The ACh-binding site is occupied by the buffer component *N*-2-hydroxyethylpiperazine-*N*′-2 ethanesulfonic acid (HEPES). The adjacent disulfide-linked cysteine pair corresponding to α1 192–193, which is characteristic of all AChR α-subunits, is on a projection of what can be defined as the "+" side of the subunit. It intercalates with the "−" side of the adjacent subunit to form the ACh-binding site. As expected from studies of AChRs [27, 63], the sequence corresponding to the MIR of α1 subunits is located at the extracellular tip and oriented out away from the central axis of the subunit. The Cys-loop linked by a disulfide bond corresponding to that between cysteines 128 and 142 of α1 subunits, the signature loop characteristic of all subunits of the gene superfamily of which AChRs are a member, is located in the AChBP at the base near what would be the transmembrane portion of an AChR or the lipid bilayer. This loop sequence shows little homology with that of AChRs and is more hydrophilic than the sequences characteristic of AChRs. This loop contributes to the water solubility of AChBP but is at the interface between the extracellular and transmembrane domains of AChRs. Reprinted by permission from Macmillan Publishers Ltd: Nature, Brejc K, van Dijk WJ, Klaassen RV, Schuurmans M, van der Oost J, et al., Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors, 411, copyright 2001. (**b**) Shows a top view of an AChBP in its resting and active conformations. The C-loop is open in the resting state and closed in the active and desensitized states. Reprinted from Hansen SB, Sulzenbacher G, Huxford T, Marchot P, Taylor P, Bourne Y. Structures of aplysia AChBP complexes with nicotinic agonists and antagonists reveal distinctive binding interfaces and conformations. EMBO J 2005; 24: 3635–3646, with permission from John Wiley and Sons. Large movement of the C loop at the ACh-binding sites is propagated through the AChR to produce movement of the M2 transmembrane domain and ultimately opening of the channel gate located at the middle or bottom of the transmembrane domain [64]

α7 AChR subunits primarily form homomeric AChRs. The extracellular domain cleaved by protein engineering from α7 assembles into water-soluble pentamers with the ligand-binding properties of native α7 AChRs, but it does so inefficiently [67]. Thus, the molluscan AChBP probably contains sequence adaptations for efficient assembly and secretion as a water-soluble protein. Based on this hypothesis, a chimeric α7 ligand-binding domain, which shares 64% sequence identity and 71% similarity with native α7, was generated by combining sequences from α7 AChR with those from AChBP, and crystal structures of the resulting pentamer and its complexes with epibatidine and α-bungarotoxin were determined [68, 69]. This and other features of the structure will be discussed in more detail in subsequent sections.

Structures of AChR Subunits

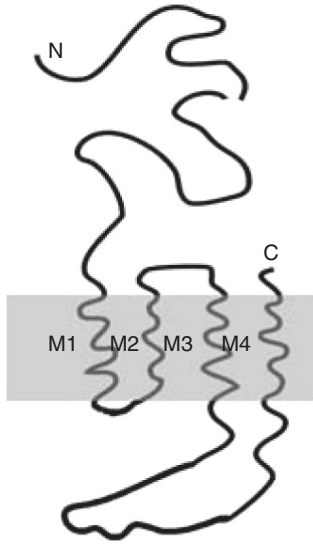
All AChR subunits share several features. Figure 2.3 diagrammatically shows the transmembrane orientation of the mature polypeptide chain of a generic AChR subunit. To produce the mature polypeptide sequence, a signal sequence of about 20 amino acids is removed from the N-terminus of each subunit during translation as the N-terminal domain crosses the membrane into the lumen of the endoplasmic reticulum.

The large N-terminal extracellular domain of each subunit, which consists of about 210 amino acids, contains a disulfide-linked loop (the Cys-loop) which is characteristic of all receptors in this superfamily. In α1 subunits, it extends from cysteine 128 to cysteine 142. This sequence is among the most conserved of all AChR subunit sequences. In the AChBP (see Fig. 2.2), this loop is located near what would be the lipid bilayer or extracellular surface of the transmembrane regions [59]. It is hydrophobic in AChRs but hydrophilic in the binding protein. A proline in the loop, which is conserved in all AChR subunits, is missing in the binding protein. Mutating this proline to glycine disrupts assembly of AChR subunits and prevents transport to the surface of assembled AChRs [72]. The extracellular domains of AChR subunits contain one or more glycosylation sites, and in all but the α7–9 subunits, which can form homomeric AChRs, there is an N-glycosylation site at position 141 adjacent to the disulfide bond of the signature loop.

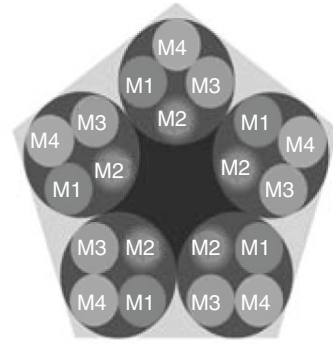
Three closely spaced, highly conserved, largely α-helical transmembrane sequences (M1–M3) corresponding approximately to amino acids 220–310 extend between the large extracellular domain and the largest cytoplasmic domain. The N-terminal third of M1 and the hydrophilic side of M2 form each subunit's contribution to the lining of the channel [1, 43, 70]. This will be described in slightly more detail in a subsequent section on the channel and gate.

The large cytoplasmic domain between the transmembrane sequences M3 and M4 comprises 110–270 amino acids (α4 having by far the largest). This is the most variable region in sequence between subunits and between species. Consequently, many subunit-specific antibodies bind in this region [73]. The large cytoplasmic domain, in contrast with the extracellular domain, is quite flexible in structure. Consequently, many antibodies to this region bind to both native and denatured protein, and visualization of this disordered region is difficult with crystallographic methods. The large cytoplasmic region of muscle AChRs interacts with rapsyn, a protein which links it to the cytoskeleton and thus helps to position it appropriately in the neuromuscular junction [54]. Proteins other than rapsyn are probably involved in interacting with the large cytoplasmic domain of neuronal AChRs to help transport them to and localize them at their various pre-, post-, and extrasynaptic sites of action, e.g., members of the PSD-95 family, but these proteins have not been characterized in detail [74]. The chaperone protein 14-3-3η binds to α4 at serine 441 in the large cytoplasmic domain, especially when it is phosphorylated, helping to increase conformational maturation or assembly [75]. Several chaperone proteins are known to participate in the conformational maturation and assembly of muscle AChR subunits [76–79]. For example, COPI interacts at lysine 314 of α1 subunits. The transmembrane portion of M1 in α1 subunits contains an endoplasmic reticulum

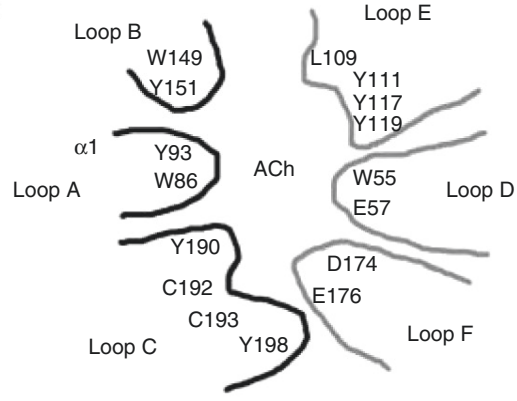
Transmembrane Orientation of an AChR Subunit



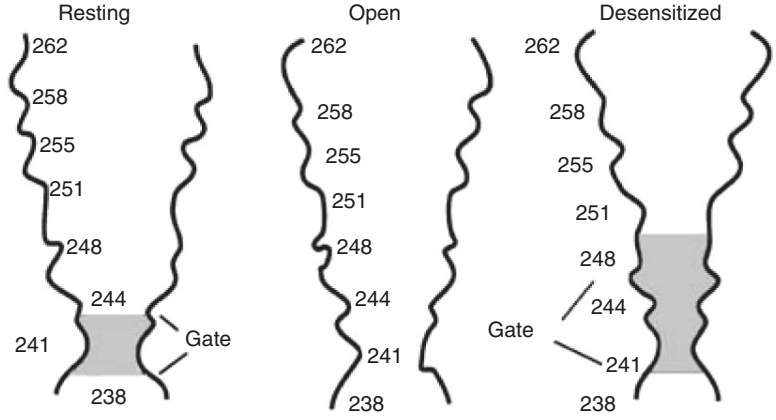
Organization of Subunits Around the Ion Channel



Amino Acids Forming the ACh Binding Site



α1 M2 Amino Acids Lining the Cation Channel



retention sequence that is obscured only after complete assembly of AChR pentamers [79]. The large cytoplasmic domain contains phosphorylation sites and perhaps other sequences thought to be involved in regulating the rate of desensitization [80, 81] and other properties, such as intracellular transport [82]. Surprisingly, it even contains sequences that contribute to channel gating kinetics [83]. This is because the rigid amphipathic α-helices in the large cytoplasmic domain, which immediately precede M4, form intracellular portals that regulate access of ions to the central cation channel through the AChR [50, 84].

A fourth transmembrane domain (M4), of about 20 amino acids, extends from the large cytoplasmic domain to the extracellular surface, leading to a 10–20 amino acid extracellular sequence. In the case of human α4 subunits, the C-terminal end of the α4 sequence has been found to form a site through which binding of estrogen enhances AChR function three- to seven-fold, while it inhibits the response of α3β2 AChRs [85, 86]. No functional role for the C-terminal sequence is yet known for other AChR subunits.

α-subunits are defined by the presence of a disulfide-linked adjacent cysteine pair in the large extracellular domain homologous to α192 and 193 of α1 subunits. In all α-subunits, other than α5 and perhaps α10, this is thought to contribute to the ACh-binding site. These cysteines, after reduction of the disulfide bond between them, were the targets of the first affinity labels developed for AChRs [43]. In the AChBP (see Fig. 2.2), this cysteine pair is located at the tip of a protruding C loop on what can be defined as the "+" side of the subunit. The ACh-binding site is formed at the interface between the + side of an α-subunit and the "−" side of an adjacent subunit, as will be discussed in somewhat more detail in a subsequent section.

Organization of Subunits in AChR Subtypes

Homologous rodlike AChR subunits are organized in a pentagonal array around the central cation channel [50, 55]. In the muscle-type AChRs of *Torpedo* electric organ, the order of subunits around the channel is α1, γ, α1, δ, and

Fig. 2.3 Aspects of AChR structure. The transmembrane orientation of a generic AChR subunit is depicted in this diagram. The actual structure of the AChR shown in Fig. 2.1b and the large extracellular domain of the AChBP shown in Fig. 2.2 provide more details. The transmembrane domains M1–4 are depicted as largely α-helical. The overall shape of the subunit is depicted as rodlike. Five of these rods assemble in a pentagonal array to form the AChR shown in Fig. 2.1. The subunits are organized around the ion channel so that the amphipathic M2 transmembrane domain from each subunit contributes to the lining of the channel. In muscle AChRs, (e.g., with the subunit arrangement α1γα1δβ), and other heteromeric AChRs (e.g., with the subunit arrangement α4β2α4β2β2), there are only two ACh-binding sites at interfaces between the + side of α-subunits and complementary subunits, but small concerted conformation changes of all subunits are involved in activation and desensitization [55, 65, 70]. Thus, all subunits contribute to the conductance and gating of the channel, even if they are not part of an ACh-binding site. The amino acids lining the ACh-binding site have been identified by affinity labeling and mutagenesis studies [43, 66] and have been found to correspond well to those identified in the crystal structure of the AChBP and α7/AChBP chimera [60, 61, 68, 69]. Notice the predominance of aromatic amino acids in this region. As in ACh esterase [71], the quaternary amine group of ACh is thought to be bound through interactions with π electrons of these aromatic amino acids rather than ionic interactions with acidic amino acids. Note also that the ACh-binding site is formed from amino acids from three different parts of the extracellular domain of the α-subunit interacting with three parts of the complementary subunit and that the interaction is at the interface between the + side of the α-subunit and the − side of the complementary subunit. Thus, the site is ideally positioned to trigger small concerted conformation changes between subunits, thereby permitting low-energy binding events in the extracellular domain to regulate opening, closing, and desensitization of the ion channel gate near the cytoplasmic vestibule of the channel [50, 65, 71]. The amino acids lining the cation channel and accessible either from the extracellular or cytoplasmic surface have been determined largely by the substituted cysteine accessibility method (SCAM) [70]. The channel lining is thought to be formed by M2. The figure depicts the M1–M2 linker at the cytoplasmic end of M1 and M2. In the closed, resting state of the channel, only a short region is occluded and inaccessible to labeling. In the closed desensitized state, a larger region is occluded

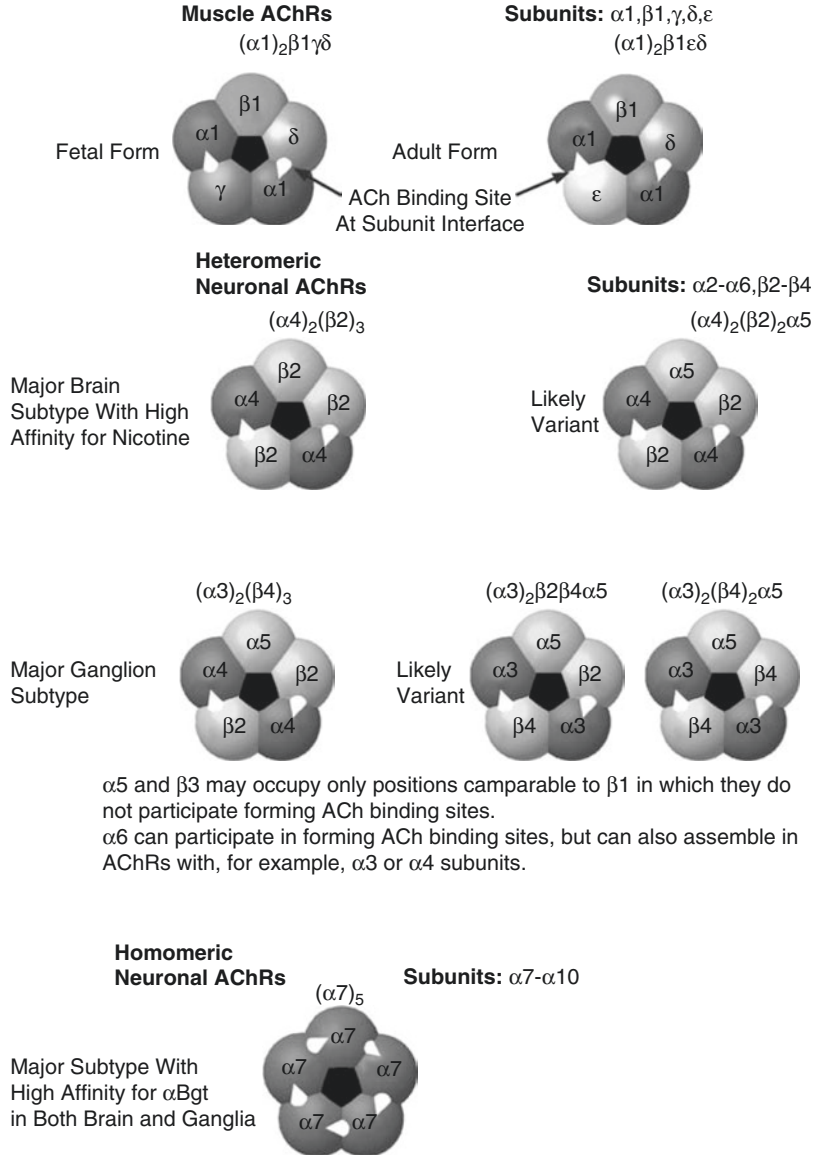


Fig. 2.4 Prominent AChR subtypes. AChR subtypes are defined by their subunit compositions. The arrangement of AChR subunits around the channel is known for *Torpedo* electric organ muscle type AChRs [43] and inferred for other types based on this example. The subunit stoichiometry of some neuronal AChR subtypes is known [90, 91], and it is known that ACh-binding sites can be formed at specific interfaces of α1–4 or α6 with β2 or β4 subunits [85, 92, 93], but recently a third ACh-binding site has been found at α4/α4, α5/α4, β3/α4, and α4/α5 interfaces, termed unorthodox sites [94–98]. Stoichiometries such as $(\alpha4)_3$ $(\beta2)_2$ have been found in cell lines and may exist in vivo [99–101]. All subunits are expected to participate in the structure of the channel and in conformation changes associated with activation and desensitization. Modified from Lindstrom [27]

β1 [43]. In the adult muscle AChR subtype, ε substitutes for γ. Reflecting their similar functional roles and evolutionary origins [87], γ, δ, and ε have especially similar sequences, as do β1, β2, and β4. In cDNA expression systems, β2 and β4 can substitute for β1 in muscle AChR [88]. α7 can also substitute for α1 [89], revealing basic homologies between AChR subunits rather than an AChR subtype, which is thought to be important in vivo. The arrangement of subunits in major AChR subtypes is depicted in Fig. 2.4.

The primordial AChR was presumably homomeric, and heteromeric AChRs evolved subsequent to duplication of the primordial subunit [87]. AChR subunits are lettered in order of increasing molecular weight as they were discovered in the AChRs first purified from *Torpedo* electric organ, and they are numbered in the order in which their cDNAs were cloned [48].

Homomeric AChRs can be formed by α7, α8, and α9 subunits [1, 3]. In mammals, α7 is the predominant homomeric AChR. Like muscle AChRs (though with much lower affinity), α7, α8, and α9

AChRs bind α-bungarotoxin (α-Bgt) and related snake venom peptide toxins to their ACh-binding sites. Heteromeric neuronal AChRs containing α1–6 in combination with β2–4 subunits do not bind α-Bgt. Recently, α7 has also been found to be co-assembled with β2 to form a heteromeric AChR in human brain [102]. α8 has been found only in chickens [103]. It can form heteromeric AChRs with α7. α9 can form heteromeric AChRs with α10 [104, 105].

Heteromeric neuronal AChRs are formed from α-subunit 2, 3, 4, 6, or 7 in combination with β2 or β4 [1, 3, 102]. α5 and β3 are present as additional subunits in AChRs in which ACh-binding sites are formed by other α- and β-subunits [92, 93, 106–110]. The AChR subunit pairs α2, α4; α3, α6; β2, β4; and α5, β3 are closely related in sequence, reflecting their origins by gene duplication and their similar functional roles [87]. The subunit compositions of these heteromeric neuronal AChRs can be as simple as α3β4α3β4β4 or α4β2α4β2β2, with two identical ACh-binding sites. They can be more complex, involving three (e.g., α3β4α3β4α5) or more types of AChR subunits (α3β4α6β2β3). These can have two identical ACh-binding sites (α4β2α4β2β2); two different ACh-binding sites with one kind of α-subunit (α3β2α3β4α5), as in muscle AChRs (α1εα1δβ1); or two different ACh-binding sites with two different α-subunits (α3β2α6β2β3). Many neurons express complex mixtures of ACh subunits [111–114]. Ganglionic neurons usually express α3, α5, α7, β2, and β4 subunits, which could potentially form many subtypes, but the function of α3β4 tends to predominate postsynaptically [115, 116]. α7 assembles into homomers or heteromers with β2 in these neurons. Adjacent neurons, within the same nucleus, can express different complex combinations of AChR subunits [113, 117]. It remains to be determined which AChR subtypes are expressed from these subunit combinations, where they are located within the neurons, and what functional roles they play in postsynaptic transmission, presynaptic modulation, and extrasynaptic plasticity. What is clear is that AChRs can play many, much more complex, regulatory roles than their classic postsynaptic role in high-safety factor neuromuscular transmission might suggest.

In muscle AChRs, ACh-binding sites are formed at the interfaces of the + side of α1 with the –side of γ-, δ-, or ε-subunits, and β1 subunits do not participate in forming ACh-binding sites [43]. In heteromeric neuronal AChRs, α5, β3 and other subunits can occupy positions comparable to β1 and are referred to as accessory subunits [110, 118]. Accessory subunits can be involved in forming ACh sites. For example, α4β2 AChRs can exist in two stoichiometries $(\alpha4\beta2)_2\beta2$ and $(\alpha4\beta2)_2\alpha4$. In addition to the two α4/β2 ACh sites present in both stoichiometries, an α4 accessory subunit allows a formation of a third α4/α4 ACh-binding site that increases the response four- to five-fold [94, 95]. An α5 or β3 accessory subunit can similarly form an α5/α4 or β3/α4 ACh-binding site [96]. AChR subunits contribute to both ion channel properties and agonist potency not only because they contribute to the lining of the channel but also because the ease with which the agonist-induced conformation changes of the whole AChR molecule take place in the course of activation or desensitization depends on the structures of all moving parts of the AChR [119].

Acetylcholine-Binding Sites

ACh-binding sites are formed at the interfaces between subunits. This is shown in high resolution in the case of the AChBP in Fig. 2.2 and diagrammatically in Fig. 2.3. Both affinity labeling studies of native AChRs [43, 66] and the structure of the AChBP [60, 61] reveal that the ACh-binding site does not contain negatively charged amino acids to bind positively charged ACh. The ACh-binding site contains many aromatic amino acids, and it is thought that interactions between the π electrons of these amino acids and the quaternary amine play an important role in binding. The ACh-binding site of ACh esterase also works this way [71].

Tetramethylammonium is the simplest form of an agonist. The small movements initiated by the low-affinity binding at just this portion of the site must be sufficient to trigger channel opening. The fundamental features of an AChR agonist are a quaternary or tertiary amine which binds to the

site and permits closure of the C loop over the site [61] (see Fig. 2.2).

By contrast with small agonists, antagonists tend to be larger and are often multivalent. These prevent closing of the C loop. In the extreme case of large snake venom toxins like α-bungarotoxin, binding of the toxin leaves the C loop propped open even more than in the resting conformation [61] (see Fig. 2.2). This also helps to explain the slow rate of binding of snake venom toxins. They can only fit into the site when it assumes a relatively rare especially wide-open conformation.

Snake venom toxins such as α-bungarotoxin and α-cobratoxin have been extremely important for identifying, localizing, quantifying, purifying, and characterizing AChRs [48, 89]. These are peptides of ~9000 mw with a flat three-fingered structure. Only the tip of the longest finger enters the ACh-binding site of muscle AChRs [61, 120]. Snake venom toxins evolved from families of proteins of similar structure, some of which have recently been found to interact competitively or allosterically with AChRs. Lynx1 is tethered to membranes by a glycophosphatidyl anchor, colocalizes with α4β2 AChRs in the brain, promotes expression, and enhances desensitization of these AChRs [121]. SLURP-1 is found in both anchored and soluble forms, is secreted by keratinocytes, and potentiates responses of α7 AChRs found in keratinocytes [122]. Mutations in SLURP-1 cause Mal de Meleda, an inflammatory skin disease characterized by hypoproliferation and release of TNFα from macrophages. SLURP-1 binds competitively to keratinocyte AChRs [123]. SLURP-2 is secreted from keratinocytes and acts as an agonist on keratinocyte α3 AChRs [124]. SLURP-2 delays keratinocyte differentiation and prevents apoptosis, whereas SLURP-1 facilitates keratinization and programmed cell death. This suggests that the cholinergic signaling through AChR subtypes was involved in intracellular communication before it evolved to specialized use in rapid signaling with the evolution of the nervous system. The regulation of gene expression evident in cholinergic effects on keratinocytes may well be reflected in trophic effects of AChRs within the nervous system. Within the primitive nervous system of *C. elegans*, for example, there are many more AChR-like subunits than are found in humans [125]. Even the puffer fish genome has more AChR subunits than humans, reflecting this evolutionary trend [126]. Simple precursors of AChR family have been identified in bacteria [127] and have proven extremely useful for crystal structure determination of these ion channels [128–131]. AChBP secreted by mollusk glia is also very useful for structural studies of the extracellular domain of AChRs and the structures of their ACh-binding sites [59–62].

The ACh-binding sites are located halfway up the extracellular domain [60, 61], far removed from the gate at the cytoplasmic end of the channel whose opening they regulate [70]. This regulation is thought to occur by means of small global conformation changes of the AChR protein, which may involve slight changes in the angles of the rodlike subunits to produce an iris-like regulation of channel opening [55, 64]. Both the ACh-binding site [132] and the cation channel [70] change conformation between the resting, open, and desensitized states. The amino acids lining the ACh-binding site come from three parts of the + side of the α-subunit and three parts of the – side of the complementary subunit [43, 60, 61]. It would seem that such an arrangement would be ideal for initiating and communicating small motions involved in ligand binding to motions along subunit interfaces throughout the molecule. The binding energies involved are small, as must be the differences between the resting and open conformations as the channel flickers open.

It is argued that the two ACh-binding sites in muscle AChRs should differ in affinity for ACh (K_D = μM versus mM) in order to ensure the rapid opening and closing of the channel [1, 46]. The properties of the two ACh-binding sites in muscle AChRs differ because they are formed at the interface of α1 with γ-, δ-, or ε-subunits [133]. The α1γ sites have higher affinity, producing longer channel opening at low ACh concentrations, as may be appropriate for fetal AChRs during synapse development (and perhaps useful when fetal AChRs help to compensate for AChR loss after autoimmune or genetic damage). The

actual kinetics of gating, surprisingly, depend not just on properties of the ACh site or gate but also on sequences in the large cytoplasmic domain [83]. During an end plate potential, ACh is present in the synaptic cleft at concentrations in the mM range for less than a millisecond [134]. Normally, this ensures a substantial safety factor for transmission because the ACh saturates the AChRs and the AChRs are in excess over what is needed to provide sufficient current to trigger an action potential (Chap. 1). When the number of AChRs is reduced in autoimmune or congenital myasthenia, the current may be insufficient, or become insufficient on successive stimuli as fewer vesicles of ACh are released and AChRs accumulate in the desensitized state. Then some synthesis of the fetal form of AChR with higher affinity for ACh and consequent longer burst and channel open times may be critical for achieving sufficient current to more nearly ensure transmission.

Competitive antagonists ideally keep AChRs in the resting conformation. They are usually multivalent quaternary or tertiary amines thought to interact both at the ACh site and peripheral sites to stabilize the resting state. Some, like curare, may actually be very low-efficacy agonists [135]. α-Bungarotoxin and related snake venom peptides of 8000–9000 Da are large flat molecules that might cover 800–1200 $Å^2$ of AChR surface [136], corresponding perhaps to a 30Å × 30 Å square centered on the ACh-binding site. Only the tip of one finger of this structure enters the actual ACh-binding site [61, 136], but this occurs with high affinity and stabilizes the AChR in a resting state.

Exposure to ACh or other agonists for long periods causes desensitization, which is a conformation (or perhaps a set of conformations) characterized by a closed channel and higher affinity for agonists. In the normal course of neuromuscular transmission, desensitization is not a limiting factor. However, ACh esterase inhibitors given to treat MG (by increasing the concentration and duration of ACh to compensate for the loss of AChRs) can, at excessive doses, further impair transmission by causing accumulation of desensitized AChRs [137]. Nerve gases and insecticides that act as esterase inhibitors can have similar effects [138]. The depolarizing block surgical muscle relaxant succinylcholine should also have a similar component to its action in addition to producing a depolarizing blockade of action potential generation. Nicotine in tobacco users is present for many hours ($t_{1/2}$ for clearance = 2 h) at an average concentration around 0.2 μM and rises to nearly 1 μM briefly after inhalation of smoke [139]. The low affinity of muscle AChRs for nicotine normally prevents much effect on neuromuscular transmission. Nicotine, acting on several neuronal AChR subtypes with a wide range of affinities for nicotine, produces many behavioral effects (addiction, tolerance, anxiolysis, cognitive enhancement, anti-nociception) resulting from a complex mixture of activation, desensitization, and upregulation effects on the AChRs [3, 140, 141]. AChRs containing α4 and β2 subunits, which account for most high-affinity nicotine binding in the brain, can account for the most prominent effects of nicotine: reward, tolerance, and sensitization [32].

Binding of agonists at two ACh-binding sites is required for efficient activation of AChRs [133]. Antagonist blockage of any one site is usually sufficient to prevent activation [142]. Homomeric AChRs, such as α7 AChRs, have five ACh-binding sites. Desensitization at any one of these may be sufficient to prevent activation, probably accounting for the very rapid desensitization characteristic of such AChRs [143].

Cation Channel and Its Gate

The cation channel is lined by amino acids from the M2 transmembrane domains, as depicted in Fig. 2.3. Much of the identification of amino acids lining the channel pore has been achieved using the substituted-cysteine accessibility method (SCAM) [43, 70]. In this method, successive amino acids along a putative transmembrane domain such as M1 or M2 are replaced by cysteine. This introduces a free thiol group, usually without greatly altering AChR function.

Then a thiol alkylating agent which contains a positively charged amino group (e.g., 2-aminoethyl methanethiosulfonate (MTSEA)) is applied from outside or inside the cell in which the mutated AChR is expressed. If MTSEA covalently reacts and blocks the channel, it is assumed that the substituted cysteine was exposed on the interior of the channel. From the periodicity at which cysteines are exposed, it can be inferred whether the domain has α-helical or another secondary conformation, and accessibility of the cysteine in the resting, open, or desensitized conformation determines the outer limits of the channel gate. Most of M1 and M2 appear to be in an α-helical conformation. In the resting state, the open lumen of the channel extends to nearly the cytoplasmic surface with only a small-occluded region between α1 G240 and T244. The highly conserved sequence α1 G240, E241, and K242, in the M1–M2 linker immediately preceding the cytoplasmic end of M2, lines the narrowest part of the channel in which the gate is located. Small motions in this region could open and close the channel. In the desensitized state, nearly half of the channel is occluded over a region extending from G240 to L251. Other mutagenesis studies have also helped define the structure of the channel [144]. Several polar or charged rings of amino acids lining the channel formed by homologous amino acids from each subunit form the selectivity filter. Electron crystallography images the channel as lined by a cylinder of α-helical M2 sequences, and this structure is surrounded by an arrangement of the other three transmembrane domains [145]. Both the extracellular and intracellular vestibules are strongly electronegative, which would provide a stable environment for cations passing through the channel [50]. Access of cations to the inner vestibule is through narrow lateral portals formed by cytoplasmic α-helices immediately preceding M4 [50]. Electrophysiological studies suggest that three or four polar amino acids lining these portals are critical for allowing passage of ions into the inner lumen of the channel in all members of the Cys-loop family of receptors [84].

Antigenic Structure and the Main Immunogenic Region (MIR)

Immunization with native α1 AChRs provokes antibodies directed primarily at the extracellular surface [73, 146]. This is because more than half of the antibodies to AChR in an immunized animal or an MG patient are directed at the MIR [52, 53, 147–149]. This is a group of highly conformation-dependent epitope; thus, immunization with denatured AChRs or subunits, by default, provokes antibodies to the cytoplasmic surface, whose epitopes are much less conformation-dependent [73]. Synthetic peptides can be used to induce antibodies to many parts of the AChR sequence, but many of the antibodies will not bind to AChRs in their native conformation [73].

mAbs from rats and mice immunized with AChRs have provided excellent model autoantibodies and structural probes for AChRs [3, 52, 139]. Half or more of the mAbs to native α1 AChRs are directed at the MIR [53, 148]. Such antibodies compete with each other for binding and prevent the binding of more than half of the autoantibodies from MG patients [52, 147, 148]. Thus, the antibodies bind to overlapping regions, but not necessarily to identical epitopes.

The structure of the MIR is being defined with increasing precision. Both absolutely conformation-dependent mAbs to the MIR, and others which also bind to denatured α1 subunits with lower affinity, depend critically on the MIR loop (α1 66–76), especially α1 amino acids 68 and 71, as shown by mutagenesis studies [150]. The dependence on the native conformation of the AChR for binding of rat mAbs to the MIR and MG patient autoantibodies to the MIR suggests that several sequences which are adjacent only in the native conformation contribute to the formation of the cluster of MIR epitopes recognized by rat mAbs and MG patient autoantibodies. In the AChBP, the sequence which would form the MIR in an α1 subunit is located at the extracellular tip of the subunit near the N-terminus in a loop directed away from axis of the subunit, as shown in Fig. 2.2 [59]. The sequence of this region in the AChBP is not closely homologous to that of α1 subunits. By making chimeras of

human muscle AChR α1 subunits with AChBP, the native conformation of the MIR was determined to be formed by two discontiguous sequences of the extracellular domain of the α1 subunit (N-terminal α-helix and the MIR loop), which are adjacent only in the native conformation [148, 151]. The chimera, incorporating both the structural elements, exhibits high affinity for rat and human mAbs to the MIR; reacts with autoantibodies from cat, dog, and human MG patients; and induces EAMG in rats. The MIR was located at the extracellular tip of α1 subunits and angled away from the central axis of the AChR. An antibody bound to one α1 subunit cannot also bind the other α1 subunit in the same AChR but can efficiently cross-link adjacent AChRs [148]. An antibody bound to the MIR would cover a large area of the α1 surface. Thus, several adjacent epitopes could be obscured by a single-bound mAb. A crystal structure of the AChR α1 subunit bound by the Fab fragment of a mAb to the MIR confirms these conclusions [152].

The separation between the MIR at the tip of the subunit and the ACh-binding site halfway down the side of the subunit reveals why both antibodies to the MIR and α-bungarotoxin can bind simultaneously to α1 AChRs. Few autoantibodies in MG are directed at the ACh-binding site [153]. These facts form the structural basis of the basic immunodiagnostic assay for MG in which ^{125}I-labeled α-bungarotoxin is used to specifically label detergent-solubilized human α1 AChRs to permit quantitation of MG patient autoantibodies in an immune precipitation assay [154].

The fundamental pathological significance of the MIR derives from its basic structure. This is represented in Fig. 2.5. The MIR is highly immunogenic. This may be because it is easily accessible on the protein surface and is present in two copies per AChR to permit multivalent binding and cross-linking of receptor immunoglobulins to efficiently stimulate B cells. Because the MIR is easily accessible on the extracellular surface, antibodies can easily bind to it and fix complement. α1 AChRs are densely packed in a semicrystalline array at the tips of folds in the postsynaptic membrane, promoting high-avidity binding of antibodies and focal lysis of the postsynaptic membrane but allowing the postsynaptic membrane to reseal after the membrane fragments containing antibodies, AChR, and complement are shed [155]. This process contributes to impairment of transmission both by destroying AChRs and by disrupting the morphology of the synapse in which presynaptic active zones of ACh release are normally located near postsynaptic patches of AChRs and also release endogenous AChRs that drive a feed-forward cycle of autoimmune stimulation to AChRs, thereby sustaining the autoimmune response [156, 157]. The MIR is oriented at an

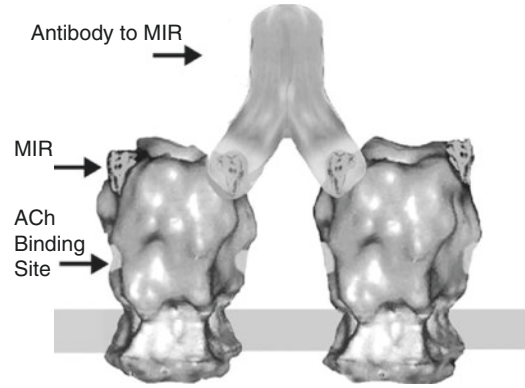


Fig. 2.5 Structural basis of the pathological significance of the MIR. It is important to remember that the MIR is a region of closely spaced epitopes, not a single epitope. The MIR is easily accessible on the extracellular surface. This permits binding of autoantibodies and complement. The MIR has a highly immunogenic conformation. There are two copies of the MIR. This contributes to its immunogenicity by permitting aggregation of receptor immunoglobulins on B cells. It contributes to synaptic pathology by permitting cross-linking of AChRs by antibodies to cause antigenic modulation. The MIR is oriented to facilitate cross-linking of adjacent AChRs by antibody while not permitting cross-linking of α1 subunits within an AChR. The MIR is away from the ACh-binding site and subunit interfaces. Thus, antibodies to the MIR do not inhibit AChR function competitively or allosterically. Also, this permits binding of both antibodies and α-bungarotoxin simultaneously, so that immunodiagnostic assays can be done by indirect immunoprecipitation of detergent solubilized AChRs labeled with ^{125}I-α-bungarotoxin. AChRs are densely packed in a semicrystalline array at the tips of folds in the postsynaptic membrane. Thus, bound antibody and complement can cause focal lysis and shedding of AChR-rich membrane fragments, while the postsynaptic membrane can reseal. The result is loss of AChR and disrupted synaptic morphology, but not lethal lysis of the muscle fiber

angle which promotes cross-linking of AChR by antibodies. This triggers antigenic modulation, which is a cross-linking-induced increase in the rate of AChR internalization and lysosomal destruction [158–160]. mAbs to the MIR and most serum antibodies do not impair AChR function [161]. This is probably because the antibodies do not interfere with ACh binding.

The MIR plays important functional roles in maintaining the structure and function of AChRs. Transplanting the α1 MIR into human α7 AChR results in a 53-fold increase in expression of mature chimeric AChRs over wild-type α7 [148]. The interaction between the two structural elements of the MIR promotes efficient assembly of the AChRs by nucleating conformational maturation of the subunits. This functional role applies to all subunits of AChRs and related receptors (e.g., 5-HT_{3A}) [162]. Incorporating an additional α1 sequence, the loop region (α1 15–30) between the N-terminal helix and the first β-strand, into this chimera leads to an increase of over two orders of magnitudes in sensitivity to activation by ACh [148]. There must be long-range communications mediated by cooperative motions between the MIR and the ACh-binding sites.

Some rat mAbs to the MIR on α1 bind well to human α1, as well as α3, and α5 subunits (which have similar MIR sequences) [3, 110]. These mAbs do not recognize denatured α3 even though they do recognize denatured α1. MG patient antisera do not bind significantly to human α3 AChRs, and human autoantibodies to α3 do not bind to α1 [29]. Thus, the MIR epitopes recognized by rats and humans are not identical, even though they are close enough that antibodies to them compete for binding.

Prominent antibody epitopes on the cytoplasmic surfaces of α1 subunits and other subunits have been determined using antisera and antibodies [52, 73]. These epitopes are pathologically irrelevant because autoantibodies cannot bind to them in vivo.

The spectrum of autoantibodies to AChRs produced in MG patients is dominated by the MIR but includes other parts of the AChR and closely resembles the spectrum seen in animals with EAMG or in dogs with idiopathic MG [147, 163]. Many MG sera react better with extrajunctional AChRs [164]. This may reflect that the endogenous immunogen in MG has γ-subunits, and these may be more immunogenic because they are not normally expressed in adults. One unusual MG patient serum was found to be specific for γ and occluded the α1γ ACh-binding site, thereby inhibiting function [165]. Rare mothers have been found who make autoantibodies only to γ-subunits [166]. These have no effect on the mother (whose AChRs have only ε-subunits), but they paralyze or kill fetuses, causing arthrogryposis multiplex congenita.

α1 subunits also predominate in the T-cell response to AChRs, but epitopes have been found on all subunits [167, 168]. Several α1 T-cell epitopes seem to predominate in MG, but there is disagreement between laboratories on quite which sequences comprise these epitopes. In Lewis rats, α1 100–116 is a dominant T-cell epitope, but in brown Norway rats, α172–205 predominates, as does α1 52–70 in buffalo rats [169]. There is probably similar diversity in human groups. Pathologically significant T-cell epitopes could derive from any part of an AChR subunit because the T cells do not bind to native AChRs in vivo but to peptide fragments digested by "professional" antigen-presenting cells like dendritic cells or "interested amateurs" like AChR-reactive B cells.

Before an AChR epitope can be recognized by the antigen receptor of a T-helper cell that will collaborate with an AChR-stimulated B lymphocyte to produce plasma cells secreting autoantibodies to AChRs, the AChR peptide must first be bound by an MHC class II antigen-presenting protein [170]. The proteolytic processing mechanisms of the antigen-presenting cell and the binding properties of the various MHC class II proteins restrict the peptides which can be recognized by T cells [171]. This is probably reflected in part in the higher incidence of HLA-A1, B8, and DR3 class II MHC determinants in young-onset Caucasian MG patients and different MHC determinants in other groups [172]. Inbred mice become resistant to EAMG subsequent to a single amino acid change in the I-A_B protein [173, 174]. The human α1 sequence 144–156 is recognized only when it is presented by HLA-DR4

class II protein variants with glycine at position 86 and not by a variant with valine at this position [172]. If humans were as inbred as these mouse strains, and if AChRs had only a few T-cell epitopes, MG would be much more genetically constrained than is actually the case.

Induction of the Autoimmune Response to AChRs in MG

The mechanisms which induce and sustain the autoimmune response to α1 AChRs in MG are not known. These mechanisms may differ in various forms of human MG, and mechanisms may differ between species. Human MG is a remitting and exacerbating disease in which the autoimmune response to α1 AChRs persists for years [27, 158]. Feline MG has many similarities with human MG [175], while canine MG is an acute disease in which the autoimmune response to AChRs and muscle weakness usually remit within 6 months, except when the MG is associated with a neoplastic growth [176]. This could suggest that in humans and cats, a source of immunogen persists, which does not usually happen in dogs. Another possibility is that human and cat patients share common susceptibility genes for MG, but dogs do not.

MG development following virus infection, e.g., West Nile virus [177, 178] and Zika virus [179], has been described in patients without any earlier evidence of MG. Recent studies found evidence for persistent Epstein-Barr virus (EBV) infection in the thymus of MG patients, suggesting that EBV might contribute to the onset or maintenance of the autoimmune response in MG thymus [180, 181]. One possible mechanism by which viruses break down self-tolerance is cross-reactive B- and T-cell recognitions, known as molecular mimicry, between virus proteins and AChR subunits. The fact that wild-type AChBP, a protein distantly related to muscle AChR, induces EAMG supports that some cases of MG might be initiated by molecular mimicry of AChR by infectious agents [157]. Another possibility is that a chronic occult infection in humans might involve a tissue (e.g., thymus), which expresses α1 AChR in an unusual amount, place, or state of posttranslational modification, causing it to be immunogenic. However, many observations indicate that EBV contributes also to the pathological mechanisms of multiple sclerosis [182–184], systemic lupus erythematosus [185–187], and rheumatoid arthritis [188, 189]. Thus, EBV infection could be one of the environmental factors that facilitate the development of autoimmune diseases in genetically susceptible individuals [190].

MG in humans can be initiated and then terminated when the immunogen is removed. Rheumatoid arthritis patients treated with penicillamine sometimes develop autoimmune MG [191, 192]. It is thought that, in these patients with a predisposition to autoimmune responses, covalent reaction of penicillamine with thiol groups on α1 AChRs haptenizes them to produce new antigenic sites and provoke an autoimmune response [193]. Ending the penicillamine treatment ends the MG within a couple of months. Neonatal MG occurs in babies of mothers with MG as a result of passive transfer of autoantibodies from the mother [194, 195]. This too spontaneously remits within a couple of months. These may be results of lack of genetic susceptibility to MG in these patients. If active EAMG is not sufficiently severe to be lethal, the animals remain abnormally fatigable even 30 weeks after the initial immunization and have chronic remitting-relapsing course like humans do [196]. This suggests that an autoimmune assault on neuromuscular junction AChRs may be sufficient to present enough AChR to the immune system to sustain the autoimmune response. Rats with EAMG induced by wild-type or chimeric AChBP developed antibodies to AChR cytoplasmic domains, though these antigens lack cytoplasmic domains [156, 157]. In rats with EAMG induced by electric organ AChR, there is a continued increase in the level of antibodies to rat muscle AChR 30 days following the initial immunization, a time when the level of antibodies to electric organ AChRs decreases [197]. These observations illustrate that complement-mediated attack on the postsynaptic membrane causes shedding AChR-rich membrane fragments that drive a feed-forward cycle of autoimmune stimulation to muscle AChRs. The spectrum of autoantibody specifici-

ties in MG and EAMG is very similar [147, 163]. Therefore, immune stimulation by the endogenous AChRs may sustain the autoimmune response in both EAMG and MG [198].

A paraneoplastic autoimmune response may be present in some MG patients. Twelve percent of MG patients have thymoma, and 35% of thymoma patients have MG [199]. These patients characteristically make high levels of autoantibodies to AChRs and to several structural proteins found within muscle cells, e.g., titin [200–202]. Thymic tumor cells do not usually express α1 AChRs [167], but traces of AChR have been detected in thymus myoid cells, thymic epithelia, and dendritic cells [203, 204]. The autoantibodies to AChRs might result from a bystander adjuvant effect of the disruption caused by the tumor to an immune regulatory organ which contains traces of AChRs. Alternatively, viruses or other factors which cause the tumor might also cause disruption of AChR-expressing cells. In Lambert Eaton myasthenic syndrome (LEMS), tumor immunogens play a much more obvious role in provoking the autoimmune response. Sixty percent of these patients have small-cell lung carcinomas [205] (as a result of addiction to tobacco through neuronal AChRs) [3, 141]. These carcinomas express voltage-sensitive calcium channels, which are the target of the autoimmune response in LEMS. The muscular weakness which these patients exhibit due to autoantibody impairment of ACh release [205], often for years before the tumor is discovered, is the tradeoff for development of a successful immune response to the tumor voltage-gated calcium channel, which holds the otherwise rapidly fatal tumor in check.

Autoimmune Mechanisms Which Impair Neuromuscular Transmission in MG and EAMG

EAMG has been induced in many species by immunization with purified *Torpedo* electric organ AChR [153]. Native AChR is quite immunogenic, and EAMG has been induced with syngeneic AChR [206], even in the absence of adjuvants [207]. The most detailed studies have been in Lewis rats [153, 155, 208]. Some other rat strains produce high levels of antibodies to AChRs but are resistant to EAMG [209]. Some mouse strains are more resistant than others, but all are more resistant than are Lewis rats [174].

Among MG patients, the absolute concentration of antibodies to AChR does not correlate closely with severity [210–212]. Patients with only ocular signs have lower concentrations than do patients with generalized MG, and antibody concentrations fall in almost all patients who clinically improve, but also in most patients who do not [211, 213]. Thus, absolute antibody levels cannot be used as a biomarker of improvement in MG.

The basic mechanisms by which autoantibodies to AChRs impair neuromuscular transmission appear to be very similar in MG and chronic EAMG [153, 155].

There are forms of both MG and EAMG which are passively transferred by autoantibodies to AChRs. Passive transfer of EAMG is associated with an antibody and complement-dependent, transient phagocyte-mediated attack on the postsynaptic membrane which greatly amplifies the pathological potency of the bound antibodies [214, 215]. In neither chronic EAMG nor MG is antibody-dependent phagocytic attack or cytotoxic T-lymphocyte attack on the postsynaptic membrane observed [155]. Repeated injection of mice with large amounts of MG patient IgG causes a mild form of muscular weakness [216, 217]. Mothers with MG can passively transfer MG to their babies [194, 195]. Usually this is mild and transient, ending as maternal IgG is cleared from the baby.

Active immunization of Lewis rats with AChR in adjuvant results in chronic EAMG after about 30 days [206]. If sufficient AChR and adjuvants are used, this can be preceded by an acute phase of EAMG 8–11 days after immunization, which involves antibody and complement-dependent phagocytes, as in passive EAMG [214, 218–220]. In both chronic EAMG and MG, ACh sensitivity is decreased, and decrementing electromyogram responses are observed as transmission becomes more likely to fail on successive stimuli [221,

222]. This is because decreased postsynaptic sensitivity to ACh reduces the safety factor for transmission, so failure becomes more likely as fewer vesicles of ACh are released on successive stimuli due to depletion of docked vesicles. Serum autoantibody concentration is high, there is loss of more than half of muscle AChRs, and many of those which remain have antibodies bound [214, 223]. The structure of the postsynaptic membrane is disrupted: the folded structure is simplified, AChR content is reduced, antibodies and complement are bound, and focal lysis occurs with shedding of membrane fragments into the synaptic cleft [219, 224, 225]. In addition to AChR loss through complement-mediated lysis, loss occurs due to antigenic modulation following cross-linking of AChRs by antibody [159, 160, 226]. Only a small fraction of antibodies to AChR are capable of inhibiting AChR function [227–232]. The lack of direct effect on AChR function by most antibodies to AChR is illustrated by the observation that if rats are depleted by the C3 component of complement to prevent both lysis and phagocytic invasion, then passive transfer of IgG from rats with chronic EAMG can label at least 67% of the AChRs without causing weakness [233].

Effects of AChR Mutations in Congenital Myasthenic Syndromes

The structure of muscle AChRs is optimized for its functional role [27]. Studies of congenital myasthenic syndromes due to mutations in AChR subunits [1, 10, 128] have clearly demonstrated that it is detrimental to either increase [234] or decrease [235, 236] affinity for ACh or increase [237] or decrease [238, 239] the channel opening time. Such changes often result in large pleotropic changes in the number of AChRs and the morphology of the neuromuscular junction, further contributing to impairment of neuromuscular transmission. Below, some examples of the many congenital myasthenic syndromes elegantly characterized by Andrew Engel [240] and his coworkers will be described briefly with the intention of relating defects in aspects of the AChR structures previously described to specific functional defects and the resulting medical effects (see Chap. 16).

Synthesis of AChR subunits can be impaired or prevented by mutations in the promotor region or the MIR or frameshift mutations that result in truncation of the subunit [239, 241–244]. These sorts of mutations are more frequent in ε-subunits than others because, in humans, expression of γ is induced, which partially compensates for the loss of ε. Expansion of the end plate area also helps to compensate for loss of AChRs. In mice, knockout of the ε-subunit is perinatal lethal [245]. Deletion mutants of α1 subunits are not seen, presumably because this would be lethal.

Mutations both within and outside the ACh-binding region alter agonist potency and efficacy. Reciprocally, these mutations alter channel-opening kinetics. A mutation which replaces α1 glycine 153 with a serine (in loop B of the ACh-binding site) decreases the rate of ACh dissociation, resulting in repeated channel opening during prolonged ACh binding and increased desensitization [234]. The resulting 100-fold increase in affinity which prevents the normal rapid termination of transmission results in excitotoxic damage, including reduced AChR amount, increased desensitization, and morphological alterations of the postsynaptic region due to overloading with cations. ACh esterase inhibitors would not be beneficial therapy. This mutation was less disabling to the patient than were some mutations in the M2 channel-lining region which, by increasing the stability of the open state of the channel, prolonged channel opening fivefold longer than did this increase in affinity for ACh [237]. A similar increase in agonist-binding affinity, activation, and desensitization was also caused by a mutation in the α1 M2 domain V249F at a position that is not on the hydrophilic lining of the channel [246]. A mutation which replaces valine 188 with a methionine in the C loop of the agonist-binding domain of the α1 subunit decreases gating efficiency 70-fold and reduces affinity for ACh two-fold [239]. An ε P121L mutation near the E loop of the ACh-binding site reduced the affinity for ACh in the open and

desensitized states, resulting in infrequent and brief episodes of channel opening and, consequently, impaired transmission causing weakness [235]. The amount of AChR and synaptic morphology was normal due to the absence of excitotoxicity in this mutation. An ε W55R mutation at the α-/ε-subunit interface of the agonist binding site decreases agonist affinity 30-fold and gating efficiency 75-fold and substantially reduces channel opening probability [236]. An α1 N217K mutation in the M1 region was found to cause a 20-fold increase in the affinity of ACh for the resting state of the AChR [247]. This N is a conserved amino acid in the synaptic third of the M1 transmembrane domain, i.e., not a contact amino acid in the ACh-binding site but part of the lumen of the channel, between the ACh-binding site and the conserved P121 C122 in the middle of M1, a region which is known to move during the process of AChR activation [248]. Efficacy and potency of agonists are effected by mutations in the M2 channel-forming part of the AChR because some of the mutations so destabilize the resting conformation that even binding of choline (usually not an effective agonist) is sufficient to activate the AChR [249]. Thus, the levels of choline in serum are sufficient to cause "spontaneous" activation of AChRs and contribute to excitotoxic damage in these long-channel congenital myasthenic syndromes. Experimental mutations in similar regions in M2 of α7 AChRs cause some antagonists to behave as agonists [250] by facilitating the transition from the resting to the open state. Choline is selective as an agonist, though a weak one, for α7 AChRs [251]. This may be because the five ACh-binding sites on α7 homomers permit the low-affinity binding of a sufficient number of choline molecules to trigger activation by this very weak agonist.

Mutations in the M2 channel-forming part of the AChR frequently affect the duration of channel opening, usually causing prolonged channel opening resulting in excitotoxicity. For example, the T264P mutation in the εM2 hydrophilic surface causes prolonged channel openings [237]. The α-V249F mutation on the hydrophobic surface of M2 also causes an autosomal dominant slow-channel myasthenic syndrome [246]. Agonist potency is increased, and desensitization is enhanced sufficiently to impair function at physiological rates of stimulation. Excitotoxic effects include loss of AChR, degeneration of junctional folds, cluttering of an enlarged synaptic cleft with bits of membranous debris, degeneration of synaptic mitochondria and other organelles, and apoptosis of some junctional nuclei. Mutations in the M2 regions of several AChR subunits cause slow-channel syndromes [241]. Other mutations can cause excessively rapid closure of the channel, but also excessive conductance, so that the net effect is also excitotoxic [238].

Myasthenic syndromes have also been identified in other parts of AChR subunits. One was found to involve two recessive mutations in ε [252]. The C128S mutation at one end of the disulfide-linked signature loop in the extracellular domain inhibits AChR assembly. Then the ε 1245 ins 18 mutation, which duplicates six amino acids in the C-terminal half of the large cytoplasmic domain, surprisingly, forms AChRs with altered opening kinetics and reduced net openings. That mutations in and out of the ACh-binding site effect agonist potency, and that mutations in and out of the channel effect gating, are all evidence that activation and desensitization of AChRs involve both local and global conformational changes and that mutations in distant parts of the AChR protein can effect the stability of various conformation states and the kinetics of transition between them. Clinically, it is interesting that the patient and her similarly affected siblings with these two mutations went through life-exhibiting muscle weakness and fatigue, but the crisis which provoked detailed study was when the 36-year-old patient underwent surgery and was given a curariform muscle relaxant which resulted in paralysis lasting several hours due to her low safety factor for transmission.

Another striking feature of mutations causing congenital myasthenic syndromes is that there are a lot of these mutations. Often a recessive mutation does not produce a phenotype until two occur simultaneously. Thus far, more than 60 such mutations have been found [22]. It seems likely that similar varieties of mutations will be found in other receptors and channels.

Neuronal AChR Subtypes and Functional Roles

There are many potential and a few known prominent neuronal AChR subtypes, as indicated in Fig. 2.4 [2]. α4β2 AChRs account for most of the high-affinity nicotine binding in the brain. α2 AChRs in primate brain may assume some of the roles of α4 AChRs in rodent brains [253]. Some of these also have α5 or perhaps β3 subunits replacing the β2 subunit equivalent to β1 in muscle AChRs [119, 254]. Autonomic ganglia postsynaptic AChRs are predominantly α3β4 AChRs, but α3β4α5 AChRs and subtypes containing β2 are also present. In brain aminergic neurons, α6 in combination often with β3 and β2 or β4 and sometimes α3 or α4 can form a variety of complex subtypes [93, 112–114, 255, 256]. α7 homomers are found both in autonomic ganglia and in brain. Heteromeric α7β2 AChRs are expressed in basal forebrain and cerebral cortical neurons [257, 258]. Neurons often contain complex mixtures of AChR subtypes [111, 113]. In autonomic ganglia, α3 AChRs perform a postsynaptic role in transmission similar to that of muscle AChRs, but many brain AChRs are involved in pre- and extrasynaptic modulation of the release of many transmitters [259], and some extrasynaptic α7 AChRs may play roles in trophic regulation [116].

A good way to get an overview of the functional roles of neuronal AChRs is to examine the features of mice in which a particular subunit has been knocked out or replaced with a hyperactive mutant form. For example, knockout of α3 subunits is neonatal lethal [260]. They lack autonomic transmission and die as a result of a distended and infected bladder.

Knockout of α4 subunits causes loss of most high-affinity nicotine binding in the brain, loss of nicotine-induced antinociception, and increased anxiety [261, 262]. Replacement of α4 with excitotoxic M2 mutation was neonatal lethal, but a heterozygote survived which had reduced expression of the mutation due to some expression of the neo cassette involved in making the mutant mouse [263]. These mice lost dopaminergic neurons in the substantia nigra, exhibited altered motor behavior and learning, and increased anxiety. Knock-in of hyperactive α4 subunits was used to show that AChRs containing α4 subunits can account for the rewarding, tolerance, and sensitizing effects of nicotine [32]. These hypersensitive AChRs also cause changes in the sleep-wake cycle and increase sensitivity to nicotine-induced seizures [264].

Knockout of α6 subunits does not reduce the total number of AChRs in dopaminergic nerve endings in the striatum because of a compensatory increase in the α4 subunits which are expressed in these neurons to compensate for the loss of α6 in α6β2β3 and α6α4β2β3 AChRs [265, 266]. α6-containing AChRs are selectively expressed in these nerve endings, while α4 AChRs are selectively expressed in the cell bodies of these neurons in the ventral tegmental area. α6 AChRs are selectively lost in MPTP-induced animal models of Parkinson's disease [267] and may be target for drug therapy in this disease [37].

Knockout of α7, surprisingly, produced few evident problems [268, 269]. The most evident phenotype is reduced fertility. Knock-in of an excitotoxic M2 mutant α7 was neonatally lethal [270]. A problem in interpretation of knockout mice is that developmental compensation may minimize the apparent functional importance of the knockout subunit. The problem with knocking-in a lethally excitotoxic mutant subunit is that any neuron which at any time in its development expresses some of the subunit risks dying, thus exaggerating the apparent functional importance of the subunit. Multinucleate muscle cells are large and rather resilient to the lethal excitotoxic effects of mutant AChRs, but smaller neurons may not be, especially early in development when their ability to buffer excess calcium ions entering through hyperactive AChRs is low [271].

Knockout of α9 prevented cochlear efferent stimulation [272], as expected from its postsynaptic role there. α10 subunits are functionally equivalent to β-subunits, and α9α10 AChRs usually have the stoichiometry $(\alpha9)_2(\alpha10)_3$ [273]. These AChRs are unusual in their physiological role in that entry of Ca^{++}

through these AChRs triggers Ca^{2+}-sensitive K^+ channels resulting in a net inhibitory postsynaptic response [274].

Knockout of β2 altered some learning behaviors, caused increased neuronal death with aging, prevented nicotine-induced antinociception, and prevented nicotine reinforcement (i.e., addiction) [275]. Selective expression of β2 only in the ventral tegmental area of β2 knockout mice results in nicotine-induced dopamine release and nicotine self-administration [275]. Nicotine induces wakefulness in wild type but not β2 knockout mice, and knockout mice exhibit disrupted sleep patterns [276]. Wild-type mouse pups exposed to nicotine exhibit reduced growth, unstable breathing, as well as impaired arousal and catecholamine synthesis [277]. β2 knockout pups exhibit the same symptoms. This suggests that these effects of nicotine result from desensitization of AChRs containing β2 subunits. Deletion of the β2 subunit altered development of tolerance to nicotine and eliminated nicotine-induced upregulation of the amount of brain AChR [278]. Nicotine induces/increases the amount of AChRs [279–281]. This is much more prominent with β2-containing than β4-containing AChRs [281, 282]. Upregulation results primarily from nicotine binding to assembly intermediates, thereby causing a conformation change which promotes their assembly [99, 283]. Nicotine also slows the rate of AChR destruction [99, 280, 281]. High concentrations of nicotine can increase the expression of human muscle AChRs in cell lines [284], but in vivo smoking-associated concentrations of nicotine only upregulate brain [285, 286] not muscle AChRs.

Knockout of β3 subunits [287] selectively reduces, but does not eliminate, expression of the α6-containing nigrostriatal AChRs [255] with which they are associated. This alters striatal dopamine release, locomotor activity, and acoustic startle behaviors.

Knockout of β4 produced viable mice, indicating that β2 expression must have compensated for loss of β4 in α3 AChRs in autonomic neurons [288]. When both β2 and β4 were knocked out, the effects were even more lethally severe than the knockout of the α3 subunit.

Neuronal nicotinic AChRs are directly responsible for addiction to the nicotine in tobacco [3, 140, 141] and are more peripherally involved in many other neurological problems [3, 36].

Autoimmune Impairment of Neuronal AChRs

Low levels of autoantibodies to α3 AChRs were found in 41% of patients with idiopathic or paraneoplastic dysautonomias and 9% of patients with postural tachycardia syndrome, idiopathic gastrointestinal dysmotility, or diabetic autonomic neuropathy [29]. Antibody levels were higher in the more severely affected and decreased with improvement. Low levels of autoantibodies to α3 or α7 AChRs have also been found in a few patients with MG, LEMS, Guillain-Barre syndrome, or chronic inflammatory demyelinating polyneuropathy who also exhibited symptoms of autonomic dysfunction [289]. Unlike human MG, but like canine MG [176], these autoimmune dysautonomias are often monophasic illnesses [29]. Passive transfer of serum IgG purified from patients with autoimmune autonomic ganglionopathy [AAG] causes an impairment of autonomic ganglionic synaptic transmission in mice [290]. Patient IgG, rather than Fab fragments prepared from patient IgG, decreases whole-cell neuronal AChR current in cultured human IMR-32 cells [291]. This antibody-mediated inhibition of AChR is not readily reversed by removing antibody and does not require complement or any other immune mediators. Thus, the effect of antibodies on the AChR does not result from interference with agonist-binding or complement-mediated focal lysis but primarily from antigenic modulation as has been described for muscle AChR antibodies in MG and EAMG. As with MG, plasmapheresis of AAG produces dramatic improvement [292]. Experimental AAG (EAAG) can be induced by immunization of rabbits with the bacterially expressed extracellular domain of the α3 subunit [293, 294], and serum from these rabbits can passively transfer EAAG to mice [28].

It is interesting that rat mAbs to the MIR react well with human α3 AChRs [110], yet MG patient antisera in general do not react with human α3 AChRs [29], AAG patient autoantibodies do not react with muscle AChRs [29], and rats with EAMG do not exhibit obvious autonomic symptoms [27]. Thus, despite the similarities in primary sequences in the 66–76 regions of α1, α3, α5, and β3 subunits, the conformation dependence of MIR antibody binding and the large areas of a protein occluded by a bound antibody can account for the apparent differences in MIR antibody specificity between rats and humans, despite mutual competition for binding to α1 subunits.

Low levels of AChR α3, α5, α7, α9, and β2 and β4 AChR subunits have been reported in human keratinocytes, and ACh has been reported to modulate keratinocyte adhesion, proliferation, migration, and differentiation [6]. Autoantibodies in pemphigus patients have been found directed at not only desmoglein but also at α9 AChRs and a keratinocyte ACh-binding protein termed pemphaxin [31]. Pemphaxin is not the equivalent of the snail AChBP [59, 295]. Autoimmune responses to neuronal AChRs found in other non-neuronal tissues have not been reported yet.

A priori, antibody-mediated autoimmune responses to receptors in the brain would seem unlikely due to the presence of the blood-brain barrier. However, the field of neuronal autoantibody-associated diseases of the central nervous system has expanded dramatically recently. Autoantibodies to a *N*-methyl-D-aspartate (NMDA) receptor subunit GluRε2 and to α7 AChR have been found in some Rasmussen's encephalitis patients and certain forms of cerebellar degeneration [24, 296, 297]. Recent studies reported the presence of autoantibodies against the NMDA receptor [298], $GABA_A$ receptor [299], $GABA_B$ receptor [300], glycine receptor [301], contactin-associated protein-like 2 (CASPR2) [302], and leucine-rich glioma-inactivated 1 (LGI1) [302] in patients with autoimmune encephalitis. Thus, there is precedent for antibody-mediated autoimmune responses to brain neuronal AChRs, and it is possible that such responses could involve neuronal AChRs [303].

Effects of Human Neuronal AChR Mutations

Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is a rare form of epilepsy [resembling night terrors] which is caused by mutations in either α4 or β2 subunits in the M2 region [24, 304, 305]. There are mutations known in both α4 and in β2. A conundrum is that the α4 mutations involve loss of function, whereas the β2 mutations involve gain of function. For example, replacement of α4 serine 247 in the M2 channel lining with a phenylalanine reduced channel function as a result of use-dependent functional upregulation, faster desensitization, slower recovery from desensitization, less inward rectification, and virtual elimination of Ca^{++} permeability [306]. Although many functions of α4β2 AChRs in brain are unknown, α4β2 AChRs have been shown to promote the release of the inhibitory transmitter GABA [307]. Thus, reduced function of α4β2 AChRs might produce the excessive activation characteristic of epilepsy, if under the right circumstances in the wake/sleep cycle, reduced AChR function caused reduced inhibition. However, ADNFLE mutations in β2 subunits cause excessive function when expressed with α4 [304, 305]. As an example, a V287M mutation in a conserved valine near the C-terminal end of M2 causes a ten-fold increase in ACh potency and reduced desensitization [305]. Since β2 can also function in combinations with α2, α3, and α6, it is possible that one of these combinations or α4β2 AChRs in a different circuit could account for ADNFLE. It has been proposed that a common feature shared by all ADNFLE mutations is reduced Ca^{++} potentiation of the response to ACh [308]. Clearly, it is much more difficult to study neuronal AChR mutations than muscle AChRs because the many complex functions of neuronal AChRs in many overlapping circuits are not well known and because biopsy material is not usually available or easy to study.

The autonomic dysfunction and neonatal lethality of α3 or β2 plus β4 knockouts in mice [260, 288] resemble the human disease megacystis-microcolon-intestinal hypoperistalsis syndrome. No AChR mutations have yet been

found to account for this disease [309], although there is some evidence for loss of α3 AChRs in these patients [310]. Rare missense variants within the intracellular loop of α3, α4, and β4 AChR subunits are significantly overrepresented in sporadic amyotrophic lateral sclerosis (SALS) patients and alter receptor functions [311].

The large number of mutations found in muscle AChRs [1, 22] makes it seem likely that there are many neuronal AChR mutation syndromes to be discovered. Instead of affecting a single subtype of AChR, and producing symptoms of one basic type, varying degrees or features of myasthenia, congenital neuronal AChR mutation syndromes will probably be discovered which affect a wide range of AChR subtypes. These mutations might produce hyper- and hypofunction and result in a wide range of pleotropic phenotypes affecting both the central and peripheral nervous systems as well as the non-neuronal tissues in which these AChRs are expressed.

Acknowledgment The Lindstrom laboratory is supported by grants from the NIH.

References

1. Sine SM, Engel AG. Recent advances in Cys-loop receptor structure and function. Nature. 2006;440:448–55.
2. Berg DK, Shoop RD, Chang KT. Nicotinic acetylcholine receptors in ganglionic transmission. In: Clementi F, Gotti C, Fornasari D, editors. Neuronal nicotinic receptors. New York: Springer; 2000. p. 247–67.
3. Lindstrom J. The structures of neuronal nicotinic receptors. In: Clementi F, Gotti C, Fornasari D, editors. Neuronal nicotinic receptors. New York: Springer; 2000. p. 101–62.
4. Kaiser S, Soliakov L, Wonnacott S. Presynaptic neuronal nicotinic receptors: pharmacology, heterogeneity, and cellular mechanisms. In: Clementi F, Gotti C, Fornasari D, editors. Neuronal nicotinic receptors. New York: Springer; 2000. p. 193–211.
5. Zoli M. Distribution of cholinergic neurons in the mammalian brain with special reference to their relationship with neuronal nicotinic receptors. In: Clementi F, Gotti C, Fornasari D, editors. Neuronal nicotinic receptors. New York: Springer; 2000. p. 13–30.
6. Grando SA. Cholinergic control of epidermal cohesion. Exp Dermatol. 2006;15:265–82.
7. Sekhon HS, Jia Y, Raab R, Kuryatov A, Pankow JF, Whitsett JA, Lindstrom J, Spindel ER. Prenatal nicotine increases pulmonary α7 nicotinic receptor expression and alters fetal lung development in monkeys. J Clin Invest. 1999;103:637–47.
8. Macklin KD, Maus AD, Pereira EF, Albuquerque EX, Conti-Fine BM. Human vascular endothelial cells express functional nicotinic acetylcholine receptors. J Pharmacol Exp Ther. 1998;287:435–9.
9. Maus AD, Pereira EF, Karachunski PI, Horton RM, Navaneetham D, Macklin K, Cortes WS, et al. Human and rodent bronchial epithelial cells express functional nicotinic acetylcholine receptors. Mol Pharmacol. 1998;54:779–88.
10. Hamano R, Takahashi HK, Iwagaki H, Yoshino T, Nishibori M, Tanaka N. Stimulation of α7 nicotinic acetylcholine receptor inhibits CD14 and the toll-like receptor 4 expression in human monocytes. Shock. 2006;26:358–64.
11. Rosas-Ballina M, Goldstein RS, Gallowitsch-Puerta M, Yang L, Valdes-Ferrer SI, Patel NB, et al. The selective α7 agonist GTS-21 attenuates cytokine production in human whole blood and human monocytes activated by ligands for TLR2, TLR3, TLR4, TLR9, and RAGE. Mol Med. 2009;15:195–202.
12. Yoshikawa H, Kurokawa M, Ozaki N, Nara K, Atou K, Takada E, Kamochi H, Suzuki N. Nicotine inhibits the production of proinflammatory mediators in human monocytes by suppression of I-κB phosphorylation and nuclear factor-κB transcriptional activity through nicotinic acetylcholine receptor α7. Clin Exp Immunol. 2006;146:116–23.
13. Sato K, Nagayama H, Tadokoro K, Juji T, Takahashi TA. Extracellular signal-regulated kinase, stress-activated protein kinase/c-Jun N-terminal kinase, and p38mapk are involved in IL-10-mediated selective repression of TNF-α-induced activation and maturation of human peripheral blood monocyte-derived dendritic cells. J Immunol. 1999;162:3865–72.
14. Borovikova LV, Ivanova S, Zhang M, Yang H, Botchkina GI, Watkins LR, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature. 2000;405:458–62.
15. de Lucas-Cerrillo AM, Maldifassi MC, Arnalich F, Renart J, Atienza G, Serantes R, et al. Function of partially duplicated human α7 nicotinic receptor subunit CHRFAM7A gene: potential implications for the cholinergic anti-inflammatory response. J Biol Chem. 2011;286:594–606.
16. Wang H, Liao H, Ochani M, Justiniani M, Lin X, Yang L, et al. Cholinergic agonists inhibit HMGB1 release and improve survival in experimental sepsis. Nat Med. 2004;10:1216–21.
17. Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, et al. Nicotinic acetylcholine receptor α7 subunit is an essential regulator of inflammation. Nature. 2003;421:384–8.
18. De Rosa MJ, Dionisio L, Agriello E, Bouzat C, Esandi Mdel C. α7 nicotinic acetylcholine recep-

tor modulates lymphocyte activation. Life Sci. 2009;85:444–9.
19. Razani-Boroujerdi S, Boyd RT, Davila-Garcia MI, Nandi JS, Mishra NC, Singh SP, et al. T cells express α7-nicotinic acetylcholine receptor subunits that require a functional TCR and leukocyte-specific protein tyrosine kinase for nicotine-induced Ca^{2+} response. J Immunol. 2007;179:2889–98.
20. Kawashima K, Yoshikawa K, Fujii YX, Moriwaki Y, Misawa H. Expression and function of genes encoding cholinergic components in murine immune cells. Life Sci. 2007;80:2314–9.
21. Skok MV, Kalashnik EN, Koval LN, Tsetlin VI, Utkin YN, Changeux JP, et al. Functional nicotinic acetylcholine receptors are expressed in B lymphocyte-derived cell lines. Mol Pharmacol. 2003;64:885–9.
22. Engel AG, Ohno K, Sine SM. Sleuthing molecular targets for neurological diseases at the neuromuscular junction. Nat Rev Neurosci. 2003;4:339–52.
23. Combi R, Dalpra L, Tenchini ML, Ferini-Strambi L. Autosomal dominant nocturnal frontal lobe epilepsy--a critical overview. J Neurol. 2004;251:923–34.
24. Steinlein OK. Neuronal nicotinic receptors in human epilepsy. Eur J Pharmacol. 2000;393:243–7.
25. Becchetti A, Aracri P, Meneghini S, Brusco S, Amadeo A. The role of nicotinic acetylcholine receptors in autosomal dominant nocturnal frontal lobe epilepsy. Front Physiol. 2015;6:22.
26. Ferini-Strambi L, Sansoni V, Combi R. Nocturnal frontal lobe epilepsy and the acetylcholine receptor. Neurologist. 2012;18:343–9.
27. Lindstrom JM. Acetylcholine receptors and myasthenia. Muscle Nerve. 2000;23:453–77.
28. Vernino S, Ermilov LG, Sha L, Szurszewski JH, Low PA, Lennon VA. Passive transfer of autoimmune autonomic neuropathy to mice. J Neurosci. 2004;24:7037–42.
29. Vernino S, Low PA, Fealey RD, Stewart JD, Farrugia G, Lennon VA. Autoantibodies to ganglionic acetylcholine receptors in autoimmune autonomic neuropathies. N Engl J Med. 2000;343:847–55.
30. Vernino S, Hopkins S, Wang Z. Autonomic ganglia, acetylcholine receptor antibodies, and autoimmune ganglionopathy. Auton Neurosci. 2009;146:3–7.
31. Grando SA. Autoimmunity to keratinocyte acetylcholine receptors in pemphigus. Dermatology. 2000;201:290–5.
32. Tapper AR, McKinney SL, Nashmi R, Schwarz J, Deshpande P, Labarca C, Whiteaker P, et al. Nicotine activation of α4* receptors: sufficient for reward, tolerance, and sensitization. Science. 2004;306:1029–32.
33. Peto R, Lopez AD, Boreham J, Thun M, Heath C Jr. Mortality from tobacco in developed countries: indirect estimation from national vital statistics. Lancet. 1992;339:1268–78.
34. Heeschen C, Jang JJ, Weis M, Pathak A, Kaji S, Hu RS, et al. Nicotine stimulates angiogenesis and promotes tumor growth and atherosclerosis. Nat Med. 2001;7:833–9.
35. Hurst RS, Hajos M, Raggenbass M, Wall TM, Higdon NR, Lawson JA, et al. A novel positive allosteric modulator of the α7 neuronal nicotinic acetylcholine receptor: in vitro and in vivo characterization. J Neurosci. 2005;25:4396–405.
36. Lloyd GK, Williams M. Neuronal nicotinic acetylcholine receptors as novel drug targets. J Pharmacol Exp Ther. 2000;292:461–7.
37. Quik M, McIntosh JM. Striatal α6* nicotinic acetylcholine receptors: potential targets for Parkinson's disease therapy. J Pharmacol Exp Ther. 2006;316:481–9.
38. Jurado-Coronel JC, Avila-Rodriguez M, Capani F, Gonzalez J, Moran VE, Barreto GE. Targeting the nicotinic acetylcholine receptors (nAChRs) in astrocytes as a potential therapeutic target in Parkinson's disease. Curr Pharm Des. 2016;22:1305–11.
39. Haydar SN, Dunlop J. Neuronal nicotinic acetylcholine receptors – targets for the development of drugs to treat cognitive impairment associated with schizophrenia and Alzheimer's disease. Curr Top Med Chem. 2010;10:144–52.
40. Quik M, Bordia T, Zhang D, Perez XA. Nicotine and nicotinic receptor drugs: potential for Parkinson's disease and drug-induced movement disorders. Int Rev Neurobiol. 2015;124:247–71.
41. Coe JW, Brooks PR, Wirtz MC, Bashore CG, Bianco KE, Vetelino MG, et al. 3,5-Bicyclic aryl piperidines: a novel class of α4β2 neuronal nicotinic receptor partial agonists for smoking cessation. Bioorg Med Chem Lett. 2005;15:4889–97.
42. Cahill K, Lindson-Hawley N, Thomas KH, Fanshawe TR, Lancaster T. Nicotine receptor partial agonists for smoking cessation. Cochrane Database Syst Rev. 2016;(5):CD006103.
43. Karlin A, Akabas MH. Toward a structural basis for the function of nicotinic acetylcholine receptors and their cousins. Neuron. 1995;15:1231–44.
44. Galzi JL, Devillers-Thiery A, Hussy N, Bertrand S, Changeux JP, Bertrand D. Mutations in the channel domain of a neuronal nicotinic receptor convert ion selectivity from cationic to anionic. Nature. 1992;359:500–5.
45. Eisele JL, Bertrand S, Galzi JL, Devillers-Thiery A, Changeux JP, Bertrand D. Chimaeric nicotinic-serotonergic receptor combines distinct ligand binding and channel specificities. Nature. 1993;366:479–83.
46. Jackson MB. Perfection of a synaptic receptor: kinetics and energetics of the acetylcholine receptor. Proc Natl Acad Sci U S A. 1989;86:2199–203.
47. Kummer TT, Misgeld T, Sanes JR. Assembly of the postsynaptic membrane at the neuromuscular junction: paradigm lost. Curr Opin Neurobiol. 2006;16:74–82.
48. Lindstrom J. Purification and cloning of nicotinic acetylcholine receptors. In: Arneric SP, Brioni D, editors. Neuronal nicotinic receptors: pharmacology

and therapeutic opportunities. New York: Wiley; 1999. p. 3–23.
49. Changeux J-P, Edelstein SJ. Nicotinic acetylcholine receptors: from molecular biology to cognition. New York: Odile Jacob; 2005.
50. Unwin N. Refined structure of the nicotinic acetylcholine receptor at 4Å resolution. J Mol Biol. 2005;346:967–89.
51. Patrick J, Lindstrom J. Autoimmune response to acetylcholine receptor. Science. 1973;180:871–2.
52. Tzartos SJ, Barkas T, Cung MT, Mamalaki A, Marraud M, Orlewski P, Papanastasiou D, et al. Anatomy of the antigenic structure of a large membrane autoantigen, the muscle-type nicotinic acetylcholine receptor. Immunol Rev. 1998;163:89–120.
53. Tzartos SJ, Lindstrom JM. Monoclonal antibodies used to probe acetylcholine receptor structure: localization of the main immunogenic region and detection of similarities between subunits. Proc Natl Acad Sci U S A. 1980;77:755–9.
54. Maimone MM, Merlie JP. Interaction of the 43 kd postsynaptic protein with all subunits of the muscle nicotinic acetylcholine receptor. Neuron. 1993;11:53–66.
55. Unwin N. The Croonian Lecture 2000. Nicotinic acetylcholine receptor and the structural basis of fast synaptic transmission. Philos Trans R Soc Lond B Biol Sci. 2000;355:1813–29.
56. Unwin N, Fujiyoshi Y. Gating movement of acetylcholine receptor caught by plunge-freezing. J Mol Biol. 2012;422:617–34.
57. Du J, Lu W, Wu S, Cheng Y, Gouaux E. Glycine receptor mechanism elucidated by electron cryomicroscopy. Nature. 2015;526:224–9.
58. Morales-Perez CL, Noviello CM, Hibbs RE. X-ray structure of the human α4β2 nicotinic receptor. Nature. 2016;538:411–5.
59. Brejc K, van Dijk WJ, Klaassen RV, Schuurmans M, van Der Oost J, Smit AB, et al. Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. Nature. 2001;411:269–76.
60. Celie PH, van Rossum-Fikkert SE, van Dijk WJ, Brejc K, Smit AB, Sixma TK. Nicotine and carbamylcholine binding to nicotinic acetylcholine receptors as studied in AChBP crystal structures. Neuron. 2004;41:907–14.
61. Hansen SB, Sulzenbacher G, Huxford T, Marchot P, Taylor P, Bourne Y. Structures of Aplysia AChBP complexes with nicotinic agonists and antagonists reveal distinctive binding interfaces and conformations. EMBO J. 2005;24:3635–46.
62. Smit AB, Syed NI, Schaap D, van Minnen J, Klumperman J, Kits KS, et al. A glia-derived acetylcholine-binding protein that modulates synaptic transmission. Nature. 2001;411:261–8.
63. Beroukhim R, Unwin N. Three-dimensional location of the main immunogenic region of the acetylcholine receptor. Neuron. 1995;15:323–31.
64. Lee WY, Sine SM. Principal pathway coupling agonist binding to channel gating in nicotinic receptors. Nature. 2005;438:243–7.
65. Bouzat C, Gumilar F, Spitzmaul G, Wang HL, Rayes D, Hansen SB, et al. Coupling of agonist binding to channel gating in an ACh-binding protein linked to an ion channel. Nature. 2004;430:896–900.
66. Grutter T, Changeux JP. Nicotinic receptors in wonderland. Trends Biochem Sci. 2001;26:459–63.
67. Wells GB, Anand R, Wang F, Lindstrom J. Water-soluble nicotinic acetylcholine receptor formed by α7 subunit extracellular domains. J Biol Chem. 1998;273:964–73.
68. Huang S, Li SX, Bren N, Cheng K, Gomoto R, Chen L, Sine SM. Complex between α-bungarotoxin and an α7 nicotinic receptor ligand-binding domain chimaera. Biochem J. 2013;454:303–10.
69. Li SX, Huang S, Bren N, Noridomi K, Dellisanti CD, Sine SM, et al. Ligand-binding domain of an α7-nicotinic receptor chimera and its complex with agonist. Nat Neurosci. 2011;14:1253–9.
70. Wilson G, Karlin A. Acetylcholine receptor channel structure in the resting, open, and desensitized states probed with the substituted-cysteine-accessibility method. Proc Natl Acad Sci U S A. 2001;98:1241–8.
71. Sussman JL, Harel M, Frolow F, Oefner C, Goldman A, Toker L, et al. Atomic structure of acetylcholinesterase from Torpedo californica: a prototypic acetylcholine-binding protein. Science. 1991;253:872–9.
72. Fu DX, Sine SM. Asymmetric contribution of the conserved disulfide loop to subunit oligomerization and assembly of the nicotinic acetylcholine receptor. J Biol Chem. 1996;271:31479–84.
73. Das MK, Lindstrom J. Epitope mapping of antibodies to acetylcholine receptor α subunits using peptides synthesized on polypropylene pegs. Biochemistry. 1991;30:2470–7.
74. Conroy WG, Liu Z, Nai Q, Coggan JS, Berg DK. PDZ-containing proteins provide a functional postsynaptic scaffold for nicotinic receptors in neurons. Neuron. 2003;38:759–71.
75. Jeanclos EM, Lin L, Treuil MW, Rao J, DeCoster MA, Anand R. The chaperone protein 14-3-3η interacts with the nicotinic acetylcholine receptor α4 subunit. Evidence for a dynamic role in subunit stabilization. J Biol Chem. 2001;276:28281–90.
76. Keller SH, Lindstrom J, Ellisman M, Taylor P. Adjacent basic amino acid residues recognized by the COP I complex and ubiquitination govern endoplasmic reticulum to cell surface trafficking of the nicotinic acetylcholine receptor α-Subunit. J Biol Chem. 2001;276:18384–91.
77. Keller SH, Lindstrom J, Taylor P. Involvement of the chaperone protein calnexin and the acetylcholine receptor β-subunit in the assembly and cell surface expression of the receptor. J Biol Chem. 1996;271:22871–7.

78. Keller SH, Taylor P. Determinants responsible for assembly of the nicotinic acetylcholine receptor. J Gen Physiol. 1999;113:171–6.
79. Wang JM, Zhang L, Yao Y, Viroonchatapan N, Rothe E, Wang ZZ. A transmembrane motif governs the surface trafficking of nicotinic acetylcholine receptors. Nat Neurosci. 2002;5:963–70.
80. Fenster CP, Beckman ML, Parker JC, Sheffield EB, Whitworth TL, Quick MW, et al. Regulation of α4β2 nicotinic receptor desensitization by calcium and protein kinase C. Mol Pharmacol. 1999;55:432–43.
81. Miles K, Huganir RL. Regulation of nicotinic acetylcholine receptors by protein phosphorylation. Mol Neurobiol. 1988;2:91–124.
82. Williams BM, Temburni MK, Levey MS, Bertrand S, Bertrand D, Jacob MH. The long internal loop of the α 3 subunit targets nAChRs to subdomains within individual synapses on neurons in vivo. Nat Neurosci. 1998;1:557–62.
83. Bouzat C, Bren N, Sine SM. Structural basis of the different gating kinetics of fetal and adult acetylcholine receptors. Neuron. 1994;13:1395–402.
84. Hales TG, Dunlop JI, Deeb TZ, Carland JE, Kelley SP, Lambert JJ, et al. Common determinants of single channel conductance within the large cytoplasmic loop of 5-hydroxytryptamine type 3 and α4β2 nicotinic acetylcholine receptors. J Biol Chem. 2006;281:8062–71.
85. Curtis L, Buisson B, Bertrand S, Bertrand D. Potentiation of human α4β2 neuronal nicotinic acetylcholine receptor by estradiol. Mol Pharmacol. 2002;61:127–35.
86. Paradiso K, Zhang J, Steinbach JH. The C terminus of the human nicotinic α4β2 receptor forms a binding site required for potentiation by an estrogenic steroid. J Neurosci. 2001;21:6561–8.
87. Le Novere N, Changeux JP. Molecular evolution of the nicotinic acetylcholine receptor: an example of multigene family in excitable cells. J Mol Evol. 1995;40:155–72.
88. Duvoisin RM, Deneris ES, Patrick J, Heinemann S. The functional diversity of the neuronal nicotinic acetylcholine receptors is increased by a novel subunit: β 4. Neuron. 1989;3:487–96.
89. Couturier S, Bertrand D, Matter JM, Hernandez MC, Bertrand S, Millar N, et al. A neuronal nicotinic acetylcholine receptor subunit (α 7) is developmentally regulated and forms a homo-oligomeric channel blocked by α-BTX. Neuron. 1990;5:847–56.
90. Anand R, Conroy WG, Schoepfer R, Whiting P, Lindstrom J. Neuronal nicotinic acetylcholine receptors expressed in Xenopus oocytes have a pentameric quaternary structure. J Biol Chem. 1991;266:11192–8.
91. Cooper E, Couturier S, Ballivet M. Pentameric structure and subunit stoichiometry of a neuronal nicotinic acetylcholine receptor. Nature. 1991;350:235–8.
92. Boorman JP, Groot-Kormelink PJ, Sivilotti LG. Stoichiometry of human recombinant neuronal nicotinic receptors containing the β3 subunit expressed in Xenopus oocytes. J Physiol. 2000;529(Pt 3):565–77.
93. Kuryatov A, Olale F, Cooper J, Choi C, Lindstrom J. Human α6 AChR subtypes: subunit composition, assembly, and pharmacological responses. Neuropharmacology. 2000;39:2570–90.
94. Harpsoe K, Ahring PK, Christensen JK, Jensen ML, Peters D, Balle T. Unraveling the high- and low-sensitivity agonist responses of nicotinic acetylcholine receptors. J Neurosci. 2011;31:10759–66.
95. Mazzaferro S, Benallegue N, Carbone A, Gasparri F, Vijayan R, Biggin PC, et al. Additional acetylcholine (ACh) binding site at α4/α4 interface of (α4β2)2α4 nicotinic receptor influences agonist sensitivity. J Biol Chem. 2011;286:31043–54.
96. Jain A, Kuryatov A, Wang J, Kamenecka TM, Lindstrom J. Unorthodox acetylcholine binding sites formed by α5 and β3 accessory subunits in α4β2* nicotinic acetylcholine receptors. J Biol Chem. 2016;291:23452–63.
97. Jin X, Bermudez I, Steinbach JH. The nicotinic α5 subunit can replace either an acetylcholine-binding or nonbinding subunit in the α4β2* neuronal nicotinic receptor. Mol Pharmacol. 2014;85:11–7.
98. Wang J, Kuryatov A, Sriram A, Jin Z, Kamenecka TM, Kenny PJ, et al. An accessory agonist binding site promotes activation of α4β2* nicotinic acetylcholine receptors. J Biol Chem. 2015;290:13907–18.
99. Kuryatov A, Luo J, Cooper J, Lindstrom J. Nicotine acts as a pharmacological chaperone to upregulate human α4β2 acetylcholine receptors. Mol Pharmacol. 2005;68:1839–51.
100. Nelson ME, Kuryatov A, Choi CH, Zhou Y, Lindstrom J. Alternate stoichiometries of α4β2 nicotinic acetylcholine receptors. Mol Pharmacol. 2003;63:332–41.
101. Zhou Y, Nelson ME, Kuryatov A, Choi C, Cooper J, Lindstrom J. Human α4β2 acetylcholine receptors formed from linked subunits. J Neurosci. 2003;23:9004–15.
102. Wu J, Liu Q, Tang P, Mikkelsen JD, Shen J, Whiteaker P, Yakel JL. Heteromeric α7β2 nicotinic acetylcholine receptors in the brain. Trends Pharmacol Sci. 2016;37:562–74.
103. Schoepfer R, Conroy WG, Whiting P, Gore M, Lindstrom J. Brain α-bungarotoxin binding protein cDNAs and MAbs reveal subtypes of this branch of the ligand-gated ion channel gene superfamily. Neuron. 1990;5:35–48.
104. Elgoyhen AB, Vetter DE, Katz E, Rothlin CV, Heinemann SF, Boulter J. α10: a determinant of nicotinic cholinergic receptor function in mammalian vestibular and cochlear mechanosensory hair cells. Proc Natl Acad Sci U S A. 2001;98:3501–6.
105. Lustig LR, Peng H, Hiel H, Yamamoto T, Fuchs PA. Molecular cloning and mapping of the human nicotinic acetylcholine receptor α10 (CHRNA10). Genomics. 2001;73:272–83.

106. Forsayeth JR, Kobrin E. Formation of oligomers containing the β3 and β4 subunits of the rat nicotinic receptor. J Neurosci. 1997;17:1531–8.
107. Fucile S, Barabino B, Palma E, Grassi F, Limatola C, Mileo AM, et al. α5 Subunit forms functional α3 β4 α5 nAChRs in transfected human cells. Neuroreport. 1997;8:2433–6.
108. Groot-Kormelink PJ, Luyten WH, Colquhoun D, Sivilotti LG. A reporter mutation approach shows incorporation of the "orphan" subunit β3 into a functional nicotinic receptor. J Biol Chem. 1998;273:15317–20.
109. Vailati S, Hanke W, Bejan A, Barabino B, Longhi R, Balestra B, et al. Functional α6-containing nicotinic receptors are present in chick retina. Mol Pharmacol. 1999;56:11–9.
110. Wang F, Gerzanich V, Wells GB, Anand R, Peng X, Keyser K, et al. Assembly of human neuronal nicotinic receptor α5 subunits with α3, β2, and β4 subunits. J Biol Chem. 1996;271:17656–65.
111. Conroy WG, Berg DK. Neurons can maintain multiple classes of nicotinic acetylcholine receptors distinguished by different subunit compositions. J Biol Chem. 1995;270:4424–31.
112. Gotti C, Moretti M, Zanardi A, Gaimarri A, Champtiaux N, Changeux JP, et al. Heterogeneity and selective targeting of neuronal nicotinic acetylcholine receptor (nAChR) subtypes expressed on retinal afferents of the superior colliculus and lateral geniculate nucleus: identification of a new native nAChR subtype α3β2(α5 or β3) enriched in retinocollicular afferents. Mol Pharmacol. 2005;68:1162–71.
113. Lena C, de Kerchove D'Exaerde A, Cordero-Erausquin M, Le Novere N, del Mar Arroyo-Jimenez M, et al. Diversity and distribution of nicotinic acetylcholine receptors in the locus ceruleus neurons. Proc Natl Acad Sci U S A. 1999;96:12126–31.
114. Zoli M, Moretti M, Zanardi A, McIntosh JM, Clementi F, Gotti C. Identification of the nicotinic receptor subtypes expressed on dopaminergic terminals in the rat striatum. J Neurosci. 2002;22:8785–9.
115. Nelson ME, Wang F, Kuryatov A, Choi CH, Gerzanich V, Lindstrom J. Functional properties of human nicotinic AChRs expressed by IMR-32 neuroblastoma cells resemble those of α3β4 AChRs expressed in permanently transfected HEK cells. J Gen Physiol. 2001;118:563–82.
116. Shoop RD, Chang KT, Ellisman MH, Berg DK. Synaptically driven calcium transients via nicotinic receptors on somatic spines. J Neurosci. 2001;21:771–81.
117. Klink R, de Kerchove d'Exaerde A, Zoli M, Changeux JP. Molecular and physiological diversity of nicotinic acetylcholine receptors in the midbrain dopaminergic nuclei. J Neurosci. 2001;21:1452–63.
118. Ramirez-Latorre J, Yu CR, Qu X, Perin F, Karlin A, Role L. Functional contributions of α5 subunit to neuronal acetylcholine receptor channels. Nature. 1996;380:347–51.
119. Gerzanich V, Wang F, Kuryatov A, Lindstrom J. α 5 Subunit alters desensitization, pharmacology, Ca^{++} permeability and Ca^{++} modulation of human neuronal α 3 nicotinic receptors. J Pharmacol Exp Ther. 1998;286:311–20.
120. Tsetlin VI, Hucho F. Snake and snail toxins acting on nicotinic acetylcholine receptors: fundamental aspects and medical applications. FEBS Lett. 2004;557:9–13.
121. Ibanez-Tallon I, Miwa JM, Wang HL, Adams NC, Crabtree GW, Sine SM, et al. Novel modulation of neuronal nicotinic acetylcholine receptors by association with the endogenous prototoxin lynx1. Neuron. 2002;33:893–903.
122. Chimienti F, Hogg RC, Plantard L, Lehmann C, Brakch N, Fischer J, et al. Identification of SLURP-1 as an epidermal neuromodulator explains the clinical phenotype of Mal de Meleda. Hum Mol Genet. 2003;12:3017–24.
123. Arredondo J, Chernyavsky AI, Webber RJ, Grando SA. Biological effects of SLURP-1 on human keratinocytes. J Invest Dermatol. 2005;125:1236–41.
124. Arredondo J, Chernyavsky AI, Jolkovsky DL, Webber RJ, Grando SA. SLURP-2: a novel cholinergic signaling peptide in human mucocutaneous epithelium. J Cell Physiol. 2006;208:238–45.
125. Jones AK, Sattelle DB. Functional genomics of the nicotinic acetylcholine receptor gene family of the nematode, Caenorhabditis elegans. BioEssays. 2004;26:39–49.
126. Jones AK, Elgar G, Sattelle DB. The nicotinic acetylcholine receptor gene family of the pufferfish, Fugu rubripes. Genomics. 2003;82:441–51.
127. Bocquet N, Prado de Carvalho L, Cartaud J, Neyton J, Le Poupon C, Taly A, et al. A prokaryotic proton-gated ion channel from the nicotinic acetylcholine receptor family. Nature. 2007;445:116–9.
128. Hilf RJ, Dutzler R. X-ray structure of a prokaryotic pentameric ligand-gated ion channel. Nature. 2008;452:375–9.
129. Hilf RJ, Dutzler R. Structure of a potentially open state of a proton-activated pentameric ligand-gated ion channel. Nature. 2009;457:115–8.
130. Basak S, Schmandt N, Gicheru Y, Chakrapani S. Crystal structure and dynamics of a lipid-induced potential desensitized-state of a pentameric ligand-gated channel. Elife. 2017;6:6.
131. Pan J, Chen Q, Willenbring D, Mowrey D, Kong XP, Cohen A, et al. Structure of the pentameric ligand-gated ion channel GLIC bound with anesthetic ketamine. Structure. 2012;20:1463–9.
132. Bohler S, Gay S, Bertrand S, Corringer PJ, Edelstein SJ, Changeux JP, et al. Desensitization of neuronal nicotinic acetylcholine receptors conferred by N-terminal segments of the β 2 subunit. Biochemistry. 2001;40:2066–74.
133. Prince RJ, Sine SM. Acetylcholine and epibatidine binding to muscle acetylcholine receptors distinguish between concerted and uncoupled models. J Biol Chem. 1999;274:19623–9.

134. Kuffler SW, Yoshikami D. The number of transmitter molecules in a quantum: an estimate from iontophoretic application of acetylcholine at the neuromuscular synapse. J Physiol. 1975;251:465–82.
135. Steinbach JH, Chen Q. Antagonist and partial agonist actions of d-tubocurarine at mammalian muscle acetylcholine receptors. J Neurosci. 1995;15:230–40.
136. Malany S, Osaka H, Sine SM, Taylor P. Orientation of α-neurotoxin at the subunit interfaces of the nicotinic acetylcholine receptor. Biochemistry. 2000;39:15388–98.
137. Magleby KL. Neuromuscular transmission. In: Engel AG, Franzini-Armstrong C, editors. Myology: basic and clinical, vol. 1994. 2nd ed. New York: McGraw-Hill; 1994. p. 442–63.
138. Gunderson CH, Lehmann CR, Sidell FR, Jabbari B. Nerve agents: a review. Neurology. 1992;42:946–50.
139. Benowitz NL. Pharmacology of nicotine: addiction and therapeutics. Annu Rev Pharmacol Toxicol. 1996;36:597–613.
140. Dani JA, Ji D, Zhou FM. Synaptic plasticity and nicotine addiction. Neuron. 2001;31:349–52.
141. Picciotto MR, Caldarone BJ, King SL, Zachariou V. Nicotinic receptors in the brain. Links between molecular biology and behavior. Neuropsychopharmacology. 2000;22:451–65.
142. Kopta C, Steinbach JH. Comparison of mammalian adult and fetal nicotinic acetylcholine receptors stably expressed in fibroblasts. J Neurosci. 1994;14:3922–33.
143. Papke RL, Meyer E, Nutter T, Uteshev VV. α7 receptor-selective agonists and modes of α7 receptor activation. Eur J Pharmacol. 2000;393:179–95.
144. Corringer PJ, Le Novere N, Changeux JP. Nicotinic receptors at the amino acid level. Annu Rev Pharmacol Toxicol. 2000;40:431–58.
145. Miyazawa A, Fujiyoshi Y, Unwin N. Structure and gating mechanism of the acetylcholine receptor pore. Nature. 2003;423:949–55.
146. Froehner SC. Identification of exposed and buried determinants of the membrane-bound acetylcholine receptor from Torpedo californica. Biochemistry. 1981;20:4905–15.
147. Tzartos SJ, Seybold ME, Lindstrom JM. Specificities of antibodies to acetylcholine receptors in sera from myasthenia gravis patients measured by monoclonal antibodies. Proc Natl Acad Sci U S A. 1982;79:188–92.
148. Luo J, Taylor P, Losen M, de Baets MH, Shelton GD, Lindstrom J. Main immunogenic region structure promotes binding of conformation-dependent myasthenia gravis autoantibodies, nicotinic acetylcholine receptor conformation maturation, and agonist sensitivity. J Neurosci. 2009;29:13898–908.
149. Lindstrom J, Luo J, Kuryatov A. Myasthenia gravis and the tops and bottoms of AChRs: antigenic structure of the MIR and specific immunosuppression of EAMG using AChR cytoplasmic domains. Ann N Y Acad Sci. 2008;1132:29–41.
150. Saedi MS, Anand R, Conroy WG, Lindstrom J. Determination of amino acids critical to the main immunogenic region of intact acetylcholine receptors by in vitro mutagenesis. FEBS Lett. 1990;267:55–9.
151. Luo J, Lindstrom J. Antigenic structure of the human muscle nicotinic acetylcholine receptor main immunogenic region. J Mol Neurosci. 2010;40:217–20.
152. Noridomi K, Watanabe G, Hansen MN, Han GW, Chen L. Structural insights into the molecular mechanisms of myasthenia gravis and their therapeutic implications. Elife. 2017;25:6.
153. Lindstrom J, Shelton D, Fujii Y. Myasthenia gravis. Adv Immunol. 1988;42:233–84.
154. Lindstrom J. An assay for antibodies to human acetylcholine receptor in serum from patients with myasthenia gravis. Clin Immunol Immunopathol. 1977;7:36–43.
155. Engel AG. Myasthenia gravis and myasthenic disorders. New York: Oxford University Press; 2012.
156. Lindstrom J, Luo J. Myasthenogenicity of the main immunogenic region. Ann N Y Acad Sci. 2012;1274:9–13.
157. Luo J, Lindstrom J. Myasthenogenicity of the main immunogenic region and endogenous muscle nicotinic acetylcholine receptors. Autoimmunity. 2012;45:245–52.
158. Drachman DB. The biology of myasthenia gravis. Ann Rev Neurosci. 1981;4:195–225.
159. Drachman DB, Angus CW, Adams RN, Michelson JD, Hoffman GJ. Myasthenic antibodies cross-link acetylcholine receptors to accelerate degradation. N Engl J Med. 1978;298:1116–22.
160. Heinemann S, Bevan S, Kullberg R, Lindstrom J, Rice J. Modulation of acetylcholine receptor by antibody against the receptor. Proc Natl Acad Sci U S A. 1977;74:3090–4.
161. Blatt Y, Montal MS, Lindstrom JM, Montal M. Monoclonal antibodies specific to the β and γ subunits of the Torpedo acetylcholine receptor inhibit single-channel activity. J Neurosci. 1986;6:481–6.
162. Criado M, Mulet J, Castillo M, Gerber S, Sala S, Sala F. The loop between β-strands β 2 and β 3 and its interaction with the N-terminal α-helix is essential for biogenesis of α 7 nicotinic receptors. J Neurochem. 2010;112:103–11.
163. Shelton GD, Cardinet GH, Lindstrom JM. Canine and human myasthenia gravis autoantibodies recognize similar regions on the acetylcholine receptor. Neurology. 1988;38:1417–23.
164. Weinberg CB, Hall ZW. Antibodies from patients with myasthenia gravis recognize determinants unique to extrajunctional acetylcholine receptors. Proc Natl Acad Sci U S A. 1979;76:504–8.
165. Burges J, Wray DW, Pizzighella S, Hall Z, Vincent A. A myasthenia gravis plasma immunoglobulin reduces miniature endplate potentials at human endplates in vitro. Muscle Nerve. 1990;13:407–13.
166. Vincent A, Newland C, Brueton L, Beeson D, Riemersma S, Huson SM, et al. Arthrogryposis mul-

tiplex congenita with maternal autoantibodies specific for a fetal antigen. Lancet. 1995;346:24–5.
167. Beeson D, Bond AP, Corlett L, Curnow SJ, Hill ME, Jacobson LW, et al. Thymus, thymoma, and specific T cells in myasthenia gravis. Ann N Y Acad Sci. 1998;841:371–87.
168. Conti-Fine BM, Navaneetham D, Karachunski PI, Raju R, Diethelm-Okita B, Okita D, et al. T cell recognition of the acetylcholine receptor in myasthenia gravis. Ann N Y Acad Sci. 1998;841:283–308.
169. Fujii Y, Lindstrom J. Specificity of the T cell immune response to acetylcholine receptor in experimental autoimmune myasthenia gravis. Response to subunits and synthetic peptides. J Immunol. 1988;140:1830–7.
170. Hohlfeld R, Wekerle H. The immunopathogenesis of myasthenia gravis. In: Engel AG, editor. Myasthenia gravis and myasthenic disorders. New York: Oxford University Press; 1999. p. 87–104.
171. Raju R, Spack EG, David CS. Acetylcholine receptor peptide recognition in HLA DR3-transgenic mice: in vivo responses correlate with MHC-peptide binding. J Immunol. 2001;167:1118–24.
172. Ong B, Willcox N, Wordsworth P, Beeson D, Vincent A, Altmann D, et al. Critical role for the Val/Gly86 HLA-DR β dimorphism in autoantigen presentation to human T cells. Proc Natl Acad Sci U S A. 1991;88:7343–7.
173. Christadoss P, Lindstrom JM, Melvold RW, Talal N. Mutation at I-A β chain prevents experimental autoimmune myasthenia gravis. Immunogenetics. 1985;21:33–8.
174. Christadoss P, Poussin M, Deng C. Animal models of myasthenia gravis. Clin Immunol. 2000;94:75–87.
175. Hague DW, Humphries HD, Mitchell MA, Shelton GD. Risk factors and outcomes in cats with acquired myasthenia gravis (2001-2012). J Vet Intern Med. 2015;29:1307–12.
176. Shelton GD, Lindstrom JM. Spontaneous remission in canine myasthenia gravis: implications for assessing human MG therapies. Neurology. 2001;57:2139–41.
177. Greco M, Cofano P, Lobreglio G. Seropositivity for West Nile virus antibodies in patients affected by myasthenia gravis. J Clin Med Res. 2016;8:196–201.
178. Leis AA, Szatmary G, Ross MA, Stokic DS. West Nile virus infection and myasthenia gravis. Muscle Nerve. 2014;49:26–9.
179. Molko N, Simon O, Guyon D, Biron A, Dupont-Rouzeyrol M, Gourinat AC. Zika virus infection and myasthenia gravis: report of 2 cases. Neurology. 2017;88:1097–8.
180. Cavalcante P, Maggi L, Colleoni L, Caldara R, Motta T, Giardina C, Antozzi C, Berrih-Aknin S, Bernasconi P, Mantegazza R. Inflammation and Epstein-Barr virus infection are common features of myasthenia gravis thymus: possible roles in pathogenesis. Autoimmune Dis. 2011;2011:213092.
181. Cavalcante P, Serafini B, Rosicarelli B, Maggi L, Barberis M, Antozzi C, et al. Epstein-Barr virus persistence and reactivation in myasthenia gravis thymus. Ann Neurol. 2010;67:726–38.
182. Ascherio A, Munger KL. 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: Epstein-Barr virus and multiple sclerosis: epidemiological evidence. Clin Exp Immunol. 2010;160:120–4.
183. Fraser KB, Haire M, Millar JH, McCrea S. Increased tendency to spontaneous in-vitro lymphocyte transformation in clinically active multiple sclerosis. Lancet. 1979;2:175–6.
184. Sumaya CV, Myers LW, Ellison GW, Ench Y. Increased prevalence and titer of Epstein-Barr virus antibodies in patients with multiple sclerosis. Ann Neurol. 1985;17:371–7.
185. Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med. 2003;349:1526–33.
186. James JA, Kaufman KM, Farris AD, Taylor-Albert E, Lehman TJ, Harley JB. An increased prevalence of Epstein-Barr virus infection in young patients suggests a possible etiology for systemic lupus erythematosus. J Clin Invest. 1997;100:3019–26.
187. Zandman-Goddard G, Berkun Y, Barzilai O, Boaz M, Blank M, Ram M, et al. Exposure to Epstein-Barr virus infection is associated with mild systemic lupus erythematosus disease. Ann N Y Acad Sci. 2009;1173:658–63.
188. Ferrell PB, Aitcheson CT, Pearson GR, Tan EM. Seroepidemiological study of relationships between Epstein-Barr virus and rheumatoid arthritis. J Clin Invest. 1981;67:681–7.
189. Klatt T, Ouyang Q, Flad T, Koetter I, Buhring HJ, Kalbacher H, et al. Expansion of peripheral $CD8^+$ $CD28^-$ T cells in response to Epstein-Barr virus in patients with rheumatoid arthritis. J Rheumatol. 2005;32:239–51.
190. Fust G. The role of the Epstein-Barr virus in the pathogenesis of some autoimmune disorders – similarities and differences. Eur J Microbiol Immunol (Bp). 2011;1:267–78.
191. Russell AS, Lindstrom JM. Penicillamine-induced myasthenia gravis associated with antibodies to acetylcholine receptor. Neurology. 1978;28:847–9.
192. Vincent A, Newsom-Davis J, Martin V. Anti-acetylcholine receptor antibodies in D-penicillamine-associated myasthenia gravis. Lancet. 1978;1:1254.
193. Penn AS, Low BW, Jaffe IA, Luo L, Jacques JJ. Drug-induced autoimmune myasthenia gravis. Ann N Y Acad Sci. 1998;841:433–49.
194. Keesey J, Lindstrom J, Cokely H. Anti-acetylcholine receptor antibody in neonatal myasthenia gravis. N Engl J Med. 1977;296:55.
195. Vernet-der Garabedian B, Lacokova M, Eymard B, Morel E, Faltin M, Zajac J, et al. Association of neonatal myasthenia gravis with antibodies against the fetal acetylcholine receptor. J Clin Invest. 1994;94:555–9.

196. Luo J, Lindstrom J. Antigen-specific immunotherapeutic vaccine for experimental autoimmune myasthenia gravis. J Immunol. 2014;193:5044–55.
197. Lindstrom JM, Lennon VA, Seybold ME, Whittingham S. Experimental autoimmune myasthenia gravis and myasthenia gravis: biochemical and immunochemical aspects. Ann N Y Acad Sci. 1976;274:254–74.
198. Luo J, Lindstrom J. AChR-specific immunosuppressive therapy of myasthenia gravis. Biochem Pharmacol. 2015;97:609–19.
199. Willcox N. Thymic tumors with myasthenia gravis or bone marrow dyscrasias. In: Peckham M, Pinedo H, Veronesi U, editors. Oxford textbook of oncology, vol. 1995. Oxford: Oxford University Press; 1995. p. 1562–8.
200. Baggi F, Andreetta F, Antozzi C, Simoncini O, Confalonieri P, Labeit S, et al. Anti-titin and anti-ryanodine receptor antibodies in myasthenia gravis patients with thymoma. Ann N Y Acad Sci. 1998;841:538–41.
201. Cikes N, Momoi MY, Williams CL, Howard FM Jr, Hoagland HC, Whittingham S, et al. Striational autoantibodies: quantitative detection by enzyme immunoassay in myasthenia gravis, thymoma, and recipients of D-penicillamine or allogeneic bone marrow. Mayo Clin Proc. 1988;63:474–81.
202. Vincent A, Newsom-Davis J. Acetylcholine receptor antibody characteristics in myasthenia gravis. I. Patients with generalized myasthenia or disease restricted to ocular muscles. Clin Exp Immunol. 1982;49:257–65.
203. Wakkach A, Guyon T, Bruand C, Tzartos S, Cohen-Kaminsky S, Berrih-Aknin S. Expression of acetylcholine receptor genes in human thymic epithelial cells: implications for myasthenia gravis. J Immunol. 1996;157:3752–60.
204. Zheng Y, Wheatley LM, Liu T, Levinson AI. Acetylcholine receptor α subunit mRNA expression in human thymus: augmented expression in myasthenia gravis and upregulation by interferon-γ. Clin Immunol. 1999;91:170–7.
205. Vincent A, Lang B, Newsom-Davis J. Autoimmunity to the voltage-gated calcium channel underlies the Lambert-Eaton myasthenic syndrome, a paraneoplastic disorder. Trends Neurosci. 1989;12:496–502.
206. Lindstrom JM, Einarson BL, Lennon VA, Seybold ME. Pathological mechanisms in experimental autoimmune myasthenia gravis. I. Immunogenicity of syngeneic muscle acetylcholine receptor and quantitative extraction of receptor and antibody-receptor complexes from muscles of rats with experimental automimmune myasthenia gravis. J Exp Med. 1976;144:726–38.
207. Jermy A, Beeson D, Vincent A. Pathogenic autoimmunity to affinity-purified mouse acetylcholine receptor induced without adjuvant in BALB/c mice. Eur J Immunol. 1993;23:973–6.
208. Lindstrom J. Experimental induction and treatment of myasthenia gravis. In: Engel AG, editor. Myasthenia gravis and myasthenic disorders. New York: Oxford University Press; 1999. p. 111–30.
209. Zoda TE, Krolick KA. Antigen presentation and T cell specificity repertoire in determining responsiveness to an epitope important in experimental autoimmune myasthenia gravis. J Neuroimmunol. 1993;43:131–8.
210. Lindstrom JM, Seybold ME, Lennon VA, Whittingham S, Duane DD. Antibody to acetylcholine receptor in myasthenia gravis. Prevalence, clinical correlates, and diagnostic value. Neurology. 1976;26:1054–9.
211. Sanders DB, Burns TM, Cutter GR, Massey JM, Juel VC, Hobson-Webb L, Muscle Study Group. Does change in acetylcholine receptor antibody level correlate with clinical change in myasthenia gravis? Muscle Nerve. 2014;49:483–6.
212. Vincent A, Newsom-Davis J. Acetylcholine receptor antibody as a diagnostic test for myasthenia gravis: results in 153 validated cases and 2967 diagnostic assays. J Neurol Neurosurg Psychiatry. 1985;48:1246–52.
213. Seybold ME, Lindstrom JM. Patterns of acetylcholine receptor antibody fluctuation in myasthenia gravis. Ann N Y Acad Sci. 1981;377:292–306.
214. Lindstrom JM, Engel AG, Seybold ME, Lennon VA, Lambert EH. Pathological mechanisms in experimental autoimmune myasthenia gravis. II. Passive transfer of experimental autoimmune myasthenia gravis in rats with anti-acetylcholine recepotr antibodies. J Exp Med. 1976;144:739–53.
215. Tzartos S, Hochschwender S, Vasquez P, Lindstrom J. Passive transfer of experimental autoimmune myasthenia gravis by monoclonal antibodies to the main immunogenic region of the acetylcholine receptor. J Neuroimmunol. 1987;15:185–94.
216. Toyka KV, Birnberger KL, Anzil AP, Schlegel C, Besinger U, Struppler A. Myasthenia gravis: further electrophysiological and ultrastructural analysis of transmission failure in the mouse passive transfer model. J Neurol Neurosurg Psychiatry. 1978;41:746–53.
217. Toyka KV, Brachman DB, Pestronk A, Kao I. Myasthenia gravis: passive transfer from man to mouse. Science. 1975;190:397–9.
218. Engel AG, Sakakibara H, Sahashi K, Lindstrom JM, Lambert EH, Lennon VA. Passively transferred experimental autoimmune myasthenia gravis. Sequential and quantitative study of the motor endplate fine structure and ultrastructural localization of immune complexes (IgG and C3), and of the acetylcholine receptor. Neurology. 1979;29:179–88.
219. Engel AG, Tsujihata M, Lambert EH, Lindstrom JM, Lennon VA. Experimental autoimmune myasthenia gravis: a sequential and quantitative study of the neuromuscular junction ultrastructure and electrophysiologic correlations. J Neuropathol Exp Neurol. 1976;35:569–87.
220. Engel AG, Tsujihata M, Lindstrom JM, Lennon VA. The motor end plate in myasthenia gravis and

in experimental autoimmune myasthenia gravis. A quantitative ultrastructural study. Ann N Y Acad Sci. 1976;274:60–79.
221. Bevan S, Heinemann S, Lennon VA, Lindstrom J. Reduced muscle acetylcholine sensitivity in rats immunised with acetylcholine receptor. Nature. 1976;260:438–9.
222. Lambert EH, Lindstrom JM, Lennon VA. End-plate potentials in experimental autoimmune myasthenia gravis in rats. Ann N Y Acad Sci. 1976;274:300–18.
223. Lindstrom JM, Lambert EH. Content of acetylcholine receptor and antibodies bound to receptor in myasthenia gravis, experimental autoimmune myasthenia gravis, and Eaton-Lambert syndrome. Neurology. 1978;28:130–8.
224. Sahashi K, Engel AG, Lambert EH, Howard FM Jr. Ultrastructural localization of the terminal and lytic ninth complement component (C9) at the motor end-plate in myasthenia gravis. J Neuropathol Exp Neurol. 1980;39:160–72.
225. Sahashi K, Engel AG, Linstrom JM, Lambert EH, Lennon VA. Ultrastructural localization of immune complexes (IgG and C3) at the end-plate in experimental autoimmune myasthenia gravis. J Neuropathol Exp Neurol. 1978;37:212–23.
226. Appel SH, Anwyl R, McAdams MW, Elias S. Accelerated degradation of acetylcholine receptor from cultured rat myotubes with myasthenia gravis sera and globulins. Proc Natl Acad Sci U S A. 1977;74:2130–4.
227. Bufler J, Pitz R, Czep M, Wick M, Franke C. Purified IgG from seropositive and seronegative patients with mysasthenia gravis reversibly blocks currents through nicotinic acetylcholine receptor channels. Ann Neurol. 1998;43:458–64.
228. Burges J, Vincent A, Molenaar PC, Newsom-Davis J, Peers C, Wray D. Passive transfer of seronegative myasthenia gravis to mice. Muscle Nerve. 1994;17:1393–400.
229. Donnelly D, Mihovilovic M, Gonzalez-Ros JM, Ferragut JA, Richman D, Martinez-Carrion M. A noncholinergic site-directed monoclonal antibody can impair agonist-induced ion flux in Torpedo californica acetylcholine receptor. Proc Natl Acad Sci U S A. 1984;81:7999–8003.
230. Fels G, Plumer-Wilk R, Schreiber M, Maelicke A. A monoclonal antibody interfering with binding and response of the acetylcholine receptor. J Biol Chem. 1986;261:15746–54.
231. Gomez CM, Richman DP. Anti-acetylcholine receptor antibodies directed against the α-bungarotoxin binding site induce a unique form of experimental myasthenia. Proc Natl Acad Sci U S A. 1983;80:4089–93.
232. Lang B, Richardson G, Rees J, Vincent A, Newsom-Davis J. Plasma from myasthenia gravis patients reduces acetylcholine receptor agonist-induced Na^+ flux into TE671 cell line. J Neuroimmunol. 1988;19:141–8.
233. Lennon VA, Seybold ME, Lindstrom JM, Cochrane C, Ulevitch R. Role of complement in the pathogenesis of experimental autoimmune myasthenia gravis. J Exp Med. 1978;147:973–83.
234. Sine SM, Ohno K, Bouzat C, Auerbach A, Milone M, Pruitt JN, et al. Mutation of the acetylcholine receptor α subunit causes a slow-channel myasthenic syndrome by enhancing agonist binding affinity. Neuron. 1995;15:229–39.
235. Ohno K, Wang HL, Milone M, Bren N, Brengman JM, Nakano S, et al. Congenital myasthenic syndrome caused by decreased agonist binding affinity due to a mutation in the acetylcholine receptor ε subunit. Neuron. 1996;17:157–70.
236. Shen XM, Brengman JM, Edvardson S, Sine SM, Engel AG. Highly fatal fast-channel syndrome caused by AChR ε subunit mutation at the agonist binding site. Neurology. 2012;79:449–54.
237. Ohno K, Hutchinson DO, Milone M, Brengman JM, Bouzat C, Sine SM, et al. Congenital myasthenic syndrome caused by prolonged acetylcholine receptor channel openings due to a mutation in the M2 domain of the ε subunit. Proc Natl Acad Sci U S A. 1995;92:758–62.
238. Engel AG, Uchitel OD, Walls TJ, Nagel A, Harper CM, Bodensteiner J. Newly recognized congenital myasthenic syndrome associated with high conductance and fast closure of the acetylcholine receptor channel. Ann Neurol. 1993;34:38–47.
239. Shen XM, Brengman JM, Sine SM, Engel AG. Myasthenic syndrome AChR α C-loop mutant disrupts initiation of channel gating. J Clin Invest. 2012;122:2613–21.
240. Engel AG. Current status of the congenital myasthenic syndromes. Neuromuscul Disord. 2012;22:99–111.
241. Engel AG, Ohno K, Milone M, Wang HL, Nakano S, Bouzat C, et al. New mutations in acetylcholine receptor subunit genes reveal heterogeneity in the slow-channel congenital myasthenic syndrome. Hum Mol Genet. 1996;5:1217–27.
242. Ohno K, Anlar B, Engel AG. Congenital myasthenic syndrome caused by a mutation in the Ets-binding site of the promoter region of the acetylcholine receptor ε subunit gene. Neuromuscul Disord. 1999;9:131–5.
243. Ohno K, Anlar B, Ozdirim E, Brengman JM, DeBleecker JL, Engel AG. Myasthenic syndromes in Turkish kinships due to mutations in the acetylcholine receptor. Ann Neurol. 1998;44:234–41.
244. Ohno K, Quiram PA, Milone M, Wang HL, Harper MC, Pruitt JN II, et al. Congenital myasthenic syndromes due to heteroallelic nonsense/missense mutations in the acetylcholine receptor ε subunit gene: identification and functional characterization of six new mutations. Hum Mol Genet. 1997;6:753–66.
245. Missias AC, Mudd J, Cunningham JM, Steinbach JH, Merlie JP, Sanes JR. Deficient development and maintenance of postsynaptic specializations in

mutant mice lacking an 'adult' acetylcholine receptor subunit. Development. 1997;124:5075–86.
246. Milone M, Wang HL, Ohno K, Fukudome T, Pruitt JN, Bren N, et al. Slow-channel myasthenic syndrome caused by enhanced activation, desensitization, and agonist binding affinity attributable to mutation in the M2 domain of the acetylcholine receptor α subunit. J Neurosci. 1997;17:5651–65.
247. Wang HL, Auerbach A, Bren N, Ohno K, Engel AG, Sine SM. Mutation in the M1 domain of the acetylcholine receptor α subunit decreases the rate of agonist dissociation. J Gen Physiol. 1997;109:757–66.
248. Lo DC, Pinkham JL, Stevens CF. Role of a key cysteine residue in the gating of the acetylcholine receptor. Neuron. 1991;6:31–40.
249. Zhou M, Engel AG, Auerbach A. Serum choline activates mutant acetylcholine receptors that cause slow channel congenital myasthenic syndromes. Proc Natl Acad Sci U S A. 1999;96:10466–71.
250. Bertrand D, Devillers-Thiery A, Revah F, Galzi JL, Hussy N, Mulle C, et al. Unconventional pharmacology of a neuronal nicotinic receptor mutated in the channel domain. Proc Natl Acad Sci U S A. 1992;89:1261–5.
251. Alkondon M, Pereira EF, Eisenberg HM, Albuquerque EX. Choline and selective antagonists identify two subtypes of nicotinic acetylcholine receptors that modulate GABA release from CA1 interneurons in rat hippocampal slices. J Neurosci. 1999;19:2693–705.
252. Milone M, Wang HL, Ohno K, Prince R, Fukudome T, Shen XM, et al. Mode switching kinetics produced by a naturally occurring mutation in the cytoplasmic loop of the human acetylcholine receptor ε subunit. Neuron. 1998;20:575–88.
253. Han ZY, Le Novere N, Zoli M, Hill JA Jr, Champtiaux N, Changeux JP. Localization of nAChR subunit mRNAs in the brain of Macaca mulatta. Eur J Neurosci. 2000;12:3664–74.
254. Vailati S, Moretti M, Balestra B, McIntosh M, Clementi F, Gotti C. β3 subunit is present in different nicotinic receptor subtypes in chick retina. Eur J Pharmacol. 2000;393:23–30.
255. Gotti C, Moretti M, Clementi F, Riganti L, McIntosh JM, Collins AC, et al. Expression of nigrostriatal α 6-containing nicotinic acetylcholine receptors is selectively reduced, but not eliminated, by β 3 subunit gene deletion. Mol Pharmacol. 2005;67:2007–15.
256. Vailati S, Moretti M, Longhi R, Rovati GE, Clementi F, Gotti C. Developmental expression of heteromeric nicotinic receptor subtypes in chick retina. Mol Pharmacol. 2003;63:1329–37.
257. Moretti M, Zoli M, George AA, Lukas RJ, Pistillo F, Maskos U, Whiteaker P, Gotti C. The novel α7β2-nicotinic acetylcholine receptor subtype is expressed in mouse and human basal forebrain: biochemical and pharmacological characterization. Mol Pharmacol. 2014;86:306–17.
258. Thomsen MS, Zwart R, Ursu D, Jensen MM, Pinborg LH, Gilmour G, et al. α7 and β2 Nicotinic acetylcholine receptor subunits form heteromeric receptor complexes that are expressed in the human cortex and display distinct pharmacological properties. PLoS One. 2015;10:e0130572.
259. Wonnacott S. Presynaptic nicotinic ACh receptors. Trends Neurosci. 1997;20:92–8.
260. Xu W, Gelber S, Orr-Urtreger A, Armstrong D, Lewis RA, Ou CN, et al. Megacystis, mydriasis, and ion channel defect in mice lacking the α3 neuronal nicotinic acetylcholine receptor. Proc Natl Acad Sci U S A. 1999;96:5746–51.
261. Marubio LM, del Mar Arroyo-Jimenez M, Cordero-Erausquin M, Lena C, Le Novere N, de Kerchove d'Exaerde A, et al. Reduced antinociception in mice lacking neuronal nicotinic receptor subunits. Nature. 1999;398:805–10.
262. Ross SA, Wong JY, Clifford JJ, Kinsella A, Massalas JS, Horne MK, et al. Phenotypic characterization of an α 4 neuronal nicotinic acetylcholine receptor subunit knock-out mouse. J Neurosci. 2000;20:6431–41.
263. Labarca C, Schwarz J, Deshpande P, Schwarz S, Nowak MW, Fonck C, Nashmi R, Kofuji P, Dang H, Shi W, Fidan M, Khakh BS, Chen Z, Bowers BJ, Boulter J, Wehner JM, Lester HA. Point mutant mice with hypersensitive α 4 nicotinic receptors show dopaminergic deficits and increased anxiety. Proc Natl Acad Sci U S A. 2001;98:2786–91.
264. Fonck C, Nashmi R, Deshpande P, Damaj MI, Marks MJ, Riedel A, et al. Increased sensitivity to agonist-induced seizures, straub tail, and hippocampal theta rhythm in knock-in mice carrying hypersensitive α 4 nicotinic receptors. J Neurosci. 2003;23:2582–90.
265. Champtiaux N, Gotti C, Cordero-Erausquin M, David DJ, Przybylski C, Lena C, et al. Subunit composition of functional nicotinic receptors in dopaminergic neurons investigated with knock-out mice. J Neurosci. 2003;23:7820–9.
266. Champtiaux N, Han ZY, Bessis A, Rossi FM, Zoli M, Marubio L, et al. Distribution and pharmacology of α 6-containing nicotinic acetylcholine receptors analyzed with mutant mice. J Neurosci. 2002;22:1208–17.
267. Quik M, Vailati S, Bordia T, Kulak JM, Fan H, McIntosh JM, Clementi F, Gotti C. Subunit composition of nicotinic receptors in monkey striatum: effect of treatments with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine or L-DOPA. Mol Pharmacol. 2005;67:32–41.
268. Franceschini D, Orr-Urtreger A, Yu W, Mackey LY, Bond RA, Armstrong D, et al. Altered baroreflex responses in α7 deficient mice. Behav Brain Res. 2000;113:3–10.
269. Orr-Urtreger A, Goldner FM, Saeki M, Lorenzo I, Goldberg L, De Biasi M, et al. Mice deficient in the α7 neuronal nicotinic acetylcholine receptor lack α-bungarotoxin binding sites and hippocampal fast nicotinic currents. J Neurosci. 1997;17:9165–71.

270. Orr-Urtreger A, Broide RS, Kasten MR, Dang H, Dani JA, Beaudet AL, et al. Mice homozygous for the L250T mutation in the α7 nicotinic acetylcholine receptor show increased neuronal apoptosis and die within 1 day of birth. J Neurochem. 2000;74:2154–66.
271. Berger F, Gage FH, Vijayaraghavan S. Nicotinic receptor-induced apoptotic cell death of hippocampal progenitor cells. J Neurosci. 1998;18:6871–81.
272. Vetter DE, Liberman MC, Mann J, Barhanin J, Boulter J, Brown MC, et al. Role of α9 nicotinic ACh receptor subunits in the development and function of cochlear efferent innervation. Neuron. 1999;23:93–103.
273. Plazas PV, Katz E, Gomez-Casati ME, Bouzat C, Elgoyhen AB. Stoichiometry of the α9α10 nicotinic cholinergic receptor. J Neurosci. 2005;25:10905–12.
274. Fuchs PA. Synaptic transmission at vertebrate hair cells. Curr Opin Neurobiol. 1996;6:514–9.
275. Maskos U, Molles BE, Pons S, Besson M, Guiard BP, Guilloux JP, et al. Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. Nature. 2005;436:103–7.
276. Lena C, Popa D, Grailhe R, Escourrou P, Changeux JP, Adrien J. β2-containing nicotinic receptors contribute to the organization of sleep and regulate putative micro-arousals in mice. J Neurosci. 2004;24:5711–8.
277. Cohen G, Roux JC, Grailhe R, Malcolm G, Changeux JP, Lagercrantz H. Perinatal exposure to nicotine causes deficits associated with a loss of nicotinic receptor function. Proc Natl Acad Sci U S A. 2005;102:3817–21.
278. McCallum SE, Collins AC, Paylor R, Marks MJ. Deletion of the β 2 nicotinic acetylcholine receptor subunit alters development of tolerance to nicotine and eliminates receptor upregulation. Psychopharmacology (Berl). 2006;184:314–27.
279. Peng X, Gerzanich V, Anand R, Wang F, Lindstrom J. Chronic nicotine treatment up-regulates α3 and α7 acetylcholine receptor subtypes expressed by the human neuroblastoma cell line SH-SY5Y. Mol Pharmacol. 1997;51:776–84.
280. Peng X, Gerzanich V, Anand R, Whiting PJ, Lindstrom J. Nicotine-induced increase in neuronal nicotinic receptors results from a decrease in the rate of receptor turnover. Mol Pharmacol. 1994;46:523–30.
281. Wang F, Nelson ME, Kuryatov A, Olale F, Cooper J, Keyser K, et al. Chronic nicotine treatment up-regulates human α3 β2 but not α3 β4 acetylcholine receptors stably transfected in human embryonic kidney cells. J Biol Chem. 1998;273:28721–32.
282. Sallette J, Bohler S, Benoit P, Soudant M, Pons S, Le Novere N, et al. An extracellular protein microdomain controls up-regulation of neuronal nicotinic acetylcholine receptors by nicotine. J Biol Chem. 2004;279:18767–75.
283. Sallette J, Pons S, Devillers-Thiery A, Soudant M, Prado de Carvalho L, Changeux JP, et al. Nicotine upregulates its own receptors through enhanced intracellular maturation. Neuron. 2005;46:595–607.
284. Luther MA, Schoepfer R, Whiting P, Casey B, Blatt Y, Montal MS, et al. A muscle acetylcholine receptor is expressed in the human cerebellar medulloblastoma cell line TE671. J Neurosci. 1989;9:1082–96.
285. Marks MJ, Pauly JR, Gross SD, Deneris ES, Hermans-Borgmeyer I, Heinemann SF, et al. Nicotine binding and nicotinic receptor subunit RNA after chronic nicotine treatment. J Neurosci. 1992;12:2765–84.
286. Perry DC, Davila-Garcia MI, Stockmeier CA, Kellar KJ. Increased nicotinic receptors in brains from smokers: membrane binding and autoradiography studies. J Pharmacol Exp Ther. 1999;289:1545–52.
287. Cui C, Booker TK, Allen RS, Grady SR, Whiteaker P, Marks MJ, et al. The β3 nicotinic receptor subunit: a component of α-conotoxin MII-binding nicotinic acetylcholine receptors that modulate dopamine release and related behaviors. J Neurosci. 2003;23:11045–53.
288. Xu W, Orr-Urtreger A, Nigro F, Gelber S, Sutcliffe CB, Armstrong D, et al. Multiorgan autonomic dysfunction in mice lacking the β2 and the β4 subunits of neuronal nicotinic acetylcholine receptors. J Neurosci. 1999;19:9298–305.
289. Balestra B, Moretti M, Longhi R, Mantegazza R, Clementi F, Gotti C. Antibodies against neuronal nicotinic receptor subtypes in neurological disorders. J Neuroimmunol. 2000;102:89–97.
290. Wang Z, Low PA, Vernino S. Antibody-mediated impairment and homeostatic plasticity of autonomic ganglionic synaptic transmission. Exp Neurol. 2010;222:114–9.
291. Wang Z, Low PA, Jordan J, Freeman R, Gibbons CH, Schroeder C, et al. Autoimmune autonomic ganglionopathy: IgG effects on ganglionic acetylcholine receptor current. Neurology. 2007;68:1917–21.
292. Schroeder C, Vernino S, Birkenfeld AL, Tank J, Heusser K, Lipp A, et al. Plasma exchange for primary autoimmune autonomic failure. N Engl J Med. 2005;353:1585–90.
293. Lennon VA, Ermilov LG, Szurszewski JH, Vernino S. Immunization with neuronal nicotinic acetylcholine receptor induces neurological autoimmune disease. J Clin Invest. 2003;111:907–13.
294. Vernino S, Low PA, Lennon VA. Experimental autoimmune autonomic neuropathy. J Neurophysiol. 2003;90:2053–9.
295. Nguyen VT, Ndoye A, Grando SA. Pemphigus vulgaris antibody identifies pemphaxin. A novel keratinocyte annexin-like molecule binding acetylcholine. J Biol Chem. 2000;275:29466–76.
296. Takahashi Y, Mori H, Mishina M, Watanabe M, Kondo N, Shimomura J, et al. Autoantibodies and cell-mediated autoimmunity to NMDA-type GluRε2 in patients with Rasmussen's encephalitis and chronic progressive epilepsia partialis continua. Epilepsia. 2005;46(Suppl 5):152–8.

297. Watson R, Jepson JE, Bermudez I, Alexander S, Hart Y, McKnight K, et al. α7-acetylcholine receptor antibodies in two patients with Rasmussen encephalitis. Neurology. 2005;65:1802–4.
298. Dalmau J, Tuzun E, Wu HY, Masjuan J, Rossi JE, Voloschin A, et al. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. Ann Neurol. 2007;61:25–36.
299. Petit-Pedrol M, Armangue T, Peng X, Bataller L, Cellucci T, Davis R, et al. Encephalitis with refractory seizures, status epilepticus, and antibodies to the $GABA_A$ receptor: a case series, characterisation of the antigen, and analysis of the effects of antibodies. Lancet Neurol. 2014;13:276–86.
300. Lancaster E, Lai M, Peng X, Hughes E, Constantinescu R, Raizer J, et al. Antibodies to the $GABA_B$ receptor in limbic encephalitis with seizures: case series and characterisation of the antigen. Lancet Neurol. 2010;9:67–76.
301. Hutchinson M, Waters P, McHugh J, Gorman G, O'Riordan S, Connolly S, et al. Progressive encephalomyelitis, rigidity, and myoclonus: a novel glycine receptor antibody. Neurology. 2008;71:1291–2.
302. Irani SR, Alexander S, Waters P, Kleopa KA, Pettingill P, Zuliani L, et al. Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactivated 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan's syndrome and acquired neuromyotonia. Brain. 2010;133:2734–48.
303. Baker SK, Morillo C, Vernino S. Autoimmune autonomic ganglionopathy with late-onset encephalopathy. Auton Neurosci. 2009;146:29–32.
304. De Fusco M, Becchetti A, Patrignani A, Annesi G, Gambardella A, Quattrone A, et al. The nicotinic receptor β2 subunit is mutant in nocturnal frontal lobe epilepsy. Nat Genet. 2000;26:275–6.
305. Phillips HA, Favre I, Kirkpatrick M, Zuberi SM, Goudie D, Heron SE, et al. CHRNB2 is the second acetylcholine receptor subunit associated with autosomal dominant nocturnal frontal lobe epilepsy. Am J Hum Genet. 2001;68:225–31.
306. Kuryatov A, Gerzanich V, Nelson M, Olale F, Lindstrom J. Mutation causing autosomal dominant nocturnal frontal lobe epilepsy alters Ca^{2+} permeability, conductance, and gating of human α4β2 nicotinic acetylcholine receptors. J Neurosci. 1997;17:9035–47.
307. Alkondon M, Pereira EF, Eisenberg HM, Albuquerque EX. Nicotinic receptor activation in human cerebral cortical interneurons: a mechanism for inhibition and disinhibition of neuronal networks. J Neurosci. 2000;20:66–75.
308. Rodrigues-Pinguet NO, Pinguet TJ, Figl A, Lester HA, Cohen BN. Mutations linked to autosomal dominant nocturnal frontal lobe epilepsy affect allosteric Ca^{2+} activation of the α 4 β 2 nicotinic acetylcholine receptor. Mol Pharmacol. 2005;68:487–501.
309. Lev-Lehman E, Bercovich D, Xu W, Stockton DW, Beaudet AL. Characterization of the human β4 nAChR gene and polymorphisms in CHRNA3 and CHRNB4. J Hum Genet. 2001;46:362–6.
310. Richardson CE, Morgan JM, Jasani B, Green JT, Rhodes J, Williams GT, et al. Megacystis-microcolon-intestinal hypoperistalsis syndrome and the absence of the α3 nicotinic acetylcholine receptor subunit. Gastroenterology. 2001;121:350–7.
311. Sabatelli M, Eusebi F, Al-Chalabi A, Conte A, Madia F, Luigetti M, et al. Rare missense variants of neuronal nicotinic acetylcholine receptor altering receptor function are associated with sporadic amyotrophic lateral sclerosis. Hum Mol Genet. 2009;18:3997–4006.

3 Immunopathogenesis of Myasthenia Gravis

Rozen Le Panse and Sonia Berrih-Aknin

Introduction

Autoimmune myasthenia gravis (MG) is due to pathogenic antibodies targeting molecules of the neuromuscular junction. MG is characterized by an abnormal fatigability, and the clinical symptoms are fluctuating with a course of remissions and relapses. The manifestations are often ocular at the onset, but all muscles could be affected.

The first evidence that the acetylcholine receptor (AChR) was involved in the disease was demonstrated by the development of muscle weakness in rabbits immunized with AChRs purified from torpedo fish [1]. The main implication of the AChR was demonstrated by the finding of a reduced number of AChRs on the muscle endplates of MG patients [2]. The target of the antibodies are AChR in more than 80% of MG patients [3, 4], muscle-specific kinase (MuSK) [5], or low-density lipoprotein receptor-related protein 4 (LRP4) [6, 7] in a minority. In this review, we will identify the subgroups of patients as AChR-MG, MuSK-MG, and LRP4-MG.

MG is a prototypic autoimmune disorder and shares many common mechanisms with other autoimmune diseases [8]. The precise etiological mechanism is unknown, but a combination of immune dysregulation, genetic background, hormones, and environmental factors takes part in the onset and progression of the disease. In AChR-MG, MuSK-MG, and LRP4-MG, the muscle is the target of the antibodies, but in AChR-MG, the thymus plays an important role as it displays frequent histological abnormalities and that thymectomy has a favorable clinical effect [9].

Heterogeneity of Myasthenia Gravis

MG classification is often based on the age of onset of symptoms, which can arise early, before the age of 50 (EOMG), or late after the age of 50 (LOMG), and the autoantibody profile. Rarely, more than one subtype of antibodies can be found in a patient [10]. The classification also takes into account the affected muscles as ocular or generalized. The heterogeneity of MG is illustrated in Fig. 3.1.

Targets of the Autoimmune Response

The autoantibodies are all directed against molecules located at the postsynaptic membrane of the neuromuscular junction (NMJ) that is a complex structure whose role is to transduce the

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© Springer International Publishing AG, part of Springer Nature 2018
H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*, Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_3

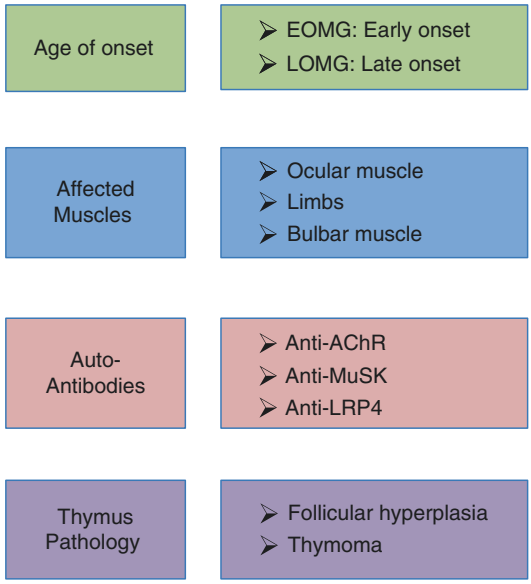

Fig. 3.1 Heterogeneity of MG diseases. The main epidemiological and clinical features of MG disease involve the age of onset, the type of affected muscles, the nature of the antibodies, and the type of thymic pathology. The age of onset could be early (defined between 40 and 50 years) or late. The disease could affect ocular muscle only or could be generalized; bulbar muscles could also be affected, thus resulting in a severe disease. Three main types of antibodies are helpful for the diagnosis. The AChR antibodies could be quantified by the regular radioimmunoassay, but in the case of a negative result, the cell-based assay could detect antibodies that recognize the clustered receptors; MuSK and LRP4 antibodies are also used for the diagnosis. The thymus is involved essentially in the form with AChR antibodies and could be hyperplastic in young female patients or a tumor in older patients

electrical impulse from the motor neuron to the skeletal muscle to allow the muscle to contract:

- AChR, the main antigen in MG, is a neurotransmitter-gated ion channel, composed of five polypeptide chains, organized in a ring around a narrow membrane pore [11]. AChRs are expressed on the muscle endplates and are clustered under the nerve terminals. When acetylcholine released by the nerve ending binds to AChR, the channel opens for the influx of sodium ions, and the subsequent endplate depolarization leads to muscle contraction [12].
- MuSK plays a major role in the development of the neuromuscular junction as mutation of this protein leads to impaired NMJ [13]. MuSK is required for the formation of AChR clusters [14].
- LRP4 forms a complex with the protein MuSK. LRP4 is the receptor for the neural agrin that can activate MuSK [15]. It has a critical role in the development of endplates since mice mutated for LRP4 die at birth of respiratory distress, as do mice with mutated forms of MuSK or agrin [15].

In support of the major role of these three proteins, mutations in AChR, MuSK, or LRP4 result in alterations of the neuromuscular transmission causing congenital myasthenias [16, 17]. Patients with manifestations of MG that do not display AChR, MuSK, or LRP4 antibodies are termed seronegative, but other potential antibody targets are searched.

Typical Features of MG Subtypes

AChR-MG

AChR is the main antigen in MG as AChR antibodies are found in 80–90% of patients. However, a significant number of MG patients have AChR antibodies not detectable by the classical immunoprecipitation assay, but identified in a cell-based assay (CBA) using cells expressing the AChR in its native configuration and clustered on the cell surface [18, 19]. Whether these antibodies have a low affinity for the solubilized receptor or recognize only the clustered AChR is not clear. It appears that the CBA is much more sensitive than the classical immunoprecipitation assay [20].

AChR-MG is associated with thymus pathology. Thymic follicular hyperplasia containing lymphoid follicles with germinal centers (GCs) is frequently found in young patients, especially women (15–40 years), while thymoma is found in a minority of patients commonly between the age of 40 and 60 years [21]. Interestingly patients with anti-clustered antibodies also display thymic hyperplasia [18]. The analysis of thymic pathologies in more than 1000 MG patients shows that women mainly have follicular hyperplasia while men have both pathologies (hyperplasia and thymoma) to a similar extent. One

main difference between the two thymic pathologies is the age of occurrence, as thymoma occurs later than follicular hyperplasia [22].

The mechanisms of action of the antibodies are well known (review in [23]). Most AChR antibodies are IgG1 and IgG3 and can bind the complement. The antibodies can damage the postsynaptic membrane by the complement pathway activation, which results in the generation of the membrane attack complex (review in [24]). The damage results in a lower density of AChR at the NMJ, associated with decreased endplate potentials. The disease severity is correlated with the loss of AChRs [25], suggesting that the loss of AChR is responsible for the clinical symptoms. Also, the postsynaptic region displays a simplification of the synaptic folds [26]. At the molecular level, the reduced expression in the AChR number is compensated by the active synthesis of the different AChR subunits [27, 28], suggesting that the muscle is not a passive target of autoimmune attack. The interpretation of these data is that the level of AChR expression at the muscle endplate is the result of its degradation by AChR antibodies and its synthesis via compensatory mechanisms.

MuSK-MG Patients

This category of patients was described for the first time in 2001 by Hoch et al. [5]. Clinical symptoms in MuSK-MG are frequently severe, and the ocular form is rare. Muscle atrophy, which is uncommon in AChR-MG patients, is frequent in MuSK-MG patients [29]. Among MG patients with generalized symptoms and without AChR antibodies, about 40% have antibodies against MuSK. The frequency of women is greater than 80% [30, 31]. Thymic follicular hyperplasia is generally not observed in this subgroup of patients [29, 32].

MuSK antibodies are of the IgG4 isotype and, therefore, do not bind the complement. The MuSK antibodies induce muscle weakness in mice [33], and the pathogenicity was shown to be due to the IgG4 but not IgG1–3 [17]. The pathogenic mechanisms include a defective clustering of the AChR [5], and the MuSK antibodies reduce the proliferation of rhabdomyosarcoma TE671 cells [34]. The effect of MuSK antibodies on the clustering of the AChR was confirmed in animal models, in which NMJs were fragmented with partial destruction of the postsynaptic muscle endplates [35]. Interestingly, MuSK antibodies seem to affect not only the postsynaptic components of the NMJ but also presynaptic molecules [29].

LRP4-MG Patients

The presence of LRP4 antibodies in MG patients were described in 2011–2012 by three groups [6, 7, 36]. LRP4-MG patients are generally young females with a mild disease. This group of patients represents approximately 20% of patients with a generalized form of MG without AChR or MuSK antibodies [6, 7]. Similarly to MuSK antibodies, LRP4 antibodies can interfere with the clustering of the AChR [7, 36]. When injected in mice, LRP4 induces myasthenic symptoms [37]. LRP4 antibodies are predominantly of the IgG1 and IgG2 subtypes and can, therefore, bind to complement [10]. Interestingly, some of the LRP4-MG patients seem to develop thymic hyperplasia [10].

Other Targets

Other molecules of the endplate appear to be the target of antibodies in MG. For example, collagen Q and agrin have been described as autoantigens [38, 39], although their specificity to MG and pathogenicity are still unknown. Also, cortactin, a protein acting downstream from LRP4/MuSK, has recently been described as a target of autoantibodies in MG. These patients frequently have a mild disease [40].

Altogether, autoantibodies to AChR, MuSK, and LRP4 are all key pathogenic factors in the different forms of MG. Although the percentage of seronegative MG patients is very low (less than 10%), there are still MG patients with unknown antibodies. Seronegative patients are still heterogeneous and may have a pure ocular or a generalized form of MG [41].

Thymus Pathology

The thymus is the principal organ of central tolerance but is not known to be altered in AIDs except in MG. In AChR-MG, the thymus dis-

plays histological changes, and thymectomy is an effective therapy [8]. The main thymic pathologies are follicular hyperplasia characterized by a large number of B cells often organized in lymphoid follicles with GCs and, thymoma, a tumor of thymic epithelial cells [42, 43].

Thymomas

Thymomas are due to the abnormal growth of epithelial cells. Ten to 20% of generalized AChR-MG patients display a thymoma that appears in patients older than 40 years of age. There is a relation between autoimmune mechanisms and thymoma development as 80% of thymoma patients have autoimmune syndromes, including mainly MG disease [44]. In thymomas associated with MG, cortical areas are generally expanded, and thymopoiesis is sustained, while the medullary area is reduced. As a result, molecules located in the medulla that are important for tolerance are deficient in thymomas. That includes FoxP3, the master gene for Treg cell function, the autoimmune regulator factor (AIRE), and the major histocompatibility complex (MHC) class II antigens (reviewed in [42] and Chap. 8).

Thymic Follicular Hyperplasia

Follicular hyperplasia is characterized by the presence of GCs (Chap. 8). Among patients with a thymic follicular hyperplasia, 80% are women. However, the sexual bias is also related to the age since the female predominance is essentially observed before the age of 40 [22]. It is clear that patients over 50 have a low risk of developing follicular hyperplasia, likely because the thymus at this age is essentially involuted [45]. Hyperplastic MG thymuses are also characterized by active neoangiogenesis processes with an ectopic development of high endothelial venules and lymphatic vessels that expressed chemokines to attract peripheral cells [46, 47].

The potential associations of thymic hyperplasia with AChR antibodies have been suggested by several findings: (1) the nature of the thymus pathology is related to the level of AChR antibodies; patients with thymic hyperplasia have the highest titers, and thymoma have intermediate titers, while involuted thymuses have the lowest titers [48]. Also, the serum level of AChR antibodies is related to the degree of hyperplasia, and most seronegative MG patients do not have GCs [22]. (2) B cells from MG thymus release in vitro AChR antibodies [49, 50]. Also thymic B cells have an activated status and do not need a step of activation to synthesize AChR antibodies [51]. (3) When MG thymic tissue or dissociated thymic cells are grafted in immunodeficient mice, AChR antibodies can be found in the serum [52, 53], and in some conditions, myasthenic symptoms appear [54].

Together, these findings support the idea that the thymus is a source of AChR antibodies. Since glucocorticoids, a common therapy in MG, reduce significantly the number of GCs [22], it is possible that the relationship between the degree of hyperplasia and the AChR antibody level is lost in corticosteroid-treated MG patients.

Effects of Thymectomy

Thymectomy has been a main treatment of MG although the evidence of its benefit (Chap. 13) was not proven until the recent multicenter, randomized trial that compared the effects of thymectomy plus prednisone with prednisone alone [55]. This trial performed over a period of 3 years shows that thymectomy associated with prednisone results in a more favorable outcome than prednisone alone. The two main outcome measures were quantitative MG score and the required prednisone dose and were both more favorable in the group which underwent thymectomy [55]. However, the results were the strongest in patients under the age of 50 years, supporting a favorable effect of thymectomy only in patients below the age of 50 years. This could be related to the pathology of the thymus that does not generally contain GCs after the age of 50 [22]. Compatible with these findings, another study with a follow-up of 123 MG

patients showed that the best prognostic factors of thymectomy are the age of the onset and duration of disease [56]. Indeed, patients with disease duration less than 12 months had significantly greater clinical improvement than those with longer disease duration, suggesting that thymic pathogenic cells could migrate to the peripheral organs.

In conclusion, the frequently observed functional and morphological changes of the thymus, the correlation between the level of AChR antibodies and the score of follicular hyperplasia, as well as the favorable clinical effects of thymectomy suggest a causal relationship between thymic pathology and MG.

Dysregulation of the Immune System

Similarly to other autoimmune diseases, MG is characterized by defects of immune cell regulation [57]. However, in MG, these defects concern not only the peripheral blood but also the thymus. We will describe here the role of B cells, the imbalance between Treg and Th17 cells, and the implication of T follicular helper cells.

The Role of B Cells

Since autoantibodies play a major pathogenic role in MG, B cells represent a main actor in the disease. Treatments based on the elimination of autoantibodies and of B cells are efficient. Plasma exchanges that result in removal of AChR antibodies induce short-term clinical improvement in MG [58]. The recent use of a monoclonal antibody against B cells, such as rituximab, is efficient, particularly in the MuSK-MG form [59].

In the thymus, the number of thymic B cells is abnormally high, and these cells are activated [51]. The increased number of B cells is likely related to the high level in the thymus of CXCL13, the main B-cell chemoattractant. Indeed CXCL13 is overexpressed in all MG subgroups, suggesting that B-cell infiltration in the thymus is not limited to the thymus with follicular hyperplasia [60]. The CXCL13 expression is also increased in the sera of glucocorticoid-untreated patients and normalized in response to treatment in correlation with clinical improvement [61]. Recently, a novel transgenic (Tg) mouse overexpressing CXCL13 in the thymus was established to mimic the thymic MG pathology. These mice are more susceptible to experimental myasthenia and develop B-cell clusters in their thymus [62].

In the periphery, the total number of B cells does not appear to be increased. However, some qualitative alterations of the B cells have been described. (1) The number of Breg cells is decreased in both AChR-MG [63] and MuSK-MG [64] patients. The frequency of B10 cells is inversely correlated with disease severity [65]. (2) The B-cell repertoire is altered. AChR-MG and MuSK-MG patients have different gene usage biases in both VH and VL sequences within the naive and memory cell subsets [66]. (3) The B-cell-activating factor (BAFF), a cytokine that plays an important role in activation, proliferation, and differentiation of B cells, is increased in the sera of AChR-MG [67, 68] and MuSK-MG [64] patients as well as in the thymus of AChR-MG patients [67, 68].

Treg and Th17 Cells

Regulatory T (Treg) cells play a central function in the control of the immune response [69]. In most autoimmune disorders, defects in the number or function of Treg cells is observed, while Th1 and Th17 cells overproduce pro-inflammatory cytokines and may be in excess [70]. The frequency of Treg cells is not altered in the thymuses of MG patients [71, 72] and the periphery [73, 74]. However the function of Treg cells is flawed in MG patients, whether measured in the thymus or the periphery [71, 75, 76]. In the thymus, conventional T (Tconv) cells are also defective as they are unable to be suppressed by normal Treg cells [77]. Using microarrays, a Th17 signature was identified in Treg cells from MG patients, while a Th1 signature was found in both Treg and Tconv cells. TNF-α likely plays a functional role in the chronic inflammation

appearing in the MG thymus [77]. It is possible that IL-17 contributes to the thymic follicular changes, as IL-17A promotes a stable interaction between B and T cells in GCs, through the upregulation of regulators of G protein signaling [78].

Follicular Helper T Cells

Follicular helper T cells provide T cell help to B cells and have a key role in GC formation. These cells express CXCR5 that is the single receptor of CXCL13 [79, 80]. CXCR5+ T cells are found in GCs as well as in peripheral blood [81], and circulating CD4+CXCR5+ T cells are described as T follicular helper cells.

In PBMC of MG patients, a high frequency of CXCR5+ CD4+ T cells was observed in correlation with the disease severity. Interestingly, the percentage of CXCR5+ CD4+ T cells decreases after therapy [82]. A recent study also shows a correlation between the frequency of these cells and the serum level of AChR Ab [83].

In the experimental model of MG (EAMG), the role of CD4+CXCR5+ was also confirmed [84], by showing a similar correlation between the frequency of CD4+CXCR5+ cells and the levels of AChR antibodies in the serum. Also, the silencing of Bcl-6 gene expression decreases the CD4+CXCR5+ cell number, IL-21 expression, as well as AChR antibody levels [84] resulting in improvement of EAMG.

Recent studies have highlighted that among follicular helper T cells expressing CXCR5, coexist the real follicular cells (Tfh) and a small subset of follicular regulatory cells (Tfr). The distinction between these two subsets is possible by the use of FoxP3 that is expressed in Tfr only. The analysis of these subsets in MG shows an imbalance in the Tfh/Tfr ratio, with a decreased Tfr number; the Tfh/Tfr ratio is linked to the disease manifestations in MG patients. In addition, the patients who received glucocorticoids displayed a normalized frequency of Tfh cells, in addition to attenuated disease activity [85, 86].

Together, these data strengthen the hypothesis of a role of Tfh cells in MG pathogenesis and highlight a link between the disease manifestations in MG patients and the disequilibrium in the Tfh/Tfr ratio.

What Are the Causes of MG?

Irrespective of the clinical form, MG is a multifactorial disease. Similarly to the other autoimmune diseases, the onset of MG is due to a combination of predisposing and triggering environmental factors, and epigenetics make the link between these two components [8]. Although the predisposing background is necessary, it is not sufficient. The main predisposing factors are the genetic, but the level of vitamin D, the microbiota, or the level of sexual hormones could also be predisposing [8]. Indeed most autoimmune diseases are more frequent in women, in which sexual hormones could alter the tolerance mechanism via the downregulation of AIRE expression [87]. In this chapter, we focus on the contribution of genetics, the role of miRNA, and among the triggering factors we will discuss the viral hypothesis.

Contribution of Genetics

The concordance rate in monozygotic twins is useful to define the contribution of the genetics in the susceptibility of MG. A study that compiled 31 pairs of monozygotic twins showed a concordance of MG in 35% of cases, while the concordance was approximately 4–5% among heterozygous twins [88], highlighting the important role of genetics in susceptibility to MG. A recent study aimed to identify novel disease-associated genes in immune cells, using the unique situation of monozygotic (MZ) twins. The analysis of the transcriptome and methylome of monocytes showed a high similarity between the MG discordant twin and his healthy twin suggesting that genetic predisposition may have a stronger contribution than previously assumed [89]. Also, several transcripts associated with immune homeostasis and inflammation resolution were dysregulated in the MG twin compared to the healthy twin, including the

TREM-1 signaling pathway, as well as EGR2 and EGR3 genes known to induce the expression of suppressor of cytokine signaling-1 (SOCS1) and SOCS3, inhibitors of STAT1 and STAT3, which mediate IFN-I antiviral and inflammatory immune responses [90, 91]. These findings suggest a novel role of the innate immune system and circulating monocytes in MG [89].

The genes most commonly associated with MG include the human leukocyte antigen (HLA) class I and class II genes [92]. In the EOMG form, HLA-B8 (MHC class I) and HLA-DR3 (MHC class II) are highly associated [93]. A GWAS association study confirmed the strongest association with HLA-B*08 [94]. In LOMG, a GWAS study revealed the most solid association with HLA-DQA1*05:01 and minimal with HLA-B*08:01, but they are both protective [95]. In MuSK-MG patients, an association with the HLA-DR14-DQ5 was observed [96].

Other susceptibility genes include genes involved in the immune responses, namely, PTPN22 that interferes with signaling in T cells, CTLA-4 that limits the excessive activation of T cells, and several cytokines such as interleukin (IL)-1β, IL-10, tumor necrosis factor (TNF)-α, and (interferon) IFN-γ [97]. In the EOMG form, a new association with the TNFAIP3-interacting protein 1 (TNIP1) confers greater risk than PTPN22 [94]. Another recent analysis in 1200 EOMG patients described association with CD86, AKAP12, VAV1, BAFF, and with the previously known TNF-α gene [97]. The α-AChR promoter has also been shown to be genetically associated with autoimmune MG [98].

The comparison of the GWAS studies in EOMG and LOMG reveals striking differences in the genetic susceptibility between the two groups. Only PTPN22 was associated with both groups. In LOMG an association with TNFRSF11A was confirmed, and a novel candidate gene, ZBTB10, was identified, while TNIP1 (associated with EOMG) showed no association in LOMG [95]. As indicated above, differences are also observed in the HLA region; HLA-B*08 is protective in LOMG, while it is strongly predisposing in the EOMG subgroup. Together, these findings highlight the requirement of subgrouping MG patients for biological and clinical studies.

Most of the genes associated with MG are linked to the regulation of the immune system. Since some specific alleles are associated with an altered level of the protein expression, it is possible that subjects with alleles associated with low immunoregulatory capacity are at highest risk to develop an autoimmune disease, particularly MG.

Role of the MicroRNA

MicroRNAs (miRNAs) are small noncoding RNAs (20–25 nucleotides length) acting as post-transcriptional regulators. They target mRNAs and lead to their degradation or the inhibition of their translation, according to the perfect or imperfect miRNA-mRNA matching, respectively [99]. miRNAs have a role in pathophysiological processes, including autoimmune diseases [100]. The implication of miRNAs in MG has been mainly investigated as potential biomarkers [101–104], but the implication of specific miRNAs on regulating gene involved in MG starts to be described. For example, serum levels of miR-20b are decreased in EOMG patients with AChR antibodies and correlate negatively with the circulating levels of IL-8 and IL-25 and a quantitative MG score [105]. A downregulation of miR-20b has also been observed in thymoma tissues and serum from patients with thymoma-associated MG in which miR-20b could act as a tumor suppressor in the development of thymoma-associated MG by inhibiting the activation of molecules involved in the NFAT signaling pathway [106]. As another example, an increased expression of miR-146a has been described in PBMCs from MG patients with AChR antibodies, as observed in other autoimmune disorders [8]. Authors suggest that miR-146 could regulate the activation of AChR-specific B cells and contribute to the pathogenesis of MG [107]. Compatible with a role of miR-146 in MG, silencing miR-146a in a mouse MG model influences B cells and ameliorates symptoms [108]. Ongoing studies investigating the implication of

miRNAs will better define their specific impact in MG and their potential interest as therapeutic targets.

Triggering Factors

Whether an infectious agent could be at the initiation of MG is still a supposition. It is well known that a viral infection results in a massive production of IFN-I [109]. The role of IFN-I has been described in MG. MG could develop after IFN-α- or IFN-β-based therapy [110–113], showing that IFN-I increases the risk of developing MG [114]. Also, the levels of IFN-I and numerous IFN-I-induced genes are increased in the thymus of MG patients [60, 115]. Among the different subtypes of IFN-I, IFN-β appears the most important. IFN-β is increased in the thymuses of MG patients and can upregulate the expression of molecules with a key role in MG: (1) the autoantigen (α-AChR) in thymic epithelial cells (TECs); (2) the chemokines CXCL13 and CCL21, which play a major role in the chemotaxis of B cells, T cells, and dendritic cells [47]; and (3) B-cell-activating factor (BAFF), which promotes the development of autoreactive B cells [68] are all upregulated by IFN-β. Finally, a link between increased expression of IFN-I and the onset of MG has been shown in a murine model treated with poly(I:C), which mimics viral double-stranded RNA (dsRNA); not only the production of thymic IFN-β was increased, but the animals developed AChR antibodies and mild clinical MG manifestations [116].

A potential scenario includes a viral infection as a first step, followed by increased production of IFN-I and IFN-II in the thymus that will result in an overexpression of chemokines, of pro-inflammatory cytokines, and of α-AChR as well as a release of α-AChR fragments due to increased TEC death [68]. B-cell chemoattractant attracts B cells from the periphery via HEV that are numerous [47]. In this context, B cells and DC in the thymus could present fragments of AChR to T cells that are activated, leading to the autoimmune response against AChR. In this thymic environment, Treg cells are not efficient, and Th17 cells are numerous, that results in ineffective immune regulation [77]. The chronicity of the autoimmune response could be explained by the inability of T cells to be suppressed [77] and by the possible continuous release of new AChR molecules because of the antibodies attack on thymic myoid cells [117].

This scenario that suggests the implication of a viral infection in MG development is supported by other findings. An association between viral infection, such as EBV, and thymic pathology has been proposed [118]. Also, other viruses, such as cytomegalovirus, human foamy virus, and Nile virus, could also be associated with MG [114, 119]. The abnormal expression of various toll-like receptors (TLRs) has been demonstrated in PBMCs from MG [107, 120] but also in MG thymuses [116, 121–123]. However, since possible triggering infection occurs a long time before the development of MG symptoms, the direct link between MG and an infection is difficult to assess. As EBV in its latent form is localized in B cells, its presence in the MG thymus could reflect the high level of B-cell infiltration in the MG thymus. However, Cavalcante et al. demonstrated the reactivation of EBV in MG thymus [122]. Is EBV playing a role in the onset of the disease, or is EBV reactivated in the inflammatory environment of the MG thymus and playing a role in the chronicity of the disease, is not yet defined.

Viral infections have not only been associated with thymic hyperplasia but also with thymoma. Indeed, patients with thymoma have high levels of IFN-I neutralizing antibodies [124]. The presence of these antibodies could be due to the high level of IFN-I produced in the thymomas. Compatible with this hypothesis, thymuses from MG patients with thymoma exhibit high levels of IFN-I, particularly IFN-α2, IFN-α8, IFN-ω, and IFN-β [125]. Altogether these findings propose that a viral infection could be responsible for the development of thymoma in MG patients.

Conclusion

Since the 1970s, MG has been investigated subsequently to the discovery of AChR autoantibodies. Several other antigenic targets have been identified, but so far the AChR-MG form

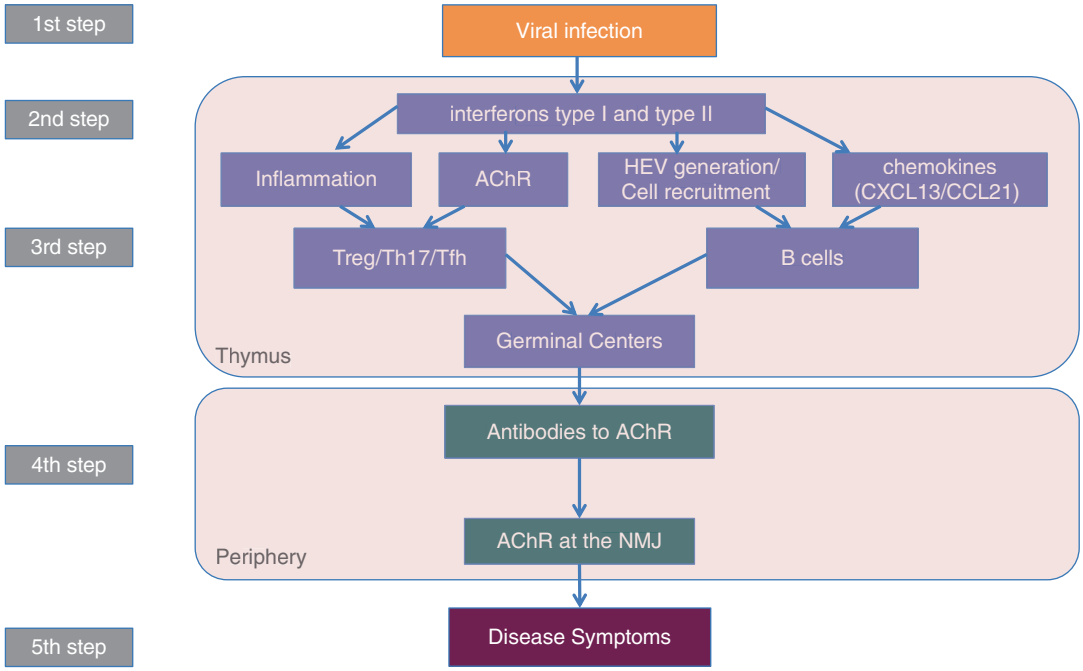

Fig. 3.2 The scenario of the pathophysiology of AChR-MG. Five main steps are described: (1) A viral infection occurs. (2) It will result in increased production of interferons type I and type II. (3) These molecules act by increasing the expression of the autoantigen (AChR), the cell recruitment, via generation of new vessels (viz., HEV), increased chemokines, and inflammation. This environment is favorable for Th17 development and defective Treg cells. Together with the increased level of chemokines, Tfh, and B cells, and in response to AChR, the germinal centers could develop and will produce AChR antibodies. (4) The antibodies will be released in the periphery where they can reach the NMJ. (5) The binding of the antibodies to the NMJ could lead to MG symptoms

is the best known. MG disease looks more heterogeneous and composite than believed. Thus a better classification for the different MG subtypes and their distinct physiopathological mechanisms have been proposed.

Broad biological investigations including transcriptomic analyses have improved our knowledge on MG and underlined new theories about the development of MG. Autoimmune mechanisms are common between autoimmune and inflammatory diseases including deficiencies in immune regulation, especially regarding the balance between Treg cells and inflammatory Th17 cells and between Tfh and Tfr cells. The contribution of other components is also strongly suspected in the autoimmunity such as the influence of the genetic background, sexual hormones, the microbiota, and the level of vitamin D. Although these components have not all been studied in MG, it is likely that they contribute to the etiological and pathological mechanisms involved in MG.

A scheme of physiopathology is shown in Fig. 3.2. A triggering factor such as an infection could result in inflammation and IFN-I production that subsequently will lead to significant changes in α-AChR expression in the thymus, as well as the overproduction of pro-inflammatory cytokines and chemokines attracting B cells. These complex interactions could result in AChR antibody production. These antibodies will then circulate to the NMJ, where they impair the neuromuscular transmission, inducing myasthenic clinical manifestations.

References

1. Patrick J, Lindstrom J. Autoimmune response to acetylcholine receptor. Science. 1973;180:871–2.
2. Fambrough DM, Drachman DB, Satyamurti S. Neuromuscular junction in myasthenia gravis: decreased acetylcholine receptors. Science. 1973;182:293–5.
3. Appel SH, Almon RR, Levy N. Acetylcholine receptor antibodies in myasthenia gravis. N Engl J Med. 1975;293:760–1.
4. Aharonov A, Abramsky O, Tarrab-Hazdai R, Fuchs S. Humoral antibodies to acetylcholine receptor in patients with myasthenia gravis. Lancet. 1975;2:340–2.
5. Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A. Auto-antibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. Nat Med. 2001;7:365–8.
6. Higuchi O, Hamuro J, Motomura M, Yamanashi Y. Autoantibodies to low-density lipoprotein receptor-related protein 4 in myasthenia gravis. Ann Neurol. 2011;69:418–22.
7. Zhang B, Tzartos JS, Belimezi M, Ragheb S, Bealmear B, Lewis RA, et al. Autoantibodies to lipoprotein-related protein 4 in patients with double-seronegative myasthenia gravis. Arch Neurol. 2012;69:445–51.
8. Berrih-Aknin S. Myasthenia Gravis: paradox versus paradigm in autoimmunity. J Autoimmun. 2014;52:1–28.
9. Berrih-Aknin S, Le Panse R. Myasthenia gravis: a comprehensive review of immune dysregulation and etiological mechanisms. J Autoimmun. 2014;52:90–100.
10. Zisimopoulou P, Evangelakou P, Tzartos J, Lazaridis K, Zouvelou V, Mantegazza R, et al. A comprehensive analysis of the epidemiology and clinical characteristics of anti-LRP4 in myasthenia gravis. J Autoimmun. 2014;52:139–45.
11. Unwin N. Nicotinic acetylcholine receptor and the structural basis of neuromuscular transmission: insights from Torpedo postsynaptic membranes. Q Rev Biophys. 2013;46:283–322.
12. Martyn JA, Fagerlund MJ, Eriksson LI. Basic principles of neuromuscular transmission. Anaesthesia. 2009;64(Suppl 1):1–9.
13. DeChiara TM, Bowen DC, Valenzuela DM, Simmons MV, Poueymirou WT, Thomas S, et al. The receptor tyrosine kinase MuSK is required for neuromuscular junction formation in vivo. Cell. 1996;85:501–12.
14. Hesser BA, Henschel O, Witzemann V. Synapse disassembly and formation of new synapses in postnatal muscle upon conditional inactivation of MuSK. Mol Cell Neurosci. 2006;31:470–80.
15. Kim N, Stiegler AL, Cameron TO, Hallock PT, Gomez AM, Huang JH, et al. Lrp4 is a receptor for Agrin and forms a complex with MuSK. Cell. 2008;135:334–42.
16. Beeson D. Congenital myasthenic syndromes: recent advances. Curr Opin Neurol. 2016;29:565–71.
17. Kucukerden M, Huda R, Tuzun E, Yilmaz A, Skriapa L, Trakas N, et al. MuSK induced experimental autoimmune myasthenia gravis does not require IgG1 antibody to MuSK. J Neuroimmunol. 2016;295–s296:84–92.
18. Leite MI, Jacob S, Viegas S, Cossins J, Clover L, Morgan BP, et al. IgG1 antibodies to acetylcholine receptors in 'seronegative' myasthenia gravis. Brain. 2008;131:1940–52.
19. Binks S, Vincent A, Palace J. Myasthenia gravis: a clinical-immunological update. J Neurol. 2016;263:826–34.
20. Hong Y, Zisimopoulou P, Trakas N, Karagiorgou K, Stergiou C, Skeie GO, et al. Multiple antibody detection in 'seronegative' myasthenia gravis patients. Eur J Neurol. 2017;24:844–50.
21. Berrih-Aknin S. Role of the thymus in autoimmune myasthenia gravis. Clin Exp Neuroimmunol. 2016;7:226–37.
22. Truffault F, de Montpreville V, Eymard B, Sharshar T, Le Panse R, Berrih-Aknin S. Thymic Germinal Centers and Corticosteroids In Myasthenia Gravis: An Immunopathological Study in 1035 cases and a critical review. Clin Rev Allergy Immunol. 2017;52:108–24.
23. Le Panse R, Berrih-Aknin S. Autoimmune myasthenia gravis: autoantibody mechanisms and new developments on immune regulation. Curr Opin Neurol. 2013;26:569–76.
24. Tuzun E, Christadoss P. Complement associated pathogenic mechanisms in myasthenia gravis. Autoimmun Rev. 2013 Jul;12(9):904–11.
25. Pestronk A, Drachman DB, Self SG. Measurement of junctional acetylcholine receptors in myasthenia gravis: clinical correlates. Muscle Nerve. 1985;8:245–51.
26. Nakano S, Engel AG. Myasthenia gravis: quantitative immunocytochemical analysis of inflammatory cells and detection of complement membrane attack complex at the end-plate in 30 patients. Neurology. 1993;43:1167–72.
27. Guyon T, Levasseur P, Truffault F, Cottin C, Gaud C, Berrih-Aknin S. Regulation of acetylcholine receptor alpha subunit variants in human myasthenia gravis. Quantification of steady-state levels of messenger RNA in muscle biopsy using the polymerase chain reaction. J Clin Invest. 1994;94:16–24.
28. Guyon T, Wakkach A, Poea S, Mouly V, Klingel-Schmitt I, Levasseur P, et al. Regulation of acetylcholine receptor gene expression in human myasthenia gravis muscles. Evidences for a compensatory mechanism triggered by receptor loss. J Clin Invest. 1998;102:249–63.
29. Evoli A, Tonali PA, Padua L, Monaco ML, Scuderi F, Batocchi AP, et al. Clinical correlates with anti-MuSK antibodies in generalized seronegative myasthenia gravis. Brain. 2003;126:2304–11.

30. Guptill JT, Sanders DB, Evoli A. Anti-MuSK antibody myasthenia gravis: clinical findings and response to treatment in two large cohorts. Muscle Nerve. 2011;44:36–40.
31. Lavrnic D, Losen M, Vujic A, De Baets M, Hajdukovic LJ, Stojanovic V, et al. The features of myasthenia gravis with autoantibodies to MuSK. J Neurol Neurosurg Psychiatry. 2005;76:1099–102.
32. Leite MI, Strobel P, Jones M, Micklem K, Moritz R, Gold R, et al. Fewer thymic changes in MuSK antibody-positive than in MuSK antibody-negative MG. Ann Neurol. 2005;57:444–8.
33. Huijbers MG, Zhang W, Klooster R, Niks EH, Friese MB, Straasheijm KR, et al. MuSK IgG4 autoantibodies cause myasthenia gravis by inhibiting binding between MuSK and Lrp4. Proc Natl Acad Sci U S A. 2013;110:20783–8.
34. Boneva N, Frenkian-Cuvelier M, Bidault J, Brenner T, Berrih-Aknin S. Major pathogenic effects of anti-MuSK antibodies in myasthenia gravis. J Neuroimmunol. 2006;177:119–31.
35. Morsch M, Reddel SW, Ghazanfari N, Toyka KV, Phillips WD. Muscle specific kinase autoantibodies cause synaptic failure through progressive wastage of postsynaptic acetylcholine receptors. Exp Neurol. 2012;237:286–95.
36. Pevzner A, Schoser B, Peters K, Cosma NC, Karakatsani A, Schalke B, et al. Anti-LRP4 autoantibodies in AChR- and MuSK-antibody-negative myasthenia gravis. J Neurol. 2012;259:427–35.
37. Shen C, Lu Y, Zhang B, Figueiredo D, Bean J, Jung J, et al. Antibodies against low-density lipoprotein receptor-related protein 4 induce myasthenia gravis. J Clin Invest. 2013;123:5190–202.
38. Cossins J, Belaya K, Zoltowska K, Koneczny I, Maxwell S, Jacobson L, et al. The search for new antigenic targets in myasthenia gravis. Ann N Y Acad Sci. 2012;1275:123–8.
39. Gasperi C, Melms A, Schoser B, Zhang Y, Meltoranta J, Risson V, et al. Anti-agrin autoantibodies in myasthenia gravis. Neurology. 2014;82:1976–83.
40. Cortes-Vicente E, Gallardo E, Martinez MA, Diaz-Manera J, Querol L, Rojas-Garcia R, et al. Clinical characteristics of patients with double-seronegative myasthenia gravis and antibodies to cortactin. JAMA Neurol. 2016;73:1099–104.
41. Jacob S, Viegas S, Leite MI, Webster R, Cossins J, Kennett R, et al. Presence and pathogenic relevance of antibodies to clustered acetylcholine receptor in ocular and generalized myasthenia gravis. Arch Neurol. 2012;69:994–1001.
42. Marx A, Pfister F, Schalke B, Saruhan-Direskeneli G, Melms A, Strobel P. The different roles of the thymus in the pathogenesis of the various myasthenia gravis subtypes. Autoimmun Rev. 2013 Jul;12(9):875–84.
43. Berrih-Aknin S, Morel E, Raimond F, Safar D, Gaud C, Binet JP, et al. The role of the thymus in myasthenia gravis: immunohistological and immunological studies in 115 cases. Ann N Y Acad Sci. 1987;505:50–70.
44. Filosso PL, Galassi C, Ruffini E, Margaritora S, Bertolaccini L, Casadio C, et al. Thymoma and the increased risk of developing extrathymic malignancies: a multicentre study. Eur J Cardiothorac Surg. 2013;44:219–24; discussion 224.
45. Bertho JM, Demarquay C, Moulian N, Van Der Meeren A, Berrih-Aknin S, Gourmelon P. Phenotypic and immunohistological analyses of the human adult thymus: evidence for an active thymus during adult life. Cell Immunol. 1997;179:30–40.
46. Berrih-Aknin S, Ruhlmann N, Bismuth J, Cizeron-Clairac G, Zelman E, Shachar I, et al. CCL21 overexpressed on lymphatic vessels drives thymic hyperplasia in myasthenia. Ann Neurol. 2009;66:521–31.
47. Weiss JM, Cufi P, Bismuth J, Eymard B, Fadel E, Berrih-Aknin S, et al. SDF-1/CXCL12 recruits B cells and antigen-presenting cells to the thymus of autoimmune myasthenia gravis patients. Immunobiology. 2013;218:373–81.
48. Berrih S, Morel E, Gaud C, Raimond F, Le Brigand H, Bach JF. Anti-AChR antibodies, thymic histology, and T cell subsets in myasthenia gravis. Neurology. 1984;34:66–71.
49. Willcox HN, Newsom-Davis J, Calder LR. Greatly increased autoantibody production in myasthenia gravis by thymocyte suspensions prepared with proteolytic enzymes. Clin Exp Immunol. 1983;54:378–86.
50. Lisak RP, Levinson AI, Zweiman B, Kornstein MJ. Antibodies to acetylcholine receptor and tetanus toxoid: in vitro synthesis by thymic lymphocytes. J Immunol. 1986;137:1221–5.
51. Leprince C, Cohen-Kaminsky S, Berrih-Aknin S, Vernet-Der Garabedian B, Treton D, Galanaud P, et al. Thymic B cells from myasthenia gravis patients are activated B cells. Phenotypic and functional analysis. J Immunol. 1990;145:2115–22.
52. Schonbeck S, Padberg F, Hohlfeld R, Wekerle H. Transplantation of thymic autoimmune microenvironment to severe combined immunodeficiency mice. A new model of myasthenia gravis. J Clin Invest. 1992;90:245–50.
53. Aissaoui A, Klingel-Schmitt I, Couderc J, Chateau D, Romagne F, Jambou F, et al. Prevention of autoimmune attack by targeting specific T-cell receptors in a severe combined immunodeficiency mouse model of myasthenia gravis. Ann Neurol. 1999;46:559–67.
54. Sudres M, Maurer M, Robinet M, Bismuth J, Truffault F, Girard D, et al. Preconditioned mesenchymal stem cells treat myasthenia gravis in a humanized preclinical model. JCI Insight. 2017;2:e89665.
55. Wolfe GI, Kaminski HJ, Aban IB, Minisman G, Kuo HC, Marx A, et al. Randomized trial of thymectomy in myasthenia gravis. N Engl J Med. 2016;375:511–22.
56. Yang J, Liu C, Li T, Li C. Prognosis of thymectomy in myasthenia gravis patients with thymus hyperplasia. Int J Neurosci. 2017;127(9):785–9.
57. Anaya JM. Common mechanisms of autoimmune diseases (the autoimmune tautology). Autoimmun Rev. 2012;11:781–4.

58. Newsom-Davis J, Wilson SG, Vincent A, Ward CD. Long-term effects of repeated plasma exchange in myasthenia gravis. Lancet. 1979;1:464–8.
59. Diaz-Manera J, Martinez-Hernandez E, Querol L, Klooster R, Rojas-Garcia R, Suarez-Calvet X, et al. Long-lasting treatment effect of rituximab in MuSK myasthenia. Neurology. 2012;78:189–93.
60. Le Panse R, Cizeron-Clairac G, Bismuth J, Berrih-Aknin S. Microarrays reveal distinct gene signatures in the thymus of seropositive and seronegative myasthenia gravis patients and the role of CC chemokine ligand 21 in thymic hyperplasia. J Immunol. 2006;177:7868–79.
61. Meraouna A, Cizeron-Clairac G, Panse RL, Bismuth J, Truffault F, Tallaksen C, et al. The chemokine CXCL13 is a key molecule in autoimmune myasthenia gravis. Blood. 2006;108:432–40.
62. Weiss JM, Robinet M, Aricha R, Cufi P, Villeret B, Lantner F, et al. Novel CXCL13 transgenic mouse: inflammation drives pathogenic effect of CXCL13 in experimental myasthenia gravis. Oncotarget. 2016;7:7550–62.
63. Karim MR, Zhang HY, Yuan J, Sun Q, Wang YF, Regulatory B. Cells in seropositive myasthenia gravis versus healthy controls. Front Neurol. 2017;8:43.
64. Guptill JT, Yi JS, Sanders DB, Guidon AC, Juel VC, Massey JM, et al. Characterization of B cells in muscle-specific kinase antibody myasthenia gravis. Neurol Neuroimmunol Neuroinflamm. 2015;2:e77.
65. Yi JS, Russo MA, Massey JM, Juel V, Hobson-Webb LD, Gable K, et al. B10 cell frequencies and suppressive capacity in myasthenia gravis are associated with disease severity. Front Neurol. 2017;8:34.
66. Vander Heiden JA, Stathopoulos P, Zhou JQ, Chen L, Gilbert TJ, Bolen CR, et al. Dysregulation of B cell repertoire formation in myasthenia gravis patients revealed through deep sequencing. J Immunol. 2017;198:1460–73.
67. Kang SY, Kang CH, Lee KH. B-cell-activating factor is elevated in serum of patients with myasthenia gravis. Muscle Nerve. 2016;54:1030–3.
68. Cufi P, Dragin N, Ruhlmann N, Weiss JM, Fadel E, Serraf A, et al. Central role of interferon-beta in thymic events leading to myasthenia gravis. J Autoimmun. 2014;52:44–52.
69. Hori S, Takahashi T, Sakaguchi S. Control of autoimmunity by naturally arising regulatory CD4+ T cells. Adv Immunol. 2003;81:331–71.
70. Bedoya SK, Lam B, Lau K, Larkin J III. Th17 cells in immunity and autoimmunity. Clin Dev Immunol. 2013;2013:986789.
71. Balandina A, Lecart S, Dartevelle P, Saoudi A, Berrih-Aknin S. Functional defect of regulatory CD4(+) CD25+ T cells in the thymus of patients with autoimmune myasthenia gravis. Blood. 2005;105:735–41.
72. Matsui N, Nakane S, Saito F, Ohigashi I, Nakagawa Y, Kurobe H, et al. Undiminished regulatory T cells in the thymus of patients with myasthenia gravis. Neurology. 2010;74:816–20.
73. Battaglia A, Di Schino C, Fattorossi A, Scambia G, Evoli A. Circulating CD4+CD25+ T regulatory and natural killer T cells in patients with myasthenia gravis: a flow cytometry study. J Biol Regul Homeost Agents. 2005;19:54–62.
74. Thiruppathi M, Rowin J, Li Jiang Q, Sheng JR, Prabhakar BS, Meriggioli MN. Functional defect in regulatory T cells in myasthenia gravis. Ann N Y Acad Sci. 2012;1274:68–76.
75. Luther C, Adamopoulou E, Stoeckle C, Brucklacher-Waldert V, Rosenkranz D, Stoltze L, et al. Prednisolone treatment induces tolerogenic dendritic cells and a regulatory milieu in myasthenia gravis patients. J Immunol. 2009;183:841–8.
76. Zhang Y, Wang HB, Chi LJ, Wang WZ. The role of FoxP3+CD4+CD25hi Tregs in the pathogenesis of myasthenia gravis. Immunol Lett. 2009;122:52–7.
77. Gradolatto A, Nazzal D, Truffault F, Bismuth J, Fadel E, Foti M, et al. Both Treg cells and Tconv cells are defective in the Myasthenia gravis thymus: roles of IL-17 and TNF-alpha. J Autoimmun. 2014;52:53–63.
78. Mountz JD, Wang JH, Xie S, Hsu HC. Cytokine regulation of B-cell migratory behavior favors formation of germinal centers in autoimmune disease. Discov Med. 2011;11:76–85.
79. Schaerli P, Willimann K, Lang AB, Lipp M, Loetscher P, Moser B. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. J Exp Med. 2000;192:1553–62.
80. Breitfeld D, Ohl L, Kremmer E, Ellwart J, Sallusto F, Lipp M, et al. Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. J Exp Med. 2000;192:1545–52.
81. Forster R, Emrich T, Kremmer E, Lipp M. Expression of the G-protein--coupled receptor BLR1 defines mature, recirculating B cells and a subset of T-helper memory cells. Blood. 1994;84:830–40.
82. Saito R, Onodera H, Tago H, Suzuki Y, Shimizu M, Matsumura Y, et al. Altered expression of chemokine receptor CXCR5 on T cells of myasthenia gravis patients. J Neuroimmunol. 2005;170:172–8.
83. Luo C, Li Y, Liu W, Feng H, Wang H, Huang X, et al. Expansion of circulating counterparts of follicular helper T cells in patients with myasthenia gravis. J Neuroimmunol. 2013;256:55–61.
84. Xin N, Fu L, Shao Z, Guo M, Zhang X, Zhang Y, et al. RNA interference targeting Bcl-6 ameliorates experimental autoimmune myasthenia gravis in mice. Mol Cell Neurosci. 2014;58:85–94.
85. Wen Y, Yang B, Lu J, Zhang J, Yang H, Li J. Imbalance of circulating CD4(+)CXCR5(+)FOXP3(+) Tfr-like cells and CD4(+)CXCR5(+)FOXP3(-) Tfh-like cells in myasthenia gravis. Neurosci Lett. 2016;630:176–82.
86. Zhang CJ, Gong Y, Zhu W, Qi Y, Yang CS, Fu Y, et al. Augmentation of circulating follicular helper T Cells and their impact on autoreactive B cells in myasthenia gravis. J Immunol. 2016;197(7):2610.

87. Dragin N, Bismuth J, Cizeron-Clairac G, Biferi MG, Berthault C, Serraf A, et al. Estrogen-mediated downregulation of AIRE influences sexual dimorphism in autoimmune diseases. J Clin Invest. 2016;126:1525–37.
88. Ramanujam R, Pirskanen R, Ramanujam S, Hammarstrom L. Utilizing twins concordance rates to infer the predisposition to myasthenia gravis. Twin Res Hum Genet. 2011;14:129–36.
89. Mamrut S, Avidan N, Truffault F, Staun-Ram E, Sharshar T, Eymard B, et al. Methylome and transcriptome profiling in Myasthenia Gravis monozygotic twins. J Autoimmun. 2017;82:62–73.
90. Sumitomo S, Fujio K, Okamura T, Yamamoto K. Egr2 and Egr3 are the unique regulators for systemic autoimmunity. JAKSTAT. 2013;2:e23952.
91. Li S, Miao T, Sebastian M, Bhullar P, Ghaffari E, Liu M, et al. The transcription factors Egr2 and Egr3 are essential for the control of inflammation and antigen-induced proliferation of B and T cells. Immunity. 2012;37:685–96.
92. Vandiedonck C, Giraud M, Garchon HJ. Genetics of autoimmune myasthenia gravis: the multifaceted contribution of the HLA complex. J Autoimmun. 2005;25(Suppl):6–11.
93. Giraud M, Vandiedonck C, Garchon HJ. Genetic factors in autoimmune myasthenia gravis. Ann N Y Acad Sci. 2008;1132:180–92.
94. Gregersen PK, Kosoy R, Lee AT, Lamb J, Sussman J, McKee D, et al. Risk for myasthenia gravis maps to a (151) Pro→Ala change in TNIP1 and to human leukocyte antigen-B*08. Ann Neurol. 2012;72:927–35.
95. Seldin MF, Alkhairy OK, Lee AT, Lamb JA, Sussman J, Pirskanen-Matell R, et al. Genome-wide Association study of late-onset myasthenia gravis: confirmation of TNFRSF11A, and identification of ZBTB10 and Three Distinct HLA Associations. Mol Med. 2015;21:769–81.
96. Meriggioli MN, Sanders DB. Autoimmune myasthenia gravis: emerging clinical and biological heterogeneity. Lancet Neurol. 2009;8:475–90.
97. Avidan N, Le Panse R, Berrih-Aknin S, Miller A. Genetic basis of myasthenia gravis – a comprehensive review. J Autoimmun. 2014;52:146–53.
98. Giraud M, Beaurain G, Eymard B, Tranchant C, Gajdos P, Garchon HJ. Genetic control of autoantibody expression in autoimmune myasthenia gravis: role of the self-antigen and of HLA-linked loci. Genes Immun. 2004;5:398–404.
99. Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. Cell. 2009;136:642–55.
100. Chen JQ, Papp G, Szodoray P, Zeher M. The role of microRNAs in the pathogenesis of autoimmune diseases. Autoimmun Rev. 2016;15:1171–80.
101. Punga T, Le Panse R, Andersson M, Truffault F, Berrih-Aknin S, Punga AR. Circulating miRNAs in myasthenia gravis: miR-150-5p as a new potential biomarker. Ann Clin Transl Neurol. 2014;1:49–58.
102. Punga T, Bartoccioni E, Lewandowska M, Damato V, Evoli A, Punga AR. Disease specific enrichment of circulating let-7 family microRNA in MuSK+ myasthenia gravis. J Neuroimmunol. 2016;292:21–6.
103. Punga AR, Andersson M, Alimohammadi M, Punga T. Disease specific signature of circulating miR-150-5p and miR-21-5p in myasthenia gravis patients. J Neurol Sci. 2015;356:90–6.
104. Nogales-Gadea G, Ramos-Fransi A, Suarez-Calvet X, Navas M, Rojas-Garcia R, Mosquera JL, et al. Analysis of serum miRNA profiles of myasthenia gravis patients. PLoS One. 2014;9:e91927.
105. Chunjie N, Huijuan N, Zhao Y, Jianzhao W, Xiaojian Z. Disease-specific signature of serum miR-20b and its targets IL-8 and IL-25, in myasthenia gravis patients. Eur Cytokine Netw. 2015;26:61–6.
106. Xin Y, Cai H, Lu T, Zhang Y, Yang Y, Cui Y. miR-20b Inhibits T cell proliferation and activation via NFAT signaling pathway in thymoma-associated myasthenia gravis. Biomed Res Int. 2016;2016:9595718.
107. Lu J, Yan M, Wang Y, Zhang J, Yang H, Tian FF, et al. Altered expression of miR-146a in myasthenia gravis. Neurosci Lett. 2013;555:85–90.
108. Zhang J, Jia G, Liu Q, Hu J, Yan M, Yang B, et al. Silencing miR-146a influences B cells and ameliorates experimental autoimmune myasthenia gravis. Immunology. 2015;144:56–67.
109. Gilliet M, Cao W, Liu YJ. Plasmacytoid dendritic cells: sensing nucleic acids in viral infection and autoimmune diseases. Nat Rev Immunol. 2008;8:594–606.
110. Piccolo G, Franciotta D, Versino M, Alfonsi E, Lombardi M, Poma G. Myasthenia gravis in a patient with chronic active hepatitis C during interferon-alpha treatment. J Neurol Neurosurg Psychiatry. 1996;60:348.
111. Mase G, Zorzon M, Biasutti E, Vitrani B, Cazzato G, Urban F, et al. Development of myasthenia gravis during interferon-alpha treatment for anti-HCV positive chronic hepatitis. J Neurol Neurosurg Psychiatry. 1996;60:348–9.
112. Dionisiotis J, Zoukos Y, Thomaides T. Development of myasthenia gravis in two patients with multiple sclerosis following interferon beta treatment. J Neurol Neurosurg Psychiatry. 2004;75:1079.
113. Batocchi AP, Evoli A, Servidei S, Palmisani MT, Apollo F, Tonali P. Myasthenia gravis during interferon alfa therapy. Neurology. 1995;45:382–3.
114. Cavalcante P, Cufi P, Mantegazza R, Berrih-Aknin S, Bernasconi P, Le Panse R. Etiology of myasthenia gravis: innate immunity signature in pathological thymus. Autoimmun Rev. 2013;12:863–74.
115. Cizeron-Clairac G, Le Panse R, Frenkian-Cuvelier M, Meraouna A, Truffault F, Bismuth J, et al. Thymus and myasthenia gravis: what can we learn from DNA microarrays? J Neuroimmunol. 2008;201–202:57–63.
116. Cufi P, Dragin N, Weiss JM, Martinez-Martinez P, De Baets MH, Roussin R, et al. Implication of double-stranded RNA signaling in the etiology

of autoimmune myasthenia gravis. Ann Neurol. 2013;73:281–93.
117. Leite MI, Jones M, Strobel P, Marx A, Gold R, Niks E, et al. Myasthenia gravis thymus: complement vulnerability of epithelial and myoid cells, complement attack on them, and correlations with autoantibody status. Am J Pathol. 2007;171:893–905.
118. Cavalcante P, Barberis M, Cannone M, Baggi F, Antozzi C, Maggi L, et al. Detection of poliovirus-infected macrophages in thymus of patients with myasthenia gravis. Neurology. 2010;74:1118–26.
119. Leis AA, Szatmary G, Ross MA, Stokic DS. West nile virus infection and myasthenia gravis. Muscle Nerve. 2014;49(1):26–9.
120. Wang YZ, Yan M, Tian FF, Zhang JM, Liu Q, Yang H, et al. Possible involvement of toll-like receptors in the pathogenesis of myasthenia gravis. Inflammation. 2013;36:121–30.
121. Bernasconi P, Barberis M, Baggi F, Passerini L, Cannone M, Arnoldi E, et al. Increased toll-like receptor 4 expression in thymus of myasthenic patients with thymitis and thymic involution. Am J Pathol. 2005;167:129–39.
122. Cavalcante P, Galbardi B, Franzi S, Marcuzzo S, Barzago C, Bonanno S, et al. Increased expression of Toll-like receptors 7 and 9 in myasthenia gravis thymus characterized by active Epstein-Barr virus infection. Immunobiology. 2016;221:516–27.
123. Robinet M, Maillard S, Cron MA, Berrih-Aknin S, Le Panse R. Review on Toll-Like receptor activation in myasthenia gravis: application to the development of new experimental models. Clin Rev Allergy Immunol. 2017;52:133–47.
124. Meager A, Wadhwa M, Dilger P, Bird C, Thorpe R, Newsom-Davis J, et al. Anti-cytokine autoantibodies in autoimmunity: preponderance of neutralizing autoantibodies against interferon-alpha, interferon-omega and interleukin-12 in patients with thymoma and/or myasthenia gravis. Clin Exp Immunol. 2003;132:128–36.
125. Cufi P, Soussan P, Truffault F, Fetouchi R, Robinet M, Fadel E, et al. Thymoma-associated myasthenia gravis: On the search for a pathogen signature. J Autoimmun. 2014;52:29–35.

4 Animal Models of Myasthenia Gravis for Preclinical Evaluation

Linda L. Kusner, Rozen Le Panse, Mario Losen, and William D. Phillips

Anti-AChR-Experimental Autoimmune MG (AChR-EAMG)

Myasthenia gravis (MG) results in weakness due to autoantibodies that target the neuromuscular junction (NMJ) causing dysfunction. Development of new therapeutics that are specific, potent, and safe is necessary to improve quality of life in MG patients. Animal models can accelerate the preclinical evaluation of new or repurposed therapeutics for MG. The animal models are specific for the targeted antigen at the NMJ. The models are produced by antigen immunization as in the experimental autoimmune MG (EAMG) or by specific antibodies as in the passive transfer MG (PTMG) (Table 4.1). Standard procedures for animal care, sampling and randomization of animals, experimental design, and outcome measures will clarify the interpretation of preclinical testing and improve our ability to navigate potential new therapeutics toward clinical trials.

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Classical EAMG Mouse Model

The acetylcholine receptor-experimental autoimmune MG (AChR-EAMG) model can be triggered by active immunization with AChR peptides, whole AChR subunits, and recombinant fragments of muscle AChR or, more often, with purified AChR isolated from the electrical organs of the marine ray, Torpedo (T-AChR) [1, 2]. Mouse strains carrying the H-2b haplotype are more susceptible to AChR-EAMG, and the C57BL6 mouse strain is the wild-type mouse strain conventionally used in most studies [3, 4].

T-AChR is emulsified with an equal volume of complete Freund's adjuvant (CFA, incomplete Freund's adjuvant (IFA) supplemented with 1 mg/mg heat-inactivated *Mycobacterium tuberculosis*). Mice aged between 6 and 9 weeks receive several subcutaneous injections with a total of 20–30 μg T-AChR: in the hind footpads and at several sites in the back and tail base. After 25–30 days, mice are immunized a second time with T-AChR emulsified in IFA. If, after the sec-

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H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*, Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_4

Table 4.1 Animal models of MG

MG model	Induction method	Mode of action	Complement dependent
AChR-EAMG	Injection of CFA + AChR	Production of antibody to AChR	Yes
AChR-EAMG	Injection of antibody to AChR	Binding of antibody	Yes
Mg-NSG	Implant MG thymus	Production of antibody and localization of human lymphocytes	Yes
MuSK-EAMG	Injection of CFA + MuSK	Production of antibody to MuSK	No
MuSK-EAMG	Injection of antibody to MuSK	Binding of antibody	No

ond immunization, the incidence of AChR-EAMG is less than 50%, a third immunization with T-AChR in IFA is advised [2]. To evaluate the efficiency of the experimental model, AChR-EAMG mice and a control group (injected with CFA but no immunogen) are run in parallel.

During the immunization series, mice are inspected for welfare, weighed, and graded for weakness, especially after boosters when weakness may occur. Mice displaying any particular signs of severe weakness or sores must be euthanized. All mice are euthanized at the end of the experiments for assessment of immune-pathological parameters. In the context of preclinical studies to evaluate efficacy, treatments are started after the second or the third immunization, once the majority of mice have developed symptoms.

Classical AChR-EAMG Rat Model: Induction

Strain, sex, and age of the rats are contributing factors in susceptibility to AChR-EAMG [5, 6]. Lewis female rats, 7–10 weeks of age, have been commonly used in both discovery phase and preclinical testing [7–13]. Differences in major histocompatibility haplotypes between strains can affect outcomes [14]. AChR-EAMG is induced in rats by injection with T-AChR emulsified in CFA at the base of the tail. Weakness in the rats is observed in the acute phase, 7–10 days after injection, and the chronic phase, 30–45 days after injection [15]. For therapeutic assessment, treatments begin after the observance of weakness in the animals.

Outcome Measurements for AChR-EAMG Models

Health Score

To assess a health score, each animal is inspected on a regular basis based for weakness. For consistency of scoring, the same investigator should test all the animals and remain blinded to treatment group. A global health score can be calculated taking into account different tests to evaluate animal behavior and associated muscle weaknesses, as detailed below [2, 16].

- *Measurement of body weight*: 6- to 9-week-old mice and 7–10-week-old rats normally gain weight until the end of the experiment. Upon immunization with T-AChR, animals that are weak may stop gaining weight, lose weight, or gain less weight than control animals.
- *Measurement of grip strength*: For objective measurement of muscle strength, animals are first exercised to tire them with 20 paw grips on a cage top wire grid or with a run on a treadmill. Each animal is then allowed to grasp a grid attached to a dynamometer using its forepaws and is then gently and steadily pulled by its tail away from the grid until it loses its grip. Forelimb muscle strength is determined by the mean maximum tension from several measurements.

- *Inverted grid test*: For mice only, the animal is placed in the center of the grid, and the grid is inverted. The grid is held inverted steadily 30–50 cm above a padded surface such that the mouse hangs on by all four paws. For each mouse, the time at which the mouse falls off is documented.
- *Observation*: Animals are assessed at rest and after exercise for activity. A clinical score is assigned as follows: grade 0, normal muscle strength and no muscle weakness, even after exercise; grade 1, normal at rest but weak after exercise, with the chin on the floor and inability to raise the head, hunched back, and reduced mobility; grade 2, weakness at rest with inability to raise the head, hunched back, and reduced mobility; grade 3, severe weakness, dehydrated, and paralyzed (quadriplegic) and loss of significant weight; and grade 4, moribund or found dead in the cage. For many experiments, a humane endpoint of 3 is sufficient to evaluate drug efficacy. The use of grade 3 and grade 4 as an outcome measure would require a stricter ethical argumentation, for example, if a drug has a specific effect in a myasthenic crisis.

Anti-AChR Antibody Measurement

AChR antibody levels in the serum of mice can be measured: (1) by radioimmunoassay (RIA), using murine AChR coupled to ^{125}I-α-bungarotoxin as described previously [1], or (2) by enzyme-linked immunosorbent assay (ELISA), using plates coated with T-AChR or mouse membrane extracts containing AChR. It is important to note that in AChR-EAMG, a large fraction of the antibodies raised against T-AChR will not cross-react with rodent AChR and therefore will not contribute to endplate pathology [17]. The levels of all anti-AChR antibodies are measured using a secondary antibody for total immunoglobulin G (IgG) or an isotype-specific antibody.

Measurement of Muscle AChR Concentration

The extent of neuromuscular junction (NMJ) pathology can be assessed by quantifying muscle AChR. This is done biochemically by measuring the binding of ^{125}I-conjugated α-bungarotoxin. Different techniques can be used to assess the amount of AChR, either in the whole carcasses of immunized animal or just in the muscle diaphragm or tibialis anterior muscle [1, 2, 6, 18, 19].

Muscle Histopathology

The structure of the NMJ and AChR content (together with IgG and complement deposits in the EAMG model) are relevant to assess the impact of the anti-AChR antibodies. The AChR organization at the NMJ can be visualized with fluorescently conjugated α-bungarotoxin and immunofluorescence to localize IgG, complement factors (C3, C9), or membrane attack complex. Immunofluorescence can be used in double-labeled micrographs to assess co-localization of AChR to IgG and complement deposits [1]. As a reference, a presynaptic marker such as the vesicular acetylcholine transporter (VAChT) or the synaptic vesicle protein 2 (SV2) can be used to immunolabel the NMJ.

Electromyography

Decrement of compound muscle action potential (CMAP) can be measured in the tibialis anterior of AChR-EAMG animals. At healthy NMJs, each nerve impulse releases many vesicle-loads (quanta) of acetylcholine, and each quantum produces a strong quantal response. When the nerve is stimulated, many simultaneous quantal responses add together to generate an endplate potential that is normally more than enough to trigger a muscle action potential (the safety factor). However, during muscle contraction (tetanus), successive nerve impulses deplete the number of synaptic vesicles that are immediately available to release acetylcholine. This decline in quantal number during tetanus is not a problem for the healthy NMJ, but in MG the reduced postsynaptic response to each quantum of acetylcholine makes neuromuscular transmission susceptible to fatigue. This is revealed by a decrement in the amplitude of the CMAP when the nerve is repetitively stimulated at low frequency (typically three impulses/sec). Stimulation and recording can be performed with Electromyography

(EMG) systems used in clinical practice. To detect a decremental response, the nerve is given a series of ten supramaximal stimuli at a frequency of 3/s. A 10% decrease in amplitude and area of the negative peak of the CMAP can be taken as a positive result for CMAP decrement [20]; usually the difference between first and the fourth CMAP is used. Curare challenge may be used to determine animals that are subclinical in disease weakness [7].

Acetylcholine Receptor Antibody-Specific Passive Transfer MG (AChR-PTMG)

The AChR-PTMG model involves the intravenous or intraperitoneal injection of AChR antibodies directly into the animal. The animal model bypasses the cellular adaptive immune response requirement of autoimmunity and instead assesses the antibody effector response at the target skeletal muscle synaptic membrane. The signs of weakness in AChR-PTMG are dependent upon both anti-AChR dose and IgG isotype. Discovery studies using AChR-PTMG revealed that the influx of mononuclear cells was dependent on initiation of complement by antibody binding, and the loss of AChR and postsynaptic folds was dependent on deposition of IgG and complement components [9, 21]. Moreover, the amount of complement damage was influenced by complement inhibitors within the muscle [22, 23]. Susceptibility to AChR-PTMG was shown to be dependent upon animal strain and age [24]. AChR-PTMG studies have also shown that expression levels of rapsyn and other AChR-associated proteins influence susceptibility of animals to AChR-PTMG [6] and the severity of weakness that ensues.

Preclinical testing using an AChR-PTMG model requires the antibodies to have high affinity to the AChR of the recipient species, activation of complement in the host, and availability of the antibodies to the scientific community for use. At present, only the AChR mAb35 (rat IgG1 isotype, http://dshb.biology.uiowa.edu/acetylcholine-nicotinic) fulfills all these criteria to produce a rat model. While mAb35 activates complement at the NMJ in rats, which produces weakness, injection of mAb35 in mice does not induce weakness. A possible explanation is that the rat IgG1 isotype does not efficiently activate mouse complement. Inducing AChR-PTMG with polyclonal antibodies from AChR+ MG patients brings many complexities to preclinical testing. The use of these antibodies for preclinical assessment of a therapeutic would be difficult in terms of reproducibility and multicenter verification. However, the polyclonal nature of these antibodies may make them suited to testing therapeutic compounds such as blocking antibodies or drugs that target the antibody half-life (its stability in the blood).

Outcome Measurements for AChR-PTMG

The clinical score of the animal is a necessary outcome measurement in AChR-PTMG. Onset of weakness is usually rapid with slight weakness observable at 24 h after injection. Documentation of health scores is undertaken every 12 h (or less) once the animals begin to demonstrate weakness. Dose of the antibody should be determined based on whether the animals demonstrate clear weakness by 48 h (but without inducing respiratory problems or death). For some studies, lower doses of antibodies can be useful [25]. Study design should never use death as an endpoint since careful dosing of antibodies allows clear-cut and reproducible generation of moderate disease.

The cellular or molecular pathway targeted by the therapeutic will determine which other secondary outcome measurements are relevant. Histology of the NMJ by hematoxylin and eosin stain can identify inflammatory cellular infiltrates. Immunohistochemistry of NMJ with antibodies to complement components or electron microscopy of the NMJ can verify complement damage to the endplate. ELISA or RIA of the serum or plasma can confirm the concentration of circulating AChR antibody. RIA with radiolabeled α-bungarotoxin of muscles can determine muscle AChR concentration.

Experimental Humanized Mouse Model (MG-NSG)

The classical rat and mouse EAMG models are perfectly relevant to study the autoantibody attack but are not ideal to model the human disease with respect to thymic involvement. Moreover, the EAMG models may not be suitable for preclinical studies involving human cell therapies or humanized monoclonal antibodies. To overcome these limitations, MG thymus tissues or cells can be engrafted into immunodeficient mice [18, 26]. A new humanized MG mouse model has recently been developed for such studies [27].

MG-NSG Protocol

Immunodeficient NOD-scid IL-2Rγnull (NSG) mice are used between 8 and 14 weeks of age. They are engrafted with small human thymic biopsies (obtained with the informed consent of MG patients after thymectomy or from control patients undergoing corrective heart surgery). Four fragments of around 5 mm per side are transplanted subcutaneously into the back of anesthetized NSG mice. Incisions are closed by stitches with nylon suture, and the skin is disinfected with Betadine. Usually wound healing is effective after 1 week. After engraftment, mice are carefully monitored to follow wound healing and the appearance of MG symptoms. At the end of the experiments, mice are euthanized for assessment of immune-pathological parameters.

Outcome Measurements for MG-NSG

Health Score

Mice are monitored to assess the health score, usually every week. MG-like clinical signs usually appear 2–3 weeks after engraftment and for preclinical studies. Treatments should be started when the majority of mice develop symptoms. Assessments of animals are performed in a blinded fashion, each time by the same investigator for consistency of measurements.

- *Measurement of body weight*: Upon engraftment mice that become sick may lose weight, or gain less weight, than control mice.
- *Health score*: Mice display quite evident MG symptoms, and a MG-like clinical score is established by observing mouse behavior and is graded on a scale of 0–4, as follows: score 0, no sign; score 1, abnormal movements (walking with head and tail down); score 2, reduced motility; score 3, hunched posture; and score 4, paralysis, dehydration, or death. The animals are considered to be sick when they reached score of 1 and sacrificed if they reach a score of 4.

Human Anti-AChR Antibody Measurement

Usually human anti-AChR antibodies can be detected in the serum of mice 2 weeks after engraftment with small delay from mouse to mouse. Human anti-AChR antibodies produced in MG-NSG mice are measured via a classical RIA as done in clinical laboratories. There is a correlation between the titer in MG patients at the time of thymectomy and the titer subsequently measured in the engrafted mice. However, as with the MG patients themselves, there is a lack of correlation between the level of anti-AChR antibodies and the MG-like clinical score [27].

Measurement of Muscle AChR Concentration

The loss of AChR at the NMJ can be assessed by biochemically quantifying the amount of mouse muscle AChR, using ^{125}I-conjugated α-bungarotoxin as a probe. The amount of radioactivity bound by the muscle (relating to the endplate AChR content) is inversely correlated with MG-like clinical scores [27].

Animal Models of MuSK Myasthenia

Muscle-specific kinase (MuSK) is a receptor tyrosine kinase that is vital for clustering and stabilizing AChR at the motor endplate. MuSK autoantibodies are predominantly of the IgG4 isotype: considered functionally monovalent and

do not activate complement. By binding the ectodomain of MuSK, IgG4 anti-MuSK prevents the activation of the agrin-LRP4-MuSK kinase complex [28–32]. As a result postsynaptic AChR become less stable, leading to failure of neurotransmission and muscle weakness. This understanding of the pathogenesis of MuSK myasthenia gravis depends largely upon a combination of experimental animal models and cell culture studies (reviewed in [33]). The pathogenic effects of the MuSK IgG1–3 fractions from anti-MuSK myasthenia gravis patients require further study. Here we outline the active immunization and passive transfer rodent models of MuSK-MG.

Active Immunization Models of MuSK Myasthenia (MuSK-EAMG)

Rabbits, rats, and mice have each been shown to develop myasthenia after active immunization with MuSK [34–40]. In recent years, mice have most commonly been used for MuSK-EAMG studies. Mice are injected with recombinant MuSK extracellular domain (10–30 μg/mouse or 100 μg/rat), in CFA emulsion. Control mice receive injections of CFA emulsion (no immunogen protein). Induction of myasthenic-like weakness requires one or more booster immunizations with MuSK (and adjuvant) spread out over several months before clinical weakness develops. During the immunization series, mice should be inspected for welfare (see AChR-EAMG section), weighed, and graded for weakness twice weekly.

Passive IgG Transfer Mouse Model of MuSK Myasthenia (MuSK-PTMG)

Anti-MuSK positive myasthenia gravis has been modeled in mice by injecting IgG from anti-MuSK-seropositive patients. Mice must receive daily intraperitoneal injections of a large quantity (20–40 mg) of total IgG or smaller amounts (as little as 4 mg) of the IgG4 fraction of patient plasma [38, 41–44]. To avoid an active immune response against the human immunoglobulin, C57BL6J mice were given a single, immunosuppressive injection of cyclophosphamide (300 mg/kg) 24 h after their first IgG injection [41]. Alternatively NOD/scid mice, which do not develop antibody responses, can be used for MuSK-PTMG experiments [43]. Total IgG from severely affected patients (MGFA 3B or 4) caused weakness in mice after 12–14 days of daily injections. The IgG4 fraction caused weakness after as little as six daily injections.

Weakness was always found to be associated with a reduction in the amount of AChRs at the motor endplate, as assessed by fluorescence microscopy (reviewed in [33]). During the course of the anti-MuSK injection series, the endplate potential amplitude declined progressively. This could be explained by impaired MuSK function at the endplate and the accelerated loss of AChRs from the postsynaptic membrane [30, 44, 45]. One peculiarity of mouse models of MuSK-MG (found in both EAMG and PTMG models) was the failure of the nerve terminal to increase the number of acetylcholine quanta released in response to this postsynaptic deficit [46]. This failure of presynaptic adaptation suggests that MuSK function may be necessary for feedback control of acetylcholine release from the motor nerve terminal.

MuSK-PTMG is a model with both advantages and disadvantages. The key advantage is that it allows us to test the in vivo pathogenic effects MuSK autoantibodies from patients (the actual disease-causing agent). On the other hand, the injection series requires as much as 600 mg of total patient IgG per mouse. It is important to remember that pathogenic autoantibodies generally represent only a tiny fraction of total plasma IgG from a myasthenia gravis patient. Such large amounts of total IgG are only available when a patient undergoes therapeutic plasmapheresis. Even then, the amount of IgG that can be purified severely limits the number of treatment groups and mouse replicates (mice per group) that can be included in any MuSK-PTMG experiment. This limitation might be overcome in the future by cloning the genes for pathogenic anti-MuSK IgG4 from patients, so as to generate large amounts of (MuSK-function blocking) recombinant IgG.

Outcome Measurements for Rodent Models of MuSK Myasthenia

The primary outcome measures for rodent models of MuSK-MG have mostly involved assessing limb muscle weakness and reductions in body weight (body weight loss might be exacerbated by difficulties in swallowing). Since the onset of weakness can be rapid (and cause suffering), mice must be graded regularly. For MuSK-EAMG mice or rats should be inspected twice weekly after the first booster immunization. The time course of MuSK-PTMG is briefer (6–15 days) so mice should be weighed and inspected for weakness each day (before they receive their daily IgG injection). The kinds of criteria routinely used for grading myasthenic mice include body weight, posture/mobility observations (before and after exercise), forelimb grip strength, and hang time in the inverted grid test (how long a mouse can cling to an inverted grid using all four limbs). CMAP recordings are made from the shank muscles at the endpoint for evidence of impaired neuromuscular transmission. Preclinical researchers have yet to agree upon how some or all of these tests might be combined to generate a consensus composite weakness scale appropriate as a primary outcome measure for grading myasthenic impairment in mice. A well-defined composite scale, if widely adopted, might increase the statistical power and reproducibility for preclinical testing of therapeutics.

As noted above, loss of AChRs from the motor endplate is the most consistent pathological observation from animal studies of MuSK myasthenia gravis. In mouse models of MuSK myasthenia gravis, impaired MuSK signaling function is thought to cause disassembly of the NMJ. For this reason quantitative confocal microscopic analysis of the NMJ is particularly relevant. Fluorescently labeled α-bungarotoxin provides a convenient and sensitive tag to assess the distribution of AChRs in cryostat sections or whole mounts of muscles from experimental animals. Fluorescence microscopy reveals reductions in the density of endplate AChR and can also reveal the fragmentation and shrinkage of the AChR-rich area at each endplate, compared to non-myasthenic controls. In order to assess the relative intensity of endplate α-bungarotoxin labeling, cryosections of myasthenic and control muscles must all be processed and imaged together as a single batch. Longitudinal cryosections or muscle whole mounts can be double labeled for AChR and with immunofluorescence for synaptic vesicle marker proteins to assess the alignment of nerve terminals with endplate AChRs. Detailed methods for measuring the area and relative intensity of endplate AChRs and nerve terminals have been published recently [47]. Loss of endplate AChRs provides a direct measure of the primary deficit responsible for failure of neuromuscular transmission (and weakness) in animal models of MuSK-MG.

Animal Care

Consistency of animal care at institutions may vary based on ethical, environmental, and safety regulations. Repeated use of CFA and footpad injections can raise animal welfare concerns, and ethics panels in some jurisdictions would require extraordinary argumentation before approving such protocols. Animal care involves the cage type and size, ambient temperature, number of animals per cage, bedding material, diet, and environmental enrichment supplements [48]. It is important to note that enrichment items that encourage or facilitate exercise, such as running wheels, can affect the size of the NMJ [49]. The reproducibility in the MG animal models requires the animal care to be consistent between research settings. To ensure consistency, documentation in the methods should include the care of animals. Differences in the environment housing of the animals may alter outcome of therapeutic evaluation. Study design should seek to lower stress levels for experimental animals. Chronic stress in animals has been linked to a decrease in immune response, lower cytokine expression, and a delay in antibody production [50]. Reducing stress in the animals can be achieved by minimizing handling, limiting the

number of personnel in contact with animals, reducing ambient noise by the experimenters and facility staff, avoiding unnecessary animal suffering, and providing environmental enrichment items to the cage [51]. Study design should also state the endpoints at which an animal will be terminated and/or excluded from study. Documentation of animal care is recorded throughout the study.

Rigor of Study Design

Experimental design of a preclinical study requires specific criteria to promote reproducibility and rigor. Guidelines identify four specific areas: randomization of animals, blind assessments, power calculations for study size, and data handling including use of both sexes in study [52]. Prior to initiation of the study, primary and secondary outcome measurements should be identified. To ensure sufficient number of animals per group, power analysis based on the primary outcome measurement are performed to determine the sample size needed for statistical significance (with acceptable levels of type I and type II statistical error). Randomization of animals into groups and blinding of the observer to these groups will reduce subconscious observer bias. Therapeutic and control agents that are to be delivered should have a coded label to ensure that the operator remains blinded to the treatment each animal receives. The criteria for excluding an animal from the study should be predefined before commencing the study, and any animal that is subsequently lost or excluded must be documented. Both male and female mice can be used, but outcome measurements regarding body weight and muscle strength are different for males and females so they should be grouped separately for assessment of outcomes [2]. The power analysis, experimental design, and randomization need to take gender differences into account. The criteria required for a rigorous study are based upon the experience in human trials and reflect the need to increase reproducibility in preclinical setting.

References

1. Wu B, Goluszko E, Huda R, Tuzun E, Christadoss P. Experimental autoimmune myasthenia gravis in the mouse. Curr Protoc Immunol. 2013;Chapter 15:Unit 15.8.
2. Tuzun E, Berrih-Aknin S, Brenner T, Kusner LL, Le Panse R, Yang H, et al. Guidelines for standard preclinical experiments in the mouse model of myasthenia gravis induced by acetylcholine receptor immunization. Exp Neurol. 2015;270:11–7.
3. Fuchs S, Nevo D, Tarrab-Hazdai R, Yaar I. Strain differences in the autoimmune response of mice to acetylcholine receptors. Nature. 1976;263(5575):329–30.
4. Berman PW, Patrick J. Experimental myasthenia gravis. A murine system. J Exp Med. 1980;151(1):204–23.
5. Hoedemaekers A, Graus Y, van Breda Vriesman P, de Baets M. Age- and sex-related resistance to chronic experimental autoimmune myasthenia gravis (EAMG) in Brown Norway rats. Clin Exp Immunol. 1997;107(1):189–97.
6. Losen M, Stassen MH, Martinez-Martinez P, Machiels BM, Duimel H, Frederik P, et al. Increased expression of rapsyn in muscles prevents acetylcholine receptor loss in experimental autoimmune myasthenia gravis. Brain. 2005;128(Pt 10):2327–37.
7. Gomez AM, Vrolix K, Martinez-Martinez P, Molenaar PC, Phernambucq M, van der Esch E, et al. Proteasome inhibition with bortezomib depletes plasma cells and autoantibodies in experimental autoimmune myasthenia gravis. J Immunol. 2011;186(4):2503–13.
8. Lennon VA, Seybold ME, Lindstrom JM, Cochrane C, Ulevitch R. Role of complement in the pathogenesis of experimental autoimmune myasthenia gravis. J Exp Med. 1978;147(4):973–83.
9. Lindstrom JM, Engel AG, Seybold ME, Lennon VA, Lambert EH. Pathological mechanisms in experimental autoimmune myasthenia gravis. II. Passive transfer of experimental autoimmune myasthenia gravis in rats with anti-acetylcholine recepotr antibodies. J Exp Med. 1976;144(3):739–53.
10. van der Neut Kolfschoten M, Schuurman J, Losen M, Bleeker WK, Martinez-Martinez P, Vermeulen E, et al. Anti-inflammatory activity of human IgG4 antibodies by dynamic fab arm exchange. Science. 2007;317(5844):1554–7.
11. Okumura S, McIntosh K, Drachman DB. Oral administration of acetylcholine receptor: effects on experimental myasthenia gravis. Ann Neurol. 1994;36(5):704–13.
12. Soltys J, Kusner LL, Young A, Richmonds C, Hatala D, Gong B, et al. Novel complement inhibitor limits severity of experimentally myasthenia gravis. Ann Neurol. 2009;65(1):67–75.
13. de Silva S, Blum JE, McIntosh KR, Order S, Drachman DB. Treatment of experimental myasthenia gravis with total lymphoid irradiation. Clin Immunol Immunopathol. 1988;48(1):31–41.

14. Fujii Y, Lindstrom J. Specificity of the T cell immune response to acetylcholine receptor in experimental autoimmune myasthenia gravis. Response to subunits and synthetic peptides. J Immunol. 1988;140(6):1830–7.
15. Losen M, Martinez-Martinez P, Molenaar PC, Lazaridis K, Tzartos S, Brenner T, et al. Standardization of the experimental autoimmune myasthenia gravis (EAMG) model by immunization of rats with Torpedo Californica acetylcholine receptors—recommendations for methods and experimental designs. Exp Neurol. 2015;270:18–28.
16. Weiss JM, Robinet M, Aricha R, Cufi P, Villeret B, Lantner F, et al. Novel CXCL13 transgenic mouse: inflammation drives pathogenic effect of CXCL13 in experimental myasthenia gravis. Oncotarget. 2016;7(7):7550–62.
17. Lennon VA, Lindstrom JM, Seybold ME. Experimental autoimmune myasthenia: a model of myasthenia gravis in rats and guinea pigs. J Exp Med. 1975;141(6):1365–75.
18. Aissaoui A, Klingel-Schmitt I, Couderc J, Chateau D, Romagne F, Jambou F, et al. Prevention of autoimmune attack by targeting specific T-cell receptors in a severe combined immunodeficiency mouse model of myasthenia gravis. Ann Neurol. 1999;46(4):559–67.
19. Martinez-Martinez P, Losen M, Duimel H, Frederik P, Spaans F, Molenaar P, et al. Overexpression of rapsyn in rat muscle increases acetylcholine receptor levels in chronic experimental autoimmune myasthenia gravis. Am J Pathol. 2007;170(2):644–57.
20. Kimura J. Electrodiagnosis in disease of nerve and muscle: principles and practice. Oxford: Oxford University Press; 2001.
21. Engel AG, Sakakibara H, Sahashi K, Lindstrom JM, Lambert EH, Lennon VA. Passively transferred experimental autoimmune myasthenia gravis. Sequential and quantitative study of the motor end-plate fine structure and ultrastructural localization of immune complexes (IgG and C3), and of the acetylcholine receptor. Neurology. 1979;29(2):179–88.
22. Morgan BP, Chamberlain-Banoub J, Neal JW, Song W, Mizuno M, Harris CL. The membrane attack pathway of complement drives pathology in passively induced experimental autoimmune myasthenia gravis in mice. Clin Exp Immunol. 2006;146(2):294–302.
23. Kusner LL, Halperin JA, Kaminski HJ. Cell surface complement regulators moderate experimental myasthenia gravis pathology. Muscle Nerve. 2013;47(1):33–40.
24. Hoedemaekers A, Bessereau JL, Graus Y, Guyon T, Changeux JP, Berrih-Aknin S, et al. Differential susceptibility of young and old rat neuromuscular junctions to antibody-mediated AChR degradation in experimental autoimmune myasthenia gravis. Ann N Y Acad Sci. 1998;841:550–4.
25. Gomez AM, Stevens JA, Mane-Damas M, Molenaar P, Duimel H, Verheyen F, et al. Silencing of Dok-7 in adult rat muscle increases susceptibility to passive transfer myasthenia gravis. Am J Pathol. 2016;186(10):2559–68.
26. Schonbeck S, Padberg F, Hohlfeld R, Wekerle H. Transplantation of thymic autoimmune microenvironment to severe combined immunodeficiency mice. A new model of myasthenia gravis. J Clin Invest. 1992;90(1):245–50.
27. Sudres M, Maurer M, Robinet M, Bismuth J, Truffault F, Girard D, et al. Preconditioned mesenchymal stem cells treat myasthenia gravis in a humanized preclinical model. JCI Insight. 2017;2(7):e89665.
28. Koneczny I, Cossins J, Waters P, Beeson D, Vincent A. MuSK myasthenia gravis IgG4 disrupts the interaction of LRP4 with MuSK but both IgG4 and IgG1-3 can disperse preformed agrin-independent AChR clusters. PLoS One. 2013;11:e80695.
29. Huijbers MG, Zhang W, Klooster R, Niks EH, Friese MB, Straasheijm IR, et al. MuSK IgG4 auto-antibodies cause myasthenia gravis by inhibiting binding between MuSK and Lrp4. PNAS. 2013;110:20783–8.
30. Ghazanfari N, Morsch M, Reddel SW, Liang SX, Phillips WD. Muscle specific kinase autoantibodies suppress the MuSK pathway and ACh receptor retention at the mouse neuromuscular junction. J Physiol. 2014;592(13):2881–97.
31. Ghazanfari N, Linsao ELTB, Trajanovska S, Morsch M, Gregorevic P, Liang SX, et al. Forced expression of muscle specific kinase slows postsynaptic acetylcholine receptor loss in a mouse model of MuSK myasthenia gravis. Physiol Rep. 2015;3(12):12658.
32. Otsuka K, Ito M, Ohkawara B, Masuda A, Kawakami Y, Sahashi K, et al. Collagen Q and anti-MuSK autoantibody competitively suppress agrin/LRP4/MuSK signaling. Sci Rep. 2015;5:13928.
33. Phillips WD, Christadoss P, Losen M, Punga A, Shigemoto K, Verschuuren J, et al. Guidelines for pre-clinical animal and cellular models of MuSK-myasthenia gravis. Exp Neurol. 2015;270:29–40.
34. Shigemoto K, Kubo S, Maruyama N, Hato N, Yamada H, Jie C, et al. Induction of myasthenia by immunization against muscle-specific kinase. J Clin Invest. 2006;116:1016–24.
35. Jha S, Xu K, Maruta T, Oshima M, Mosier DR, Atassi MZ, et al. Myasthenia gravis induced in mice by immunization with the recombinant extracellular domain of rat muscle-specific kinase (MuSK). J Neuroimmunol. 2006;175:107–17.
36. Richman DP, Nishi K, Morell SW, Chang JM, Ferns MJ, Wollmann RL, et al. Acute severe animal model of anti-muscle-specific kinase myasthenia. Arch Neurol. 2012;69(4):453–60.
37. Punga AR, Lin S, Oliveri F, Meinen S, Ruegg MA. Muscle-selective synaptic disassembly and reorganization in MuSK antibody-positive MG mice. Exp Neurol. 2011;230:207–17.
38. Viegas S, Jacobson L, Waters P, Cossins J, Jacob S, Leite MI, et al. Passive and active immunization models of MuSK-Ab positive myasthenia: electrophysi-

ological evidence for pre and postsynaptic defects. Exp Neurol. 2012;234:506–12.
39. Ulusoy C, Kim E, Tuzun E, Huda R, Yilmaz V, Poulas K, et al. Preferential production of IgG1, IL-4 and IL-10 in MuSK-immunized mice. Clin Immunol. 2014;151(2):155–63.
40. Patel V, Oh A, Voit A, Sultatos LG, Babu GJ, Wilson BA, et al. Altered active zones, vesicle pools, nerve terminal conductivity, and morphology during experimental MuSK myasthenia gravis. PLoS One. 2014;9(12):e110571.
41. Cole RN, Reddel SW, Gervasio OL, Phillips WD. Anti-MuSK patient antibodies disrupt the mouse neuromuscular junction. Ann Neurol. 2008;63(6):782–9.
42. Kawakami Y, Ito M, Hirayama M, Sahashi K, Ohkawara B, Masuda A, et al. Anti-MuSK autoantibodies block binding of collagen Q to MuSK. Neurology. 2011;77(20):1819–26.
43. Klooster R, Plomp JJ, Huijbers MG, Niks EH, Straasheijm KR, Detmers FJ, et al. Muscle-specific kinase myasthenia gravis IgG4 autoantibodies cause severe neuromuscular junction dysfunction in mice. Brain. 2012;135(Pt 4):1081–101.
44. Morsch M, Reddel SW, Ghazanfari N, Toyka KV, Phillips WD. Muscle specific kinase autoantibodies cause synaptic failure through progressive wastage of postsynaptic acetylcholine receptors. Exp Neurol. 2012;237(2):286–95.
45. Ghazanfari N, Linsao EL, Trajanovska S, Morsch M, Gregorevic P, Liang SX, et al. Forced expression of muscle specific kinase slows postsynaptic acetylcholine receptor loss in a mouse model of MuSK myasthenia gravis. Physiol Rep. 2015;3(12):e12658.
46. Plomp JJ, Morsch M, Phillips WD, Verschuuren JJ. Electrophysiological analysis of neuromuscular synaptic function in myasthenia gravis patients and animal models. Exp Neurol. 2015;270:41–54.
47. Tse N, Morsch M, Ghazanfari N, Cole L, Visvanathan A, Leamey C, et al. The neuromuscular junction: measuring synapse size, fragmentation and changes in synaptic protein density using confocal fluorescence microscopy. J Vis Exp. 2014;94:e52220.
48. Toth LA. The influence of the cage environment on rodent physiology and behavior: implications for reproducibility of pre-clinical rodent research. Exp Neurol. 2015;270:72–7.
49. Cheng A, Morsch M, Murata Y, Ghazanfari N, Reddel SW, Phillips WD. Sequence of age-associated changes to the mouse neuromuscular junction and the protective effects of voluntary exercise. PLoS One. 2013;8(7):e67970.
50. Tournier JN, Mathieu J, Mailfert Y, Multon E, Drouet C, Jouan A, et al. Chronic restraint stress induces severe disruption of the T-cell specific response to tetanus toxin vaccine. Immunology. 2001;102(1):87–93.
51. Moncek F, Duncko R, Johansson BB, Jezova D. Effect of environmental enrichment on stress related systems in rats. J Neuroendocrinol. 2004;16(5):423–31.
52. Landis SC, Amara SG, Asadullah K, Austin CP, Blumenstein R, Bradley EW, et al. A call for transparent reporting to optimize the predictive value of preclinical research. Nature. 2012;490(7419):187–91.

5 Epidemiology and Genetics of Myasthenia Gravis

Melissa Nel and Jeannine M. Heckmann

Introduction

Myasthenia gravis (MG) represents a heterogeneous group of autoantibody-mediated diseases targeting the neuromuscular junction. Although much is known about the pathogenic mechanisms of MG at the muscle end plate, the precise etiology triggering MG remains unknown. However, there is increasing recognition that autoimmune disease is a consequence of a complex interplay between the host's genetic susceptibility and factors such as infections and other environmental influences that may impact the epigenetic regulation of genes [1]. In this chapter we discuss the epidemiological profile of MG and the genetic susceptibility of MG subtypes.

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Epidemiology of MG

The prevalence estimates of MG (i.e., number of cases present in a population at a specified time) vary depending on methodological approach but generally range between 77 and 167 per million people living in Europe [2]. A pooled estimate of 35 studies performed globally calculated an incidence rate (i.e., number of new cases per year) of 5.3 per million person-years. The global incidence rate estimates based on studies using only acetylcholine receptor (AChR)-antibody positivity as inclusion criterion ranged between 4 and 18 per million person-years [3]. While MG was originally thought to occur predominantly in young women [4], an increasing incidence of MG among the European elderly (70–90-year-olds) has been reported since the mid-1980s despite the stable incidence rates among those with earlier onset disease [5, 6]. Data from Asia (Taiwan and Japan) [7, 8], Australia [9], and Africa (South Africa) [10, 11] have shown the same trend with incidence peaking among older men. Vincent et al. suggest the sharp falloff in incidence of AChR antibody (Ab)-positive MG after the age of 80 is likely due to underdiagnosis in the very elderly [12].

The "hygiene hypothesis" linking better socioeconomic conditions and fewer infections such as tuberculosis in the developed world was proposed to explain the apparent changing epidemiology of MG [1]. However, data from Africa,

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H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*, Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_5

with similar MG age/gender incidence trends [11] despite significantly higher rates of infectious diseases such as tuberculosis [13], do not support this theory. Based on the same observations worldwide, it is likely that the apparent global changing pattern of MG from a disease of young women to predominantly that of older people is a result of improved diagnostics, an aging population as well as improved access to health care in parts of the developing world [14–17].

Epidemiology of Antigen Subtypes

In most cases of MG, the autoantibodies targeting the acetylcholine receptor (AChR) are of the IgG1 and IgG3 isotypes. In the few patients who have circulating antibodies targeting the muscle-specific kinase (MuSK) end plate protein (MuSK-MG), the antibody is mainly non-complement fixing IgG4. In recent years autoantibodies to other end plate autoantigens such as lipoprotein-related protein 4 (LRP4) (IgG1, IgG2, IgG3) [18] have been recognized. Next we discuss the incidence of MG classified according to antigen subtype.

AChR Antibody-Positive MG (AChR-MG)

Individuals with circulating antibodies to the AChR comprise 85–90% of patients with clinical and electrophysiological criteria consistent with MG. The incidence rates of AChR-MG appear to be similar across the world including Caucasian patients from different continents [19, 20] as well as those in Arab countries [21] and from Africa [10, 11]. In these studies a bimodal distribution is found with peaks representing the ages at symptom onset: early-onset MG (EOMG) in the third decade and comprising predominantly women and late-onset MG (LOMG) peaking between the eighth and ninth decades and more frequent in men [5, 22]. As mentioned, the latter group has apparently increased over the past three decades, first reported in 1991 in Denmark [23] and subsequently in European, American, Asian (including Japan), and African populations [8, 11, 22]. In addition, it has been noted that AChR-Ab titers among Caucasian and Chinese individuals with MG were similar [24].

AChR-MG represents the largest subgroup by antigen type and age at symptom onset associated with certain clinical, pathological, and genetic characteristics. Therefore, we define three groups: (1) juvenile onset (prepubertal/childhood and postpubertal between 12 and 20 years), (2) EOMG with symptoms before the age of 40 or 50 years (depending on arbitrary definition criteria), and (3) LOMG as those with symptoms after the age of 40 or later (50 or even 65 years) varying based on individual study definitions. Early studies recognized that EOMG occurs most commonly among young women who were likely to have hyperplastic thymuses and carry HLA-B8, -DR3 alleles in the major histocompatibility (MHC) region [24], whereas LOMG occurs predominantly in men with atrophic thymuses who demonstrate associations with HLA-B7, -DR2 alleles [24].

Epidemiological studies in juvenile-onset MG are relatively sparse. The annual incidence of AChR-MG in children from Canada (mixed population), South Africa (predominantly African genetic ancestry), and Turkey [11, 19, 25] may be higher than Caucasian cohorts from the United Kingdom and Norway (≈3 per million vs. <1.5 per million, respectively) [26, 27].

MuSK Antibody-Positive MG

Overall, MuSK antibody-positive MG or MuSK-MG represents about 1–4% of all subjects with the disease. The annual incidence of MuSK-MG in the Netherlands is 0.3 patients per million per year [14] with a slightly higher prevalence in Greece (2.9 vs. 1.9 per million, respectively) [3]. However, the proportion of AChR-Ab-negative myasthenics with MuSK-MG varies substantially; both African American [28] and South African [29] cohorts show 30–50% of the AChR antibody-negative group have MuSK-MG compared to European genetic

ancestry investigations which range from 20% in the Netherlands to none in Norway [30]. Two studies of Chinese populations found frequencies of 2.5–3% of MuSK-MG among AChR-Ab-negative myasthenics [31, 32]. However, approximately 10% of MuSK-MG cases, at least in patients with African genetic ancestry, were falsely negative using the MuSK radioimmunoassay and were subsequently found positive using a MuSK cell-based assay [29]. Rarely, MuSK-MG has been found among juveniles with MG [33].

LRP4 Antibody-Positive MG

LRP-4 antibodies impact end plate AChR clustering by binding to LRP4 and thereby interfering with the LRP4-agrin clustering function. LRP4-MG is estimated to be half as frequent as MuSK-MG, at least in Caucasian populations [14]. An evaluation of 635 patients from European centers, who were seronegative by radioimmunoassay for both AChR- and MuSK-Abs, detected antibodies to LRP4 in 18% using a cell-based assay. However, 3.6% of the 110 controls with other "neuro-immune disorders," mainly multiple sclerosis, also had detectable LRP4-Abs [18] as well as 7.5% of AChR-MG and 15% with MuSk-MG. The MG patients with LRP4-Abs tended to have symptom onset prior to age 40 (65%) and mild generalized MG (55%) or symptoms confined to the ocular muscles (22%) [18]. In a small study of 27 patients with African genetic ancestry and generalized MG, no sera showed reactivity to LRP4 in a cell-based assay [29].

Triple-Seronegative MG (AChR-/MuSK-/LRP4-Antibody Negative)

A small proportion of patients with generalized MG do not have detectable circulating antibodies to AChR, MuSK, or LRP4 antigens either by radioimmunoassay or cell-based assays. These triple-seronegative MG patients usually have a milder clinical phenotype than those with MuSK-MG, though there are rare patients in this group who have severe refractory disease and even muscle atrophy similar to MuSK-MG [18, 29]. A cohort study composed of European and African genetic ancestry and AChR antibody-negative MG showed proportionately more triple-seronegative MG among older Caucasians in contrast to MuSK-MG among younger patients with African genetic ancestry [29].

Epidemiology of Clinical Subtypes of MG

Ocular MG

Observational studies with prolonged follow-up found that 90% of patients with disease confined to the ocular muscles for 2 years will remain as ocular MG [14]. An early study from North America found ocular MG to be more frequent among older men [34], while a retrospective evaluation from Japan found ocular MG almost twice as frequently among individuals presenting with MG at an average age of 70 years compared to younger patients with a mean age of 27 [8]. A recent European study found almost twice as many older patients with ocular MG in the Netherlands compared to Norway [2].

An audit from China [32] found a higher incidence of childhood-onset ocular MG compared to postpubertal children although this was not observed in Japan [8] or Taiwan [7]. Nevertheless, peri-pubertal Caucasian, Asian, and African children show a lower proportion of ocular MG compared to those who are younger [33, 35]. A substantial proportion of Asian and African children with ocular MG do not respond to MG therapies, in particular pyridostigmine ± prednisone (see MG subphenotype) [35, 36].

Generalized MG

Although there are clearly two peaks in the ages at onset of generalized MG as outlined earlier and genetic associations (see later), there are no consistent reports of differences between EOMG

and LOMG with respect to clinical presentations or responses to therapies [17]. Nevertheless, the EOMG subgroup appears to have well-defined features across populations with a female predominance and thymic hyperplasia. In contrast, LOMG is predominated by men with atrophic or involuted thymus [17, 37]. Although a Japanese cohort showed lower frequencies of concomitant autoimmune disease and AChR-Ab titers among the LOMG compared to EOMG [8], a comprehensive study from the United Kingdom found similar AChR antibody titers irrespective of age [12].

Thymoma-Associated MG

Thymoma MG occurs in 10–15% of myasthenic cohorts worldwide. Thymoma MG may present at any age but with a higher frequency among those over 40 [7, 17, 38, 39]. These patients almost always have detectable AChR-Abs, although rarely patients may be AChR-Ab negative with mild ocular MG [24, 40].

Treatment-Resistant Ophthalmoplegia

Extraocular muscles are highly susceptible to development of myasthenic weakness and are frequently the first muscles to be involved [41]. While the extraocular muscles usually respond to immunosuppressive therapies in a similar manner to non-ocular muscles, a unique subphenotype of MG has been identified which presents as treatment-resistant ophthalmoplegia (OP-MG). This subphenotype is substantially more frequent among individuals with African genetic ancestry and particularly those with juvenile-onset AChR antibody-positive MG [35, 38, 42]. Although most patients with treatment-resistant ophthalmoplegia have generalized MG, African and Asian children with ocular MG are also at risk [35, 36].

This subphenotype is not confined to AChR-Ab-positive MG. Treatment-resistant myasthenic ophthalmoplegia has been reported among MuSK-MG cases, both generalized and ocular, as well as a middle-aged Caucasian woman with triple-seronegative MG [29, 43]. The treatment-resistant ophthalmoplegic subphenotype is likely the result of a complex network of dysregulated genes "activated" within the context of MG resulting in increased complement-mediated damage and altered ganglioside biosynthesis and myogenesis [42, 44, 45]. Several African-specific and functional susceptibility variants in these key pathways have been reported at a higher frequency in OP-MG compared to MG patients with treatment-responsive ocular manifestations.

Genetics of Myasthenia Gravis

Similar to other autoimmune diseases, MG has a strong heritable component. One to 7% of Caucasian, African, and Chinese MG patients have family members with MG [32, 38, 46, 47], but family members may vary in MG subtype with respect to age at onset, thymus histology, and even detectable MG-autoantibodies [48]. A MG patient may have first- or second-degree family members with other autoimmune diseases, most frequently autoimmune thyroid disease and rheumatoid arthritis (RA) [22, 29].

The gene variants that presumably make an individual susceptible or resistant to autoimmune disease development are likely a result of evolutionary pressures to adapt to the environment [49]. These variants are not necessarily rare in a population, but most individuals will carry a mix of risk and protective alleles, which together will determine the overall "autoimmune" genetic risk [49]. However, a high burden or specific combinations of different risk alleles can predispose an individual to MG.

There are several strategies to dissect the genetics of these complex disorders. Prior to high-throughput sequencing capabilities, polymorphic genetic markers were assessed for their association with MG. This strategy involved genotyping variants, usually single-nucleotide polymorphisms (SNPs) within or close to candidate genes, and determining their frequencies in a

cohort of cases vs. controls, all from the same population. In the last decade, several genome-wide linkage association studies (GWAS) have been performed using genotyping SNP arrays and sample sizes that are tenfold larger—in this approach individual SNPs spaced at regular intervals across the genome may act as a tag for a genomic region, and the frequencies can be compared with population controls. Because multiple tests are performed using this approach, a conservative genome-wide level of significance ($p < 5\times10^{-8}$) needs to be applied to reduce the number of false positives [49]. Based on algorithmic predictions of GWAS SNP genotyping data, the heritability component of AChR-MG that is captured when the common SNP variants are simultaneously considered was estimated to range between 25 and 38% [50].

Some gene associations, notably in the HLA region such as the HLA-A1-B8-DR3 haplotype, appear to be shared among different target organ-specific autoimmune diseases such as MG, thyroid disease, celiac disease, and systemic lupus erythematosus (SLE) and may signify a genetic predisposition to developing autoimmunity [22]. Not surprisingly, patients with MG may have additional autoimmune disease, such autoimmune thyroid disease or SLE [51]. Below we discuss notable findings of studies searching for MG susceptibility genes using both candidate approaches, i.e., those informed by current knowledge around autoimmunity and GWAS (summarized in Table 5.1). Although the strength of GWAS is that it is an unbiased exploration of tagged loci, there are likely many risk variants hidden below the GWAS significance threshold [49].

Table 5.1 Significant gene associations with MG by odds ratios (OR) in unbiased genome-wide association studies (GWAS)

	EOMG	LOMG	All MG (>18 years)
HLA class I: *-B*08* female; male	6.9; 3.6 [56] 4.7 [50]	–	
HLA class II: *-DRB1*, *-DQA1* (rs9271871) *-DQA1*0501*	4.9 [50]	4.3 [50] 0.5 [57]	2.3 [57]
PTPN22 R620W	1.7 [56]	1.6 [57]	
TNIP1	1.7 [56]	–	
TNFRSFIIA	–	1.4 [57] 1.6 [50]	
CTLA4	–		1.4 [50]
ZBTB10	–	0.5 [57]	–

Non-HLA Genes

Protein Tyrosine Phosphatase Non-receptor Type 22 (PTPN22)

The PTPN22 1858T (or R620W) variation has been extensively studied over the last decade in European Caucasians for association with MG after it was shown to associate with thyroid disease and RA [49]. Several groups have found modest, albeit inconsistent, associations with subgroups of MG stratified by laboratory features such as "non-thymoma with anti-titin antibodies" [52], "MG without anti-titin antibodies" [53], and late-onset AChR-Ab positive LOMG [54]. A meta-analysis of these European studies failed to show any association with MG or MG subgroups [55]. A large GWAS performed in Caucasians with EOMG found an association signal with the rs2476601 SNP encoding R620W [56] although two independent GWAS Caucasian cohorts found this SNP to associate with LOMG [50, 57]. *PTPN22* encodes a lymphocyte-specific protein which acts as a negative regulator of T-cell signaling responses. The 620W risk variant is thought to reduce the T-cell regulatory effect and impact on B-cell activation [57]. A report from Turkey found no association with this variant and MuSK-MG [58].

Cathepsin 2 (CTSL2)

Cathepsin 2 (CTSL2) encodes the cysteine protease cathepsin L2, which is involved in antigen presentation by HLA class II molecules in the thymus. Although an association was reported between a SNP upstream of CTSL2 in a small group of European subjects with EOMG, as well

as juvenile-onset type 1 diabetics, CTSL2 gene expression studies on thymic tissues were negative [59].

The BAFF and VAV1 Interaction

A SNP association study in a large multicenter cohort of Caucasians with AChR-Ab-positive EOMG analyzed several SNPs spanning 35 candidate genes. Apart from the MHC region (HLA DRA and TNF), the only significant "signal," albeit with modest association, was observed by analyzing the interplay between SNP alleles in *VAV1* and allelic variants in the *BAFF* promoter region. The authors suggest an epistatic interaction, which is stronger than either gene alone, between VAV1 involved in T-cell proliferation and BAFF involved with maturation of B-cells [60]. Caucasians with EOMG compared to population controls showed a modest negative association with SNPs tagging the coding regions of two genes, VAV1 and BAFF, both of which encode proteins involved in T-cell and B-cell functions, respectively [60]. The significance of these associations is unclear.

Cytokine Polymorphisms

Various groups have performed case-control studies assessing the association of MG with interleukin gene polymorphisms. Variations in the *interleukin-4 (IL-4) receptor alpha* (*IL4Ra*) gene were reported to show an association in a European cohort [61], although this was not reproduced [62]. Several polymorphisms in the *interleukin-10 (IL-10)* gene have been reported to be associated with RA, SLE, and inflammatory bowel disease. An association with a functional homozygous polymorphism in the *IL-10* promoter region, resulting in "low" IL-10 secretion, associated with a subgroup of late-onset MG and thymoma MG [63]. A group from Turkey assessed several polymorphisms in the *interferon-gamma*, *IL-10*, and *interleukin-12b* genes in a MG cohort comprising several subgroups but found no significant associations after correcting for multiple comparisons [64].

Another cytokine, tumor necrosis factor superfamily IIA (TNFRS11A), was found to associate with Caucasians with AChR-Ab MG and older than 50 years in two independent GWAS cohorts [50, 57]. Therefore, *TNFRS11A*, which encodes the receptor activator of nuclear factor-κB important in the regulation of immune surveillance between T-cell and dendritic cells, appears to have a role in older individuals developing MG [50].

TNFAIP3-Interacting Protein 1 (TNIP1)

A GWAS performed in Caucasians with EOMG showed a modest but significant association with *TNIP1* [56] although an independent GWAS did not confirm this signal [49]. Gregersen et al. found several non-synonymous SNPs in the *TNIP1* gene were almost twofold higher among the EOMG cases compared with controls [56]. TNIP1, a member of a family of proteins interacting with transcription factors such as NF-$_{\kappa}$B1, has an inhibitory profile. Interestingly, mice with knock-in TNIP1 mutations showed elevated immunoglobulin levels and B-cell hyperactivation phenotypes [56].

Cytotoxic T-Lymphocyte-Associated Protein 4 (CTLA4)

In a large Caucasian cohort comprising North Americans and Italians, SNPs in and flanking the *CTLA4* locus were associated with MG at genome-wide significance level [50]. Although the *CTLA4 +49A > G* association was replicated in a smaller cohort of older MG cases from Germany, the population frequency of the variant was high (MG 49% vs. controls 36%) [65]. Two candidate gene studies performed in China found associations with AChR-Ab-positive MG and *CTLA4*, although one found the association with a promoter region SNP (rs733618) in only Chinese children with MG [33], whereas the other study found the same SNP association only in a combined cohort of older- and younger-onset MG [66]. CTLA4 appears to be involved in the autoregulation or feedback control of activated T-cells and possibly B-cell expansion signals [50].

MG-Specific Candidate Genes

The predominant autoantigen in AChR-Ab-positive MG is the alpha subunit of the AChR encoded by the *CHRNA1* gene. Previously, associations with polymorphisms in the *CHRNA1* gene and MG subjects of European and African genetic ancestry were found [48, 67]. A rare functional SNP (rs16862847) in the promoter region of the *CHRNA1* gene was found to associate with early-onset Caucasian MG and more recently this association was replicated in a Chinese cohort [33, 68]. Giraud et al. showed that the alternate variant disrupted the binding of the interferon regulatory factor 8 (IRF8) thereby repressing the promoter activity of *CHRNA1* in thymic epithelial cells *in vitro*; it is speculated that the consequent repression of alpha-subunit transcription in the thymus may impact on establishing self-tolerance to the autoantigen [68].

No associations have been found for genes that encode the beta and epsilon subunits of the AChR [22], but an association has been found with variants in the gene of the delta subunit [69].

HLA Genetic Associations

The human leukocyte antigen (HLA) region on chromosome 6 was the first genetic region that was studied for association with MG in Europeans using serotyping techniques [24]. MG, like other target-organ autoimmune diseases, has been shown to associate with various class I (HLA-A, HLA-B) and class II genes (HLA-DR, HLA-DQ). These HLA genes encode molecules that present antigens to CD4+ T helper cells, which are necessary to mount an adaptive immune response specific to foreign pathogens. In the case of autoimmune disease, HLA gene polymorphisms may impact on the structure of HLA molecules, which alter the affinity and presentation of self-antigens and thereby influence tolerance to self-antigens. The HLA gene loci are difficult to study due to their highly polymorphic nature and extensive linkage disequilibrium (multiple HLA alleles which are co-inherited across different loci).

Table 5.2 presents HLA associations among MG subgroups by study population and geographical area. The HLA-A1, -B8, -DR3 haplotype (where DR3 designation implicates various DRB1 alleles based on their assigned serological specificities) is the earliest reported HLA association in AChR-Ab-positive EOMG [24]. Subsequent candidate gene association studies have replicated the association with MG and HLA-B*08 and HLA-DRB1*03/*03:01. In AChR-Ab EOMG, HLA-B*08 has the strongest association in various northern and southern European populations and has been replicated in a GWAS study comprising Caucasians with EOMG [54, 56, 70]. In this EOMG cohort, HLA-B*08 showed significantly stronger associations in women compared to men (OR = 6.9 vs. 3.6) [56]. Although the previously reported association signal for HLA-DRB1*03:01 was detected, it lost significance after conditioning on the HLA-B*08 association signal, which indicates that the two alleles are closely associated and that the true MG association signal can be attributed entirely to HLA-B*08. The study also identified an association signal for HLA-DRB*16 with AChR-Ab-positive MG, which has also been found in previous candidate association studies of both AChR-Ab positive and MuSK-MG. A subsequent GWAS, also in Caucasians, failed to replicate the established HLA-A1, -B8, -DR3 haplotype association with EOMG but instead identified a susceptibility allele in the region of HLA-DQA1 (rs601006) [50].

Numerous studies have found an association with various HLA-DQB1*05 alleles in AChR-Ab-positive MG as well as MuSK-MG. However, since the HLA-DQ and HLA-DR loci are in tight linkage disequilibrium, it is possible that this HLA-DQB1 association signal may in fact be linked to the same predisposing HLA-DRB1*03 locus particularly if the HLA-DQ genotyping was performed in isolation.

Childhood-onset ocular MG in Japanese and Chinese children, who were predominantly AChR-Ab negative, found a number associations with HLA-DR and -DQ alleles, excluding HLA-DQB1*03 (see Table 5.2) [71, 72]. These findings

Table 5.2 Positive HLA associations of myasthenia gravis subgroups[a]

	HLA gene association studies	Geographical area	GWAS	Subjects
MG (all subgroups)	**-B*08**	European [82, 83] Middle East [84]		
	-DRB1*03 [-DR3]	European [83, 85] North African [86]		
	-DQB1*05:02	European [87]		
Juvenile MG (AChR-Ab+) ≤15 years	**-DRB1*09:01** **-DRB1*13:02** -DRB3*03:01 -DRB4*01:01	Asian [71]		
	-DQA1*01:02 -DQA1*03:01/*02 **-DQB1*03:03/*01/*02** -DQB1*06:04	Asian [71, 72]#		
EOMG (AChR-Ab+)	**-B*08:01** -C*07:01	European, Turkish [54, 70]	**-B*08**	European [56]
	-DRB1*03/*03:01 [-DR3]	European [78]	-DRB1*03	
LOMG (AChR-Ab+)	**-DRB1*15:01 [-DR2]** -DQB1*05:02	European [37, 70] European [76]	-A*03:01 -DQB1*02:02	European [57]
Thymoma MG	-A*25	European [88]		
MuSK-Ab+	-DRB1*14 [-DR14] -DRB1*16	[European] [77, 79], Japanese [89], Middle East [90], Turkish [76]		
	-DQB1*05/*05:02 **[-DQ5]**	[European] [77, 79], Japanese [89], Turkish [54, 76, 78], Middle East [90]		

[a]Ab+ refers to antibody positive, MG to myasthenia gravis. The PubMed database was searched for literature relevant to HLA association with myasthenia gravis using "myasthenia gravis" and "HLA" search terms. Original research articles written in English and published between 1996 and 2017 which compared MG/MG subgroup vs. age and race matched healthy controls were selected. Data was extracted for positive individual HLA associations (excluding haplotypes) with myasthenia gravis (by subgroup if specified) which were statistically significant after correcting for multiple testing for candidate gene association studies ($p < 0.01$) or reached genome-wide significance ($p < 5 \times 10^{-8}$) for genome-wide association studies (GWAS). Both serological and molecular HLA typing methods were considered. HLA alleles derived from molecular typing are denoted with an asterisk (*). Pre-2010 allele nomenclature was updated [91], and higher allele resolutions are grouped with lower resolutions which define the same allele group. HLA alleles which have associated serological specificities according to the HLA dictionary [91, 92] are grouped with these independently associated HLA serotypes where applicable (shown in brackets). EOMG is defined as AChR-Ab + and onset <40 or 45 years. **Bold** indicates the stronger association. # denotes associations in juvenile ocular MG

are consistent with associations with HLA-DR9 serotype and ocular MG in Chinese children [73, 74]. This suggests that juvenile MG may have a distinct immunological basis.

Few studies have been performed in LOMG. In Europeans with LOMG (over the age of 50) HLA-DRB1*15, which was part of the old HLA-DR2 serotype, and HLA-DQB*05:02 [37, 75] have been found as risk alleles for MG. A GWAS in Caucasians with LOMG showed positive associations with several HLA loci although the strongest was a protective association with HLA-DQA1*05:01 [57].

Several relatively small cohorts of MuSK antibody-positive patients (n = 14–78) have been studied for HLA associations in case-control studies, but the results are strengthened by the reproducibility in populations from various geographical locations comprising cohorts from Northern and Southern Europe (including Turkey), the Middle East (Iranian), and Far East (Japanese) [54, 76–79]. Although MuSK-MG is uniquely associated with HLA-DRB1*14, it also shares allele associations with other MG subgroups (HLA-DRB1*16, HLA-DQB1*05/*05:02). Similar HLA allele associations are found across different MG subgroups, which may not necessarily negate the theory that subtypes of MG have distinct HLA-risk profiles. For example, allelic forms of the DR and DQ HLA proteins among MG patients have an epistatic relationship, either acting together or modifying the effects of the other rather than being causative alleles in isolation [80]. This indicates that defining the "true" MG genetic association signal in subtypes of MG may require consideration of a broader HLA-DR-DQ haplotype rather than individual allelic associations at isolated loci. While HLA-DRB1 alleles in the -DR3 serotype group have shown a consistent association in Caucasians, other HLA-DRB loci might be more relevant in Africans as African Americans with MG were previously reported to have an increase in DRB antigens classified in the HLA-DR5 serologic group [81]. No investigations of HLA associations have been performed in African populations.

Epigenetic Regulation of Gene Expression

Epigenetics refers to events or conditions resulting in the reversible modification of DNA which impacts gene expression, but without modifying the DNA sequence. These epigenetic regulators may include chemical modifications of DNA (methylation) and histones (acetylation) as well as noncoding RNAs [93]. The epigenome is critical in determining whether genes can be actively transcribed or silenced in a given tissue. Factors arising from outside an individual such as environmental triggers, drugs, or toxins or altered internal milieu such as hormonal influences may influence the activity of the DNA methylation machinery and consequently impact on gene expression. Indeed, women are thought to have an increased risk for developing many autoimmune diseases, and recent work suggests that estrogen may impact on the establishment of T-cell tolerance in the thymus by mediating DNA methylation changes to the *autoimmune regulator (AIRE)* gene [94]. A *-579G > T* DNA methyltransferase 3B gene (*DNMT3B)* promoter polymorphism has been implicated in impaired methylation of tumor suppressor genes and to be associated with thymoma MG but not MG without thymoma [95].

Circulating microRNA (miRNA) are small noncoding RNA species (20–22 nucleotides in length) that regulate gene expression by posttranscriptional silencing; miRNA molecules can recognize and bind to specific motifs in the untranslated region of mRNA transcripts (seed regions) and thereby target them for degradation. These molecules are encoded by the genome, may be influenced by infectious agents, and can have both widespread and tissue-specific effects. The genes encoding miRNA may also be subject to genetic variation, which may impact on pathways/networks in complex diseases [96]. Studies examining the role of miRNAs in MG are emerging. However, since miRNA profiling captures dynamic signals (as opposed to a static signal of encoded DNA variation), it is difficult to correlate and summarize the MG-associated miRNA signatures defined by small studies where the study

variables are not consistent. This is because specific miRNA signatures are likely influenced by factors such as the MG subtype and autoantibody profile, the sample origin (usually serum, peripheral blood mononuclear cells, or thymus tissue), and whether the sample was collected from treated or treatment-naïve patients. In addition, the normalization strategies applied in determining whether a particular miRNA is up- or downregulated are complex and sensitive to biases arising from small-sized cohorts which is a characteristic of many studies performed to date.

Despite these limitations, some common themes are emerging with regard to miRNA dysregulation in MG. For example, the let-7 family of miRNAs may impact lymphocyte proliferation and can be influenced by bacterial proteins [39]. This is interesting given that several lines of evidence now implicate a viral infection as a trigger for MG [39]. Punga et al. found let-7a-5p and let-7f-5p were upregulated in the sera of MuSK-MG [97]. Another study found that miR-150-5p and miR-21-5p were upregulated and miR-27a-3p was downregulated in sera of AChR-Ab-positive MG patients [98]. Both miR-150-5p and miR-21-5p are involved in the differentiation and immune response of T-cells, while miR-27a-3p influences natural killer (NK) cell cytotoxicity. A GWAS in Caucasians found a SNP variant in the zinc finger- and BTB-domain 10 (*ZBTB10*) gene which showed genome-wide significance for a negative (or protective) association with LOMG (see Table 5.1). This gene is thought to be regulated by miR-27a which in turn may influence *ZBTB10's* impact on Sp1, Sp3, and Sp4 transcription factor signaling and its indirect effect on estrogen [57].

Conclusion

The case-control GWAS association studies in MG, as is true for many other autoimmune diseases, have uncovered genetic variants associated with MG which are not uncommon in the general population. A number of candidates have emerged which show consistently strong associations with MG subtypes; HLA-B*08 strongly associates with EOMG in Caucasians and subjects from the Middle East despite occurring in 17% of the Caucasian controls [56]. MuSK-Ab-positive MG appears to associate with HLA-DRB*14 and -DQB1*05 in individuals with European, North African, and Asian genetic ancestry. As the "proportion" of MG predicted by an individual's genetic background is likely to be between 35 and 38% [50], it is postulated that several susceptibility genes with smaller effect sizes remain to be discovered.

Acknowledgments MN and JMH have received financial support from the South African (SA) Medical Research Center (MRC) and the SA National Research Fund, as well as the AFM Telethon.

References

1. Bach JF. The etiology of autoimmune diseases: the case of myasthenia gravis. Ann N Y Acad Sci. 2012;1274:33–9.
2. Boldingh MI, Maniaol AH, Brunborg C, Dekker L, Heldal AT, Lipka AF, et al. Geographical distribution of myasthenia gravis in northern Europe—results from a population-based study from two countries. Neuroepidemiology. 2015;44(4):221–31.
3. Carr AS, Cardwell CR, McCarron PO, McConville J. A systematic review of population based epidemiological studies in myasthenia gravis. BMC Neurol. 2010;10:46.
4. Draper IT. Myasthenia gravis. Postgrad Med J. 1965;41(476):356–70.
5. Somnier FE. Increasing incidence of late-onset anti-AChR antibody-seropositive myasthenia gravis. Neurology. 2005;65(6):928–30.
6. Pedersen EG, Hallas J, Hansen K, Jensen PE, Gaist D. Late-onset myasthenia not on the increase: a nationwide register study in Denmark, 1996–2009. Eur J Neurol. 2013;20(2):309–14.
7. Lai CH, Tseng HF. Nationwide population-based epidemiological study of myasthenia gravis in taiwan. Neuroepidemiology. 2010;35(1):66–71.
8. Matsui N, Nakane S, Nakagawa Y, Kondo K, Mitsui T, Matsumoto T, et al. Increasing incidence of elderly onset patients with myasthenia gravis in a local area of Japan. J Neurol Neurosurg Psychiatry. 2009;80(10):1168–71.
9. Gattellari M, Goumas C, Worthington JM. A national epidemiological study of myasthenia gravis in Australia. Eur J Neurol. 2012;19(11):1413–20.
10. Bateman KJ, Schinkel M, Little F, Liebenberg L, Vincent A, Heckmann JM. Incidence of seropositive myasthenia gravis in Cape Town and South Africa. S Afr Med J. 2007;97(10):959–62.

11. Mombaur B, Lesosky MR, Liebenberg L, Vreede H, Heckmann JM. Incidence of acetylcholine receptor-antibody-positive myasthenia gravis in South Africa. Muscle Nerve. 2015;51(4):533–7.
12. Vincent A, Clover L, Buckley C, Grimley Evans J, Rothwell PM. Evidence of underdiagnosis of myasthenia gravis in older people. J Neurol Neurosurg Psychiatry. 2003;74(8):1105–8.
13. van der Watt JJ, Harrison TB, Benatar M, Heckmann JM. Polyneuropathy, anti-tuberculosis treatment and the role of pyridoxine in the HIV/AIDS era: a systematic review. Int J Tuberc Lung Dis. 2011;15(6):722–8.
14. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. Lancet Neurol. 2015;14(10):1023–36.
15. Mombaur B, Heckmann JM. Myasthenia gravis is a rare but treatable disease. S Afr Med J. 2015;105(8):619.
16. Phillips LH II, Torner JC. Epidemiologic evidence for a changing natural history of myasthenia gravis. Neurology. 1996;47(5):1233–8.
17. Alkhawajah NM, Oger J. Late-onset myasthenia gravis: a review when incidence in older adults keeps increasing. Muscle Nerve. 2013;48(5):705–10.
18. Zisimopoulou P, Evangelakou P, Tzartos J, Lazaridis K, Zouvelou V, Mantegazza R, et al. A comprehensive analysis of the epidemiology and clinical characteristics of anti-LRP4 in myasthenia gravis. J Autoimmun. 2014;52:139–45.
19. Pakzad Z, Aziz T, Oger J. Increasing incidence of myasthenia gravis among elderly in British Columbia, Canada. Neurology. 2011;76(17):1526–8.
20. Heldal AT, Eide GE, Gilhus NE, Romi F. Geographical distribution of a seropositive myasthenia gravis population. Muscle Nerve. 2012;45(6):815–9.
21. Benamer HT, Bredan A. The epidemiology of myasthenia gravis in Arab countries: a systematic review. Muscle Nerve. 2015;51(1):144–5.
22. Avidan N, Le Panse R, Berrih-Aknin S, Miller A. Genetic basis of myasthenia gravis—a comprehensive review. J Autoimmun. 2014;52:146–53.
23. Somnier FE, Keiding N, Paulson OB. Epidemiology of myasthenia gravis in Denmark. A longitudinal and comprehensive population survey. Arch Neurol. 1991;48(7):733–9.
24. Compston DA, Vincent A, Newsom-Davis J, Batchelor JR. Clinical, pathological, HLA antigen and immunological evidence for disease heterogeneity in myasthenia gravis. Brain. 1980;103(3):579–601.
25. Haliloglu G, Anlar B, Aysun S, Topcu M, Topaloglu H, Turanli G, et al. Gender prevalence in childhood multiple sclerosis and myasthenia gravis. J Child Neurol. 2002;17(5):390–2.
26. Parr JR, Andrew MJ, Finnis M, Beeson D, Vincent A, Jayawant S. How common is childhood myasthenia? The UK incidence and prevalence of autoimmune and congenital myasthenia. Arch Dis Child. 2014;99(6):539–42.
27. Popperud TH, Boldingh MI, Brunborg C, Faiz KW, Heldal AT, Maniaol AH, et al. Juvenile myasthenia gravis in Norway: a nationwide epidemiological study. Eur J Paediatr Neurol. 2017;21(2):312–7.
28. Oh SJ, Morgan MB, Lu L, Hatanaka Y, Hemmi S, Young A, et al. Racial differences in myasthenia gravis in Alabama. Muscle Nerve. 2009;39(3):328–32.
29. Huda S, Woodhall MR, Vincent A, Heckmann JM. Characteristics of acetylcholine-receptor-antibody-negative myasthenia gravis in a South African cohort. Muscle Nerve. 2016;54(6):1023–9.
30. Niks EH, Kuks JB, Verschuuren JJ. Epidemiology of myasthenia gravis with anti-muscle specific kinase antibodies in the Netherlands. J Neurol Neurosurg Psychiatry. 2007;78(4):417–8.
31. Yeh JH, Chen WH, Chiu HC, Vincent A. Low frequency of MuSK antibody in generalized seronegative myasthenia gravis among Chinese. Neurology. 2004;62(11):2131–2.
32. Zhang X, Yang M, Xu J, Zhang M, Lang B, Wang W, et al. Clinical and serological study of myasthenia gravis in HuBei Province, China. J Neurol Neurosurg Psychiatry. 2007;78(4):386–90.
33. Hong Y, Skeie GO, Zisimopoulou P, Karagiorgou K, Tzartos SJ, Gao X, et al. Juvenile-onset myasthenia gravis: autoantibody status, clinical characteristics and genetic polymorphisms. J Neurol. 2017;264(5):955–62.
34. Grob D, Arsura EL, Brunner NG, Namba T. The course of myasthenia gravis and therapies affecting outcome. Ann N Y Acad Sci. 1987;505:472–99.
35. Heckmann JM, Hansen P, Van Toorn R, Lubbe E, Janse van Rensburg E, Wilmshurst JM. The characteristics of juvenile myasthenia gravis among South Africans. S Afr Med J. 2012;102(6):532–6.
36. Kim JH, Hwang JM, Hwang YS, Kim KJ, Chae J. Childhood ocular myasthenia gravis. Ophthalmology. 2003;110(7):1458–62.
37. Maniaol AH, Elsais A, Lorentzen AR, Owe JF, Viken MK, Saether H, et al. Late onset myasthenia gravis is associated with HLA DRB1*15:01 in the Norwegian population. PLoS One. 2012;7(5):e36603.
38. Heckmann JM, Owen EP, Little F. Myasthenia gravis in South Africans: racial differences in clinical manifestations. Neuromuscul Disord. 2007;17(11–12):929–34.
39. Berrih-Aknin S, Le Panse R. Myasthenia gravis: a comprehensive review of immune dysregulation and etiological mechanisms. J Autoimmun. 2014;52:90–100.
40. Richards J, Howard JF Jr. Seronegative myasthenia gravis associated with malignant thymoma. Neuromuscul Disord. 2017;27(5):417–8.
41. Soltys J, Gong B, Kaminski HJ, Zhou Y, Kusner LL. Extraocular muscle susceptibility to myasthenia gravis: unique immunological environment? Ann N Y Acad Sci. 2008;1132:220–4.
42. Nel M, Jalali Sefid Dashti M, Gamieldien J, Heckmann JM. Exome sequencing identifies targets in the treatment-resistant ophthalmoplegic subphe-

notype of myasthenia gravis. Neuromuscul Disord. 2017;27(9):816–25.
43. Chan JW, Orrison WW. Ocular myasthenia: a rare presentation with MuSK antibody and bilateral extraocular muscle atrophy. Br J Ophthalmol. 2007;91(6):842–3.
44. Heckmann JM, Uwimpuhwe H, Ballo R, Kaur M, Bajic VB, Prince S. A functional SNP in the regulatory region of the decay-accelerating factor gene associates with extraocular muscle pareses in myasthenia gravis. Genes Immun. 2010;11(1):1–10.
45. Nel M, Buys JM, Rautenbach R, Mowla S, Prince S, Heckmann JM. The African-387 C>T TGFB1 variant is functional and associates with the ophthalmoplegic complication in juvenile myasthenia gravis. J Hum Genet. 2016;61(4):307–16.
46. Pirskanen R. Genetic aspects in myasthenia gravis. A family study of 264 Finnish patients. Acta Neurol Scand. 1977;56(5):365–88.
47. Namba T, Brunner NG, Brown SB, Muguruma M, Grob D. Familial myasthenia gravis. Report of 27 patients in 12 families and review of 164 patients in 73 families. Arch Neurol. 1971;25(1):49–60.
48. Heckmann JM, Morrison KE, Emeryk-Szajewska B, Strugalska H, Bergoffen J, Willcox N, et al. Human muscle acetylcholine receptor alpha-subunit gene (CHRNA1) association with autoimmune myasthenia gravis in black, mixed-ancestry and Caucasian subjects. J Autoimmun. 1996;9(2):175–80.
49. Goris A, Liston A. The immunogenetic architecture of autoimmune disease. Cold Spring Harb Perspect Biol. 2012;4(3):a007260.
50. Renton AE, Pliner HA, Provenzano C, Evoli A, Ricciardi R, Nalls MA, et al. A genome-wide association study of myasthenia gravis. JAMA Neurol. 2015;72(4):396–404.
51. Berrih-Aknin S. Myasthenia gravis: paradox versus paradigm in autoimmunity. J Autoimmun. 2014;52:1–28.
52. Greve B, Hoffmann P, Illes Z, Rozsa C, Berger K, Weissert R, et al. The autoimmunity-related polymorphism PTPN22 1858C/T is associated with anti-titin antibody-positive myasthenia gravis. Hum Immunol. 2009;70(7):540–2.
53. Vandiedonck C, Capdevielle C, Giraud M, Krumeich S, Jais JP, Eymard B, et al. Association of the PTPN22*R620W polymorphism with autoimmune myasthenia gravis. Ann Neurol. 2006;59(2):404–7.
54. Saruhan-Direskeneli G, Hughes T, Yilmaz V, Durmus H, Adler A, Alahgholi-Hajibehzad M, et al. Genetic heterogeneity within the HLA region in three distinct clinical subgroups of myasthenia gravis. Clin Immunol. 2016;166–167:81–8.
55. Provenzano C, Ricciardi R, Scuderi F, Maiuri MT, Maestri M, La Carpia F, et al. PTPN22 and myasthenia gravis: replication in an Italian population and meta-analysis of literature data. Neuromuscul Disord. 2012;22(2):131–8.
56. Gregersen PK, Kosoy R, Lee AT, Lamb J, Sussman J, McKee D, et al. Risk for myasthenia gravis maps to a (151) pro-->Ala change in TNIP1 and to human leukocyte antigen-B*08. Ann Neurol. 2012;72(6):927–35.
57. Seldin MF, Alkhairy OK, Lee AT, Lamb JA, Sussman J, Pirskanen-Matell R, et al. Genome-wide association study of late-onset myasthenia gravis: confirmation of TNFRSF11A, and identification of ZBTB10 and three distinct HLA associations. Mol Med. 2015;21(1):769–81. https://doi.org/10.2119/molmed.2015.00232. [Epub ahead of print].
58. Kaya GA, Coskun AN, Yilmaz V, Oflazer P, Gulsen-Parman Y, Aysal F, et al. The association of PTPN22 R620W polymorphism is stronger with late-onset AChR-myasthenia gravis in Turkey. PLoS One. 2014;9(8):e104760.
59. Viken MK, Sollid HD, Joner G, Dahl-Jorgensen K, Ronningen KS, Undlien DE, et al. Polymorphisms in the cathepsin L2 (CTSL2) gene show association with type 1 diabetes and early-onset myasthenia gravis. Hum Immunol. 2007;68(9):748–55.
60. Avidan N, Le Panse R, Harbo HF, Bernasconi P, Poulas K, Ginzburg E, et al. VAV1 and BAFF, via NFkappaB pathway, are genetic risk factors for myasthenia gravis. Ann Clin Transl Neurol. 2014;1(5):329–39.
61. Pal Z, Varga Z, Semsei A, Remenyi V, Rozsa C, Falus A, et al. Interleukin-4 receptor alpha polymorphisms in autoimmune myasthenia gravis in a Caucasian population. Hum Immunol. 2012;73(2):193–5.
62. Huang D, Xia S, Zhou Y, Pirskanen R, Liu L, Lefvert AK. No evidence for interleukin-4 gene conferring susceptibility to myasthenia gravis. J Neuroimmunol. 1998;92(1–2):208–11.
63. Alseth EH, Nakkestad HL, Aarseth J, Gilhus NE, Skeie GO. Interleukin-10 promoter polymorphisms in myasthenia gravis. J Neuroimmunol. 2009;210(1–2):63–6.
64. Yilmaz V, Tutuncu Y, Baris Hasbal N, Parman Y, Serdaroglu P, Deymeer F, et al. Polymorphisms of interferon-gamma, interleukin-10, and interleukin-12 genes in myasthenia gravis. Hum Immunol. 2007;68(6):544–9.
65. Chuang WY, Strobel P, Bohlender-Willke AL, Rieckmann P, Nix W, Schalke B, et al. Late-onset myasthenia gravis—CTLA4(low) genotype association and low-for-age thymic output of naive T cells. J Autoimmun. 2014;52:122–9.
66. Li HF, Hong Y, Zhang X, Xie Y, Skeie GO, Hao HJ, et al. Gene polymorphisms for both auto-antigen and immune-modulating proteins are associated with the susceptibility of autoimmune myasthenia gravis. Mol Neurobiol. 2017;54(6):4771–80. [Epub ahead of print].
67. Garchon HJ, Djabiri F, Viard JP, Gajdos P, Bach JF. Involvement of human muscle acetylcholine receptor alpha-subunit gene (CHRNA) in susceptibility to myasthenia gravis. Proc Natl Acad Sci U S A. 1994;91(11):4668–72.
68. Giraud M, Taubert R, Vandiedonck C, Ke X, Levi-Strauss M, Pagani F, et al. An IRF8-binding promoter variant and AIRE control CHRNA1 promiscuous expression in thymus. Nature. 2007;448(7156):934–7.

69. Giraud M, Eymard B, Tranchant C, Gajdos P, Garchon HJ. Association of the gene encoding the delta-subunit of the muscle acetylcholine receptor (CHRND) with acquired autoimmune myasthenia gravis. Genes Immun. 2004;5(1):80–3.
70. Janer M, Cowland A, Picard J, Campbell D, Pontarotti P, Newsom-Davis J, et al. A susceptibility region for myasthenia gravis extending into the HLA-class I sector telomeric to HLA-C. Hum Immunol. 1999;60(9):909–17.
71. Shinomiya N, Nomura Y, Segawa M. A variant of childhood-onset myasthenia gravis: HLA typing and clinical characteristics in Japan. Clin Immunol. 2004;110(2):154–8.
72. Zhu WH, Lu JH, Lin J, Xi JY, Lu J, Luo SS, et al. HLA-DQA1*03:02/DQB1*03:03:02 is strongly associated with susceptibility to childhood-onset ocular myasthenia gravis in Southern Han Chinese. J Neuroimmunol. 2012;247(1–2):81–5.
73. Hawkins BR, Yu YL, Wong V, Woo E, Ip MS, Dawkins RL. Possible evidence for a variant of myasthenia gravis based on HLA and acetylcholine receptor antibody in Chinese patients. Q J Med. 1989;70(263):235–41.
74. Chen WH, Chiu HC, Hseih RP. Association of HLA-Bw46DR9 combination with juvenile myasthenia gravis in Chinese. J Neurol Neurosurg Psychiatry. 1993;56(4):382–5.
75. Testi M, Terracciano C, Guagnano A, Testa G, Marfia GA, Pompeo E, et al. Association of HLA-DQB1 *05:02 and DRB1 *16 alleles with late-onset, Nonthymomatous, AChR-Ab-positive myasthenia gravis. Autoimmune Dis. 2012;2012:541760.
76. Alahgholi-Hajibehzad M, Yilmaz V, Gulsen-Parman Y, Aysal F, Oflazer P, Deymeer F, et al. Association of HLA-DRB1 *14, -DRB1 *16 and -DQB1 *05 with MuSK-myasthenia gravis in patients from Turkey. Hum Immunol. 2013;74(12):1633–5.
77. Niks EH, Kuks JB, Roep BO, Haasnoot GW, Verduijn W, Ballieux BE, et al. Strong association of MuSK antibody-positive myasthenia gravis and HLA-DR14-DQ5. Neurology. 2006;66(11):1772–4.
78. Bartoccioni E, Scuderi F, Augugliaro A, Chiatamone Ranieri S, Sauchelli D, Alboino P, et al. HLA class II allele analysis in MuSK-positive myasthenia gravis suggests a role for DQ5. Neurology. 2009;72(2):195–7.
79. Nikolic AV, Andric ZP, Simonovic RB, Rakocevic Stojanovic VM, Basta IZ, Bojic SD, et al. High frequency of DQB1*05 and absolute absence of DRB1*13 in muscle-specific tyrosine kinase positive myasthenia gravis. Eur J Neurol. 2015;22(1):59–63.
80. Lincoln MR, Ramagopalan SV, Chao MJ, Herrera BM, Deluca GC, Orton SM, et al. Epistasis among HLA-DRB1, HLA-DQA1, and HLA-DQB1 loci determines multiple sclerosis susceptibility. Proc Natl Acad Sci U S A. 2009;106(18):7542–7.
81. Christiansen FT, Pollack MS, Garlepp MJ, Dawkins RL. Myasthenia gravis and HLA antigens in American blacks and other races. J Neuroimmunol. 1984;7(2–3):121–9.
82. Vandiedonck C, Beaurain G, Giraud M, Hue-Beauvais C, Eymard B, Tranchant C, et al. Pleiotropic effects of the 8.1 HLA haplotype in patients with autoimmune myasthenia gravis and thymus hyperplasia. Proc Natl Acad Sci U S A. 2004;101(43):15464–9.
83. Donmez B, Ozakbas S, Oktem MA, Gedizlioglu M, Coker I, Genc A, et al. HLA genotypes in Turkish patients with myasthenia gravis: comparison with multiple sclerosis patients on the basis of clinical subtypes and demographic features. Hum Immunol. 2004;65(7):752–7.
84. Hajeer AH, Sawidan FA, Bohlega S, Saleh S, Sutton P, Shubaili A, et al. HLA class I and class II polymorphisms in Saudi patients with myasthenia gravis. Int J Immunogenet. 2009;36(3):169–72.
85. Giraud M, Beaurain G, Yamamoto AM, Eymard B, Tranchant C, Gajdos P, et al. Linkage of HLA to myasthenia gravis and genetic heterogeneity depending on anti-titin antibodies. Neurology. 2001;57(9):1555–60.
86. Fekih-Mrissa N, Klai S, Zaouali J, Gritli N, Mrissa R. Association of HLA-DR/DQ polymorphism with myasthenia gravis in Tunisian patients. Clin Neurol Neurosurg. 2013;115(1):32–6.
87. Baggi F, Antozzi C, Andreetta F, Confalonieri P, Ciusani E, Begovich AB, et al. Identification of a novel HLA class II association with DQB1*0502 in an Italian myasthenic population. Ann N Y Acad Sci. 1998;841:355–9.
88. Vandiedonck C, Raffoux C, Eymard B, Tranchant C, Dulmet E, Krumeich S, et al. Association of HLA-A in autoimmune myasthenia gravis with thymoma. J Neuroimmunol. 2009;210(1–2):120–3.
89. Kanai T, Uzawa A, Kawaguchi N, Sakamaki T, Yoshiyama Y, Himuro K, et al. HLA-DRB1*14 and DQB1*05 are associated with Japanese anti-MuSK antibody-positive myasthenia gravis patients. J Neurol Sci. 2016;363:116–8.
90. Ehsan S, Amirzargar A, Yekaninejad MS, Mahmoudi M, Mehravar S, Moradi B, et al. Association of HLA class II (DRB1, DQA1, DQB1) alleles and haplotypes with myasthenia gravis and its subgroups in the Iranian population. J Neurol Sci. 2015;359(1–2):335–42.
91. Marsh SG, Albert ED, Bodmer WF, Bontrop RE, Dupont B, Erlich HA, et al. Nomenclature for factors of the HLA system, 2010. Tissue Antigens. 2010;75(4):291–455.
92. Holdsworth R, Hurley CK, Marsh SG, Lau M, Noreen HJ, Kempenich JH, et al. The HLA dictionary 2008: a summary of HLA-A, -B, -C, -DRB1/3/4/5, and -DQB1 alleles and their association with serologically defined HLA-A, -B, -C, -DR, and -DQ antigens. Tissue Antigens. 2009;73(2):95–170.
93. Klein CJ, Benarroch EE. Epigenetic regulation: basic concepts and relevance to neurologic disease. Neurology. 2014;82(20):1833–40.
94. Dragin N, Bismuth J, Cizeron-Clairac G, Biferi MG, Berthault C, Serraf A, et al. Estrogen-mediated downregulation of AIRE influences sexual dimorphism in autoimmune diseases. J Clin Invest. 2016;126(4):1525–37.

95. Coppede F, Ricciardi R, Denaro M, De Rosa A, Provenzano C, Bartoccioni E, et al. Association of the DNMT3B -579G>T polymorphism with risk of thymomas in patients with myasthenia gravis. PLoS One. 2013;8(11):e80846.
96. Huang RS, Gamazon ER, Ziliak D, Wen Y, Im HK, Zhang W, et al. Population differences in microRNA expression and biological implications. RNA Biol. 2011;8(4):692–701.
97. Punga T, Bartoccioni E, Lewandowska M, Damato V, Evoli A, Punga AR. Disease specific enrichment of circulating let-7 family microRNA in MuSK+ myasthenia gravis. J Neuroimmunol. 2016;292:21–6.
98. Punga T, Le Panse R, Andersson M, Truffault F, Berrih-Aknin S, Punga AR. Circulating miRNAs in myasthenia gravis: miR-150-5p as a new potential biomarker. Ann Clin Transl Neurol. 2014;1(1):49–58.

6 Clinical Presentations of Myasthenia Gravis

Jan B. M. Kuks

Definition and Classification

The signs and symptoms of a postsynaptic neuromuscular junction disorder as myasthenia gravis (MG) result from fluctuating strength of the voluntary muscles. The degree of weakness is partly dependent on the exertion of a muscle group but also varies spontaneously over longer periods of time influenced by the immunological or hormonal factors, stress, medication use, or without apparent cause. The course of MG is difficult to predict, especially in the first years when relapses and remissions may be frequent. Most patients have a more stable course later on, and in the long term, full remissions may occur. All voluntary muscle may be involved but usually to varying degrees. In about 15% of the patients with MG, manifestations remain confined to the ocular muscles, while among others bulbar symptoms and signs predominate, even without any ocular symptom. Usually MG begins with a few isolated signs (Table 6.1) and extends to other muscles within a few weeks to a few months, and seldom over years. Apart from a certain degree of local muscle atrophy that may occur in a small part of the patients, no other neurological abnormalities are expected.

Most patients with myasthenic symptoms suffer from the acquired autoimmune disease, myasthenia gravis with antibodies to the acetylcholine receptor (a-AChR). Another autoimmune postsynaptic neuromuscular junction disorder is myasthenia with antibodies to muscle-specific tyrosine kinase at the end plate (a-MuSK). In the remaining group of acquired MG, about 50% of the patients may have antibodies against lipoprotein-related protein (LRP4). Acquired MG, developing at any age after birth, should be distinguished from transient neonatal myasthenia, occurring in 10–15% of children of myasthenic mothers and congenital (hereditary) myasthenia, which is usually present at birth but may become apparent in the first years of life, or at times in adulthood.

Clinical Manifestation

Ocular

Ocular symptoms are the most frequent manifestations of MG. No consensus is found in the literature about the ocular symptom that most frequently appears first: ptosis or diplopia. Further, any of the extraocular muscles may be involved in isolation or in combination leading to horizontal, vertical, or diagonal double vision.

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H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*, Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_6

Table 6.1 Clinical manifestations of myasthenia gravis

	Early onset 1–39 years	Late onset 40–85 years	Thymoma	Total
Ocular diplopia	70 (14)[a]	84 (36)	17 (1)	171
Ptosis	55 (16)	36 (12)	14	105
Ptosis and diplopia	64 (10)	47 (18)	15	126
Bulbar articulation	43	5	6	54
Face	20	2		22
Chewing	1	6	3	10
Swallowing	7	4	1	12
Neck muscles	2	5	4	11
Combined	23	20	16	59
Oculo-bulbar	25	17	30	72
Limbs arms	15	3	4	22
Hands or fingers	12	1	2	15
Legs	45	6	1	52
Combined	29	5	5	39
Generalized	4	5	16	25
Respiration	4	2	4	10
Total	419 (40)	248 (66)	138 (1)	805

[a]Initial signs of MG reported by the author's patients. In parentheses are number of the patients who remained purely ocular during the follow-up. This was the case in only 1 patient with a thymoma, in 9.5% in the early-onset group, and 26.6% in the late-onset group. The ocular muscles were involved at onset in 59% of all patients, the bulbar muscles in 30%, and the ocular and bulbar muscles combined in 80%. Of the 10 patients with initial respiratory signs, 8 had a prolonged apnea without previous weakness, and 2 were children with high fever. The onset with ocular signs was more frequent in the late-onset group than in the early-onset group (74% vs. 51%). Limb muscle weakness was more frequent in the early-onset than in the late-onset group (25% vs. 8%). Patients with thymomas had the highest incidence of bulbar signs at onset (55% vs. 31% in the early-onset and 26% in the late-onset group)

The inferior oblique muscle is most frequently involved [1]; purely horizontal double vision is relatively rare and should make considering other diagnoses. Ptosis is often asymmetric and may fluctuate in degree and in response to contralateral action (see Chap. 7, Ocular Myasthenia). In cases of symmetric ptosis, blepharospasm should be excluded. The patient is immediately aware of double vision as it occurs acutely, but a mild ptosis may escape attention. Some patients appreciate fluctuation of the diplopia and may be able to characterize the nature of the double images themselves. Others only complain of blurred vision, which becomes normal when viewing with one eye. Patients may complain of worsening of their symptoms by bright light and prefer to wear sunglasses. Patients with isolated fluctuating ocular symptoms with no other evidence of weakness form a distinct group of "ocular myasthenia"; in contrast there are rare patients who never have ocular symptoms. In cases of binocular ophthalmoplegia, MG may mimic brainstem disorders, and importantly, MG should not be confused with oculopharyngeal dystrophy, thyroid, or mitochondrial myopathy.

Bulbar

In neurological jargon, "bulbar muscles" are those innervated by motor neurons originating in the pons and the medulla oblongata (cranial nerves V, VII, IX, X, XI, XII). The most frequent of the bulbar signs among patients with severe MG is facial weakness, which may be asymmetrical (in some patients the initial diagnosis is confused with Bell's palsy), but this asymmetry is rarely as pronounced as that seen with ocular signs. Facial weakness usually develops insidiously and only appreciated in retrospect. Although sensory loss is uncharacteristic for MG, some patients complain of stiffness, numbness like the sensation of dental anesthesia, and sometimes paraesthesias or even

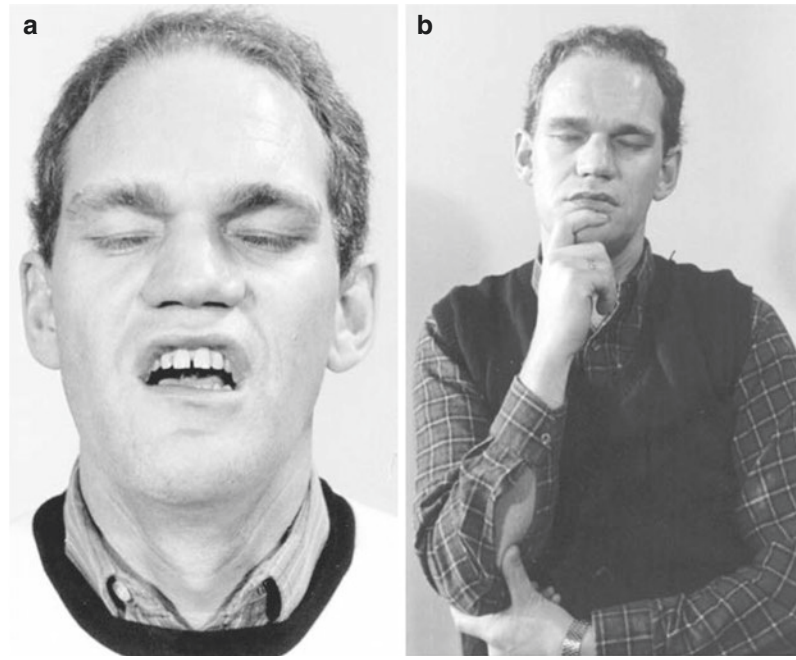

Fig. 6.1 A 36-year-old man with typical facial weakness. (**a**) In an attempt to close the eyelids firmly, the eyelashes remain visible, and the orbicularis oris weakness is evidenced by the straight smile. (**b**) He needs to support his jaw by holding his mouth closed

hypalgesia. At rest, the facial expression may be unremarkable, but any expression of emotion, and particularly laughing, betrays the loss of normal function. Observers may ask why a patient appears sad or angry, whether they show misery or laugh. A classical feature is the myasthenic sneer ("snarl" or the "rire verticale," Fig. 6.1a). Since weakness of the upper part of the face is also present, laughing makes the eyelids droop, even if ptosis is not noticeable at rest.

Orbicularis oris weakness is first noticed by the inability to whistle or kiss, in sneezing forcefully, in eating soup with a spoon, or by dysarthria. Insufficient strength of the orbicularis oculi may cause problems in keeping the eyes closed while washing the face or hair. Several of our patients complained that they could not close one eye and keep the other open while taking photographs. If the eyes are not completely closed during sleeping, they may be irritated on awakening. With severe weakness, patients may avoid social contacts because of their facial symptoms.

Speech difficulties manifesting as nasality of the voice or a difficulty in articulation are the most common at onset of MG. More than any other symptom, dysarthria is likely to occur initially under the influence of emotions. At first, it is frequently an isolated and fluctuating symptom that disappears after a period of silence; later on it may disturb intelligibility in a considerable way. Drunken speech, lisping, slurring, or nasal speech may occur. Loss of air through the nose may be heard in patients with palatal weakness. In a minority of patients, the voice first becomes weaker in volume, but not dysarthric. Some hoarseness may occur, but aphonia is not a sign of MG. Obstruction of the airways by weakness of the vocal cords has been reported [2, 3]. In general, dysarthria and nasality occur, while the volume remains normal. In the case of a hypernasality ("spastic dysarthria"), motor neuron disease should be considered, but differentiating from MG may be difficult [4].

Weakness of the tongue may lead to problems in pronunciation of d, t, and s. Some patients complain that their tongues are thick and do not fit in their mouths. The time needed for eating a meal increases, and conversation becomes difficult while eating.

Chewing may be difficult at the end of the meal, or the problem may be first appreciated when chewing bubble gum or eating peanuts. If observed while eating, some MG patients support their jaws, under the lips and the floor of their

mouth, in order to counteract gravity and to "chew with their hands." A characteristic posture is seen in Fig. 6.1b.

Swallowing difficulties may be due to weakness of the lips, the tongue or pharyngeal muscles, and, often, a combination of these. They may be worsened by incomplete crunching of food in patients with chewing problems. Swallowing in some patients improves if they turn their head. Regurgitation of fluids through the nose is a sign of palatal weakness. Insufficiency of the upper pharyngeal muscles results in the sensation that food is sticking in the throat. Weakness of lower pharyngeal muscles may cause an inability to pass ingested food with a globus sensation. Retrosternal feelings of obstruction should *not* be considered related to MG. Laryngeal weakness may lead to coughing after swallowing. Severe impairment of swallowing accompanies drooling of saliva, choking, and ventilatory insufficiency.

Patients with dysphagia commonly report a preference for cold food. This may relate to improvement in neuromuscular transmission produced by relative cooling of the muscles.

An important symptom correlating well with the severity of dysphagia is weight loss, and many of our patients with bulbar manifestations provide a history of 5–10 kg of weight in the 3–6 months prior to diagnosis. Weakness of neck muscles may result in difficulty in balancing the head, which is particularly troublesome if the patient works in a bent position. Complaints about stiffness, vague pains in the neck and the back of the head, and occasionally paraesthesias are then common, and nearly always set the doctor on the wrong track searching for cervical spine pathology.

Bulbar weakness is not always easily assessed, and quantification may be difficult. Dysphagia may be evaluated clinically and electrophysiologically. On clinical examination, the patient cannot blowout their cheeks without air escaping, if pressure is applied to the cheeks by the examiner's fingers. The closed eyes may be opened with one finger by the examiner, or the eye lids cannot be closed completely. The most sensitive functional test of the muscles of articulation is speaking aloud without interruption. An easy test is counting (l01, 102, etc.) or reading aloud. A certain degree of quantitation of examination findings is possible by noting when dysarthria or nasal twang is appreciated to the time when the patient becomes unintelligible (Fig. 6.2).

Weakness may lead to inability to protrude the tongue and reach the frenulum of the upper lip. If the tongue is pushed against the inner cheek, the degree of weakness can be appreciated directly by the examiner. Palatal weakness may be demonstrated, if peak-flow volume measures improve after pinching the nose. Weakness of the sternocleidomastoids and of the neck muscles may be

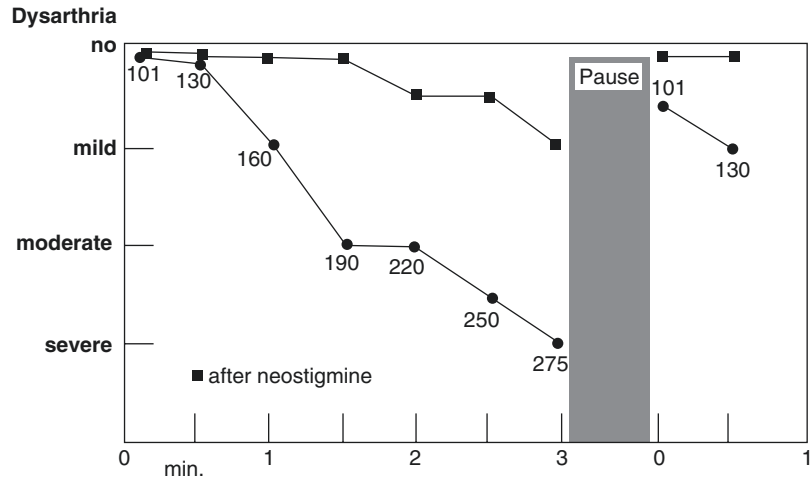

Fig. 6.2 Counting test. Improvement is appreciated by pausing and with neostigmine

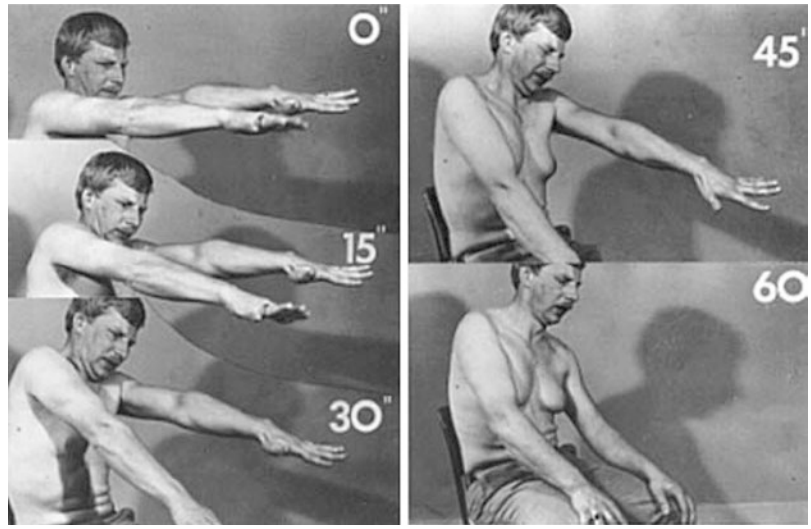

Fig. 6.3 Extended arm test. The patient is asked to lift his arms with hands in pronation. Normally, this position usually may be sustained for at least 3 min. Myasthenic weakness may lead the patient to drop one or both arms. Weakness of one of the extensor digitorum muscles (especially of the fourth finger) may become manifest with this test

appreciated on routine testing. A useful functional test is having the patient lift their head in the recumbent position, and the individual should be able to look at their toes for 60 s.

Several tests and devices that provide quantitative measures of bulbar function among MG patients have been developed [5–8].

Limb, Trunk, and Respiratory

A minority (15–20%) of the patients complain first of weakness of arms, hands, or the legs. Among patients under age 30 years, limb weakness, especially of the legs, was the first sign in one third of our patients. Perhaps, this is related to younger individuals placing these muscle groups into situations of heavier loading, for example, during sports. Inability to maintain arm position or repetitively elevate the upper extremity, e.g., when hanging laundry, hammering a nail, or washing hair, is a common complaint. Isolated weakness of one or more extensors of the fingers, usually the fourth or fifth, may be difficult for the doctor to interpret and sometimes leads to the misdiagnosis of a peripheral nerve compression. Selective triceps muscle weakness has been reported more than once [9].

Leg weakness frequently leads to falls, and several of our patients had MG diagnosed after a fall from stairs. If the first manifestations are weakness of the limbs or of the trunk muscles, most patients complain of undue fatigability and peculiar feelings of heaviness. Most patients have both arm and leg symptoms, but often one predominates, and the differential fatigability may be confirmed by examination (Fig. 6.3).

Fluctuating pain in the back and girdle muscles occurs in some patients and is readily explained as an insufficiency of the postural muscles. Chronic pain is not a feature of MG. Furthermore many patients with postsynaptic myasthenia report muscle cramps irrespective of the use of anticholinesterases or cyclosporine; this complaint may be respond to antiepileptics, such as phenytoin, without worsening the myasthenia.

Weakness of the respiratory and other trunk muscles is rarely the first, isolated sign of the disease but may be the first manifestation that brings a patient to medical care. We have only seen this in rapidly evolving generalized MG in children with coincident infection or as an effect of curare during narcosis (“prolonged apnea”). Some patients report short periods of altered awareness with inspiratory stridor, which are often the forerunners of longer-lasting and life-threatening attacks. Rarely, short episodes of unconsciousness occur, which may not be recognized as caused by sudden apneas. Ventilatory symptoms should always lead to a rapid hospitalization.

Pelvic floor weakness occurs in our experience mostly in combination with upper leg weakness and may lead to stress incontinence, especially in female patients.

Pure limb girdle myasthenia with junctional autoantibodies has been reported [10]. The diagnosis may be delayed because of resemblance to other myopathies, especially in familial forms. The Lambert-Eaton myasthenic syndrome always should be taken into the differential diagnosis of fluctuating proximal limb girdle weakness with or without cranial nerve symptoms, especially if the patients report autonomous symptoms (dry mouth). Using manual and hand-held dynamometer evaluation, nearly 30% of MG patents were found to have permanent fixed muscle weakness, especially in shoulder abductors, and more commonly among men than women [11].

Distal myasthenia gravis [12, 13] is another diagnostic challenge, which may be confused with pure motor "peripheral nerve lesions." If a patient is suspected to have MG, it is essential to test muscle strength after exercise. All tests designed to measure the capacity to do muscle work require the cooperation of the patient. In general this presents no difficulties, but the examiner must discern true weakness from a nonorganic failure to generate maximal muscle force.

The following procedures in the assessment of muscle strength and exhaustibility are useful. Firstly the strength of individual muscle groups is tested after rest. Accuracy is improved by using a hand-held dynamometer and the adoption of fixed postures. After moderate exertion, which does not decrease the strength of normal muscles, the strength of the patient is measured again. Moderate exertion may be standardized as follows [14]:

1. The arms, as well as the hands and fingers, are stretched horizontally (see Fig. 6.3). This position should be maintained for 3 min. Without trembling or shaking. The patient may require some encouragement performing the maneuver. Increasing weakness will produce some shaking or a gradual drooping of arms, hands, or fingers. If weakness is minimal, it may only be detected by measuring the strength again after 3 min of exertion. This test is sensitive but not specific. Patients with other neuromuscular diseases may not be able to maintain their arms outstretched for more than a minute, but the strength before and after this effort remains the same.
2. Grip strength on repeated contraction may be measured with a hand-held dynamometer. A simple ergometer can be made out from a blood pressure manometer.
3. Patients should be able to perform knee bends, or in older people rise, from a standard chair repeatedly without the aid of the arms 20 times.
4. Walking on tiptoes and on the heels at least 30 steps.
5. Straight leg rising to 45° for 1 min in the recumbent position.
6. Vital capacity and peak-flow measurements should give normal values five times in a row. The results of these tests may be compromised by the weakness of the lips or palate.

Among most patients with a generalized MG but without actual or previous dyspnea, a decrease of the vital capacity and other respiratory parameters is found, and even, in 40% of pure ocular cases, the vital capacity may be decreased [15]. Routine lung function tests show that the vital capacity is decreased to a greater extent than the forced expiratory volume. In most patients, peak-flow or vital capacity measurement is a valuable tool in the follow-up and is done easily at any time and circumstance. Most of these tests are quantifiable, and the patient can perform some of them at home, to obtain a reliable picture of diurnal and periodic fluctuation. According to the complaints of the individual, other tests may be appropriate. A survey of the examinations that are used in our practice is given in Table 6.2 [16]. The data acquired may show a convincing difference between the strength at rest and after exertion. They can also serve as a frame of reference in the evaluation of the effect of anticholinesterases and other treatments.

Table 6.2 Clinical investigation of the patient with myasthenia gravis[a]

Inspection of the head at rest
• Note ptosis of the eyelids (usually asymmetrical), ocular deviations, or drooping of the head
• Facial weakness may be manifested by disappearance of the nasolabial fold and loss of expression
• Weakness of the neck- or cheek muscles may be masked by supporting the jaw
• Ptosis may be compensated by lifting the eyebrows
• There may be tilting or rotation of the head to minimalize diplopia
Ocular functions
• Looking straight toward a bright light provokes ptosis
• Looking aside or upward for 60 s[b] provokes ptosis, especially on the side of abduction
• Diplopia may occur after sustained lateral/vertical gaze (maximal 60 s[b], not further than 45° from the midline)
Bulbar functions
• Test peak expiratory flow or vital lung capacity with and without nose clips to detect palatal weakness
• Repeated closing of the eyes tightly provokes weakness of the ocular orbicular muscle; eye lashes may remain visible in spite of firmly closing the eyelids
• Nasal speech may occur after counting 101–199. Note the number where dysarthria or nasality occurs
• Masseter function may be tested by biting on a spatula before and after 100x closing the mouth with click
• Swallowing a glass of water may not be possible without coughing or regurgitation through the nose
Neck musculature (tests in horizontal position)
• Keeping the head raised for 120 s[b] ("look at your feet")
• Raising the head repeatedly (20×)
Arm (test in sitting position)
• Arms stretched forward in pronate position (90°) for 240 s[b]
– Note the beginning of trembling or shaking of the arm/hand
– Note drooping of individual fingers
Hand
• Inflation of sphygmomanometer until 300 mmHg
• Fist closing/opening with fingers joined together (no digital abduction allowed) (70×)
• Hand grip with a dynamometer (dominant hand ≥45[b] (men) or ≥30[b] (women) kgW, non-dominant hand ≥35[b] and ≥25[b] kgW, respectively)
Leg
• Hip flexion (45° supine in horizontal position) for 100 s[b]
• Deep knee bends (20×)
• Rising from a standard chair without the use of the hands
Ventilatory function
• Vital capacity or peak-flow in rested condition
• Vital capacity or peak-flow may decrease after repeated testing (5–10×)

[a]Test muscle force directly before and after repetitive activity

[b]Maximal values according to recommendations of the medical scientific advisory board of the Myasthenia Gravis Foundation of America [19]

Muscle Atrophy

From a clinical viewpoint, localized muscle atrophy is detectable in about 6–10% of the patients with MG [17, 18], and a still higher percentage is reached if patients with permanent ophthalmoplegia are included. Remarkably few details about the distribution of the atrophy are found in the literature, but atrophy of the tongue is particularly appreciated (Fig. 6.4). In our patients localized muscle atrophy occurred in 9% of early-onset patients, 8% of late-onset patients, and 12% of the patients with thymomas [19]. In the limbs we found mainly shoulder muscles, forearm muscles (finger extensors), and foot extensor muscles were affected. Atrophy in bulbar muscles was more frequently found in our series; some of these patients are now recognized having a-MuSK myasthenia [20].

Non-motor Symptoms

Clinical experience with MG patients does not raise suspicion of central nervous system involvement, but some patients may have non-muscle-

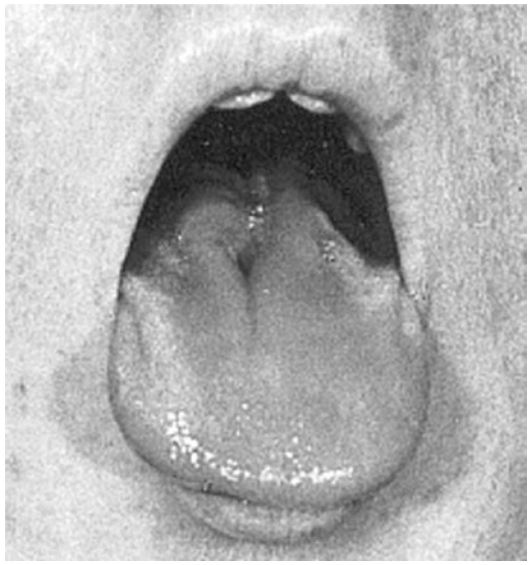

Fig. 6.4 A 60-year-old man with atrophy of the tongue. Note the presence of one medial and two lateral furrows, which define the "triple furrowed tongue." MG existed from early infancy, and was diagnosed at age 52

related complaints. Non-motor symptoms in MG can be attributed to concomitant diseases, especially in patients with a thymoma [21], or occur without a relation to other disorders. Such complaints are not related to acetylcholine receptor antibodies from MG patients, since these do not bind receptors extracted from the human brain, and, therefore, complaints do not arise from autoimmune targeting of central cholinergic receptors [22]. Chronic disease with feelings of unwellness in and of itself may lead to compromised cognitive functioning. Some studies suggest that MG patients perform worse on a range of cognitive tests [23].

Antibodies directed to the ganglionic acetylcholine receptor may cause autonomic neuropathy, and these antibodies incidentally have been found in a-AChR MG as well causing gastrointestinal to panautonomic failure, and the same was found in MG patients without these antibodies, especially in patients with a thymoma. Cross-reactivity of nicotinic AChR antibodies has been postulated [24]. Sympathetic hyperreactivity was found in a mixed group of MG patients [25] and parasympathetic cardiac impairment in patients with [26] and without a thymoma [27]. Although MG is primarily a disease of the acetylcholine receptor on the postsynaptic muscle membrane, some indications exist that somatosensory, auditory, and visual pathway involvement could occur [28].

Fatigue

Weakness, and not merely "fatigue," is a sign of MG. However, MG patients complain of a non-specific feeling of fatigue more often than control subjects [29]. This is not related to the degree of muscle weakness [30] but nevertheless may limit activity level. On the other hand, some patients appreciate that prolonged weakness after unusual activity. Although it is difficult to quantitate fatigue, we feel fatigue never should lead to start or adjust immunomodulation in a given patient. Other factors may play a role: fatigue caused by the use of steroids or adverse effects of other medications, sleepiness related to obstructive sleep apnea syndrome, depression in the context of a chronic disease, autonomic dysfunction [31], concomitant autoimmune or hormonal disorders and deconditioning.

Differential Diagnosis

Making the diagnosis of MG in the patient with a-AChR antibodies is not difficult. If no autoantibodies are found, the history, results of clinical examination, reaction to anticholinesterases, or findings on electrodiagnostic testing may strongly point to the diagnosis. Nevertheless, the diagnosis may remain difficult in a small subgroup of patients, in particular, those patients with purely ocular manifestations. If the patient has *bulbar symptoms*, one might need to consider other neuromuscular diseases such as amyotrophic lateral sclerosis, oculopharyngeal dystrophy, bulbar spinal atrophy, or myotonic dystrophy. Of course, brainstem pathology may also lead to bulbar manifestations. Some patients with isolated complaints and signs of dysarthria may have no organic disease. Isolated dysphagia may be due to a mechanical impairment or to a disturbance in

the parasympathetic innervation of the esophagus (achalasia). Focal dyskinesias may also need to be considered in certain patients.

In the patient seronegative for a-AChR and a-MuSK with *predominant limb weakness*, the possibility of Lambert-Eaton myasthenic syndrome surpasses that of MG. Other acquired diseases with more or less fluctuating weakness of the limb and trunk muscles include motor neuron disease, myositis, endocrine myopathies, and mitochondrial myopathies. The slight benefit appreciated with cholinesterase inhibitor treatment in these diseases should not be considered as evidence of MG. Neck extensor weakness may be a prominent or initial manifestation of MG but also occurs with motor neuron disease and myositis. There is also an isolated neck extensor myopathy that develops over 3 months without leading to further weakness [32]. Finally, some patients just have complaints of muscle pain or muscle fatigue without objective weakness. These symptoms are not suspicious for MG.

Clinical Subtypes

Several clinical subtypes may be recognized according to antibodies, clinical symptoms, and age at onset. These may be important for making therapeutic decisions [33, 34]. Classifications may overlap. Firstly congenital and acquired must be distinguished. Then there is the difference between juvenile-onset MG (age of onset between 1 and 16 years) and adult MG. In adult MG there is a difference between ocular and generalized myasthenia. The first being seronegative (50%) or seropositive (50%) for a-AChR antibodies. For generalized myasthenia there is difference between patients seropositive for a-AChR antibodies, seropositive for anti-MuSK antibodies, and seropositive for LRP4 antibodies or agrin antibodies and (still) seronegative patients. Anti-MuSK myasthenia is characterized by relatively severe bulbar symptoms, limited reaction to cholinesterase inhibitors, probably no effect of thymectomy and association with HLA DR14-DQ5 [35]. There is no clear clinical difference between a-AChR-positive patients and non-MuSK patients [36]; however, in the latter category, thymectomy cannot be recommended.

For a-AChR positive MG, the author finds the analysis of Compston et al. in categorization of patients with acquired MG useful [37]: (1) purely ocular MG (weakness restricted to the palpebral levator and the extraocular muscles), (2) early-onset generalized MG (clinical onset prior the age of 40 years) associated with HLA DR3-B8, (3) late-onset generalized MG (onset after age of 40 years) associated with HLA DR-B7, and (4) patients with a thymoma, with onset at any age. Nowadays most authors divide early and late onset according to an age of onset before or after the age of 50 years. Patients from the second category probably derive more benefit from thymectomy than others do. Anti-striated muscle antibodies mainly are found in the third and fourth categories.

Disease Course

The initial manifestations of MG have a highly variable pattern and evolution among patient. Patients, when treated and relieved of their symptoms, often appreciate that they have suffered from MG longer than initially perceived. There is a tendency of spread to muscle groups beyond those initially affected, and only in 10% [38] to 16% [39] of the patient's weakness confined to the ocular muscles in the first 3 years (Fig. 6.5), yet after 3 years generalization occurs in only 3–10% of these purely ocular patients [18, 38, 39]. It is rare for weakness to remain confined to the bulbar muscles or to the muscles of the extremities.

In most patients the diagnosis is made in the second year after symptom onset, but longer delays are not unusual [18, 38] especially for early-onset women. One reason for this delay found in our study of 100 consecutive patients is the more rapid progression of weakness in men than in women in the first 3 months of disease onset [40]. Patients with limited or predominant limb muscle weakness had a higher chance of a missed diagnosis. In general, severity of illness increases in the first years of the disease, with a

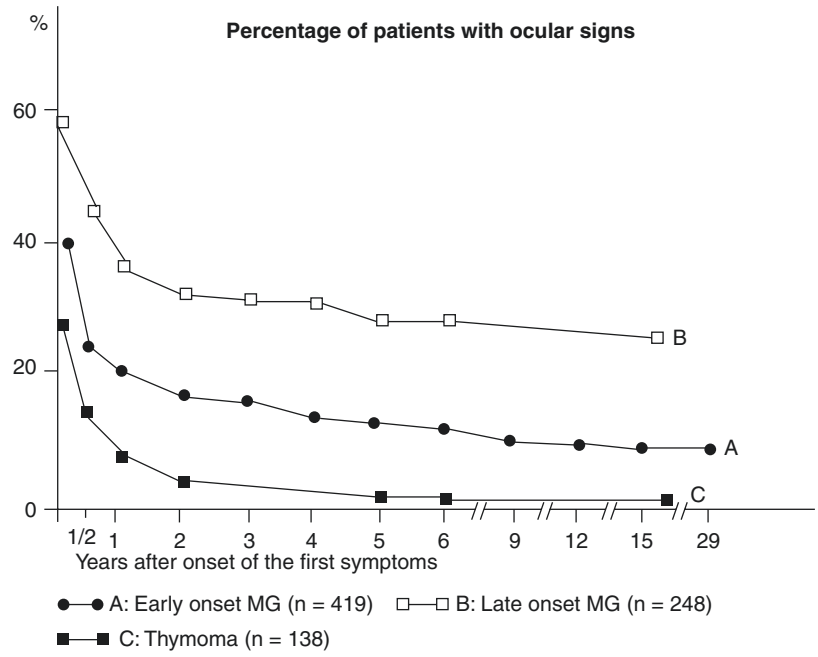

Fig. 6.5 Only ocular signs at onset were present in 39% of early-onset, 58% of late-onset, and 27% of thymoma patients. After 3 years other muscle groups were involved in 84% early-onset, 68% late-onset, and 96% thymoma patients. At the end of the follow-up, 9.6% early onset, 26.6% late-onset, and 1% of thymoma patients remained purely ocular [19]

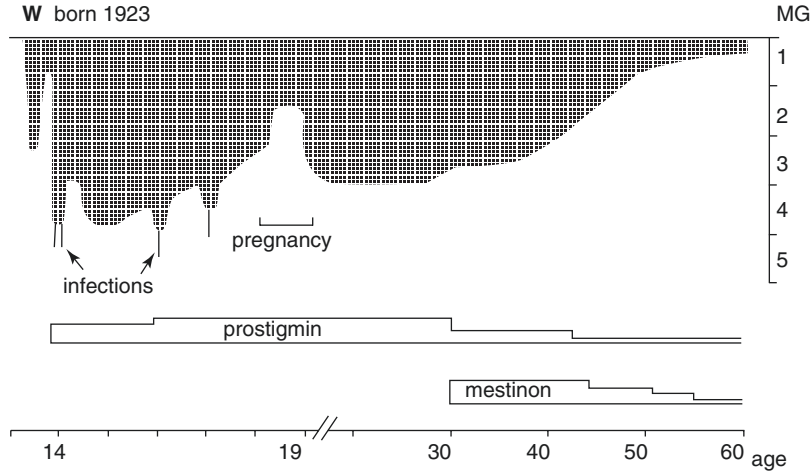

Fig. 6.6 Pictorial representation of a case history of a patient with MG. Onset of MG occurred with dysarthria during a stressful situation with generalization 6 months later, while exacerbations during infections and improvement during pregnancy were observed. During a wartime period, oral prostigmin was replaced by subcutaneous injections (0.5 mg six times a day) because tablets were scarce. The patient improved very gradually after the age of 30, and after the age of 45, only slight weakness was present. Several major operations in the last 10 years were performed without development of weakness. She has been reluctant to discontinue medication (prostigmin 7.5 mg and Mestinon 10 mg three times a day). The severity of symptoms is expressed using a six-point disability score: 0 no symptoms and 5 dependent on artificial ventilation [37, 39]

tendency to stabilize, improve, or even resolve after many years. A typical case history is schematized in Fig. 6.6.

Severe exacerbation of MG leading to myasthenic crisis occurs usually in the first years [39, 41, 42] of the disease and rarely in the first

weeks of onset. One exception is for children with MG with an infection or with rapidly progressive bulbar symptoms that lead to choking and a poor cough. In these patients crises occur more often with gradually increasing generalized weakness during respiratory infections induced by aspiration. Crisis in MG is life-threatening and demands aggressive treatment with immunomodulating strategies to minimize the duration (Chap. 12).

The mortality of MG has been reduced from 30% to zero in the past decades [37, 38, 40]. Spontaneous long-lasting remissions are rare in the first years, but in the long-term they are expected in 10–20% of patients [39–41]. Patients with ocular MG have a higher remission rate than those with the generalized disease [39, 42].

Exacerbating Factors

Certain events are recognized as unfavorably influencing the course of MG and may unmask subclinical MG. Of particular importance are infectious diseases with fever, psychological stress (but see Chap. 18), hyper- or hypothyroidism, and certain drugs such as antimalarial-drugs (quinine, chloroquine), aminoglycosides, beta-adrenergic receptor blocking drugs, and d-penicillamine [43, 44]. Some experts recommend avoidance of vaccinations; others feel they are safe [45]. Patients under immunosuppression are advised by some to stop treatment for 2–3 weeks before immunization, if appropriate [46].

The effect of pregnancy on MG is subject to conflicting reports: improvement or deterioration or no influence, each one third in all the series [47]. Most patients improve in the second part of their pregnancy [47]. In a prospective investigation of 64 pregnancies, MG relapsed in 4 of 23 asymptomatic patients who were not on therapy before conception: in patients under treatment, MG improved in 12 of 31 pregnancies, remained unchanged in 13, and deteriorated in 6. MG worsened after delivery in 15 of 54 pregnancies [48]. Minor exacerbations are experienced 3–10 days before menstruation by at least one third of the women [38, 49].

The effect of ambient temperatures has not been studied extensively. Hot weather in particular is reported to increase weakness [18, 50] and may even induce a crisis [51]. In our experience, the individual response varies considerably, and several of our patients are considerably better in warm weather.

Special Considerations

Incidence and Prevalence

The annual incidence of MG is 3–5 per million [52–54]; incidental reports mention higher incidences up to 21 [55, 56]. The prevalence is about 60 per million [52–54, 57–60], in some surveys even higher up to 150 [61]. A striking difference of prevalence was appreciated between cities and rural areas [57] but may be due to variation in populations and medical facilities. The prevalence seems to be increasing in the last decades, probably influenced by new diagnostic tests, more attentiveness by multimedia and social networks, a decrease of mortality rate, an increase of life expectancy [62], and comprehensive methods of case identification [62–67]. Nowadays, MG is rather a disease of the elderly than of young women [64]. In Virginia the incidence and prevalence were found to be higher in the African-American population than in the corresponding Caucasian population. Ocular myasthenia comprised 25% of this group of patients [58], which was higher than observed in previous series. In our own patient group, we estimate 90% of the generalized patients having a-AChR antibodies and 3% having anti-MuSK [68].

Age, Gender, and Classification

MG may have its onset at any age. In general, women are affected twice as often as men, in the childbearing period even three times as frequently, while the incidence is about equal before puberty and after the age of 40 [54, 60, 69, 70]. The relative incidence is highest in women in the

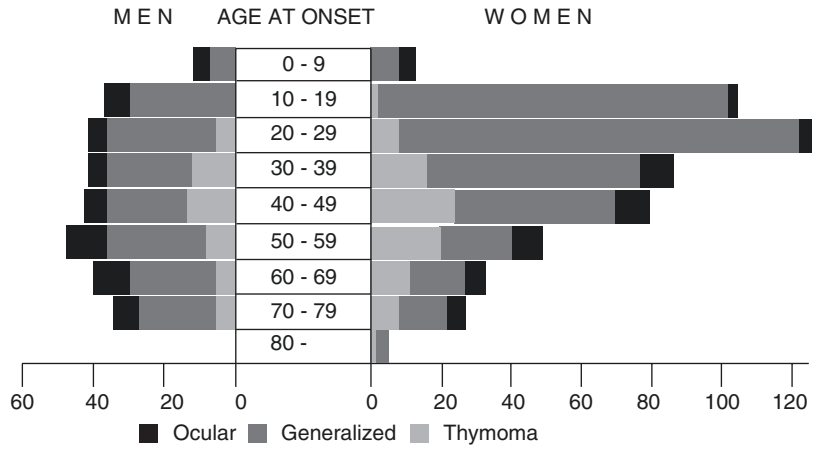

Fig. 6.7 Clinical and demographic data from 805 patients with MG evaluated between 1960 and 1994 [19]

third decade [52, 54], and in some series a late peak is found in older men [39, 54, 71]. Several authors mention an increase in incidence of elderly-onset patients [72, 73], and this is explained by population aging and probably environmental factors.

Ocular myasthenia occurs in about 15% of patients and has a higher prevalence in men, especially over the age of 40 [60, 74]. Age at onset, gender, type, and incidence of thymomas in the author's series of 800 patients are given in Fig. 6.7. A relatively higher onset (22%) in the first decade, or 36% before puberty, is reported from Chinese populations [75, 76], and the disease remains restricted to the ocular muscles in more than 50% of patients. However, the incidence of congenital cases is unknown in these series.

Acquired Juvenile MG

The prevalence of patients with disease onset in the first decade in European and American series varies from 1 to 3%, which is lower than the overall prevalence in the population. A relatively greater prevalence was found in Japanese [74] and Chinese [76] populations, with a peak at 2–3 years of age for ocular myasthenia. The relatively higher incidence of ocular myasthenia in prepubertal MG was also found in a European series [77].

The prognosis of juvenile MG is usually favorable, although exacerbations may occur with fevers. In sporadic cases, a fulminating onset with life-threatening respiratory insufficiency may occur. The incidence of a-AChR antibodies is lower than in adults, so that this test cannot be used to differentiate between congenital and autoimmune MG. The outcome of infantile (onset 1–17 years) [77] or juvenile MG (onset 1–20) [78] is comparable with the adult forms, and the same treatment modalities are used although it seems justified to be less aggressive with immunomodulation in prepubertal children [72, 79].

Myasthenia Gravis in the Elderly

There are several clinical and epidemiological reasons to differentiate between patients with late-onset and early-onset MG. Thymoma is more common in late-onset patients, the response to thymectomy is more uncertain than in younger patients, a-AChR titers are lower [80], and antibodies to other muscle components than the acetylcholine receptor are more often found among these patients [81–83]. Although there are no differences between the manifestations in patients with late-onset MG, progression to severe disease may be more common in late-onset MG. There is a somewhat increased incidence of weakness of bulbar muscles [84], which cannot be attributed to the increased incidence of a thymoma [81]. Immunomodulating therapies are effective [80], but elderly patient may be at increased risk of side effects, in particular for corticosteroids [19, 81, 84].

Myasthenia Gravis and Thymoma

The incidence of thymoma is about 15% among MG patients [60] with late-onset patients being more commonly affected (see Fig. 6.7). Generally, the course of MG is more severe, and crises occur more often than in patients without thymomas [38, 69]; however, there are contradictory studies [85]. Patients with thymomas more often have onset of MG with bulbar manifestations (see Table 6.1). Complications related to the thymoma contribute to a worse outcome, but myasthenic manifestations respond to treatment similarly in thymoma and non-thymoma patients. In our own series [19] of 138 patients observed from 1960 to 1994, 16 patients died from complications of the tumor. In the remaining group, 20 died from MG (all before 1984, only two had immunosuppressive therapy), and 55 patients went into remission with (n = 37) or without (n = 18) immunosuppression. Death related to MG occurred in close to 3% and full remission in nearly 40% of our 537 non-thymoma patients with generalized disease. Our data suggests that the natural course of MG with a thymoma is more unfavorable but that the outcome with immunomodulation is the same as in non-thymoma patients with MG.

Other Autoimmune Diseases

The hypothesis of the autoimmune pathogenesis of MG was partly based on the occurrence of other autoimmune disorders among MG patients [86]. Many associations of MG with other autoimmune diseases are reported in large series [38, 52, 54, 59, 87]. The frequencies vary from 2 to nearly 25%, with a mean of 13% compared with that of a US series of 3% [85].

Familial Myasthenia Gravis

Although MG is not considered as a hereditary disease with a definite mode of genetic transmission, familial cases are reported in 1–7% of patients [18, 59, 67, 69, 83–92]. In an analysis of 72 familial cases reported up to 1970, 39% belonged to the congenital type (onset before 2 years of age), 22% had occurred between 3 and 18 years, and 39% over the age of 18. In 76% they occurred in one generation, in 24% in two generations, but never in three. In 85% of the families, two members had MG, in 10% three and rarely more [89]. In twin studies, 6 out of 15 monozygotic twins both were affected but none out of 9 dizygotic twins [93].

Recently, 4 separate families each with 2 affected members were described out of a total of 800 patients [94]. In seven of the eight patients, a-AChR antibodies were found. No association with a single HLA haplotype was found. In another family, 5 of 10 members in 1 generation (ages 63–77) were described; no genetic abnormalities were found [95]. In our own 800 patients [19], 14 had a near family member with acquired MG (1.7%). Familial autoimmune MG seems a rarity but can be confused with congenital MG that is more often familial. For more extensive discussion of the genetics of MG, see Chap. 5.

References

1. Almog Y, Ben-David M, Nemet AY. Inferior oblique muscle paresis as a sign of myasthenia gravis. J Clin Neurosci. 2016;25:50–3.
2. Sylva M, Van der Kooi AJ, Grolman W. Dyspnoea due to vocal fold abduction paresis in anti-MuSK myasthenia gravis. J Neurol Neurosurg Psychiatry. 2008;79:1083–4.
3. Osei-Lah A, Reilly V, Capildeo BJ. Bilateral abductor vocal fold paralysis due to myasthenia gravis. J Laryngol Otol. 1999;113:678–9.
4. Huybers MG, Niks EH, Klooster R, de Visser M, Kuks JB, Velding JH, et al. Myasthenia gravis with muscle specific kinase antibodies mimicking amyotrophic lateral sclerosis. Neuromuscul Disord. 2016;26:350–3.
5. Weijnen FG, van der Bilt A, Wokke J, Kuks J, van der Glas HW, Bosman F. What's in a smile? Quantification of the vertical smile of patients with myasthenia gravis. J Neurol Sci. 2000;173:124–8.
6. Weijnen FG, Van der Bilt A, Wokke JHJ, Kuks JBM, Van der Glas HW, Bosman F. Maximal bite force and surface EMG in patients with myasthenia gravis. Muscle Nerve. 2000;23:1694–9.
7. Weijnen FG, Kuks JBM, Van der Bilt, Van der Glas HW, Wassenberg MWM, Bosman F. Tongue force in patients with myasthenia gravis. Acta Neurol Scand. 2000;102:303–8.

8. Konstantopoulos K, Christou Y-P, Vogazianos P, Zamba-Papicolaou E, Kleopa KA. A quantitative method for the assessment of dysarthrophonia in myasthenia gravis. J Neurol Sci. 2017;377:42–6.
9. Domingo CA, Landau ME, Campbell WW. Selective triceps muscle weakness in myasthenia gravis is underrecognized. J Clin Neuromuscul Dis. 2016;18:103–4.
10. Vecchio D, Varrasi C, Comi C, Ripellino P, Cantello R. A patients with autoimmune limb-girdle myasthenia, and a brief review of this treatable condition. Clin Neurol Neurosurg. 2017;58:53–5.
11. Cejvanovic S, Vissing J. Muscle strength in myasthenia gravis. Acta Neurol Scand. 2014;129:367–73.
12. Fitzgerald MG, Shafritz AB. Distal myasthenia gravis. J Hand Surg Am. 2014;39:1419–20.
13. Mongiovi PC, Elsheikh E, Lawson VH, Kissel JT, Arnold WD. Neuromuscular junction disorders mimicking myopathy. Muscle Nerve. 2014;50:854–6.
14. Oosterhuis HJGH. Myasthenia gravis. Edinburgh: Churchill Livingstone; 1984.
15. Reuther P, Hertel G, Ricker K, Bürkner R. Lungenfunktionsdiagnostik bei myasthenia gravis. Verhandlungen der Deutsche Gesellschaft für Innere Medizin. 1978;84:1579–82.
16. Kuks JBM, Oosterhuis HJGH. Disorders of the neuromuscular junction: outcome measures and clinical scales. In: Guiloff RJ, editor. Clinical trials in neurology. London: Springer; 2001. p. 485–99.
17. Oosterhuis HJGH, Bethlem J. Neurogenic muscle involvement in myasthenia gravis. J Neurol Neurosurg Psychiatry. 1973;36:244–54.
18. Osserman KE. Myasthenia gravis. New York: Grune & Stratton; 1958.
19. Oosterhuis HJGH. Myasthenia gravis. Groningen: Neurological Press; 1997.
20. Farrugia ME, Robson MD, Clover L. MRI and clinical studies of facial and bulbar muscle involvement in MuSK antibody-associated myasthenia gravis. Brain. 2006;129:1481–92.
21. Suzuki S, Utsugisawa K, Suzuki N. Overlooked non-motor symptoms in myasthenia gravis. J Neurol Neurosurg Psychiatry. 2013;84:989–94.
22. Whiting PJ, Cooper J, Lindstrom JM. Antibodies in sera from patients with myasthenia gravis do not bind with nicotinic acetylcholine receptors from human brain. J Neuroimmunol. 1987;16:205.
23. Mao Z, Yin J, Lu Z, Hu X. Association between myasthenia gravis and cognitive function: a systematic review and meta-analysis. Ann Indian Acad Neurol. 2015;18:131–7.
24. Vernino S, Cheshire WP, Lennon VA. Myasthenia gravis with autoimmune autonomic neuropathy. Auton Neurosci. 2001;88:187–92.
25. Shukla G, Gupta S, Goyal V, Singh S, Srivastava A, Behari M. Abnormal sympathetic hyper-reactivity in patients with myasthenia gravis: a prospective study. Clin Neurol Neurosurg. 2013;115:179–86.
26. Peric S, Rakocevic-Stojanovic V, Nisic T, Pavlovic S, Basta I, Popovic S, et al. Cardiac autonomic control in patients with myasthenia gravis and thymoma. J Neurol Sci. 2011;307(1–2):30–3.
27. Puneeth CS, Chandra SR, Yadav R, Sathyaprabha TN, Chandran S. Heartrate and blood pressure variability in patients with myasthenia gravis. Ann Indian Acad Neurol. 2013;16:329–32.
28. Leon-Samiento FE, Leon-Ariza JS, Prad D, Leon-Aziza DS, Rizzo-Sierra CV. Sensory aspects in myasthenia gravis: a translational approach. J Neurol Sci. 2016;368:379–88.
29. Symonette CJ, Watson BV, Koopman WJ, Micolle MW. Doherty. Muslce strength and fatigue in patients with generalized myasthenia gravis. Muscle Nerve. 2010;41:362–9.
30. Jordan B, Mehl T, Schweden TLK, Menge U, Zierz S. Assessment of physical fatigability and fatigue perception in myasthenia gravis. Nuscle Nerve. 2017;55:657–63.
31. Elsais A, Wyller VB, Loge JH, Kerty E. Fatigue in myasthenia gravis: is it more than muscular weakness? BMC Neurol. 2013;13:132–9.
32. Suarez GA, Kelly JJ. The dropped head syndrome. Neurology. 1992;42:1625–7.
33. Berrih-Aknin S, Frenkian-Cuvelier M, Eymard B. Diagnostic and clinical classification of autoimmune myasthenia gravis. J Autoimmun. 2014;48:143–8.
34. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. Lancet Neurol. 2015;14:1023–6.
35. Deymeer F, Gungor-Tuncer O, Yilmaz V, Parman Y, Serdaroglu P, Ozdemir C, et al. Clinical comparison of anti-Musk- vs. anti-AChr_positive and seronegative myasthenia gravis. Neurology. 2007;68:609–11.
36. Niks EH, Kuks JBM, Roep BO, Haasnoot GW, Verduijn W, Ballieux EPB, et al. Strong association of MuSK antibody-positive myasthenia gravis and HLA-DR14-DQ5. Neurology. 2006;66:1772–4.
37. Compston DAS, Vincent A, Newsom-Davis J, Batchelor JR. Clinical, pathological, HLA-antigen and immunological evidence for disease heterogeneity in myasthenia gravis. Brain. 1980;103:579–601.
38. Oosterhuis HJGH. Myasthenia gravis. A review. Clin Neurol Neurosurg. 1981;83:105–35.
39. Grob D, Brunner NG, Namba T. The natural course of myasthenia gravis and the effect of various therapeutic measures. Ann N Y Acad Sci. 1981;377:652–69.
40. Beekman R, Kuks JBM, Oosterhuis HJGH. Myasthenia gravis: diagnosis and follow-up of 100 consecutive patients. J Neurol Sci. 1997;244:112–8.
41. Cohen MS, Younger D. Aspects of the natural history of myasthenia gravis: crisis and death. Ann N Y Acad Sci. 1981;377:670–7.
42. Oosterhuis HJGH. Observations of the natural history of myasthenia gravis and the effect of thymectomy. Ann N Y Acad Sci. 1981;377:678–89.
43. Wittbrodt ET. Drugs and myasthenia gravis. An update. Arch Intern Med. 1997;157:399–408.

44. Myasthenia Gravis Foundation of America. Drugs to avoid. www.myasthenia.org/livingwithMG/DrugstoAvoid.aspx.
45. Auriel E, Regev K, Dori A, Karni A. Safety of influenza and H1N1 vaccinations in patients with myasthenia gravis, and patient compliance. Muscle Nerve. 2011;43:893–4.
46. Schalke B. Klinische Besonderheiten bei myasthenia gravis patienten. Akt Neurologie. 1998;25:528–9.
47. Plauché WC. Myasthenia gravis in pregnancy: an update. Am J Obstet Gynecol. 1979;135:691–7.
48. Batocchi AP, Majolini L, Evoli A, Lino MM, Minisci C, Tonali P. Course and treatment of myasthenia gravis during pregnancy. Neurology. 1999;52:447–52.
49. Leker RR, Karni A, Abramski O. Exacerbation of myasthenia during the menstrual period. J Neurol Sci. 1998;156:107–11.
50. Borenstein S, Desmedt JE. Temperature and weather correlates of myasthenia fatigue. Lancet. 1974;ii:63–6.
51. Guttman L. Heat-induced myasthenic crises. Arch Neurol. 1980;37:671–2.
52. Oosterhuis HJGH. The natural course of myasthenia gravis: a longterm follow-up study. J Neurol Neurosurg Psychiatry. 1989;52:1121–7.
53. Somnier FE, Keiding N, Paulson OB. Epidemiology of myasthenia gravis in Denmark: a longitudinal and comprehensive population survey. Arch Neurol. 1991;48:733–9.
54. Storm Mathisen A. Epidemiology of myasthenia gravis in Norway. Acta Neurol Scand. 1984;70:274–84.
55. Aragonès JM, Bolíbar I, Bonfill X, Bufill E, Mummany A, Alonso F, Illa I. Myasthenia gravis. A higher than expected incidence in the elderly. Neurology. 2003;60:1024–6.
56. Emilio Romagna Study Group on Clinical and Epidemiological Problems in Neurology. Incidence of myasthenia gravis in the Emilia-Romagna region: a prospective multicenter study. Neurology. 1998;51:255–8.
57. D'Alessandro R, Granieri E, Benassi G, Tola MR, Casmiro M, Mazzanti B, et al. Comparative study on the prevalence of myasthenia gravis in the provinces of Bologna and Ferrara, Italy. Acta Neurol Scand. 1991;2:83–8.
58. Phillips LH, Tomer JC, Anderson MS, Cox GM. The epidemiology of myasthenia gravis in central and western Virginia. Neurology. 1992;42:1888–93.
59. Pirskanen R. Genetic aspects in myasthenia gravis, a family study of 264 Finnish patients. Acta Neurol Scand. 1977;56:365–88.
60. Sorensen TT, Holm EB. Myasthenia gravis in the county of Viborg, Denmark. Eur Neurol. 1989;29:177–9.
61. Robertson NP, Deans J, Compston DAS. Myasthenia gravis: a population based epidemiological study in Cambridgeshire, England. J Neurol Neurosurg Psychiatry. 1998;65:492–6.
62. Carr AS, Cardwell CR, McCarron PO, McConvill J. A systematic review of population based epidemiological studies in myasthenia gravis. BMC Neurol. 2010;10:46–54.
63. McGrogan A, Sneddon S, De Vries CS. The incidence of myasthenia gravis: a systematic literature review. Neuroepidemiology. 2010;34:171–83.
64. Pallaver F, Riviera AP, Piffer S, Ricciard R, Roni R, Orrico D, et al. Change in myasthenia gravis epidemiology in Trento, Italy, after twenty years. Neuroepidemiology. 2011;36:282–7.
65. Mantegazza R, Baggi F, Antozzi C, Confalonieri P, Morandi L, Bernasconi P, et al. Myasthenia gravis: epidemiological data and prognostic factors. Ann N Y Acad Sci. 2003;998:413–23.
66. Casetta I, Groppo E, De Gennaro R, Cesnik E, Piccolo I, Volpato S, et al. Myasthenia gravis: a changing pattern of incidence. J Neurol. 2010;257:2015–9.
67. Breiner A, Widdifield J, Katzberg HD, Barnett C, Bril V, Tu K. Epidemiology of myasthenia gravis in Ontario, Canada. Neuromuscul Disord. 2016;26:41–6.
68. Niks EH, Kuks JBM, Verschuuren JJGM. Epidemiology of myasthenia gravis with anti-MuSK antibodies in the Netherlands. J Neurol Neursurg Psychiatry. 2007;78:417–8.
69. Grob D, Arsura EL, Brunner NG, Namba T. The course of myasthenia gravis and therapies affecting outcome. Ann N Y Acad Sci. 1987;505:472.
70. Mantegazza R, Beghi E, Pareyson D, Antozzi C, Peluchetti D, Sghirlanzoni A, et al. A multicentre follow-up study of 1152 patients with myasthenia gravis in Italy. J Neurol Sci. 1990;237:339–44.
71. Potagas C, Dellatotas G, Tavernarakis A, Molari H, Mourtzouhou P, Koutra H, et al. Myasthenia gravis: changes observed in a 30-years retrospective clinical study of a hospital-based population. J Neurol. 2004;251(1):116–7.
72. Matsuda M, Dohi-Iijima N, Nakamura A, Sekijima Y, Moriat H, Matsuzawa S, et al. Increase in incidence of elderly-onset patients with myasthenia gravis in Nagano prefecture, Japan. Intern Med. 2005;44:572–7.
73. Somnier FE. Increasing incidence of late-onset anti-AChR antibody-seropositive myasthenia gravis. Neurology. 2005;65:928–30.
74. Fukuyama Y, Hirayama Y, Osawa M. Epidemiological and clinical features of childhood myasthenia gravis in Japan. Tokyo: Japan Medical Research Foundation, University of Tokyo Press; 1981. p. 19.
75. Hawkins BR, Yu YL, Wong V, Woo E, IpM S, Dawkins RL. Possible evidence of a variant of myasthenia gravis based on HLA and acetylcholine receptor antibody in Chinese patients. Q J Med. 1989;70:235–41.
76. Wong V, Hawkins BR, Yu YL. Myasthenia gravis in Hong Kong Chinese. Acta Neurol Scand. 1992;86:68–72.
77. Batocchi AP, Evoli A, Palmisani MT, Lo Monaco M, Bartoccioni M, Tonali P. Early onset myasthenia gravis: clinical characteristics and response to therapy. Eur J Pediatr. 1990;150:66–8.

78. Lindner A, Schalke B, Toyka KV. Outcome of juvenile-onset myasthenia gravis: a retrospective study with long term follow-up of 79 patients. J Neurol. 1997;244:515–20.
79. Chiang LM, Darras BT, Kang POB. Juvenile myasthenia gravis. Muscle Nerve. 2009;39:423–31.
80. Hellmann MA, Mosberg-Galili R, Steiner I. Myasthenia gravis in the elderly. J Neurol Sci. 2013;325:1–5.
81. Antonini G, Morino S, Gragnani F, Fiorelli M. Myasthenia gravis in the elderly: a hospital based study. Acta Neurol Scand. 1996;93:260–2.
82. Aarli JA. Late-onset myasthenia gravis. Arch Neurol. 1999;56:25–7.
83. Kuks JBM, Limburg PC, Horst G, Oosterhuis HJGH. Antibodies to skeletal muscle in myasthenia gravis, part 2. Prevalence in non-thymoma patients. J Neurol Sci. 1993;120:78–81.
84. Donaldson DH, Ansher M, Horan S, Rutherford RB, Ringel SP. The relationship of age to outcome in myasthenia gravis. Neurology. 1990;40:786–90.
85. Bril V, Kojic J, Dhanani A. The long-term clinical outcome of myasthenia gravis in patients with thymoma. Neurology. 1998;51:1198–200.
86. Simpson JA. Myasthenia gravis, a new hypothesis. Scott Med J. 1960;5:419–36.
87. Thorlacius S, Aarli JA, Riise T, Matre R, Johnsen HJ. Associated disorders in myasthenia gravis: autoimmune disease and their relation to thymectomy. Acta Neurol Scand. 1989;80:290–5.
88. Jacobson DL, Gange SJ, Rose NR, Graham NMH. Epidemiology and estimated population burden of selected autoimmune disease in the United States. Clin Immunol Immunopathol. 1997;84:223–43.
89. Namba T, Brunner NG, Brown SB, Muguruma M, Grob D. Familial myasthenia gravis. Arch Neurol. 1971;25:49–60.
90. Szobor A. Myasthenia gravis: familial occurrence, a study of 1100 myasthenia gravis patients. Acta Med Hung. 1989;4:13.
91. Marrie RA, Sahlas DJ, Brftay GM. Familial autoimmune myasthenia gravis: four patients involving three generations. Can J Neurol Sci. 2000;27:307–10.
92. Chen Y, Wang W, Wei D, Yan L. Three cases of myasthenia gravis from one family with variations in clinical features and serum antibodies. Neuromuscul Disord. 2012;22:286–8.
93. Behan PO. Immune disease and HLA association with myasthenia gravis. J Neurol Neurosurg Psychiatry. 1980;4:611–21.
94. Evoli A, Batocchi AP, Zelano G, Uncini A, Palmisani MT, Tonali P. Familial autoimmune myasthenia gravis: report of four families. J Neurol Neurosurg Psychiatry. 1995;58:729–31.
95. Bergoffen J, Zmyewski CM, Fischbeck KH. Familial autoimmune myasthenia gravis. Neurology. 1994;44:551–4.

Ocular Myasthenia

7

Sui Hsien Wong and Michael Benatar

Introduction

Myasthenia gravis (MG) is a disorder of neuromuscular transmission that often manifests initially as focal weakness. The most common focal presentation is ocular, with weakness involving the extraocular muscles (EOMs), levators, or orbicularis oculi. Although there may be electrophysiological evidence of myasthenia in lower facial or limb muscles, if the weakness is limited to the ocular muscles, it is designated ocular myasthenia (OM). Eye muscle weakness at the onset of MG is evident in 85–90% of patients, with the percentage of those without clinical evidence of coexisting bulbar or limb weakness at the onset (i.e., pure OM) varying from 18 [1] to 59% [2] in different series [3–6]. The term OM has been recommended by an international consensus committee to be used to describe the current clinical state of a patient, whether or not there is generalized disease in the past [7]. However, there is some debate among neuro-ophthalmologists who may reserve the use of such terminology to patients who have never developed generalized symptoms.

Epidemiology

The epidemiology of MG is discussed in detail in Chap. 5, and here we will discuss it only briefly. Determining the relative frequencies of ocular and generalized MG is compounded by multiple factors. However, using data from several published population-based studies that defined OM on the basis of the modified Osserman classification or the criteria of the Myasthenia Gravis Foundation of American, the pooled estimate of the proportion of all patients with MG who have purely ocular disease is 35% (95% CI, 32–38%) [8–12]. The literature on childhood OM is sparse. Although he does not provide or cite any data, Fenichel states that it rarely begins prior to 6 months of age [13]. Prepubertal onset is more common in boys, and postpubertal is more common in girls.

Basis for Ocular Muscle Involvement by Myasthenia Gravis

The marked susceptibility of the extraocular muscles in MG may be explained by a variety of factors unique to these muscles [14, 15]. One fairly intui-

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H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*, Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_7

tive explanation is that ocular alignment requires a high degree of precision and a minor reduction in force generation, which would be imperceptible in a limb muscle, leads to misalignment of the visual axis and diplopia. But there are other factors, both anatomic and immunologic, which make the extraocular muscles particularly susceptible. One anatomic explanation is that these muscles differ from other skeletal muscles in that they contain both singly and multiply innervated muscle fibers. It is not simply this diversity that predisposes to myasthenia but rather that the junctional synaptic folds of the singly innervated fibers in extraocular muscles are less complex than those in other skeletal muscles [16]. Moreover, multiply innervated muscles are completely devoid of synaptic folds that serve to increase the density of acetylcholine receptors and to focus current into the depths of these folds. Voltage-gated sodium channels are located in the depths of these folds and increase the safety factor for neuromuscular transmission. The lack of synaptic fold complexity in the neuromuscular junctions of extraocular muscles lowers the safety factor, thereby increasing susceptibility to myasthenia. Extraocular muscle also differs immunologically from other skeletal muscles in that decay-accelerating factor (Daf1), a membrane-bound complement regulatory gene, is expressed at low levels in extraocular muscle [17]. Since complement regulators serve to protect cells from complement deposition and consequent membrane damage, the low level of expression of Daf1 may predispose extraocular muscles to immune-mediated damage. In addition, MG is more likely to be ocular when associated with autoimmune thyroid disease, possibly related to immunological cross-reactivity against shared thyroid and eye muscle antigens [18].

The reasons for preferential involvement of the levator palpebrae muscle are less clear but may relate to their relatively sparse junctional folds and their constant activation to keep the eyes open, which predisposes the levators to fatigue [19].

Clinical Presentation

The pattern of muscle involvement in OM ranges from weakness of a single extraocular muscle or lid to complete external ophthalmoplegia with bilateral ptosis, with any intervening combination. At times, the pattern may mimic a central motility disturbance, such as a gaze palsy or internuclear ophthalmoplegia [20]. Although accommodative weakness and fatigue may occur in OM, as may subclinical pupillary abnormalities that can only be detected accurately with quantitative techniques [21–23], we agree with Glaser and Siatkowski [24] that "if pupillary signs are present, another diagnosis must be entertained."

The diagnosis of OM should be strongly suspected by aspects of the *history*, such as (1) episodes of recurrent unilateral, alternating, or bilateral ptosis and (2) progressively increasing ptosis or diplopia during the day, with improvement upon awakening in the morning. In addition, the *examination* may mandate a diagnosis of OM. For example, whereas a third nerve palsy may begin with an isolated ptosis, involvement of extraocular muscles soon follows, and the addition of pain and pupillary dilatation is frequent. Thus, an acquired isolated painless ptosis is almost certainly diagnostic of OM, with the extraordinarily rare exceptions of isolated metastatic carcinoma to the eyelid [25], amyloid infiltration of a levator muscle [26], or unilateral painless lid myositis [27]. Similarly, ophthalmoparesis with rapid saccades within the limited range of eye movements only occurs in MG; all other conditions that restrict ocular amplitude also slow saccadic velocity [20–28]. Facial weakness limited to the orbicularis oculi, coexisting with ptosis or extraocular muscle weakness, only occurs in MG. Fatigue of extraocular muscles and lids during sustained upgaze (Fig. 7.1) also suggests OM, although this, as well as Cogan's "lid twitch"

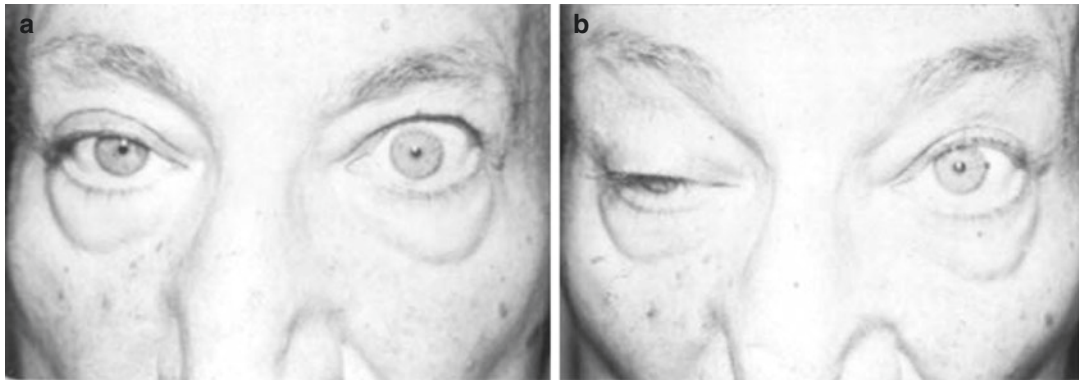

Fig. 7.1 On the left (**a**) the patient is attempting to look up, evidenced by the contraction of the frontalis muscle. Note the slight right ptosis and left lid retraction. The right panel (**b**) demonstrates the lid fatigue that developed during the sustained upward gaze. This was manifested by marked ptosis on the right and lessening of the lid retraction on the left. Photos courtesy of J. Lawton Smith, MD

sign,[1] may be secondary to midbrain lesions [29, 30].

Another OM lid sign is "enhanced" ptosis, where passive elevation of a ptotic lid causes the opposite lid to droop. The explanation is Hering's law of equal innervation; for instance, if both eyelids are ptotic due to bilateral levator weakness, and the right is more ptotic than the left, both sides will receive equivalent increased central innervation to elevate the lids. Because of the asymmetric weakness, the right lid will remain more ptotic than the left. But, if the right lid is manually elevated, the increased central innervation ceases, and the left lid becomes more ptotic [21, 31].

We must emphasize, however, that no matter how "characteristic" the history and examination are for OM, a dilated pupil or pain negates the diagnosis, unless another disorder coexists with the MG.

[1]This sign is commonly present in MG patients with unilateral ptosis. The patient is asked to look down, thereby inhibiting the levator muscles. After about 15 s, the patient looks up to the examiner's nose, or an object at eye level, and if the sign is present, the previously ptotic lid overshoots and is transiently higher than the other lid. The retracted lid then slowly drops to its previous ptotic position.

Diagnostic Testing

The methods utilized for the diagnosis of OM include the rest, ice, and edrophonium tests, acetylcholine receptor (and rarely muscle-specific kinase) antibody assays, repetitive nerve stimulation (RNS), and single-fiber electromyography (SFEMG). The methodological quality of the studies examining the diagnostic accuracy of these tests has varied widely [32]. A common limitation has been the case-control design (in which patients known to have MG are compared to healthy controls or patients with other disorders) rather than a cohort design (in which a series of patients, all of whom were referred for diagnostic evaluation for possible MG, are evaluated against some external reference standard).

The different techniques suggested for the rest and sleep tests all represent variations of the ptotic eyelid being clinically closed ("rested"). In the sleep test, the patient is placed in a quiet, darkened room with instructions to close the eyes and try to sleep for about 30 min. With both tests, a comparison is made between the degree of ptosis before and after the rest period. A case-control study of the rest test reported a sensitivity of 0.50 and a specificity of 0.97 for the diagnosis of OM

[33]. A case-control study of the sleep test, albeit of limited methodological quality, reported a sensitivity of 0.99 and a specificity of 0.91 for the diagnosis of OM [34].

Saavedra et al. first described the utility of the ice test for the diagnosis of OM [35], based on the observations by Borsenstein and Desmedt [36] that the neuromuscular block in myasthenia is ameliorated when the temperature is lowered. The ice test is typically performed by placing a surgical glove filled with ice over the ptotic eyelid for about 2 min. The degree of ptosis is compared before and after the application of ice. Three studies utilized a case-control design to determine the accuracy of the ice test for OM [37–39]. The pooled estimates of sensitivity and specificity for the diagnosis of OM were 0.94 and 0.97, respectively. Given the remote possibility of a serious allergic reaction, we suggest using latex-free surgical gloves for the ice test.

The edrophonium test, also referred to as the Tensilon test, was once regarded as the mainstay of the office diagnosis of OM. Edrophonium is a short-acting, acetylcholinesterase inhibitor with an onset of action within 10–30 secs of intravenous administration. Tensilon has since been discontinued, but edrophonium is still available in the form of Enlon (at least in the United States). While the edrophonium test seems to have fallen out of favor in part because of limited access to drug and in part because of safety considerations, the concern for serious side effects may be overstated. A questionnaire mailed to neuro-ophthalmologists yielded almost 200 respondents who had performed a combined total of over 23,000 tests in which only 37 (0.16%) were associated with a serious complication, such as bradyarrhythmia and syncope; yet 16% of the respondents preferred an alternative diagnostic procedure, such as the sleep or ice tests [40]. Although we have never personally observed any serious complications during the performance of a Tensilon test, we do recommend that atropine should always be available (in a syringe) to counter possible muscarinic side effects of Tensilon.

The most important technical aspect of the test is the need for an unequivocal end point to judge the success or failure of the test. We regard resolution of eyelid ptosis (Fig. 7.2), or the direct observation of the strengthening of at least a single paretic EOM, as the only reliable end points. Thus, patients without ptosis or discernible ophthalmoparesis have no valid end points [41] (Fig. 7.3).

Formal studies of the diagnostic accuracy of the Tensilon test are almost nonexistent [32]. The only study that we identified is a cohort study of relatively poor methodological quality that reported a sensitivity of 0.92 and a specificity of 0.97 for the diagnosis of OM [42].

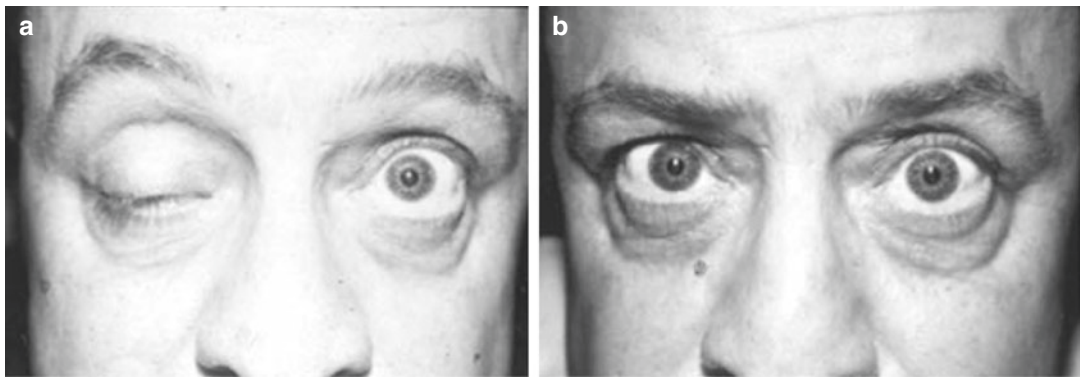

Fig. 7.2 The left panel (**a**) depicts an almost complete right ptosis in a patient with ocular myasthenia. The frontalis muscle contraction, elevating the eyebrows, reflects the patient's effort to keep the lids open. Following administration of intravenous edrophonium chloride (Tensilon), the right ptosis is resolved (**b**, right). Photos courtesy of J. Lawton Smith, MD

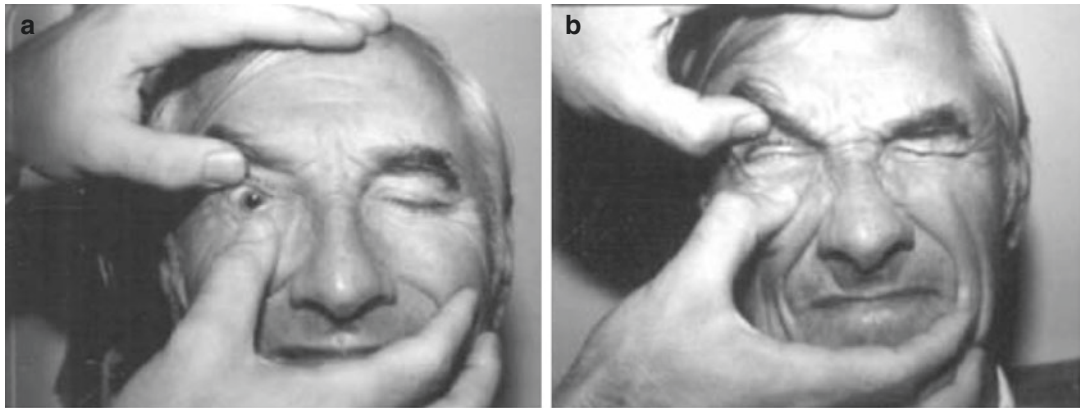

Fig. 7.3 On the left (**a**), the patient has weakness of eyelid closure, permitting easy opening by the examiner. On the right (**b**), the patient shows much stronger eyelid closure following the administration of Tensilon. This patient had myasthenia, demonstrated by EMG and the presence of antibodies. The Tensilon test, by itself, disclosing the increased strength of the orbicularis oculi, would not have been conclusive for the diagnosis of MG, since impaired volition could have explained the orbicularis weakness in part **a**. A Tensilon test with placebo control is unreliable in these circumstances, because IV saline does not cause the same systemic reaction as does Tensilon

Three case-control studies [43–45] and three cohort studies [46–48] reported the utility of acetylcholine receptor antibody testing. The pooled estimate of sensitivity for the diagnosis of OM was lower (0.44) for the higher methodological quality cohort studies [46–48] than for the less well-designed case-control studies (0.66) [43–45]. Specificity in these studies ranged from 0.95 [48] to 1.0 [48]. The consistently high specificity of elevated titers of acetylcholine receptor antibodies establishes their presence as essentially diagnostic.

The above tests refer to radioimmunoprecipitation assays detecting the presence of antibody binding to radioactively labeled AChRs in solution. In recent years the development of cell-based assays has improved serological detection rates [49]. Cell-based assays to detect antibodies on the surface of cells where AChR is clustered showed seropositivity in 50% of previously seronegative OM patients [50, 51]. Regrettably, these cell-based assays are not yet widely available and so not utilized in routine clinical practice outside of the UK.

Anti-muscle-specific receptor tyrosine kinase (MuSK) antibodies in purely OM is also seen in a small number of patients [49, 52, 53]. Newer cell-based assays including the anti-MuSK antibody tests are improving serological detection rates in OM patients (SHW) but are also not commercially available.

Antibodies to the lipoprotein receptor-related protein 4 (LRP4) are the latest pathogenic antibodies found in 2–50% of patients seronegative to both AChR and MuSK [54–56]. LRP4 forms a complex with MuSK, and thus far the clinical phenotype appears to resemble that of anti-MuSK MG [54–56]. One of us (SHW) has a small number of OM patients with anti-LRP4 antibodies.

Repetitive nerve stimulation (RNS) for the diagnosis of MG is discussed in Chap. 9. Estimates of the sensitivity of RNS for the diagnosis of OM have ranged from 0.11 (based on stimulation of the radial nerve with recording from anconeus [57]), to 0.15 (based on stimulation of the truncus primarius superior with recording from abductor digiti minimi [48]), to 0.20 (based on stimulation of the facial nerve, recording from both nasalis and frontalis) [58], to 0.39 (based on stimulation of the facial nerve with recording from orbicularis oculi) [59]. Costa et al. found that sensitivity remained around 0.33 when they performed RNS on four different nerve-muscle pairs (facial nerve-nasalis, accessory nerve-trapezius, radial nerve-anconeus, and ulnar nerve-abductor digiti minimi) [46], suggesting that the sensitivity of RNS for the diagnosis of

OM is generally poor. Specificity is better, however, ranging from 0.89 [48] to 0.97 [46, 59].

As with RNS, the diagnostic accuracy of SFEMG may vary depending upon the individual muscle examined (e.g., orbicularis oculi or frontalis), the recording technique used (e.g., stimulated or volitional), and the type of recording electrode (e.g., single fiber or concentric needle). Studies of the accuracy of SFEMG for the diagnosis of OM all employed a cohort design. Although other methodological issues raise concern, these studies paint a general picture of higher sensitivity for the diagnosis of OM when single-fiber electrodes are used to record jitter from the orbicularis oculi muscle (0.97) [46, 48] compared to studies with similar electrodes recording from the frontalis muscle (0.86) [60, 61] or concentric needles recording from the frontalis (0.62) [32]. The specificity for OM is more variable, ranging from as low as 0.66 [60] to as high as 0.98 [46].

Progression to Generalized Myasthenia Gravis

A major concern in OM is the potential spread to the generalized form of the disease. The studies reporting conversion rates from OM to secondary generalized MG (GMG) range widely, from 30 to 85% [3, 6, 12, 62–65]. This large range is likely affected by the variable duration of follow-up, different patient cohorts, retrospective methodology, and perhaps even the early use of immunosuppression, though whether such therapy does indeed reduce the risk of progression to GMG remains unresolved. In children, rates of generalization range from 39 to 49% [66].

The highest risk of conversion from OM to GMG is within the first 2 years of onset [62], although this can occur many years after the initial onset. Patients who are seropositive for anti-AChR or anti-MuSK are at higher risk of conversion to GMG [64, 67–69]. The presence (higher risk) [64] or the absence (lower risk) [70] of neurophysiological changes in the limbs or trapezius may perhaps be predictive, but this is not established.

An unresolved controversy is whether the early use of immunosuppression can reduce the risk of conversion from OM to GMG [49]. One of our recent reviews looked into this in depth [49] and concluded that although some retrospective studies suggested this possible disease-modifying effect [64, 67, 71–74], the data were flawed for reasons including different disease duration before the start of immunosuppression, possible selection bias from non-randomized sample, and inadequate matching of risk factors for immunosuppressed and control group. A randomized controlled trial is needed to address this question of risk modification with immunosuppression [69].

Spontaneous remission of disease can occur. Twenty percent of children with OM are said to go into a permanent, drug-free remission [13], compared to 10% in Bever et al.'s adult series [3]. Unfortunately, these studies regarding predictive factors or spontaneous remission rates are retrospective with their inherent limitations. At the time of writing, a prospective study (by SHW) is ongoing, which could hopefully shed further light on the risk factors and prognosis of OM.

Treatment

The goals of treating OM are to reduce symptoms. Ideally, treatment would also reduce the risk of conversion from OM to GMG, but as discussed above, there is uncertainty as to whether any of the currently available treatments impact the risk of developing GMG. The spectrum of treatments includes anticholinesterase drugs, usually pyridostigmine bromide (Mestinon®), corticosteroids and other immunosuppressants (e.g., azathioprine), and mechanical devices such as prisms or monocular occlusion for diplopia (e.g., with an eye patch) or lid props for ptosis. In children with OM, the risk of amblyopia, due to ptosis or diplopia, prompts the need for occlusion therapy to ensure the use of both eyes [75]. There are proponents and opponents of these various forms of therapy, and data are only just beginning to emerge that might permit evidence-based recommendations.

There are two randomized controlled drug trials in OM from the 1960s. One evaluated intranasal neostigmine [76] and the other corticotropin [77], but neither provided useful information for contemporary treatment. The details of these two studies, including the treatments employed and outcome measures used, are summarized in a systematic review published in the Cochrane library [78]. More recently, the results of a small randomized double-blind, placebo-controlled trial of prednisone (the EPITOME study) have been published [79, 80]. Patients whose symptoms failed to remit on pyridostigmine alone were randomized to receive placebo or prednisone, initiated at 10 mg every other day and titrated to a maximum of 40 mg/day over 16 weeks. The primary outcome measure was treatment failure, defined as failure to achieve minimal manifestation status, progression to GMG, or discontinuation of prednisone because of side effects. Of the 11 patients randomized, 9 completed 16 weeks of double-blind therapy. Treatment failure incidence was clinically and significantly different between the two groups—100% (95% CI 48–100%) in the placebo group (n = 5) compared to 17% (95% CI 0–64%) in the prednisone group (n = 6). Median time to sustained minimal manifestation status (MMS) was 14 weeks, requiring an average prednisone dose of 15 mg/day. Adverse events were infrequent and generally mild in both groups. These results support the conclusion that a strategy of low-dose prednisone with gradual escalation appears to be safe, well tolerated, and effective in treating OM.

All patients should be screened for thymoma as this can be seen in up to 5% of patients with OM [49, 81]. The data on thymectomy for non-thymomatous OM is not conclusive, and therefore it is not routinely performed, although this practice may vary globally [4, 82–86].

Based on the limited evidence available, we present our approach to the treatment of these patients. We first try Mestinon for symptomatic relief. Although it often improves ptosis and diplopia, patients may not be satisfied. For instance, patients with complete unilateral ptosis don't have diplopia; Mestinon may relieve the ptosis and unmask the diplopia. Mestinon usually increases the strength of weak extraocular muscles but may not totally correct the weakness, leaving some diplopia. A small amount of diplopia is more disconcerting than a large separation, since it is easier to ignore a diplopic image that is off in the periphery, than one close to the real image. Thus, eliminating ptosis or substantially reducing diplopia with Mestinon may be more distressing to the patient than their original symptoms.

If we cannot correct diplopia with Mestinon, we sometimes suggest patching an eye with a clip-on spectacle occluder, frosted lenses, or an occluding contact lens. Bilateral ptosis can often be improved with a "lid props" constructed by an optician (Fig. 7.4) or double adhesive tape (Clavin Non-Surgical Eye Lift) (http://www.Clavin.com/).

We will offer steroids to most patients who do not respond to the usual conservative measures described above. We favor an approach of starting with low dose and titrating up. One of us (SHW) prefers a daily, rather than an alternate day, dose of corticosteroids [87] based on feedback from patients about compliance with medication. Once remission of symptoms has been achieved and sustained for a period of time, we initiate a gradual prednisone taper. Myasthenics may "crash" with exceedingly small reductions when their alternate day dose is 20 mg or less. A "crash" in an ocular myasthenic is not particularly problematic, but we never know whether the steroids were masking generalized myasthenia, which might manifest more dramatically. We are attentive to the potential complications of steroids, such as diabetes, hypertension, glaucoma, osteoporosis, peptic ulcer disease, and weight gain. We may obtain 2-h glucose tolerance test, arrange bone density scans on patients prior to initiation of steroids, and provide gastric prophylaxis in the form of a proton pump inhibitor and prophylaxis against osteoporosis. We advise the patients to follow up regularly with their primary care physicians to monitor blood pressure and glycemic control.

Azathioprine or mycophenolate mofetil may be useful steroid-sparing agents, in patients

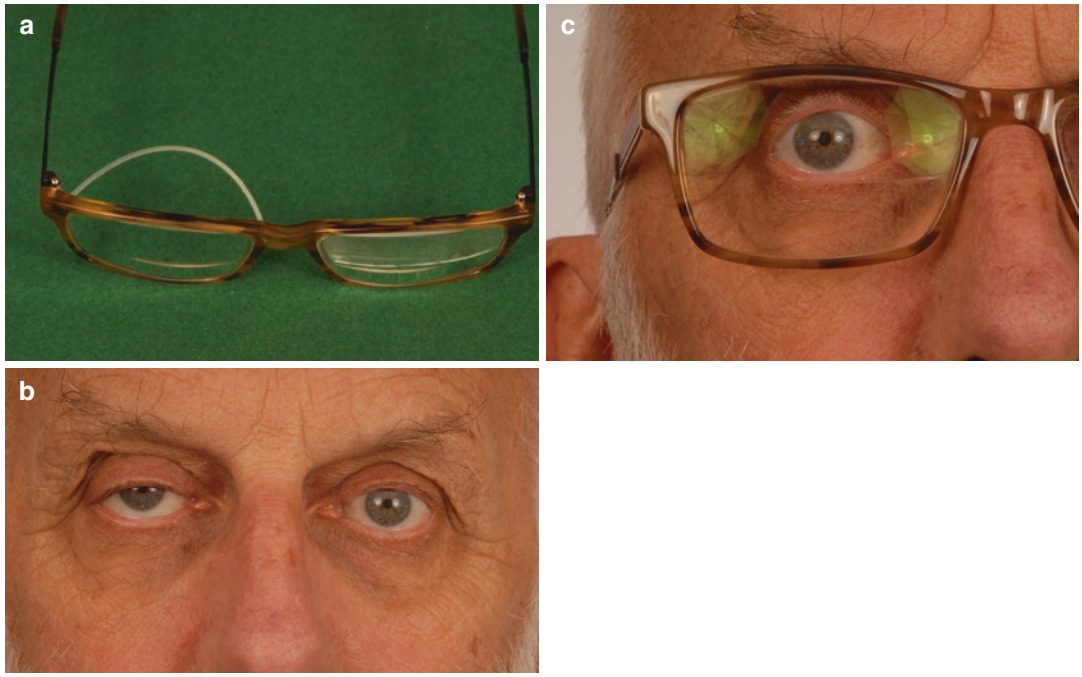

Fig. 7.4 A "lid prop" attached to the rim of the glasses can be used as a mechanical solution to improve ptosis (**a**). Right eye ptosis (**b**) is improved by the use of such a lid prop, which mechanically elevates the lid but allows blinking (**c**)

whose symptoms are responsive to corticosteroids but have persistent or recurrent symptoms when the corticosteroid dose is reduced. Intravenous immunoglobulins (IVIg) may be a useful adjunctive treatment option in refractory cases, although the published evidence suggests limited benefit from IVIg [88]. We do not use plasma exchange in OM and do not recommend elective thymectomy.

Rare patients with "fixed" (unchanged for several years) tropias may benefit from prisms or corrective strabismus surgery [89]. Lid surgery can be useful in some cases of chronic ptosis [90], although not all recommend this [91]. However, blepharoplasty to correct dermatochalasia (redundant, sagging periocular skin) coexisting with MG ptosis may increase the size of the palpebral fissure. Bentley et al. [89] used botulinum toxin in a few patients with a fixed tropia to weaken the antagonist of the paretic muscle, thereby straightening the eye. Given the generalized nature of neuromuscular dysfunction in OM, and the distant effect of locally injected botulinum [92, 93], there is a potential risk with this option.

There is an overlap of patients who have both thyroid eye disease and ocular myasthenia. Therefore, this is important to bear in mind in patients who have poor response to treatment for OM only.

At present, there is no standardized way to quantify severity of symptoms on OM. The rating scales currently used for MG studies, e.g., the MG composite score, do not adequately address this. A possible direction may be to combine ratings from patient questionnaires (e.g., MG quality of life or diplopia questionnaire) and a physician-rated questionnaire (e.g., an expanded version to include more questions on diplopia as part of the quantitative MG or MG composite scales) [94–97]. A more systematic way of quantifying severity would be important for research studies on OM and may provide another objective tool to aid management discussions between physicians and patients. At the time of writing, a

study is ongoing (by SHW) to develop newer methods of quantifying severity of OMG for research studies.

Acknowledgments We are grateful to Dr. Robert Daroff, who coauthored the original version of this chapter.

References

1. Perlo VP, Poskanzer DC, Schwab RS, Viets HR, Osserman KE, Genkins G. Myasthenia gravis: evaluation of treatment in 1,355 patients. Neurology. 1966;16:431–9.
2. Ferguson FR, Hutchinson EC, Liversedge LA. Myasthenia gravis; results of medical management. Lancet. 1955;269:636–9.
3. Bever CT, Aquino AV, Penn AS, Lovelace RE, Rowland LP. Prognosis of ocular myasthenia. Ann Neurol. 1983;14:516–9.
4. Evoli A, Tonali P, Bartoccioni E, Lo Monaco M. Ocular myasthenia: diagnostic and therapeutic problems. Acta Neurol Scand. 1988;77:31–5.
5. Grob D, Arsura EL, Brunner NG, Namba T. The course of myasthenia gravis and therapies affecting outcome. Ann N Y Acad Sci. 1987;505:472–99.
6. Simpson JF, Westerberg MR, Magee KR. Myasthenia gravis. An analysis of 295 cases. Acta Neurol Scand. 1966;42(Suppl 23):1–7.
7. Sanders DB, Wolfe GI, Benatar M, Evoli A, Gilhus NE, Illa I, et al. International consensus guidance for management of myasthenia gravis: executive summary. Neurology. 2016;87:419–25.
8. Casetta I, Fallica E, Govoni V, Azzini C, Tola M, Granieri E. Incidence of myasthenia gravis in the province of Ferrara: a community-based study. Neuroepidemiology. 2004;23:281–4.
9. Christensen PB, Jensen TS, Tsiropoulos I, Sørensen T, Kjaer M, Højer-Pedersen E, et al. Incidence and prevalence of myasthenia gravis in western Denmark: 1975 to 1989. Neurology. 1993;43:1779–83.
10. Lavrnic D, Jarebinski M, Rakocevic-Stojanovic V, Stević Z, Lavrnić S, Pavlović S, et al. Epidemiological and clinical characteristics of myasthenia gravis in Belgrade, Yugoslavia (1983-1992). Acta Neurol Scand. 1999;100:168–74.
11. Phillips LH, Torner JC, Anderson MS, Cox GM. The epidemiology of myasthenia gravis in central and western Virginia. Neurology. 1992;42:1888–93.
12. Robertson N, Deans J, Compston D. Myasthenia gravis: a population based epidemiological study in Cambridgeshire, England. J Neurol Neurosurg Psychiatry. 1998;65:492–6.
13. Fenichel G. Clinical pediatric neurology, a signs and symptoms approach. Philadelphia: Elsevier Saunders; 2005. p. 305–7.
14. Serra A, Ruff R, Kaminski H, Leigh RJ. Factors contributing to failure of neuromuscular transmission in myasthenia gravis and the special case of the extraocular muscles. Ann N Y Acad Sci. 2011;1233:26–33.
15. Serra A, Ruff RL, Leigh RJ. Neuromuscular transmission failure in myasthenia gravis: decrement of safety factor and susceptibility of extraocular muscles. Ann N Y Acad Sci. 2012;1275:129–35.
16. Spencer RF, Porter JD. Structural organization of the extraocular muscles. Rev Oculomot Res. 1988;2:33–79.
17. Porter JD, Khanna S, Kaminski HJ, Rao JS, Merriam AP, Richmonds CR, et al. Extraocular muscle is defined by a fundamentally distinct gene expression profile. Proc Natl Acad Sci U S A. 2001;98:12062–7.
18. Marino M, Barbesino G, Pinchera A, Manetti L, Ricciardi R, Rossi B, et al. Increased frequency of euthyroid ophthalmopathy in patients with Graves' disease associated with myasthenia gravis. Thyroid. 2000;10:799–802.
19. Kaminski HJ, Li Z, Richmonds C, Ruff RL, Kusner L. Susceptibility of ocular tissues to autoimmune diseases. Ann N Y Acad Sci. 2003;998:362–74.
20. Leigh R, Zee D. The neurology of eye movements. 4th ed. New York: Oxford University Press; 2006. p. 439–44.
21. Calvert P. Disorder of neuromuscular transmission. In: Miller NR, Newman N, Biousse V, Kerrison J, editors. Walsh & Hoyt's clinical neuro-ophthalmology. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 1041–84.
22. Digre K. Principles and techniques of examination of the pupils, accommodation, and lacrimation. In: Miller N, Newman N, Biousse V, Kerrison J, editors. Walsh & Hoyt's clinical neuro-opthalmology. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 715–805.
23. Loewenfeld I. The pupil: anatomy, physiology and clinical applications. Boston: Butterworth-Heinemann; 1999.
24. Glaser J, Siatkowski RM. Infranuclear disorders of eye movement. In: Glaser J, editor. Neuroophthalmology. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 1999. p. 405–9.
25. Verhagen WI, Venderink D. Ptosis due to metastasis in the levator palpebrae muscle as first symptom of an adenocarcinoma of the lung. Neuro-Ophthalmology. 2001;26:197–9.
26. Liesegang TJ. Amyloid infiltration of the levator palpebrae superioris muscle: case report. Ann Ophthalmol. 1983;15:610–3.
27. Ertas FS, Ertas NM, Gulec S, Atmaca Y, Tanju S, Sener C, et al. Unrecognized side effect of statin treatment: unilateral blepharoptosis. Ophthal Plast Reconstr Surg. 2006;22:222–4.
28. Daroff R. Ocular myasthenia: diagnosis and therapy. In: Glaser J, editor. Neuro-ophthalmology. St. Louis: Mosby; 1980. p. 62–71.
29. Bhatt A, Haviland J, Black E, Stavern G. Sensitivity and specificity of the Cogan's lid twitch for myasthenia gravis in a prospective series of patients with symptomatic ptosis. Neurology. 2005;64:A36.

30. Ragge NK, Hoyt WF. Midbrain myasthenia: fatigable ptosis, 'lid twitch' sign, and ophthalmoparesis from a dorsal midbrain glioma. Neurology. 1992;42:917–9.
31. Averbuch-Heller L, Poonyathalang A, von Maydell RD, Remler BF. Hering's law for eyelids: still valid. Neurology. 1995;45:1781–3.
32. Benatar M. A systematic review of diagnostic studies in myasthenia gravis. Neuromuscul Disord. 2006;16(7):459–67.
33. Kubis KC, Danesh-Meyer HV, Savino PJ, Sergott RC. The ice test versus the rest test in myasthenia gravis. Ophthalmology. 2000;107:1995–8.
34. Odel JG, Winterkorn JMS, Behrens MM. The sleep test for myasthenia gravis. A safe alternative to tensilon. J Clin Neuroophthalmol. 1991;11:288–92.
35. Saavedra J, Femminini R, Kochen S, deZarate JC. A cold test for myasthenia gravis. Neurology. 1979;29:1075.
36. Borenstein S, Desmedt JE. Local cooling in myasthenia. Improvement of neuromuscular failure. Arch Neurol. 1975;32:152–7.
37. Ellis FD, Hoyt CS, Ellis FJ, Jeffery AR, Sondhi N. Extraocular muscle responses to orbital cooling (ice test) for ocular myasthenia gravis diagnosis. J AAPOS. 2000;4:271–81.
38. Ertas M, Arac N, Kumral K, Tuncbay T. Ice test as a simple diagnostic aid for myasthenia gravis. Acta Neurol Scand. 1994;89:227–9.
39. Lertchavanakul A, Gamnerdsiri P, Hirunwiwatkul P. Ice test for ocular myasthenia gravis. J Med Assoc Thail. 2001;84(Suppl 1):S131–6.
40. Ing EB, Ing SY, Ing T, Ramocki JA. The complication rate of edrophonium testing for suspected myasthenia gravis. Can J Ophthalmol. 2000;35:141–4; discussion 145.
41. Daroff RB. The office Tensilon test for ocular myasthenia gravis. Arch Neurol. 1986;43:843–4.
42. Nicholson G, Nash J. Intermediate nerve conduction velocities define X-linked Charcot-Marie-tooth neuropathy families. Neurology. 1993;43:2558–64.
43. Howard FM, Lennon VA, Finley J, Matsumoto J, Elveback LR. Clinical correlations of antibodies that bind, block or modulate human acetylcholine receptors in myasthenia gravis. Ann N Y Acad Sci. 1987;505:526–38.
44. Lefvert A, Bergstrom K, Matell G, Osterman P, Pirskanen R. Determination of acetylcholine receptor antibody in myasthenia gravis: clinical usefulness and pathogenetic implications. J Neurol Neurosurg Psychiatry. 1978;41:394–403.
45. Limburg P, The T, Hummel-Tappel E, Oosterhuis H. Anti-acetylcholine receptor antibodies in myasthenia gravis. Part 1: relation to clinical parameters in 250 patients. J Neurol Sci. 1983;58:357–70.
46. Costa J, Evangelista T, Conceicão I, Carvalho MD. Repetitive nerve stimulation in myasthenia gravis—relative sensitivity of different muscles. Clin Neurophysiol. 2004;115:2776–82.
47. Nicholson GA, McLeod JG, Griffiths LR. Comparison of diagnostic tests in myasthenia gravis. Clin Exp Neurol. 1983;19:45–9.
48. Padua L, Stalberg E, LoMonaco M, Evoli A, Batocchi A, Tonali P. SFEMG in ocular myasthenia gravis diagnosis. Clin Neurophysiol. 2000;111(7):1203.
49. Wong SH, Huda S, Vincent A, Plant GT. Ocular myasthenia gravis: controversies and updates. Curr Neurol Neurosci Rep. 2014;14:421.
50. Leite MI, Jacob S, Viegas S, Cossins J, Clover L, Morgan BP, et al. IgG1 antibodies to acetylcholine receptors in 'seronegative' myasthenia gravis. Brain. 2008;131:1940–52.
51. Jacob S, Viegas S, Leite MI, Webster R, Cossins J, Kennett R, et al. Presence and pathogenic relevance of antibodies to clustered acetylcholine receptor in ocular and generalized myasthenia gravis. Arch Neurol. 2012;69:994–1001.
52. Bau V, Hanisch F, Hain B, Zierz S. Ocular involvement in MuSK antibody-positive myasthenia gravis. Klin Monatsbl Augenheilkd. 2006;223:81–3.
53. Bennett DL, Mills KR, Riordan-Eva P, Barnes PR, Rose MR. Anti-MuSK antibodies in a case of ocular myasthenia gravis. J Neurol Neurosurg Psychiatry. 2006;77:564–5.
54. Higuchi O, Hamuro J, Motomura M, Yamanashi Y. Autoantibodies to low-density lipoprotein receptor-related protein 4 in myasthenia gravis. Ann Neurol. 2011;69:418–22.
55. Pevzner A, Schoser B, Peters K, Cosma NC, Karakatsani A, Schalke B, et al. Anti-LRP4 autoantibodies in AChR- and MuSK-antibody-negative myasthenia gravis. J Neurol. 2012;259:427–35.
56. Zhang B, Tzartos JS, Belimezi M, Ragheb S, Bealmear B, Lewis RA, et al. Autoantibodies to lipoprotein-related protein 4 in patients with double-seronegative myasthenia gravis. Arch Neurol. 2012;69:445–51.
57. Kennett RP, Fawcett PRW. Repetitive nerve stimulation of anconeus in the assessment of neuromuscular transmission disorders. Electroencephalogr Clin Neurophysiol. 1993;89(3):170–6.
58. Zinman LH, O'Connor PW, Dadson KE, Leung RC, Ngo M, Bril V. Sensitivity of repetitive facial-nerve stimulation in patients with myasthenia gravis. Muscle Nerve. 2006;33:694–6.
59. Oey PL, Wieneke GH, Hoogenraad TU, van Huffelen AC. Ocular myasthenia gravis: the diagnostic yield of repetitive nerve stimulation and stimulated single fiber EMG of orbicularis oculi muscle and infrared reflection oculography. Muscle Nerve. 1993;16:142–9.
60. Rouseev R, Ashby P, Basinski A, Sharpe J. Single fiber EMG in the frontalis muscle in ocular myasthenia: specificity and sensitivity. Muscle Nerve. 1992;15:399–403.
61. Ukachoke C, Ashby P, Basinski A, Sharpe J. Usefulness of single fiber EMG for distinguishing neuromuscular weakness from other causes of ocular muscle weakness. Can J Neurol Sci. 1994;21:125–8.
62. Grob D, Brunner N, Namba T, Pagala M. Lifetime course of myasthenia gravis. Muscle Nerve. 2008;37:141–9.

63. Oosterhuis HJ. The natural course of myasthenia gravis: a long term follow up study. J Neurol Neurosurg Psychiatry. 1989;52:1121–7.
64. Sommer N, Sigg B, Melms A, Weller M, Schepelmann K, Herzau V, et al. Ocular myasthenia gravis: response to long-term immunosuppressant treatment. J Neurol Neurosurg Psychiatry. 1997;62:156–62.
65. Weizer JS, Lee AG, Coats DK. Myasthenia gravis with ocular involvement in older patients. Can J Ophthalmol. 2001;36:26–33.
66. Mullaney P, Vajsar J, Smith R, Buncic JR. The natural history and ophthalmic involvement in childhood myasthenia gravis at the hospital for sick children. Ophthalmology. 2000;107:504–10.
67. Kupersmith MJ, Latkany R, Homel P. Development of generalized disease at 2 years in patients with ocular myasthenia gravis. Arch Neurol. 2003;60:243–8.
68. Guptill JT, Sanders DB, Evoli A. Anti-MuSK antibody myasthenia gravis: clinical findings and response to treatment in two large cohorts. Muscle Nerve. 2011;44:36–40.
69. Wong SH, Petrie A, Plant GT. Ocular myasthenia gravis: toward a risk of generalization score and sample size calculation for a randomized controlled trial of disease modification. J Neuroophthalmol. 2016;36:252–8.
70. Weinberg DH, Rizzo JF 3rd, Hayes MT, Kneeland MD, Kelly JJ Jr. Ocular myasthenia gravis: predictive value of single-fiber electromyography. Muscle Nerve. 1999;22:1222–7.
71. Kupersmith M, Moster M, Bhuiyan S, Warren F, Weinberg H. Beneficial effects of corticosteroids on ocular myasthenia gravis. Arch Neurol. 1996;53:802–4.
72. Kupersmith MJ. Ocular myasthenia gravis: treatment successes and failures in patients with long-term follow-up. J Neurol. 2009;256:1314–20.
73. Mee J, Paine M, Byrne E, King J, Reardon K, O'Day J. Immunotherapy of ocular myasthenia gravis reduces conversion to generalized myasthenia gravis. J Neuroophthalmol. 2003;23:251–5.
74. Monsul NT, Patwa HS, Knorr AM, Lesser RL, Goldstein JM. The effect of prednisone on the progression from ocular to generalized myasthenia gravis. J Neurol Sci. 2004;217:131–3.
75. Kim JH, Hwang JM, Hwang YS, Kim KJ, Chae J. Childhood ocular myasthenia gravis. Ophthalmology. 2003;110:1458–62.
76. Badrising U, Brandenburg H, van Hilten J, Brietl P, Wintzen A. Intranasal neostigmine as add-on therapy in myasthenia gravis. J Neurol. 1996;243:S59.
77. Mount F. Corticotropin in treatment of ocular myasthenia—a controlled clinical trial. Arch Neurol. 1964;11:114–24.
78. Benatar M, Kaminski H. Medical and surgical treatments for ocular myasthenia. Cochrane Database Syst Rev. 2006;(2):CD005081.
79. Benatar M, McDermott MP, Sanders DB, Wolfe GI, Barohn RJ, Nowak RJ, et al. Efficacy of prednisone for the treatment of ocular myasthenia (EPITOME): a randomized controlled trial. Muscle Nerve. 2016;53(3):363–9.
80. Benatar M, Sanders D, Wolfe G, McDermott M, Tawil R. Design of the efficacy of prednisone in the treatment of ocular myasthenia (EPITOME) trial. Ann N Y Acad Sci. 2012;1275:17–22.
81. Wong SH. Screening for thymoma in suspected ocular myasthenia gravis. North American Neuro-ophthalmology Society Annual Meeting, 2016.
82. Papatestas AE, Genkins G, Kornfeld P, Eisenkraft JB, Fagerstrom RP, Pozner J, et al. Effects of thymectomy in myasthenia gravis. Ann Surg. 1987;206:79–88.
83. Hatton PD, Diehl JT, Daly BD, Rheinlander HF, Johnson H, Schrader JB, et al. Transsternal radical thymectomy for myasthenia gravis: a 15-year review. Ann Thorac Surg. 1989;47:838–40.
84. Gilbert ME, De Sousa EA, Savino PJ. Ocular myasthenia gravis treatment: the case against prednisone therapy and thymectomy. Arch Neurol. 2007;64:1790–2.
85. Kawaguchi N, Kuwabara S, Nemoto Y, Fukutake T, Satomura Y, Arimura K, et al. Treatment and outcome of myasthenia gravis: retrospective multi-center analysis of 470 Japanese patients, 1999–2000. J Neurol Sci. 2004;224(1–2):43–7.
86. Mineo TC, Ambrogi V. Outcomes after thymectomy in class I myasthenia gravis. J Thorac Cardiovasc Surg. 2013;145:1319–24.
87. Wong SH, Plant GT. Letter to Practical neurology: comment on MG: ABN's management guidelines (Sussman et al, Practical Neurology 2015). http://pn.bmj.com/content/15/3/199.responses#letter-to-practical-neurology-comment-on-mg-abns-management-guidelines-sussman-et-al-practical-neurology-2015.
88. Zinman L, Ng E, Bril V. IV immunoglobulin in patients with myasthenia gravis: a randomized controlled trial. Neurology. 2007;68:837–41.
89. Bentley CR, Dawson E, Lee JP. Active management in patients with ocular manifestations of myasthenia gravis. Eye. 2001;15:18–22.
90. Bradley EA, Bartley GB, Chapman KL, Waller RR. Surgical correction of blepharoptosis in patients with myasthenia gravis. Ophthal Plast Reconstr Surg. 2001;17:103–10.
91. Sergott RC. Ocular myasthenia. In: Lisak R, editor. Handbook of myasthenia gravis and myasthenic syndromes. New York: Marcel Dekker; 1994. p. 21–31.
92. Lange DJ, Rubin M, Greene PE, Kang UJ, Moskowitz CB, Brin MF, et al. Distant effects of locally injected botulinum toxin: a double-blind study of single fiber EMG changes. Muscle Nerve. 1991;14:672–5.
93. Sanes JR. Extracellular matrix molecules that influence neural development. Ann Rev Neurosci. 1989;12:491–516.
94. Bhanushali MJ, Wuu J, Benatar M. Treatment of ocular symptoms in myasthenia gravis. Neurology. 2008;71:1335–41.

95. Burns TM, Conaway M, Sanders DB. The MG composite: a valid and reliable outcome measure for myasthenia gravis. Neurology. 2010;74:1434–40.
96. Jaretzki A, Barohn R, Ernstoff R, Kaminski HJ, Keesey JC, Penn AS, et al. Myasthenia gravis: recommendations for clinical research standards. Task force of the Medical Scientific Advisory Board of the Myasthenia Gravis Foundation of America. Neurology. 2000;55:16–23.
97. Saleem A, Wong SH. Quantifying severity of ocular myasthenia gravis for research studies. North American Neuro-Ophthalmology Society Annual Meeting, 2017.

8 Thymoma-Associated Myasthenia Gravis

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Introduction

Since the last edition of this book, it has become clear that myasthenia gravis (MG) comprises a more complex and heterogeneous group of autoimmune diseases than previously thought. MG subtypes due to autoantibodies against the nicotinic acetylcholine receptor (AChR) and the muscle-specific kinase (MuSK) have been complemented by newly detected subtypes that have autoantibodies directed against the lipoprotein-related protein 4 (LRP4) and to the LRP4 ligand, agrin, i.e., an autoantigen that is not even strictly a "sessile" constituent of the postsynaptic signaling complex at the neuromuscular junction [1]. These MG subsets will hitherto be called AChR-MG, MuSK-MG, LRP4-MG, and Agrin-MG (Table 8.1).

Table 8.1 The various types of autoimmune myasthenia gravis and associated thymic pathologies

MG type	Thymic follicular hyperplasia	Thymic atrophy/normal for age thymic anatomy	Thymoma
AChR-MG	40%[a]	50%	10%
MuSK-MG	Single cases+[b]	>95%	Single case+[c]
LRP4-MG	30%	70%	–
Agrin-MG	nk	nk	nk
SN-MG	+	+	Single cases[d]

AChR acetylcholine receptor, *MuSK* muscle-specific kinase, *LRP4* lipoprotein-related protein 4, *SN* seronegative. Thymoma-associated MG is essentially a subtype of AChR-MG with autoantibodies that are detectable in conventional immunoprecipitation assays using *soluble* AChR

[a]A minority of these cases recognize exclusively *clustered* AChR in cell-based assays and appear "seronegative" on conventional testing by immunoprecipitation [3]

[b]Not different from controls [5]

[c]Saka E, Topcuoglu MA, Akkaya B, Galati A, Onal MZ, Vincent A. Thymus changes in anti-MuSK-positive and -negative myasthenia gravis. Neurology. 2005;65(5):782–3; author reply -3

[d]Maggi L, Andreetta F, Antozzi C, Baggi F, Bernasconi P, Cavalcante P, et al. Thymoma-associated myasthenia gravis: outcome, clinical and pathological correlations in 197 patients on a 20-year experience. Journal of neuroimmunology. 2008;201–202:237–44. Their cases were negative for anti-AChR and anti-MuSK autoantibodies

The diversification of MG subtypes has been accompanied by observations that a significant number of patients (about 10–15%) may have more than

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H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*, Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_8

one myasthenogenic autoantibody [2]. The invention of cell-based autoantibody detection assays and the possibility to engineer AChR clustering allowed the observation that patients who were previously deemed of be "seronegative" indeed have a new subtype of AChR-MG that is due to autoantibodies that exclusively recognize clustered AChR but not solubilized AChR, which is used in conventional immunoprecipitation assays [3]. Accordingly, these new biological and technical advances have dwarfed the group of true seronegative MG (SN-MG) patients [4]. Therefore, historic findings reported in connection with "seronegative" MG patients must be assessed with caution. For example, thymic follicular hyperplasia that was historically observed in a subset of AChR(−)MuSK(−) seronegative MG patients is now know to be due to AChR-MG with autoantibodies to clustered AChR [5].

No comparable breakthroughs have been achieved in the understanding of thymoma-associated MG (TAMG), except for the fact that virtually all TAMG patients show anti-AChR but no other myasthenogenic autoantibodies [6]. However, answers to several unresolved questions concerning the pathogenesis of TAMG (see below) are expected to arise from the ongoing The Cancer Genome Atlas (TCGA) project in which thymomas either with and without associated MG are subjected to an in depth multi-omics comparative analysis. Another promising development in the field concerns the recent description of a new mouse model [7] that may be a tool to study the immunological function of thymic myoid cells (TMC), i.e., cells that play a key role in the pathogenetic models of all subtypes of AChR-MG (see below).

With a focus on TAMG and thymic pathology, we compare the histological and immunological findings and current pathogenetic concepts in the AChR-MG subtypes: EOMG, LOMG, and TAMG. For epidemiological, clinical, and genetic findings, see Table 8.2.

Histopathology of the Thymus in AChR-MG

TFH (thymic follicular hyperplasia) in EOMG, so-called thymic atrophy in LOMG, and thymoma in TAMG are the pathological hallmarks of MG. Since histological abnormalities in AChR-MG patients are closely associated with typical clinical, serological, and genetic features, thymic pathology serves as a classifier of AChR-MG for clinical and scientific purposes (see Table 8.2). Of note, TFH and "thymic atrophy" are not specific for EOMG and LOMG, respectively (see below): thymuses in some SN-MG patients show TFH [5], and a single study that relied on the diagnoses of local, nonspecialized pathologists reported that LRP4-MG thymuses exhibited either TFH, "thymic atrophy," or "normal" anatomy [8]. These observations in LRP4-MG need independent confirmation. TFH is also common in the remnant thymus of TAMG patients. By contrast, thymic alterations appear to be absent in MuSK-MG [5], and thymuses from patients with pure Agrin-MG have not been studied. Since the different histological findings in AChR-MG have a bearing on pathogenetic models, they are briefly reviewed here.

Thymic Follicular Hyperplasia (TFH) in AChR-MG

Thymuses in EOMG show TFH, i.e., increased numbers of lymphoid follicles in the medulla and perivascular spaces. Size and weight of thymuses in EOMG are normal or only slightly increased for age [9] but may be reduced following corticosteroid treatment. TFH is mostly found in EOMG patients less than 50 years of age but occurs rarely till age 55 in men and 60 in women [10]. TFH also occurs in subsets (1) of SN-MG patients, (2) of remnant thymuses of TAMG patients [11], (3) of LRP4-MG patients [8], and (4) in non-myasthenic autoimmune diseases (e.g., of the thyroid) [12]. Of note, rare lymphoid follicles (typically in less than a third of thymic lobules) can also occur in healthy persons [9, 13].

Thymuses from corticosteroid-naïve patients show a normal-for-age cortex with TdT+ immature T cells, while medullary areas are expanded through diffuse B-cell infiltrates and lymphoid follicles. Follicles contain CD21+, CD23+ follicular dendritic cell (FDC) networks and often show reactive, BCL2-negative germinal centers. The number of Hassall's corpuscles is normal.

Table 8.2 Subsets of myasthenia gravis (MG) patients with anti-acetylcholine receptor autoantibodies (AChR-MG) classified according to thymic pathology and their epidemiologic, genetic, and immunological features

	TAMG/thymoma	LOMG/atrophy	EOMG/TFH
Age at onset of MG (y)[a]	4–90	(>40) >50	<10–50 (65)
Gender (male: female)	1:1	2:1	1:3
Genetic associations			
HLA association (top risk)	None[b]	MHC class II[c]	MHC class I[d,e]
PTPN22+ 1858T(+)	Increased[e]	Increased[e]	Increased[e]
CTLA4 +49A/G genotype	+49A/A increased[f]	+49G increased[e]	Like control
TNIP1 (rs2233290)	Not known	Not increased	Increased[e]
TNFRSF11A (rs4574025)	Not known	Increased	Not increased
ZBTB10 (rs6998967)	Not known	Increased[e]	Not increased
Autoantibodies to			
AChR	99%[g]	100%[g]	100%[h]
Titin	>90%	<10%	40%
Ryanodine receptor	50%	20%	<5%
IFNαs, IL12	70%, 50%	40%, 30%	<5%, <10%
T-cell export (RTE/TRECs)[i]	Increased	Decreased	Not known
Histological findings			
Myoid cells	None[j]	Few	Many
AIRE-expressing TECs[k] none[j]	Few	Many	

TAMG thymoma-associated MG, *LOMG* late-onset MG, *EOMG* early-onset MG, *TFH* thymic follicular hyperplasia, TFH is a common finding in the residual thymus of TAMG patients

[a]Gray zone in terms of age between 40 and 60 (or even 65) years concerning in EOMG and LOMG

[b]No consistent findings, never any strong association [117]

[c]HLA-DQA1 [23, 77, 78]

[d]HLA-B*08 [22, 77]

[e]Controversial (differences between studies)

[f]Increased in MG(+) as compared to MG(−) thymomas [125]

[g]Exceedingly rare thymomas have been reported to be seronegative or show anti-MuSK antibodies (see Table 8.1)

[h]By definition

[i]RTE, recent thymic emigrants as measured by "T-cell receptor excision circles" in the blood [10]

[j]50% of the rare B1 thymomas show such cells in "medullary islands"

[k]*AIRE* autoimmune regulator, *TEC* thymic epithelial cells

Lymphoid follicles disrupt the normally continuous, epithelial network and basal membrane that separate the thymic medulla from the extrathymic epithelial-free perivascular spaces, leading to fusion and expansion of both compartments in which mature CD20+ B cells and CD3+ T cells are increased [14]. Rare non-innervated myoblasts and myotubes, called thymic myoid cells (TMS), occur in the vicinity of Hassall's corpuscles and outside of lymphoid follicles, where they are intimately associated with dendritic cells [15]. TMC are MHC class II negative cells [16] that express fetal and adult-type AChRs [17, 18] and the striational autoantigen, titin [19]. Conventional corticosteroid treatment elicits a starry sky pattern and shrinkage of thymic cortex, and collapse of germinal centers, while prolonged, high-dose corticosteroid and azathioprine treatment can completely abolish cortical structures and TFH [20], inducing shrinkage of the medulla and disappearance of Hassall's corpuscles. A grading system of TFH has been proposed [21].

The triggers initiating TFH are unknown [1]. In EOMG, genetic polymorphisms of immune system-related genes [22, 23] appear to favor an abnormal inflammatory response that leads to germinal center formation and intrathymic autoantibody production [24, 25]. Also, functionally abnormal effector and regulatory T cells are involved [26]. Since production of autoantibodies in EOMG is higher inside than outside the inflamed thymus, the thymus is believed to be the primary site of autoimmunization in EOMG [27], providing the rational for thymectomy as a therapy [28].

Thymomas and Their Classification

Thymomas are rare epithelial tumors of the thymus but the most common mediastinal tumors among adults. The mean age at diagnosis is between 50 and 65 years, without gender bias. Thymomas are rare before age 30 but can occur in children. Due to their histological similarity to the thymus—as reflected by the variable content of immature, TdT(+) T cells (thymocytes)—they are unique tumors, while thymic carcinomas resemble carcinomas in other organs, are devoid of TdT+ thymocytes, and are not associated with MG [29].

Thymomas are classified according to the World Health Organization (WHO) classification [30] with the main types A, AB, B1, B2, and B3 thymomas. Other subtypes (e.g., metaplastic thymoma, pure micronodular thymoma) are disregarded here because they are not associated with MG. Histological classification criteria are the spindle (types A, AB) versus polygonal shape of tumor cells (types B1–B3), the normal (type B1) versus increased content of neoplastic epithelial cells (all others) compared to normal thymus, and the low (types A, B3) versus high content of immature, TdT+ T cells (AB, B1, B2) (Fig. 8.1). Type B1 thymoma is unique, because half of cases maintain the thymus-like epithelial expression of the autoimmune regulator protein, AIRE, while the other histotypes (i.e., 95% of all thymomas) lack this immune tolerance-related transcriptional regulator [31].

Local extension and metastasis of thymomas have traditionally been described by the Masaoka-Koga staging system [32], which reflects the propensity of thymomas for pleural metastasis (stage IVa) but rarely lymph node or distant spread (stage IVb) [33]. Recently, a TNM staging system has been introduced [34] that is being used in parallel with the Masaoka-Koga system to compare their clinical relevance. In oncological terms, invasion into adjacent organs (stage III) and metastasis (stage IV), type B2 and B3 histotypes, incomplete resection, and recurrence are unfavorable prognostic parameters, while the presence of MG is not [30].

The thymoma histotypes are associated with varying risks of MG development (Table 8.3). Lymphoid follicles are particularly common inside MG-associated thymomas, but which antibodies they produce has not been investigated. Nevertheless, it is generally accepted that relevant production of autoantibodies against the AChR inside thymomas does not occur, while anti-type I interferon antibodies are produced inside the tumor (see below). Accordingly, there is generally no short-term improvement of MG symptoms or reduction of anti-AChR autoantibody titers in the blood after surgery, while anti-interferon antibody titers drop in the postoperative period and rise again after thymoma recurrence [35].

LOMG Thymus

"Thymic atrophy" results from age-related (physiological) thymic involution or pathologically accelerated shrinkage of thymic parenchyma due to various "stressors" (including drugs). Therefore, "thymic atrophy" and "age-related normal thymus" are the common terms to describe the histological findings in LOMG. Thymic atrophy is associated with reduced numbers of the following specialized structures with a role in tolerance induction: (1) Hassall's corpuscles [9, 36] that instruct dendritic cells to induce regulatory T cells [37], (2) medullary epithelial cells expressing AIRE [own observation], and (3) thymic myoid cells (TMC) [38]. Reduced numbers of TMCs imply a reduction of bona fide skeletal muscle antigens in the thymic medulla where tolerance induction (e.g., through cross-presenting dendritic cells) takes place. However, no compelling evidence exists that thymuses with physiological involution and thymic atrophy encountered in (corticosteroid naïve) LOMG patients over 60 years of age show morphological differences [9]. This finding may not necessarily extend to the functional level: many patients with LOMG show (1) fewer recent thymic emigrant CD4+ T cells than age-match controls [10] and (2) signs of thymic AIRE deficiency as reflected by autoantibodies against type I interferons [35]. Since atrophic thymuses in LOMG are not a relevant source of AChR autoantibodies, thymectomy is usually not beneficial for LOMG [39].

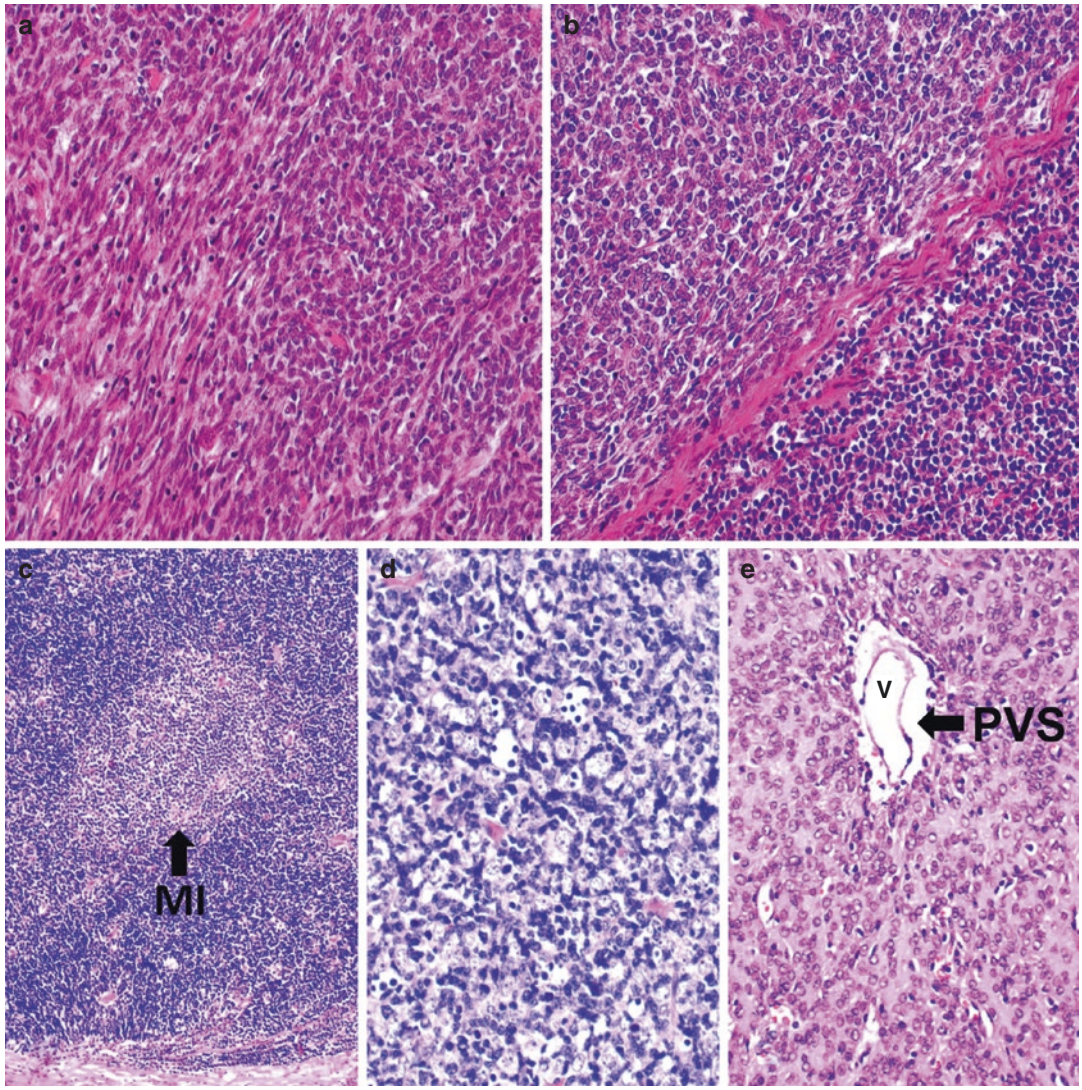

Fig. 8.1 Myasthenia gravis-associated histological thymoma subtypes according to the World Health Organization (WHO) classification [30]. (**a**) Type A thymoma, lymphocyte-poor throughout; (**b**) Type AB thymoma, focally lymphocyte rich (right lower corner); (**c**) Type B1 thymoma with typical medullary island (MI), barely recognizable epithelial cells, and abundant small (mainly immature) lymphocytes; (**d**) Type B2 thymoma with conspicuous large epithelial cells surrounded by a quantitatively dominant population of small (mainly immature) lymphocytes; (**e**) Type B3 thymoma composed of a predominant population of polygonal epithelial cells and few interstitial (mostly immature) small lymphocytes and conspicuous, optically empty perivascular space (PVS) surrounding a central capillary type vessel (v) (H&E **a**, **b**, **d**, **e** ×200, **c** ×100)

Autoantibody Spectrum and T Cells in AChR-MG

AChR-MG is defined by autoantibodies against the nicotinic postsynaptic AChR at the neuromuscular junction (NMJ) [6]. Rare AChR-MG patients have autoantibodies against other targets at the NMJ (e.g., to LRP4 or agrin but almost never MuSK) [2, 6]. Furthermore, titin, ryanodine receptors (RYR), cytokines, and some "cross-reacting" proteins will be mentioned here as autoantibody targets, because they are diagnostically relevant in TAMG and LOMG (anti-titin), are related to cardiac complications

Table 8.3 Epidemiological data of thymoma histological subtypes according to the World Health Organization (WHO) classification and proportion of cases with thymoma-associated myasthenia gravis (TAMG) [30]

WHO histological thymoma subtype	Mean relative frequency (%)	Age (y), range (average)	Gender male: female	Percentage of TAMG (+) cases, range (mean)
Type A	11.5	8–88 (64)	1:1.4	0–33 (17)
Type AB	27.5	11–89 (57)	1:1.4	6–42 (18)
Type B1	17.5	6–83 (50)	1:1.6	7–70 (44)
Type B2	26.0	4–83 (49)	1:1	24–71 (54)
Type B3	16.0	8–87 (55)	1:0.8	25–65 (50)
Others	<1.0	28–80 (60)	1:0.8	Very rare (<5%)

Due to the revised definition of type A thymoma as a consistently immature lymphocyte-poor tumor, the diagnosis of type A thymoma is now rare (<10%), and the proportion of TAMG(+) type A cases is low (<10%), while the reverse is true for type AB thymoma that is a focally or diffusely immature lymphocyte-rich tumor [30]. Thymic carcinoma is not associated with paraneoplastic myasthenia gravis

Table 8.4 Paraneoplastic diseases of presumed autoimmune pathogenesis reported to be associated with thymoma [30]

Addison's disease	Neuromyotonia
Agranulocytosis	Panhypopituitarism
Alopecia areata	Pernicious anemia
Aplastic anemia	Polymyositis
Autoimmune colitis (GvHD-like)	Pure red cell aplasia
Autoimmune autonomic neuropathy	Rheumatoid arthritis
Autoimmune gastrointestinal dysmotility (intestinal pseudo-obstruction)	Rippling muscle disease
Cushing syndrome	Sarcoidosis
Encephalitis (limbic and/or cortical)	Scleroderma
Hemolytic anemia	Sensory motor neuropathy
Hypogammaglobulinemia[a]	Stiff person syndrome
Myasthenia gravis	Systemic lupus erythematosus
Myocarditis	Thyroiditis

[a]Good syndrome

(anti-RYR), and are thought to contribute to abnormal T-cell selection in thymomas ("cross-reacting proteins" such as neurofilaments). Although there is a strong bias for muscle and cytokine autoantibody targets [40], thymomas can induce a wide spectrum of clinically important other autoimmune diseases (Table 8.4).

Acetylcholine Receptor and Autoantibodies

The nicotinic AChR of skeletal muscle is a pentameric ion channel. Its fetal isoform is composed of two α and single β, δ, and γ subunits. At birth, the γ-subunit is largely replaced by the ε-subunit to yield the adult α2βδε composition [41]. After birth, significant amounts of functional *fetal* AChR are expressed only on thymic myoid cells (TMC) [42] and the extraocular skeletal muscle NMJ [43]. The fetal AChR is reexpressed in adult muscle with denervation. The AChR α-subunit is distinguished by an extracellular loop that contains the acetylcholine binding site and the main immunogenic region (MIR) to which most AChR autoantibodies bind [44]. In contrast to skeletal muscle and TMC, medullary thymic epithelial cells (mTECs) express nonfunctional, unfolded AChR subunits [45, 46] that are the source of MHC-bound AChR peptides, which are presented by mTECs to developing T cells. This process normally induces immunological tolerance toward the AChR [46] but goes awry in EOMG [14].

Autoantibodies against solubilized AChR that is detectable by radioimmunoprecipitation assays occur in 80% in patients with generalized MG and virtually all TAMG patients. In about 5% of patients, autoantibodies bind only to clustered AChRs in cell-based assays [3, 47] and are primarily IgG1 and IgG3 antibodies that impair AChR function by complement activation and destruction of the NMJ. In addition, they increase AChR internalization following AChR cross-linking or block the ion channel [6].

Titin and Ryanodine Receptors and Autoantibodies Against Them

Titin is the giant elastic protein that spans half of the skeletal and cardiac muscle sarcomere,

connecting Z-disk and M-line. Ryanodine receptors (RYR) are calcium channels that release Ca^{2+} after activation of the sarcoplasmic reticulum in the skeletal muscle (RYR1 isoform) and heart (mainly RYR2 isoform) and play a key role in excitation-contraction coupling. Titin and RYRs are the major targets of "striational autoantibodies" that occur in 90% of TAMG and 70% of LOMG patients (anti-RYR in 50–80% of TAMG and 30–50% of LOMG) [48–50]. Because titin and RYR are intracellular antigens, autoantibodies against them are of uncertain pathogenicity in vivo [50, 51]. Nevertheless, titin antibody assays are a valuable diagnostic tool to recognize thymomas, particularly in patients under 50, even when not associated with MG, and to distinguish EOMG and LOMG, when thymectomy is considered as therapeutic option in patients over age 40 [49, 52]. In addition, titin and RYR autoantibodies have been associated with more severe myasthenic manifestations, impairment of excitation-contraction coupling, and potentially fatal cardiac dysfunction [51, 53]. Of note, striational antibodies, in contrast to cytokine antibodies, are not found in patients with congenital AIRE deficiency [54], and only exceptionally among EOMG patients [52], although TMC express AChR and titin [19] and are under immune attack in EOMG [14].

Cytokines

Interleukins (e.g., IL12, IL17, IL22), various interferons, and tumor necrosis factor α are almost unique and common targets of autoantibodies in TAMG and prevalent in 40% of LOMG patients. These autoantibodies appear not to elicit or aggravate MG but may promote immunodeficiencies due to their neutralizing capacity [55]. Their pathogenesis is poorly understood, but it is clear that most of them arise due to congenital germline mutations of the tolerogenic *AIRE* gene (see above) [56] or due to lack or paucity of AIRE expression in thymic epithelial cells of thymomas and LOMG thymuses [31].

Cross-reacting Autoantigens

Common themes in TAMG are (1) concurrence of autoantibodies against the native AChR, titin, and muscle ryanodine receptor (RYR1 and RYR2) [57–59], (2) lack of detectable protein expression of these autoantigens in thymomas [60, 61], and (3) overexpression of "cross-reacting autoantigens," i.e., of unrelated proteins with AChR, titin, and RYR epitopes in the neoplastic epithelial cells [61–64]. One of the "cross-reacting autoantigens" was identified as the medium-size neurofilament that harbors titin and the AChR alpha-subunit epitopes [65, 66]. In the absence of expressed bona fide AChR, titin, and RYRs, overexpressed "cross-reacting autoantigenic proteins" in thymoma epithelial cells are thought to bias positive selection of intratumorous maturing T cells prior to their egress from the thymoma (see below).

Autoreactive and Regulatory T Cells in AChR-MG

Autoantibody production in AChR-MG depends on AChR-reactive, MHCII-dependent CD4+ T cells [67] that recognize different epitopes of different AChR subunits in different patients. There are marked differences between EOMG and TAMG [68]. Immune tolerance toward the AChR requires thymic deletion of AChR-reactive T cells through expression of AChR peptides in mTECs and, likely, in TMCs [69]. Since deletion is not perfect even in the physiological setting, rare AChR-reactive T cells escape from the thymus to the peripheral T-cell pool of most healthy persons [70, 71]. These "natural" potentially autoreactive effector T cells are kept in check by thymus-derived FOXP3+ CD4+ CD25+ regulatory T cells (Tregs).

In EOMG, possibly LOMG, and rarely TAMG with thymopoietically *inactive* thymomas, "natural" autoreactive effector T cells are thought to become activated during MG development. Despite normal numbers of blood Tregs in EOMG, they fail to exert their function. Effector T cells in EOMG become abnormally resistant to Tregs [26], and the immunosuppres-

sive capacity of autologous Tregs per cell becomes blunted [72]. In LOMG patients, decreased FOXP3 expression and STAT5 signaling in Tregs and higher expression of PD1 on Tregs and PDL1 on effector T cells are observed [73]. Enigmatically, export of naïve CD4+ T cells from LOMG thymuses is lower than in controls [10]. Thus, there is a *qualitative* imbalance between effector and regulatory T cells in EOMG and LOMG.

In TAMG patients with thymopoietically *active* thymomas, the setting differs. Their blood T-cell repertoires contain high number of potentially autoreactive, AChR-directed naïve CD4+ effector T cells that are generated de novo in the thymoma and released to the blood [74]. By contrast, most non-myasthenic thymomas do not generate mature CD4+ T cells at all [75]. FOXP3+ CD4+ Tregs that could keep the high number of autoreactive effector T cells in check are not produced by thymomas, leading to a *quantitative* imbalance between AChR-directed CD4+ effector T cells and regulatory T cells in TAMG [76]. Although CD4+ effector T cells are key to the pathogenesis of TAMG [75], a clear MHC association is missing in TAMG [69].

Genome-wide association studies (GWAS) revealed polymorphic MHC class I genes as the major genetic risk factors in EOMG [22, 77]. MHC class I genes are also highly relevant although not as critical as MHC II genes for LOMG [23, 77, 78]. No consistent MHC class I and II risk polymorphisms have been identified in TAMG (reviewed in [69]). Nevertheless, LOMG and TAMG patients show expansion of CD8+ T cells in the blood and commonly also exhibit prominent CD8+ T cell subsets with usage of "patient-specific," single T-cell receptor Vbeta genes [79, 80]. These findings hint to an important but enigmatic role of CD8+ T cells in the pathogenesis of MG and have led to the hypothesis of a viral etiology of MG [81]. The role of EBV in the pathogenesis of EOMG is, however, a matter of controversy [82–84].

Pathogenetic Concepts

The following sections briefly discuss the pathogenetic models of EOMG and LOMG in order to highlight the peculiarities of TAMG pathogenesis.

Pathogenesis of EOMG in Relation to Thymic Follicular Hyperplasia

Early-onset MG (EOMG) is the prototypic non-thymomatous type of AChR-MG and typically shows TFH [69] (see Table 8.1). As to the term "early," there is a biological "gray zone" between 40 and 65 years of age, when patients with "early LOMG" or "late EOMG" [52, 85] can be encountered. EOMG is primarily a disease of women, strongly associated with a distinct genetic risk profile [22, 23, 78] and with other autoimmune diseases [86] (see Table 8.2). The etiology of EOMG is unknown with the exception of the D-penicillamine-induced disease [87, 88].

The intrathymic, CD4+ T cell-dependent production of autoantibodies that are mainly focused on the "main immunogenic region" of the α-subunit is characteristic of EOMG [89]. Germinal centers (GCs) are found whether autoantibodies react against soluble or clustered AChRs [3]. GCs drive the hypermutation of B-cell receptor genes and intrathymic production of AChR antibodies [90]. How GC development is triggered is unknown. Due to lack of adequate animal models [91–93] and lack of thymectomy specimens from the preclinical phase of EOMG, primary alterations that initiate GC formation cannot be distinguished from secondary changes that are elicited by the inflammation. Still, the hypothesis of an intrathymic *initiation* of EOMG [94] is supported by the following findings:

- TMCs in the medulla of normal and EOMG thymuses express adult and fetal AChR [18] and are attacked by autoantibodies and complement in EOMG [14]. Since many EOMG patients have antibodies that preferentially

recognize the *fetal* AChR that is virtually absent from extrathymic tissues, an intrathymic disease initiation is likely [95].

- Dendritic cells that show abnormally close spatial association with TMCs supposedly cross-present processed AChR peptides to adjacent autoreactive activated T cells [96].
- EOMG thymuses show intrathymic production of autoantibodies to the AChR [24], increased numbers of B cells [97], plasma cells [98, 99], and GCs [15]. GCs disrupt the normally continuous epithelial cell layer and basal lamina around expanded perivascular spaces [14, 100]. This displaces TMCs from the tolerance-inducing medulla to the inflammatory surroundings of lymphoid follicles [38]. Medullary epithelial cells [101] that express unfolded AChR subunits [102, 103] and MHC class II molecules [104, 105] are under attack by complement and may prime T cells for AChR peptides [14].
- There are increased numbers of activated lymphatic vessels [106, 107], endothelial venules [108], and toll-like receptor-bearing macrophages [109] that produce pro-inflammatory cytokines [103], chemokines [107, 108, 110], APRIL, and BAFF [111] and likely help to recruit B cells and dendritic cells to the thymus [112].
- Intrathymic regulatory T cells are functionally incompetent [72].

A Pathogenetic Model of EOMG

The original concept of intrathymic EOMG pathogenesis assumed MHC class II negative TMCs as the initial source of AChR for autoimmunization [94]. Since MHC class I and II positive medullary epithelial cells [101, 104] are now known to express unfolded AChR subunits [103] and are also attacked by autoantibodies [113] and complement [14], a two-step model of the intrathymic pathogenesis of EOMG [114] is now favored [27]. In step 1, CD4+ effector T cells are primed by unfolded AChR subunits expressed by MHC class I and II positive medullary epithelial cells (through whatever initial "trigger(s)"). In step 2, "early antibodies" that are elicited by primed T cells attack nearby AChR-bearing TMC and activate complement, entailing the release of AChR/immune complexes that, in turn, activate professional antigen-presenting cells to initiate germinal center formation. Recent genetic analyses revealed that MHC class I molecules [22, 23, 77] and TNIP1 [22] are top risk genes in EOMG. Therefore, CD8+ T cells might well play a role during the initiation of EOMG (step 1) [115], while TNIP1, a protein that is involved in the activation of toll-like receptor-bearing macrophages and dendritic cells, may play a role in step 2.

Why the intrathymic inflammatory process in EOMG is self-perpetuating is unclear but may be related to the functionally defective Tregs that have been described in EOMG thymuses [72] and abnormally active antigen-presenting cells [109]. Apparently, activated, autoreactive T and B cells ultimately egress from the thymus to seed the peripheral immune system, where skeletal muscle-derived AChR/immune complexes occur in regional lymph nodes, and functionally impaired Tregs may perpetuate EOMG even after thymectomy [69].

Pathogenesis of Thymoma-Associated MG (TAMG)

A pathogenetic model of TAMG has to take the following peculiarities of thymomas and of the genetic background of TAMG patients into account [116, 117]. (1) Autoantibodies in TAMG have a strong focus on muscle autoantigens, and these autoantibodies are not produced inside the thymoma—in sharp contrast to the preferential intrathymic AChR autoantibody production in EOMG. (2) MG-associated thymomas do not express bona fide AChR, titin, and RYRs but overexpress "cross-reacting autoantigens" with AChR, titin, and RYR epitopes in the absence of thymic myoid cells (TMC). (3) The tolerogenic autoimmune regulator protein AIRE that prevents the generation of anti-cytokine autoantibodies [118] and is normally involved in the intrathymic selection of regulatory T cells [119]

is missing in almost all thymomas whether a patient has MG or not [76]. (4) TAMG crucially depends on the intratumorous generation and subsequent export of CD4+ effector T cells from the thymoma to the peripheral immune system [75, 120]. (5) TAMG does not show a clear MHC-related genetic bias (see Table 8.2). However, hemizygosity of the MHC locus or low expression levels of MHC class II molecules have shown a strong trend for association with TAMG development in thymomas [121–123]. (6) TAMG is uniquely and "paradoxically" associated with gain-of-function genotypes of immunoregulatory genes, particularly the CTLA4^{high} genotype (+49A/A) and the PTPN22+1858T(+) genotype that are protective against a broad spectrum of other autoimmune diseases [124, 125].

Against the background of the above points, the following three-step model of TAMG pathogenesis has been suggested (Fig. 8.2): (1) Within thymopoietically active thymomas, there is a muscle-specific bias during positive selection of CD4+ T cell due to increased expression of "cross-reacting autoantigens" with AChR, titin, and RYR epitopes in conjunction with hemizygous [126] or reduced [121, 123] MHC class II expression. (2) Due to intratumorous lack of mature medullary structures, including TMC with consecutive defective cross-presentation of their muscle antigens by dendritic cells, there is impaired negative selection of T cells that are specific for muscle autoantigens, allowing for the export of non-tolerant, naïve T cells from the thymoma; negative selection might be further com-

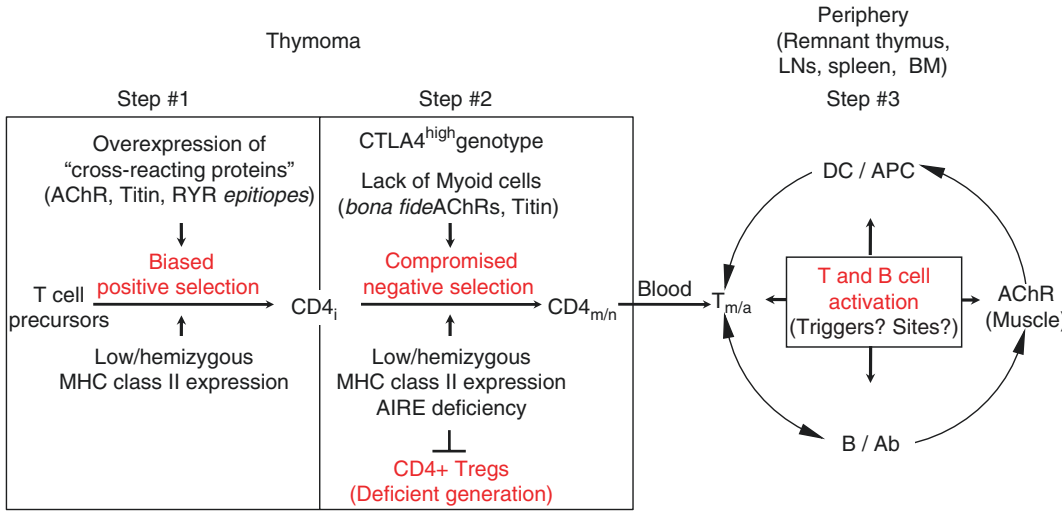

Fig. 8.2 Unified three-step model of the pathogenesis of thymoma-associated MG (TAMG). Key pathogenetic mechanisms are highlighted in red. Step #1: biased positive selection of T-cell precursors to still immature CD4+ T cells ($CD4_i$) with specificity for skeletal muscle autoantigens through epithelial overexpression of "cross-reacting proteins" with epitopes of the acetylcholine receptor (AChR), titin, and the skeletal and cardiac muscle ryanodine receptors (RYR). Abnormal "positive selection" is supported by hemizygous or reduced MHC class II expression. Step #2: failure of negative T cell selection due to largely missing medullary structures, absent thymic myoid cells, and lacking expression of the autoimmune regulator, AIRE. Also, genetic background, particularly a CTLA4^{high} phenotype, and low levels of MHC class II molecules on tumor cells may interfere with the deletion of potentially autoreactive T cells through attenuation of T-cell receptor signaling. On the other hand, lack of AIRE and attenuated T-cell receptor signaling due to low MHC class II expression levels may underlie the lacking generation of regulatory T cells (Tregs). Step #3: export of mature, naïve CD4+ T cells ($CD4_{m/n}$) through the blood to the "periphery" (residual thymus, lymph nodes, bone marrow, and eventually inflamed muscle). Following yet unknown triggers, mature naïve T cells are activated ($T_{m/a}$) by antigen-presenting dendritic cells (DC/APC) and provide help to autoantibody (Ab)-producing B cells. Whether the AChR from skeletal muscle or thymic myoid cells is the primary triggering autoantigen or gets secondarily involved is unknown. After tumor resection that usually includes thymectomy, muscle-derived AChR might fuel the self-perpetuating autoimmune process that maintains post-surgery MG symptoms

promised by hemizygous/reduced MHC class II expression and gain-of-function CTLA4 and PTPN22 genotypes. (3) After activation in the periphery by thus far unknown triggers, some of their progeny start to react against AChR, titin, and RYR and eventually stimulate B cells for autoantibody production [69].

The failure of AIRE-deficient thymomas to generate Tregs [76] may help to initiate and maintain the autoimmune process in the periphery. A scenario that is not mutually exclusive assumes that some autoreactive T cells are activated inside thymomas because of "dangerous" local expression of autoantigenic epitopes in the context of AIRE deficiency, and after their escape from thymomas, such "pre-primed" T cells could stimulate B cells in the periphery [55, 123].

Regardless, the high numbers of thymoma-derived, potentially autoreactive T cells are thought to progressively replace the patient's "historic" thymus-derived, and therefore largely tolerant, T-cell repertoire in the periphery [120]. This influence of the thymoma on the *peripheral* T-cell repertoire explains two clinical findings: first, the rare but well-documented occurrence of TAMG after thymoma removal (likely due to naïve, autoimmunity prone peripheral T cells that have been egressed from the thymoma during the months or even years of its growth and become activated postoperatively for subsequent cooperation with B cells) [75] and second, the long-lasting course of TAMG even after complete thymoma removal (likely due to self-sustaining T-cell/B-cell interactions that are likely fueled by skeletal muscle-derived AChR/autoantibody complexes that are processed in regional lymph nodes) [69, 115]. In the fewer than 5% of thymopoietically *inactive* type A thymomas with associated MG, it has been suggested that mature T cells from the "natural" AChR-directed T-cell repertoire (see section "Autoreactive and Regulatory T Cells in AChR-MG") could be activated after recirculation to the thymoma [69].

In contrast to TAMG, various other thymoma-associated autoimmune diseases, for example, autoimmune thyroiditis, type 1 diabetes, or cytopenias, and anti-cytokine autoantibody production do not depend on export of substantial numbers of CD4+ effector T cells from thymomas, since their prevalence is similar in CD4+ T-cell exporting and CD4+ non-exporting thymomas of TAMG(+) and TAMG(−) patients [75].

Pathogenesis of LOMG

Late-onset MG (LOMG) sensu stricto is a non-thymomatous subgroup of AChR-MG. A fixed age threshold is problematic, because there is a biological "gray zone" between 40 and 60 years [52] or even 65 years [85], in which patients with "late EOMG" (mainly females) and "early LOMG" (mainly males) can be encountered [10, 77]. A 50-year threshold reveals a gender bias with male predominance in LOMG compared to female predominance in EOMG [52]. A threshold at 60 years is supported by genetic data, a higher frequent of anti-striational (e.g., titin) autoantibodies and almost lacking lymphofollicular inflammatory thymic changes, particularly in males [10, 52, 77, 85].

The etiology of LOMG is unknown but could have an environmental facet considering the increase in LOMG during the last 40 years in many geographic regions [85, 127–130]. LOMG and TAMG patients resemble each other immunologically. First, autoantibodies to titin and RYR are almost as common in LOMG as in TAMG patients, while they are virtually nonexistent in other MG subtypes and other autoimmune diseases [49]. Second, cytokine autoantibody profiles (comprising IFNα2, α8, ω, and IL12 as main targets) are strongly overlapping in LOMG and TAMG [35]. Third, the proportion of supposedly naïve, CD45RA+ CD8+ T cells is increased, and CD8+ T-cell subsets with usage of individual Vbeta T-cell receptor genes are expanded in LOMG and TAMG [79, 80, 131]. On the other hand, genetic background plays a major role mainly in LOMG, while massive export of naïve CD4+ T cells from neoplastic thymic tissue to the blood is a unique feature of TAMG [10, 120].

A Pathogenetic Model of LOMG

Despite the differences in terms of genetic background and T-cell export from thymic tissue [10,

23, 78], the immunological similarities between LOMG and TAMG are so strong that the hypothesis has been proposed that aberrations in LOMG thymuses mimic abnormalities in thymomas and lead to export and possibly activation of non-tolerant T cells [69, 114]. Although export of naïve T cells from thymuses of LOMG patients at the time of diagnosis is even lower than in aged-matched control persons [10], this finding does not exclude that a small population of highly potent, AChR and titin reactive T cells generated in the near absence of thymic myoid cells (TMC) inside a largely AIRE-negative atrophic thymus could become activated after export to the periphery and initiate LOMG and that autoreactive T cells egressed from an atrophic, TMC-poor and almost AIRE-deficient thymus have accumulated in the periphery over years before the outbreak of LOMG. This is similar to rare thymoma patients who develop TAMG after thymoma resection [75]. After being triggered in the periphery [10], LOMG could become self-perpetuating as in TAMG by stimulatory AChR/autoantibody complexes in muscle-draining lymph nodes [115]. The model fits the clinical observation that thymectomy has limited impact on LOMG symptoms [132].

Major Unresolved Questions in TAMG

The pathogenetic model of TAMG does not completely resolve several observations. There is currently no evidence that proteins that contain AChR-, titin- and ryanodine receptor (RyR)-like epitopes are crucial for the development of anti-AChR, anti-titin, or anti-RyR autoimmunity in TAMG through abnormal positive selection. The Cancer Genome Atlas molecular analysis of thymic cancers may provide clues to this question through comparison of TAMG(+) and TAMG(−) thymomas. Also, it is not known whether TMCs play a physiological immune tolerance inducing role through the negative selection of muscle specific T cells. Accordingly, it is still a matter of speculation that lack of TMC in thymomas favors the escape of muscle autoantigen-specific T cells from the thymoma to the periphery. The recent description of a mouse model with TMC-deficient thymus [7] may offer a tool to address these questions. Another enigma is the occurrence of anti-titin autoimmunity in thymoma and LOMG but not EOMG patients, although TMC express both AChR and titin and are under immune attack in EOMG. Why this immune attack fosters anti-AChR but not anti-titin autoimmunity in EOMG is unclear. The mouse model [7] may help to study the different mechanisms of tolerance breakdown toward AChR and titin. In addition, in the subset of type A thymomas with virtually absent intratumorous thymopoiesis, the above pathogenetic model (see Fig. 8.2) may not apply (absence of step #1). However, thymopoiesis in type A thymomas can be very focal [30], and such tumors should be sampled completely before other pathogenetic models of TAMG are invoked for type A thymomas. Finally, it has not been elucidated which autoantigens maintain the prolonged autoantibody response after thymoma resection when the adjacent residual nonneoplastic thymus with its TMC is usually removed as well. A candidate is the AChR released from skeletal muscle end plates following destruction by autoantibodies or cytotoxic T cells, followed by presentation of AChR peptides to autoreactive T cells and B cells in regional lymph nodes. Sampling of lymph nodes in TAMG patients for in vitro autoantibody production may answer this question.

References

1. Gilhus NE. Myasthenia gravis. N Engl J Med. 2016;375(26):2570–81.
2. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. Lancet Neurol. 2015;14(10):1023–36.
3. Leite MI, Jacob S, Viegas S, Cossins J, Clover L, Morgan BP, et al. IgG1 antibodies to acetylcholine receptors in 'seronegative' myasthenia gravis. Brain. 2008;131(Pt 7):1940–52.
4. Kaminski HJ. Seronegative myasthenia gravis-a vanishing disorder? JAMA Neurol. 2016;73(9):1055–6.
5. Leite MI, Strobel P, Jones M, Micklem K, Moritz R, Gold R, et al. Fewer thymic changes in MuSK antibody-positive than in MuSK antibody-negative MG. Ann Neurol. 2005;57(3):444–8.

6. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classifications—authors' reply. Lancet Neurol. 2016;15(4):357–8.
7. Hu B, Simon-Keller K, Kuffer S, Strobel P, Braun T, Marx A, et al. Myf5 and myogenin in the development of thymic myoid cells—implications for a murine in vivo model of myasthenia gravis. Exp Neurol. 2016;277:76–85.
8. Zisimopoulou P, Evangelakou P, Tzartos J, Lazaridis K, Zouvelou V, Mantegazza R, et al. A comprehensive analysis of the epidemiology and clinical characteristics of anti-LRP4 in myasthenia gravis. J Autoimmun. 2014;52:139–45.
9. Strobel P, Moritz R, Leite MI, Willcox N, Chuang WY, Gold R, et al. The ageing and myasthenic thymus: a morphometric study validating a standard procedure in the histological workup of thymic specimens. J Neuroimmunol. 2008;201–202:64–73.
10. Chuang WY, Strobel P, Bohlender-Willke AL, Rieckmann P, Nix W, Schalke B, et al. Late-onset myasthenia gravis—CTLA4(low) genotype association and low-for-age thymic output of naive T cells. J Autoimmun. 2014;52:122–9.
11. Skeie GO, Romi F. Paraneoplastic myasthenia gravis: immunological and clinical aspects. Eur J Neurol. 2008;15(10):1029–33.
12. Dalla Costa M, Mangano FA, Betterle C. Thymic hyperplasia in patients with Graves' disease. J Endocrinol Investig. 2014;37(12):1175–9.
13. Middleton G, Schoch EM. The prevalence of human thymic lymphoid follicles is lower in suicides. Virchows Arch. 2000;436(2):127–30.
14. Leite MI, Jones M, Strobel P, Marx A, Gold R, Niks E, et al. Myasthenia gravis thymus: complement vulnerability of epithelial and myoid cells, complement attack on them, and correlations with autoantibody status. Am J Pathol. 2007;171(3):893–905.
15. Kirchner T, Schalke B, Melms A, von Kugelgen T, Muller-Hermelink HK. Immunohistological patterns of non-neoplastic changes in the thymus in myasthenia gravis. Virchows Arch B Cell Pathol Incl Mol Pathol. 1986;52(3):237–57.
16. Curnow J, Corlett L, Willcox N, Vincent A. Presentation by myoblasts of an epitope from endogenous acetylcholine receptor indicates a potential role in the spreading of the immune response. J Neuroimmunol. 2001;115(1–2):127–34.
17. Geuder KI, Marx A, Witzemann V, Schalke B, Toyka K, Kirchner T, et al. Pathogenetic significance of fetal-type acetylcholine receptors on thymic myoid cells in myasthenia gravis. Dev Immunol. 1992;2(2):69–75.
18. Schluep M, Willcox N, Vincent A, Dhoot GK, Newsom-Davis J. Acetylcholine receptors in human thymic myoid cells in situ: an immunohistological study. Ann Neurol. 1987;22(2):212–22.
19. Marx A, Osborn M, Tzartos S, Geuder KI, Schalke B, Nix W, et al. A striational muscle antigen and myasthenia gravis-associated thymomas share an acetylcholine-receptor epitope. Dev Immunol. 1992;2(2):77–84.
20. Schalke BC, Mertens HG, Kirchner T, Wegener S, Muller-Hermelink HK. Long-term treatment with azathioprine abolishes thymic lymphoid follicular hyperplasia in myasthenia gravis. Lancet. 1987;2(8560):682.
21. Marx A, Pfister F, Schalke B, Nix W, Strobel P. Thymus pathology observed in the MGTX trial. Ann N Y Acad Sci. 2012;1275:92–100.
22. Gregersen PK, Kosoy R, Lee AT, Lamb J, Sussman J, McKee D, et al. Risk for myasthenia gravis maps to a (151) Pro→Ala change in TNIP1 and to human leukocyte antigen-B*08. Ann Neurol. 2012;72(6):927–35.
23. Renton AE, Pliner HA, Provenzano C, Evoli A, Ricciardi R, Nalls MA, et al. A genome-wide association study of myasthenia gravis. JAMA Neurol. 2015;72(4):396–404.
24. Vincent A, Scadding GK, Thomas HC, Newsom-Davis J. In-vitro synthesis of anti-acetylcholine-receptor antibody by thymic lymphocytes in myasthenia gravis. Lancet. 1978;1(8059):305–7.
25. Berrih-Aknin S. Myasthenia gravis: paradox versus paradigm in autoimmunity. J Autoimmun. 2014;52:1–28.
26. Gradolatto A, Nazzal D, Truffault F, Bismuth J, Fadel E, Foti M, et al. Both Treg cells and Tconv cells are defective in the myasthenia gravis thymus: roles of IL-17 and TNF-alpha. J Autoimmun. 2014;52:53–63.
27. Hohlfeld R, Wekerle H. Reflections on the "intrathymic pathogenesis" of myasthenia gravis. J Neuroimmunol. 2008;201–202:21–7.
28. Wolfe GI, Kaminski HJ, Aban IB, Minisman G, Kuo HC, Marx A, et al. Randomized trial of thymectomy in myasthenia gravis. N Engl J Med. 2016;375(6):511–22.
29. Marx A, Chan JK, Coindre JM, Detterbeck F, Girard N, Harris NL, et al. The 2015 World Health Organization classification of tumors of the thymus: continuity and changes. J Thorac Oncol. 2015;10(10):1383–95.
30. IARC, editor. WHO classification of tumours of the lung, pleura, thymus and heart. 4th ed. Geneva: WHO; 2015.
31. Strobel P, Murumagi A, Klein R, Luster M, Lahti M, Krohn K, et al. Deficiency of the autoimmune regulator AIRE in thymomas is insufficient to elicit autoimmune polyendocrinopathy syndrome type 1 (APS-1). J Pathol. 2007;211(5):563–71.
32. Detterbeck FC, Nicholson AG, Kondo K, Van Schil P, Moran C. The Masaoka-Koga stage classification for thymic malignancies: clarification and definition of terms. J Thorac Oncol. 2011;6(7 Suppl 3):S1710–6.
33. Weis CA, Yao X, Deng Y, Detterbeck FC, Marino M, Nicholson AG, et al. The impact of thymoma histotype on prognosis in a worldwide database. J Thorac Oncol. 2015;10(2):367–72.

34. Kondo K. The International Association for the Study of Lung Cancer/the International Thymic Malignancies Interest Group proposal for the TNM staging systems for thymic epithelial tumors and large-scale retrospective data. J Thorac Dis. 2016;8(8):1856–8.
35. Meager A, Wadhwa M, Dilger P, Bird C, Thorpe R, Newsom-Davis J, et al. Anti-cytokine autoantibodies in autoimmunity: preponderance of neutralizing autoantibodies against interferon-alpha, interferon-omega and interleukin-12 in patients with thymoma and/or myasthenia gravis. Clin Exp Immunol. 2003;132(1):128–36.
36. Smith SM, Ossa-Gomez LJ. A quantitative histologic comparison of the thymus in 100 healthy and diseased adults. Am J Clin Pathol. 1981;76(5):657–65.
37. Watanabe N, Wang YH, Lee HK, Ito T, Wang YH, Cao W, et al. Hassall's corpuscles instruct dendritic cells to induce CD4+CD25+ regulatory T cells in human thymus. Nature. 2005;436(7054):1181–5.
38. Roxanis I, Micklem K, McConville J, Newsom-Davis J, Willcox N. Thymic myoid cells and germinal center formation in myasthenia gravis; possible roles in pathogenesis. J Neuroimmunol. 2002;125(1–2):185–97.
39. Romi F, Skeie GO, Aarli JA, Gilhus NE. Muscle autoantibodies in subgroups of myasthenia gravis patients. J Neurol. 2000;247(5):369–75.
40. Klein R, Marx A, Strobel P, Schalke B, Nix W, Willcox N. Autoimmune associations and autoantibody screening show focused recognition in patient subgroups with generalized myasthenia gravis. Hum Immunol. 2013;74(9):1184–93.
41. Zouridakis M, Zisimopoulou P, Poulas K, Tzartos SJ. Recent advances in understanding the structure of nicotinic acetylcholine receptors. IUBMB Life. 2009;61(4):407–23.
42. Wakkach A, Poea S, Chastre E, Gespach C, Lecerf F, De La Porte S, et al. Establishment of a human thymic myoid cell line. Phenotypic and functional characteristics. Am J Pathol. 1999;155(4):1229–40.
43. Fraterman S, Khurana TS, Rubinstein NA. Identification of acetylcholine receptor subunits differentially expressed in singly and multiply innervated fibers of extraocular muscles. Invest Ophthalmol Vis Sci. 2006;47(9):3828–34.
44. Tzartos SJ, Lindstrom JM. Monoclonal antibodies used to probe acetylcholine receptor structure: localization of the main immunogenic region and detection of similarities between subunits. Proc Natl Acad Sci U S A. 1980;77(2):755–9.
45. Siara J, Rudel R, Marx A. Absence of acetylcholine-induced current in epithelial cells from thymus glands and thymomas of myasthenia gravis patients. Neurology. 1991;41(1):128–31.
46. Giraud M, Taubert R, Vandiedonck C, Ke X, Levi-Strauss M, Pagani F, et al. An IRF8-binding promoter variant and AIRE control CHRNA1 promiscuous expression in thymus. Nature. 2007;448(7156):934–7.
47. Rodriguez Cruz PM, Al-Hajjar M, Huda S, Jacobson L, Woodhall M, Jayawant S, et al. Clinical features and diagnostic usefulness of antibodies to clustered acetylcholine receptors in the diagnosis of seronegative myasthenia gravis. JAMA Neurol. 2015;72(6):642–9.
48. Mygland A, Aarli JA, Matre R, Gilhus NE. Ryanodine receptor antibodies related to severity of thymoma associated myasthenia gravis. J Neurol Neurosurg Psychiatry. 1994;57(7):843–6.
49. Skeie GO, Aarli JA, Gilhus NE. Titin and ryanodine receptor antibodies in myasthenia gravis. Acta Neurol Scand Suppl. 2006;183:19–23.
50. Suzuki S, Utsugisawa K, Nagane Y, Suzuki N. Three types of striational antibodies in myasthenia gravis. Autoimmun Dis. 2011;2011:740583.
51. Imai T, Tsuda E, Toyoshima T, Yoshikawa H, Motomura M, Shimohama S. Anti-ryanodine receptor-positive acetylcholine receptor-negative myasthenia gravis: evidence of impaired excitation-contraction coupling. Muscle Nerve. 2011;43(2):294–5.
52. Aarli JA. Myasthenia gravis in the elderly: is it different? Ann N Y Acad Sci. 2008;1132:238–43.
53. Romi F, Skeie GO, Vedeler C, Aarli JA, Zorzato F, Gilhus NE. Complement activation by titin and ryanodine receptor autoantibodies in myasthenia gravis. A study of IgG subclasses and clinical correlations. J Neuroimmunol. 2000;111(1–2):169–76.
54. Wolff AS, Karner J, Owe JF, Oftedal BE, Gilhus NE, Erichsen MM, et al. Clinical and serologic parallels to APS-I in patients with thymomas and autoantigen transcripts in their tumors. J Immunol. 2014;193(8):3880–90.
55. Kisand K, Boe Wolff AS, Podkrajsek KT, Tserel L, Link M, Kisand KV, et al. Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. J Exp Med. 2010;207(2):299–308.
56. Meyer S, Woodward M, Hertel C, Vlaicu P, Haque Y, Karner J, et al. AIRE-deficient patients harbor unique high-affinity disease-ameliorating autoantibodies. Cell. 2016;166(3):582–95.
57. Lindstrom JM, Seybold ME, Lennon VA, Whittingham S, Duane DD. Antibody to acetylcholine receptor in myasthenia gravis. Prevalence, clinical correlates, and diagnostic value. Neurology. 1976;26(11):1054–9.
58. Aarli JA, Stefansson K, Marton LS, Wollmann RL. Patients with myasthenia gravis and thymoma have in their sera IgG autoantibodies against titin. Clin Exp Immunol. 1990;82(2):284–8.
59. Mygland A, Tysnes OB, Matre R, Volpe P, Aarli JA, Gilhus NE. Ryanodine receptor autoantibodies in myasthenia gravis patients with a thymoma. Ann Neurol. 1992;32(4):589–91.
60. Marx A, O'Connor R, Tzartos S, Kalies I, Kirchner T, Muller-Hermelink HK. Acetylcholine receptor epitope in proteins of myasthenia gravis-associated

thymomas and non-thymic tissues. Thymus. 1989;14(1–3):171–8.
61. Romi F, Bo L, Skeie GO, Myking A, Aarli JA, Gilhus NE. Titin and ryanodine receptor epitopes are expressed in cortical thymoma along with costimulatory molecules. J Neuroimmunol. 2002;128(1–2):82–9.
62. Mygland A, Kuwajima G, Mikoshiba K, Tysnes OB, Aarli JA, Gilhus NE. Thymomas express epitopes shared by the ryanodine receptor. J Neuroimmunol. 1995;62(1):79–83.
63. Kusner LL, Mygland A, Kaminski HJ. Ryanodine receptor gene expression thymomas. Muscle Nerve. 1998;21(10):1299–303.
64. Kirchner T, Hoppe F, Muller-Hermelink HK, Schalke B, Tzartos S. Acetylcholine receptor epitopes on epithelial cells of thymoma in myasthenia gravis. Lancet (London, England). 1987;1(8526):218.
65. Marx A, Wilisch A, Schultz A, Greiner A, Magi B, Pallini V, et al. Expression of neurofilaments and of a titin epitope in thymic epithelial tumors. Implications for the pathogenesis of myasthenia gravis. Am J Pathol. 1996;148(6):1839–50.
66. Schultz A, Hoffacker V, Wilisch A, Nix W, Gold R, Schalke B, et al. Neurofilament is an autoantigenic determinant in myasthenia gravis. Ann Neurol. 1999;46(2):167–75.
67. Hohlfeld R, Toyka KV, Heininger K, Grosse-Wilde H, Kalies I. Autoimmune human T lymphocytes specific for acetylcholine receptor. Nature. 1984;310(5974):244–6.
68. Nagvekar N, Moody AM, Moss P, Roxanis I, Curnow J, Beeson D, et al. A pathogenetic role for the thymoma in myasthenia gravis. Autosensitization of IL-4- producing T cell clones recognizing extracellular acetylcholine receptor epitopes presented by minority class II isotypes. J Clin Invest. 1998;101(10):2268–77.
69. Marx A, Willcox N, Leite MI, Chuang WY, Schalke B, Nix W, et al. Thymoma and paraneoplastic myasthenia gravis. Autoimmunity. 2010;43(5–6):413–27.
70. Sommer N, Harcourt GC, Willcox N, Beeson D, Newsom-Davis J. Acetylcholine receptor-reactive T lymphocytes from healthy subjects and myasthenia gravis patients. Neurology. 1991;41(8):1270–6.
71. Melms A, Malcherek G, Gern U, Wietholter H, Muller CA, Schoepfer R, et al. T cells from normal and myasthenic individuals recognize the human acetylcholine receptor: heterogeneity of antigenic sites on the alpha-subunit. Ann Neurol. 1992;31(3):311–8.
72. Balandina A, Lecart S, Dartevelle P, Saoudi A, Berrih-Aknin S. Functional defect of regulatory CD4(+)CD25+ T cells in the thymus of patients with autoimmune myasthenia gravis. Blood. 2005;105(2):735–41.
73. Alahgholi-Hajibehzad M, Oflazer P, Aysal F, Durmus H, Gulsen-Parman Y, Marx A, et al. Regulatory function of CD4+CD25++ T cells in patients with myasthenia gravis is associated with phenotypic changes and STAT5 signaling: 1,25-dihydroxyvitamin D3 modulates the suppressor activity. J Neuroimmunol. 2015;281:51–60.
74. Buckley C, Oger J, Clover L, Tuzun E, Carpenter K, Jackson M, et al. Potassium channel antibodies in two patients with reversible limbic encephalitis. Ann Neurol. 2001;50(1):73–8.
75. Strobel P, Helmreich M, Menioudakis G, Lewin SR, Rudiger T, Bauer A, et al. Paraneoplastic myasthenia gravis correlates with generation of mature naive CD4(+) T cells in thymomas. Blood. 2002;100(1):159–66.
76. Strobel P, Rosenwald A, Beyersdorf N, Kerkau T, Elert O, Murumagi A, et al. Selective loss of regulatory T cells in thymomas. Ann Neurol. 2004;56(6):901–4.
77. Maniaol AH, Elsais A, Lorentzen AR, Owe JF, Viken MK, Saether H, et al. Late onset myasthenia gravis is associated with HLA DRB1*15:01 in the Norwegian population. PLoS One. 2012;7(5):e36603.
78. Seldin MF, Alkhairy OK, Lee AT, Lamb JA, Sussman J, Pirskanen-Matell R, et al. Genome-wide Association Study of late-onset myasthenia gravis: confirmation of TNFRSF11A, and identification of ZBTB10 and three distinct HLA associations. Mol Med. 2015;21(1):769–81.
79. Hoffacker V, Schultz A, Tiesinga JJ, Gold R, Schalke B, Nix W, et al. Thymomas alter the T-cell subset composition in the blood: a potential mechanism for thymoma-associated autoimmune disease. Blood. 2000;96(12):3872–9.
80. Tackenberg B, Schlegel K, Happel M, Eienbroker C, Gellert K, Oertel WH, et al. Expanded TCR Vbeta subsets of CD8(+) T-cells in late-onset myasthenia gravis: novel parallels with thymoma patients. J Neuroimmunol. 2009;216(1–2):85–91.
81. Cavalcante P, Cufi P, Mantegazza R, Berrih-Aknin S, Bernasconi P, Le Panse R. Etiology of myasthenia gravis: innate immunity signature in pathological thymus. Autoimmun Rev. 2013;12(9):863–74.
82. Cavalcante P, Serafini B, Rosicarelli B, Maggi L, Barberis M, Antozzi C, et al. Epstein-Barr virus persistence and reactivation in myasthenia gravis thymus. Ann Neurol. 2010;67(6):726–38.
83. Meyer M, Hols AK, Liersch B, Leistner R, Gellert K, Schalke B, et al. Lack of evidence for Epstein-Barr virus infection in myasthenia gravis thymus. Ann Neurol. 2011;70(3):515–8.
84. Kakalacheva K, Maurer MA, Tackenberg B, Munz C, Willcox N, Lunemann JD. Intrathymic Epstein-Barr virus infection is not a prominent feature of myasthenia gravis. Ann Neurol. 2011;70(3):508–14.
85. Alkhawajah NM, Oger J. Late-onset myasthenia gravis: a review when incidence in older adults keeps increasing. Muscle Nerve. 2013;48(5):705–10.
86. Nacu A, Andersen JB, Lisnic V, Owe JF, Gilhus NE. Complicating autoimmune diseases in myasthenia gravis: a review. Autoimmunity. 2015;48(6):362–8.

87. Bucknall RC, Dixon ASJ, Glick EN, Woodland J, Zutshi DW. Myasthenia gravis associated with penicillamine treatment for rheumatoid arthritis. Br Med J. 1975;1(5958):600–2.
88. Poulas K, Koutsouraki E, Kordas G, Kokla A, Tzartos SJ. Anti-MuSK- and anti-AChR-positive myasthenia gravis induced by d-penicillamine. J Neuroimmunol. 2012;250(1–2):94–8.
89. Gilhus NE, Skeie GO, Romi F, Lazaridis K, Zisimopoulou P, Tzartos S. Myasthenia gravis—autoantibody characteristics and their implications for therapy. Nat Rev Neurol. 2016;12(5):259–68.
90. Sims GP, Shiono H, Willcox N, Stott DI. Somatic hypermutation and selection of B cells in thymic germinal centers responding to acetylcholine receptor in myasthenia gravis. J Immunol. 2001;167(4):1935–44.
91. Meinl E, Klinkert WE, Wekerle H. The thymus in myasthenia gravis. Changes typical for the human disease are absent in experimental autoimmune myasthenia gravis of the Lewis rat. Am J Pathol. 1991;139(5):995–1008.
92. Robinet M, Maillard S, Cron MA, Berrih-Aknin S, Le Panse R. Review on toll-like receptor activation in myasthenia gravis: application to the development of new experimental models. Clin Rev Allergy Immunol. 2017;52(1):133–47.
93. Weiss JM, Robinet M, Aricha R, Cufi P, Villeret B, Lantner F, et al. Novel CXCL13 transgenic mouse: inflammation drives pathogenic effect of CXCL13 in experimental myasthenia gravis. Oncotarget. 2016;7(7):7550–62.
94. Wekerle H, Ketelsen UP. Intrathymic pathogenesis and dual genetic control of myasthenia gravis. Lancet. 1977;1(8013):678–80.
95. Weinberg CB, Hall ZW. Antibodies from patients with myasthenia gravis recognize determinants unique to extrajunctional acetylcholine receptors. Proc Natl Acad Sci U S A. 1979;76(1):504–8.
96. Kirchner T, Hoppe F, Schalke B, Muller-Hermelink HK. Microenvironment of thymic myoid cells in myasthenia gravis. Virchows Arch B Cell Pathol Incl Mol Pathol. 1988;54(5):295–302.
97. Abdou NI, Lisak RP, Zweiman B, Abrahamsohn I, Penn AS. The thymus in myasthenia gravis. Evidence for altered cell populations. N Engl J Med. 1974;291(24):1271–5.
98. Gomez AM, Vrolix K, Martinez-Martinez P, Molenaar PC, Phernambucq M, van der Esch E, et al. Proteasome inhibition with bortezomib depletes plasma cells and autoantibodies in experimental autoimmune myasthenia gravis. J Immunol. 2011;186(4):2503–13.
99. Willcox HN, Newsom-Davis J, Calder LR. Cell types required for anti-acetylcholine receptor antibody synthesis by cultured thymocytes and blood lymphocytes in myasthenia gravis. Clin Exp Immunol. 1984;58(1):97–106.
100. Eimoto T, Kusano T, Ando K, Kikuchi M, Shirakusa T, Kawanami S. Nonneoplastic and nonhyperplastic thymus in myasthenia gravis. An immunohistochemical study with double immunoenzyme labeling of basement membrane and cellular components. Am J Clin Pathol. 1990;94(1):36–43.
101. Roxanis I, Micklem K, Willcox N. True epithelial hyperplasia in the thymus of early-onset myasthenia gravis patients: implications for immunopathogenesis. J Neuroimmunol. 2001;112(1–2):163–73.
102. Wakkach A, Guyon T, Bruand C, Tzartos S, Cohen-Kaminsky S, Berrih-Aknin S. Expression of acetylcholine receptor genes in human thymic epithelial cells: implications for myasthenia gravis. J Immunol. 1996;157(8):3752–60.
103. Poea-Guyon S, Christadoss P, Le Panse R, Guyon T, De Baets M, Wakkach A, et al. Effects of cytokines on acetylcholine receptor expression: implications for myasthenia gravis. J Immunol. 2005;174(10):5941–9.
104. Gilhus NE, Matre R. Fc gamma receptors and HLA-DR antigens on thymus cells in myasthenia gravis. J Neuroimmunol. 1986;10(3):271–8.
105. Chilosi M, Iannucci A, Fiore-Donati L, Tridente G, Pampanin M, Pizzolo G, et al. Myasthenia gravis: immunohistological heterogeneity in microenvironmental organization of hyperplastic and neoplastic thymuses suggesting different mechanisms of tolerance breakdown. J Neuroimmunol. 1986;11(3):191–204.
106. Cufi P, Dragin N, Ruhlmann N, Weiss JM, Fadel E, Serraf A, et al. Central role of interferon-beta in thymic events leading to myasthenia gravis. J Autoimmun. 2014;52:44–52.
107. Berrih-Aknin S, Ruhlmann N, Bismuth J, Cizeron-Clairac G, Zelman E, Shachar I, et al. CCL21 overexpressed on lymphatic vessels drives thymic hyperplasia in myasthenia. Ann Neurol. 2009;66(4):521–31.
108. Weiss JM, Cufi P, Bismuth J, Eymard B, Fadel E, Berrih-Aknin S, et al. SDF-1/CXCL12 recruits B cells and antigen-presenting cells to the thymus of autoimmune myasthenia gravis patients. Immunobiology. 2013;218(3):373–81.
109. Bernasconi P, Barberis M, Baggi F, Passerini L, Cannone M, Arnoldi E, et al. Increased toll-like receptor 4 expression in thymus of myasthenic patients with thymitis and thymic involution. Am J Pathol. 2005;167(1):129–39.
110. Meraouna A, Cizeron-Clairac G, Panse RL, Bismuth J, Truffault F, Tallaksen C, et al. The chemokine CXCL13 is a key molecule in autoimmune myasthenia gravis. Blood. 2006;108(2):432–40.
111. Thangarajh M, Masterman T, Helgeland L, Rot U, Jonsson MV, Eide GE, et al. The thymus is a source of B-cell-survival factors-APRIL and BAFF-in myasthenia gravis. J Neuroimmunol. 2006;178(1–2):161–6.
112. Berrih-Aknin S, Ragheb S, Le Panse R, Lisak RP. Ectopic germinal centers, BAFF and anti-B-cell therapy in myasthenia gravis. Autoimmun Rev. 2013;12(9):885–93.
113. Safar D, Aime C, Cohen-Kaminsky S, Berrih-Aknin S. Antibodies to thymic epithelial cells in myasthenia gravis. J Neuroimmunol. 1991;35(1–3):101–10.

114. Willcox N, Leite MI, Kadota Y, Jones M, Meager A, Subrahmanyam P, et al. Autoimmunizing mechanisms in thymoma and thymus. Ann N Y Acad Sci. 2008;1132:163–73.
115. Vincent A, Willcox N. The role of T-cells in the initiation of autoantibody responses in thymoma patients. Pathol Res Pract. 1999;195(8):535–40.
116. Marx A, Porubsky S, Belharazem D, Saruhan-Direskeneli G, Schalke B, Strobel P, et al. Thymoma related myasthenia gravis in humans and potential animal models. Exp Neurol. 2015;270:55–65.
117. Marx A, Pfister F, Schalke B, Saruhan-Direskeneli G, Melms A, Strobel P. The different roles of the thymus in the pathogenesis of the various myasthenia gravis subtypes. Autoimmun Rev. 2013;12(9):875–84.
118. Kisand K, Lilic D, Casanova JL, Peterson P, Meager A, Willcox N. Mucocutaneous candidiasis and autoimmunity against cytokines in APECED and thymoma patients: clinical and pathogenetic implications. Eur J Immunol. 2011;41(6):1517–27.
119. Aschenbrenner K, D'Cruz LM, Vollmann EH, Hinterberger M, Emmerich J, Swee LK, et al. Selection of Foxp3+ regulatory T cells specific for self antigen expressed and presented by Aire+ medullary thymic epithelial cells. Nat Immunol. 2007;8(4):351–8.
120. Buckley C, Douek D, Newsom-Davis J, Vincent A, Willcox N. Mature, long-lived CD4+ and CD8+ T cells are generated by the thymoma in myasthenia gravis. Ann Neurol. 2001;50(1):64–72.
121. Strobel P, Chuang WY, Chuvpilo S, Zettl A, Katzenberger T, Kalbacher H, et al. Common cellular and diverse genetic basis of thymoma-associated myasthenia gravis: role of MHC class II and AIRE genes and genetic polymorphisms. Ann N Y Acad Sci. 2008;1132:143–56.
122. Berezovsky IN, Esipova NG, Tumanyan VG. Hierarchy of regions of amino acid sequence with respect to their role in the protein spatial structure. J Comput Biol. 2000;7(1–2):183–92.
123. Willcox N, Schluep M, Ritter MA, Schuurman HJ, Newsom-Davis J, Christensson B. Myasthenic and nonmyasthenic thymoma. An expansion of a minor cortical epithelial cell subset? Am J Pathol. 1987;127(3):447–60.
124. Chuang WY, Strobel P, Belharazem D, Rieckmann P, Toyka KV, Nix W, et al. The PTPN22gain-of-function+1858T(+) genotypes correlate with low IL-2 expression in thymomas and predispose to myasthenia gravis. Genes Immun. 2009;10(8):667–72.
125. Chuang WY, Strobel P, Gold R, Nix W, Schalke B, Kiefer R, et al. A CTLA4 high genotype is associated with myasthenia gravis in thymoma patients. Ann Neurol. 2005;58(4):644–8.
126. Zettl A, Strobel P, Wagner K, Katzenberger T, Ott G, Rosenwald A, et al. Recurrent genetic aberrations in thymoma and thymic carcinoma. Am J Pathol. 2000;157(1):257–66.
127. Carr AS, Cardwell CR, McCarron PO, McConville J. A systematic review of population based epidemiological studies in Myasthenia Gravis. BMC Neurol. 2010;10:46.
128. Casetta I, Groppo E, De Gennaro R, Cesnik E, Piccolo L, Volpato S, et al. Myasthenia gravis: a changing pattern of incidence. J Neurol. 2010;257(12):2015–9.
129. Pedersen EG, Hallas J, Hansen K, Jensen PE, Gaist D. Late-onset myasthenia not on the increase: a nationwide register study in Denmark, 1996–2009. Eur J Neurol. 2013;20(2):309–14.
130. Somnier FE. Increasing incidence of late-onset anti-AChR antibody-seropositive myasthenia gravis. Neurology. 2005;65(6):928–30.
131. Tackenberg B, Nitschke M, Willcox N, Ziegler A, Nessler S, Schumm F, et al. CD45 isoform expression in autoimmune myasthenia gravis. Autoimmunity. 2003;36(2):117–21.
132. Romi F, Gilhus NE, Varhaug JE, Myking A, Skeie GO, Aarli JA. Thymectomy and anti-muscle autoantibodies in late-onset myasthenia gravis. Eur J Neurol. 2002;9(1):55–61.

9 Electrodiagnosis of Neuromuscular Junction Disorders

Christopher David Geiger and Bashar Katirji

Introduction

The electrodiagnostic (EDX) examination in patients with suspected neuromuscular junction (NMJ) disorders requires a sound working knowledge of the physiology and pathophysiology of neuromuscular transmission. The EDX studies that are useful in the evaluation of such patients include (1) motor nerve conduction studies (NCSs), (2) conventional needle electromyography (EMG), (3) repetitive nerve stimulation (RNS), and (4) single-fiber EMG. This chapter reviews the basics of neuromuscular transmission, as it relates to the EDX studies, and discusses in detail the EDX studies and findings in various NMJ disorders.

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Basic Concepts of Neuromuscular Transmission

Performing EDX studies in NMJ disorders requires understanding of few important concepts inherent to neuromuscular transmission. These physiologic facts dictate the type of RNS and single-fiber EMG needed for the accurate diagnosis of NMJ disorders. The physiology of neuromuscular transmission is discussed in detail in Chap. 1 and is addressed briefly here [1–4].

Quantum

A quantum is the amount on acetylcholine (ACh) packaged in a single vesicle, accounting for approximately 5000–10,000 ACh molecules. Each quantum (vesicle) that is released into the synaptic cleft results in a 1-mV change of postsynaptic membrane potential. This phenomenon occurs spontaneously and continuously at rest, forming the basis of the miniature end plate potential (MEPP).

The number of quanta released as the result of a nerve action potential depends on the number of quanta in the *primary* (*immediately available) store* and the release probability of the quanta. This can be defined by the equation $m = p \times n$, where m is the number of quanta released during each stimulation, p is the probability of release (effectively proportional to the concentration of

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H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*, Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_9

calcium and typically about 0.2 or 20%), and *n* is the number of quanta in the immediately available store. Under normal conditions, a single nerve action potential triggers the release of 50–300 quanta (vesicles) with an average of about 60 quanta.

In addition to the immediately available store of ACh-containing synaptic vesicles located beneath the presynaptic nerve terminal membrane, *a secondary (or mobilization) store* starts to replenish the immediately available store after 1–2 s of sequential nerve action potentials. A large *tertiary (or reserve) store* is also available in the axon and cell body.

End Plate Potential

The end plate potential (EPP) is the potential generated at the postsynaptic membrane following a presynaptic nerve action potential and neuromuscular transmission. Since each released quantum results in a 1 mV change in the postsynaptic membrane potential, the ACh release after a nerve action potential results in about a 60 mV change in the amplitude of the membrane potential. The resultant electrical-chemical-electrical transmission is fleeting, as ACh is quickly broken down by the enzyme acetylcholinesterase at the postsynaptic basal lamina.

Safety Factor

Under normal conditions, the number of quanta (vesicles) released at the NMJ by the presynaptic terminal (about 60 vesicles) far exceeds the postsynaptic membrane potential change required to reach the *threshold* needed to generate a postsynaptic muscle action potential (7–20 mV). The safety factor allows for an EPP to always reach threshold, results in an all-or-none muscle fiber action potential (MFAP), and prevents neuromuscular transmission failure despite the depletion of ACh stores with repetitive action potentials. In addition to quantal release, several other factors contribute to the safety factor and EPP including ACh receptor conduction properties, ACh receptor density, cholinesterase activity, synaptic architecture, and sodium channel density at the NMJ.

Calcium Influx into the Terminal Axon

Following depolarization of the presynaptic terminal, voltage-gated calcium channels (VGCCs) open leading to calcium influx. Through a calcium-dependent intracellular cascade, vesicles are docked at active release sites (called active zones) and release ACh molecules. Calcium then diffuses slowly away from the vesicle release site in 100–200 ms. The rate at which motor nerves are repetitively stimulated in the electrodiagnostic laboratory dictates whether calcium accumulation plays a role in enhancing the release of ACh or not, effecting increasing "*p*" in $m = p \times n$.

Compound Muscle Action Potential

The compound muscle action potential (CMAP) is obtained, during motor NCS, with supramaximal stimulation of a motor nerve while recording via a surface electrode placed over the belly of a muscle. The CMAP represents the summation of all MFAPs generated in a muscle following stimulation of all motor axons in its supplying nerve. This waveform is the culmination of all preceding electrochemical events, and it is the subject of interest in the electrodiagnostic evaluation of neuromuscular transmission disorders.

Electrodiagnostic Tests in Neuromuscular Junction Disorders

Routine Motor Nerve Conduction Studies

Sensory NCSs are always normal in NMJ disorders, unless there is an additional entrapment mononeuropathy or peripheral polyneuropathy. Motor NCSs are, however, helpful in the evaluation of all disorders affecting the motor

unit, including NMJ disorders. CMAP amplitude is the most useful parameter in NMJ disorders since the motor distal latencies, conduction velocities, F waves minimal latencies, and H-reflexes are normal.

The CMAP amplitude is usually normal in postsynaptic disorders (such as MG), following a single supramaximal stimulus of all motor nerves, due to the effect of the safety factor. Despite partial ACh receptor blockade, EPPs achieve threshold and generate MFAPs in all muscle fibers, resulting in normal CMAP. Occasionally, such as during myasthenic crisis, the CMAP amplitudes may be borderline or slightly diminished due to severe postsynaptic neuromuscular blockade. In contrast, the CMAP amplitudes, following a single supramaximal stimulus of motor nerves during routine NCSs, are often low in presynaptic disorders (such as Lambert-Eaton myasthenic syndrome—LEMS) since many EPPs do not reach threshold and many muscle fibers do not depolarize.

Conventional Needle Electromyography

The needle EMG is usually normal in NMJ disorders. However, nonspecific changes, more commonly encountered in myopathies or neurogenic disorders, may occasionally be associated with NMJ disorders, particularly when chronic and severe. These relatively rare findings include:

Moment-to-Moment Variation and Instability of Motor Unit Action Potentials

In healthy subjects, individual motor unit action potential (MUAP) amplitude, duration, and phases are stable with little, if any, morphology variation. However, individual MUAP amplitude and morphology in NMJ disorders may vary significantly during activation due to intermittent NMJ blockade, slowing, or both. During needle EMG recording, moment-to-moment instability and variation should be distinguished from MUAP overlap. This can be achieved by using an amplitude trigger to record from a single MUAP at a time.

Short-Duration, Low-Amplitude, and Polyphasic MUAPs

These MUAPs are seen primarily in proximal muscles and are similar in morphology to those seen in myopathies. In NMJ disorders, "myopathic" MUAPs are caused by physiological blocking and slowing of neuromuscular transmission at end plates during voluntary activation. This leads to exclusion of MFAPs from the MUAP (hence the short duration and low amplitude) and asynchrony of neuromuscular transmission of muscle fibers (hence the polyphasia).

Fibrillation Potentials

Except in botulism, fibrillation potentials are rarely encountered in NMJ disorders [5, 6]. They are usually inconspicuous and present mostly in proximal muscles. The mechanism of fibrillation potentials in NMJ disorders is not clear but may be related to chronic neuromuscular transmission blockade, loss of end plates, or loss of presynaptic terminal. This results in "effective" denervation of individual muscle fibers, hence the fibrillation potentials. In botulism, fibrillation potentials are commonly seen in weak muscles when needle EMG is performed several weeks after exposure [7]. Since fibrillation potentials are rare in the rest of the NMJ disorders, their presence should always raise the suspicion of an alternate diagnosis or associated illness.

Repetitive Nerve Stimulation

Principles

Motor nerves may be repetitively stimulated and the resultant CMAPs evaluated for evidence of amplitude decrement or increment. It is the rate at which a motor nerve is stimulated which dictates whether calcium accumulation plays a role in enhancing ACh release. Since calcium diffuses out of the presynaptic terminal in about 100–200 ms after a single stimulus, a slow rate of RNS (i.e., a stimulus every 200 ms or more or a stimulation rate of <5 Hz) does not effectively increase

the concentration of calcium in the terminal button. In contrast, rapid RNS (i.e., a stimulus every 100 ms or less or stimulation rate > 10 Hz) greatly enhances calcium influx and the probability of ACh release. This allows for more EEPs to reach threshold and is ultimately responsible for the CMAP increment which is typically seen in presynaptic disorders. However, slow RNS results in depletion of primary quantal stores of acetylcholine without mobilizing secondary stores. This, in turn, results in fewer EPPs reaching the threshold needed to generate an action potential and leading to the typical CMAP decrement seen in postsynaptic disorders (see section "Findings in Neuromuscular Junction Disorders" below, findings in neuromuscular disorders).

Techniques

In the EMG laboratory, RNSs are often conducted following the performance of motor NCSs. Electromyographers and EDX technologists should master the various motor NCSs and RNSs techniques to avoid technical factors that may result in false positive and false negative studies. There are certain prerequisites that are essential for performing reliable and reproducible RNSs.

- *Acetylcholinesterase inhibitors.* If medically not contraindicated, patients on acetylcholinesterase inhibitors, such as pyridostigmine, should withhold their medication, preferably for 12–24 h before RNS. These agents improve neuromuscular transmission and may mask a CMAP decrement resulting in a false negative RNS.
- *Limb temperature control*. Limb temperature should be maintained at approximately 33 °C at the recording site. A cool limb enhances neuromuscular transmission and may mask a CMAP decrement resulting in a false negative RNS. The exact reason for this effect is not well understood, but it may be due to decreased functional effectiveness of the acetylcholinesterase, resulting in increased amount of ACh available at the NMJ.
- *Limb immobilization.* The limb tested should be immobilized as best as possible to prevent movement, particularly at the stimulation or recording sites. These sites should be well secured with tape or to a board using tape or Velcro. Movement at either the stimulation or recording site may result in a variable waveform baseline, giving the appearance of a CMAP amplitude decay or increment. This type of error could potentially lead to a false diagnosis of a NMJ disorder.
- *Stimulation intensity.* Supramaximal stimulation (i.e., 10–20% above the intensity level needed for a maximal response) is needed to assure that all nerve fibers are activated and a supramaximal CMAP is obtained. Unnecessarily high-intensity or long-duration stimuli should be avoided to prevent movement artifacts and excessive pain.
- *Stimulation frequency and number of stimuli.* The stimulus rate and number of stimuli applied during RNS depend on the clinical problem and the working diagnosis.
  (a) Slow RNS is usually done at a rate 2–3 Hz; this rate is low enough to prevent calcium accumulation but high enough to deplete the quanta in the immediately available stores before the secondary (mobilization) stores start to replenish it. A total of five stimuli is adequate since the maximal decrease in ACh release occurs after the first four stimuli. There is nothing to be gained in exceeding nine to ten stimuli.
  (b) Rapid RNS is done with a frequency of 20–50 Hz to ensure accumulation of calcium in the presynaptic terminal. Since this is extremely painful, a train of 3–5 s is sufficient. A brief (10 s) period of maximal isometric voluntary exercise has the same effect as rapid RNS at 20–50 Hz. This is much less painful than rapid RNS and, given its tolerability, allows examination of multiple motor nerves. Brief exercise could substitute for rapid RNS in most cooperative subjects. However, rapid RNS is necessary in patients who cannot produce a strong isometric exercise (e.g., young children, comatose patients, or patients with severe weakness).

- *Nerve selection.* The choice of nerve to be stimulated and the muscle to be recorded depends on the patient's symptom manifestations. Useful nerves for RNS are the median and ulnar nerves, recording abductor pollicis brevis, and abductor digiti minimi, respectively. Since the upper limb is easily immobilized, these RNSs are well tolerated and accompanied by minimal movement artifact. However, since distal muscles are often spared in postsynaptic NMJ disorders (such as MG), recording from a proximal muscle is often necessary. Slow RNS of the spinal accessory nerve, recording the trapezius muscle, is the most common study of a proximal nerve. It is relatively well tolerated and subject to less movement artifact when compared to RNS of other proximal nerves such as the musculocutaneous or axillary nerves, recording the biceps or deltoid muscles, respectively. Finally, facial slow RNS, recording nasalis or orbicularis oculi muscles, is indicated in patients with ocular, bulbar, or facial weakness when MG is suspected and other RNS are normal or equivocal. However, the normal facial CMAP is often low in amplitude and often plagued by large stimulation artifacts. This renders measurement of facial CMAP decrement more difficult and subject to error.

Measurements. After establishing a supramaximal CMAP, slow RNS is usually performed by applying three to five stimuli to a mixed or motor nerve at a rate of 2–3 Hz. Calculation of the decrement with slow RNS is accomplished by comparing the baseline (first) CMAP amplitude to the lowest CMAP amplitude. In NMJ disorders, the CMAP decrement is usually maximal at the third or fourth CMAP which then plateaus or begins to improve by the fifth or sixth response, due to the mobilization store resupplying the immediately available store (Fig. 9.1). The CMAP decrement is expressed as a percentage and calculated as follows:

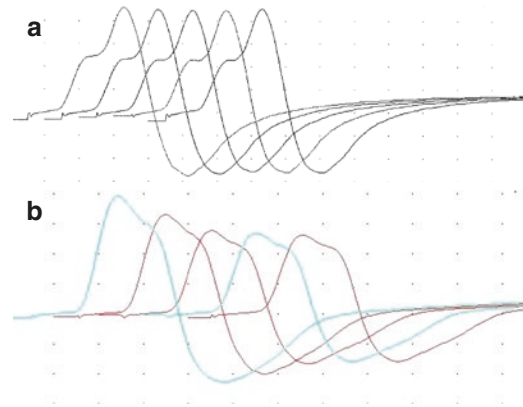


Fig. 9.1 Slow repetitive nerve stimulation (RNS) at 3 Hz of the ulnar nerve (recording hypothenar muscles). (**a**) Normal RNS. (**b**) CMAP decrement in a patient with generalized myasthenia gravis. The largest decrement is between the first and the second CMAPs, while the maximal decrement is between the first and fourth CMAPs (25%)

$$\%\ \text{decrement} = \frac{\text{Amplitude}\ (\text{1st response}) - \text{Amplitude}\ (\text{3rd or 4th response})}{\text{Amplitude}\ (\text{1st response})} \times 100$$

Slow RNS at rest should be repeated after an interval of 1–2 min to confirm a normal or abnormal response. A reproducible decrement of more than 10% is considered abnormal and eliminates false positives. Decrement of 5–10% is considered equivocal and not diagnostic. If there is a reproducible decrement at rest (≥10%), slow RNS should be repeated after the patient exercises for 10 s to demonstrate repair of the decrement (*post-exercise facilitation*). If there is no or equivocal decrement (<10%) with slow RNS at rest, the patient should perform maximal voluntary exercise for 1 min (exercise for 30 s, rest for 5 s, and exercise for another 30 s). Immediately

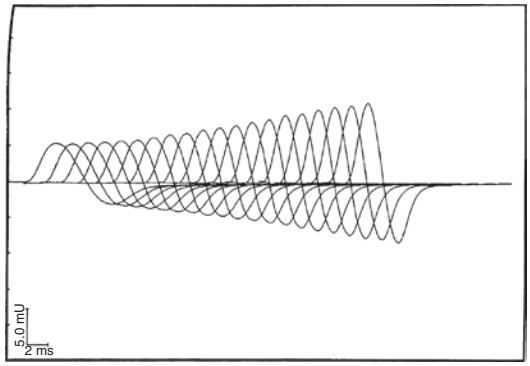


Fig. 9.2 Rapid repetitive nerve stimulation (30 Hz) of the median nerve (recording thenar muscles) in an infant with botulism. There is a 110% increment of CMAP amplitude between the baseline (1st) and last (20th) responses

following the period of exercise, slow RNS is repeated during the subsequent 1, 2, 3, 4, and 5 min. Since the amount of ACh released with each stimulus is at its minimum 2–5 min after exercise, slow RNS after exercise provides a high chance of detecting a defect in NMJ by demonstrating a worsening CMAP decrement (*post-exercise exhaustion*).

Rapid RNS is most useful in patients with suspected presynaptic NMJ disorders such as LEMS or botulism. The optimal frequency is 20–50 Hz for 2–10 s (Fig. 9.2). A typical rapid RNS applies 200 stimuli at a rate of 50 Hz (i.e., 50 Hz for 4 s). Calculation of CMAP increment after rapid RNS is as follows:

$$\%\ \text{increment} = \frac{\text{Amplitude}\ (\text{Highest response}) - \text{Amplitude}\ (\text{1st response})}{\text{Amplitude}\ (\text{1st response})} \times 100$$

A CMAP increment of more than 50–100% is considered abnormal. A modest increment of 25–40% may occur in normal individuals. This is likely caused by increased synchrony of MFAPs following tetanic stimulation (*physiologic posttetanic facilitation or pseudofacilitation*) [3, 4]. Brief periods (10 s) of maximal voluntary isometric exercise have the same effect as rapid RNS is much less painful and a good substitute in cooperative subjects [8, 9]. A single supramaximal stimulus is applied to generate a baseline CMAP, then the patient performs a 10 s maximal isometric voluntary contraction which is followed by another stimulus that produces a post-exercise CMAP. Calculation of CMAP increment after brief (10 s) voluntary contraction is similar to the calculation of the increment following rapid RNS, as follows:

$$\%\ \text{increment} = \frac{\text{Amplitude of postexercise response} - \text{Amplitude of preexercise response}}{\text{Amplitude of preexercise response}} \times 100$$

Single-Fiber EMG

Principles

Single-fiber EMG (SFEMG) is the selective recording of a small number (usually two or three) of MFAPs innervated by a single motor unit. The study aim is to analyze the effects of the variation in the time it takes the EPPs to reach threshold and generate MFAPs. With disorders of the NMJ, there is an increased variation in the time taken to attain an EPP capable of reaching threshold. SFEMG recording requires special expertise and understanding of the micro-environment of motor unit physiology. Although the examination may be applied to many neuromuscular disorders, SFEMG jitter study is most useful in clinical practice in the diagnosis of NMJ disorders, particularly myasthenia gravis [10–12].

Techniques

SFEMG jitter studies have traditionally been performed with a specialized single-fiber concentric EMG needle. These needles have a small circular recording surface (25 μm) which is located on a side port and has a recording volume of approxi-

mately 300 μm^3 (Fig. 9.3). Reference values for these electrodes were established (Table 9.1) [13]. However, there are several limitations to these electrodes. First, the cost is prohibitive with a single needle costing upwards of several hundred dollars. Second, while there have been no reported incidents, the use of reusable single-fiber electrodes also raises concerns regarding the risk of infection. Third, with multiple uses, the needles may become dull, affecting patient comfort.

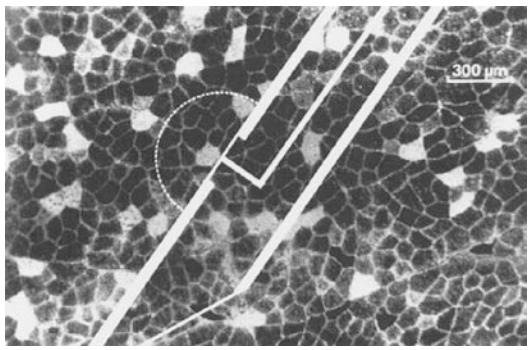


Fig. 9.3 Cross section of a muscle stained for glycogen after stimulating an isolated motor axon to show the muscle fiber distribution of one motor unit (the fibers of the motor unit become depleted of glycogen and therefore appear pale). A single-fiber EMG electrode is superimposed to show the uptake area. The strategy of recording jitter is to position the electrode (as shown) in order to record from two muscle fibers belonging to the same motor unit (Reproduced with permission from Stålberg E, Trontelj JV, Single Fiber Electromyography, Studies in Healthy and Diseased Muscle, 2nd ed., Wolters Kluwer, 1994)

For these reasons, many laboratories favor the use of standard, disposable concentric needles which have been shown to have the same sensitivity and specificity as single-fiber electrodes when assessing neuromuscular disease [14, 15]. It should be appreciated that the smallest commercially available electrode (often referred to as "facial" electrode) has an oval recording surface of 80 × 300 μm and a larger catchment area of roughly 1 cm^3 (Fig. 9.4). Given the standard distance between muscle fibers (200 μm), this creates the possibility that a captured potential is not a true representation of a single muscle fiber but possibly a summation of two to three neighboring fibers.

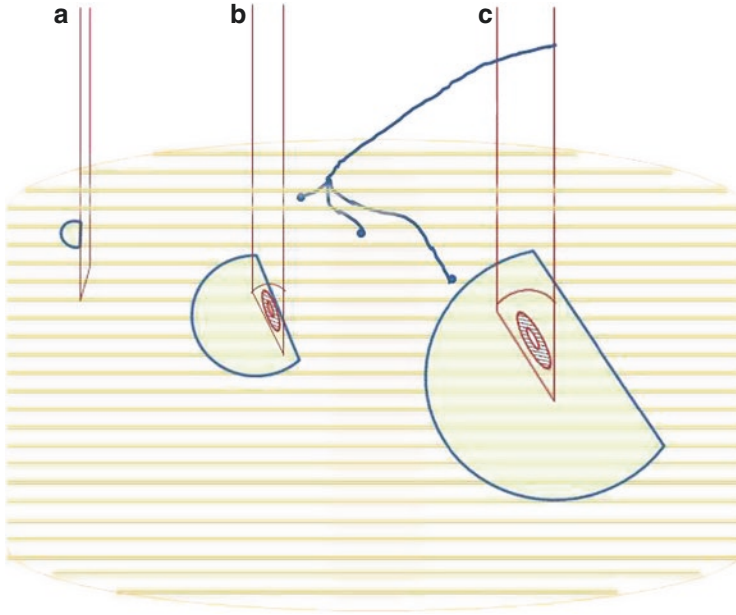
There are certain other requirements, which are essential for the accurate interpretation of jitter studies using a disposable concentric needle electrode [4, 12, 16]:

1. Use of standardized muscles including orbicularis oculi, frontalis, and extensor digitorum.
2. Filter settings should be set at 1000 Hz for the high-pass filter and 10,000 Hz for the low-pass filter (filter settings should be set at 500 Hz for the high-pass filter when using a single-fiber electrode).
3. Selected single MFAPs should have mainly one positive and one negative peak, a rise time of 300 μs with a constant slope, and, preferably, a peak-to-peak amplitude of 200 μV or more.

Table 9.1 Reference values for jitter measurements during voluntary muscle activation (μs): 95% confidence limits for upper limit of mean jitter/95% confidence limits for jitter values of individual fiber pairs[a]

Muscle/age	10 years	20 years	30 years	40 years	50 years	60 years	70 years	80 years	90 years
Frontalis	33.6/49.7	33.9/50.1	34.4/51.3	35.5/53.5	37.3/57.5	40.0/63.9	43.8/74.1		
Orbicularis oculi	39.8/54.6	39.8/54.7	40.0/54.7	40.4/54.8	40.9/55.0	41.8/55.3	43.0/55.8		
Orbicularis oris	34.7/52.5	34.7/52.7	34.9/53.2	35.3/54.1	36.0/55.7	37.0/58.2	38.3/61.8	40.2/67.0	42.5/74.2
Tongue	32.8/48.6	33.0/49.0	33.6/50.2	34.8/52.5	36.8/56.3	39.8/62.0	44.0/70.0		
Sternocleidomastoid	29.1/45.4	29.3/45.8	29.8/46.8	30.8/48.8	32.5/52.4	34.9/58.2	38.4/62.3		
Deltoid	32.9/44.4	32.9/44.5	32.9/44.5	32.9/44.6	33.0/44.8	33.0/45.1	33.1/45.6	33.2/46.1	33.3/46.9
Biceps	29.5/45.2	29.6/45.2	29.6/45.4	29.8/45.7	30.1/46.2	30.5/46.9	31.0/48.0		
Extensor digitorum communis	34.9/50.0	34.9/50.1	35.1/50.5	35.4/51.3	35.9/52.5	36.6/54.4	37.7/57.2	39.1/61.1	40.9/66.5
Abductor digiti minimi	44.4/63.5	44.7/64.0	45.2/65.5	46.4/68.6	48.2/73.9	51.0/82.7	54.8/96.6		
Quadriceps	35.9/47.9	36.0/48.0	36.5/48.2	37.5/48.5	39.0/49.1	41.3/50.0	44.6/51.2		
Tibialis anterior	49.4/80.0	49.3/79.8	49.2/79.3	48.9/78.3	48.5/76.8	47.9/74.5	47.0/71.4	45.8/67.5	44.3/62.9

[a]Adapted from [18]



Needle	Width (μm)	Area (mm^2)
a. SFEMG	25	0.0005
b. CNE facial	80	0.019
c. CNE	150	0.07

Fig. 9.4 Relative sizes and recording areas of the needle EMG electrodes. (**a**) Traditional single-fiber EMG electrode (SFEMG); (**b**) Small (facial) concentric electrode (CNE facial); (**c**) Common concentric electrode (CNE). The recording area and the number of muscle fibers for each needle are approximate and not exactly to scale (From Neuromuscular Disorders in Clinical Practice, 2nd edition, 2014, Katirji B, Kaminski HJ, Ruff RL (editors). With permission of Springer)

4. An amplitude threshold triggers and delays line to allow recording from a single MFAP by triggering on it on a screen with a delay line capability.
5. Computerized equipment assists in calculating individual and mean interpotential intervals (IPIs) and jitters (see below).

Volitional (recruitment) SFEMG is a common method for recording MFAPs, in which the patient activates and maintains the firing rate of the motor unit. This technique is not possible if the patient cannot cooperate (e.g., a child or a patient with dementia, encephalopathy, coma, or severe weakness) and is difficult if the patient is unable to maintain a constant and stable voluntary muscle contraction (e.g., in patients with Parkinsonism, tremor, dystonia, or spasticity). An amplitude threshold is used to trigger the oscilloscope trace on the closest MFAP (the action potential with the sharpest rise time and the greatest amplitude), while MFAPs from other motor units are excluded from the oscilloscope screen. With minimal voluntary activation, the needle is positioned until two muscle potentials (a pair) from a single motor unit are recognized (see Fig. 9.3). When a muscle fiber pair is identified, one fiber triggers the oscilloscope (*triggering potential*), and the second precedes or follows the first (*slave potential*). After recording multiple consecutive firings of these two muscle fibers (usually about 50–100 consecutive discharges), the electromyographer determines, usually with the aid of a computerized system, the consecutive interpotential intervals (IPIs) and calculates the difference between consecutive IPIs (Fig. 9.5). Comparison of IPIs illustrates the slight variation in transmission time at the NMJ, named the *neuromuscular jitter*. Jitter is most accurately determined by calculating a *mean consecutive difference (MCD)* (see measurements). Although jitter analysis may be obtained from any skeletal muscle, the most common muscles examined by voluntary SFEMG are the extensor digitorum, frontalis, and orbicularis oculi. These muscles are ideal because of their frequent involvement

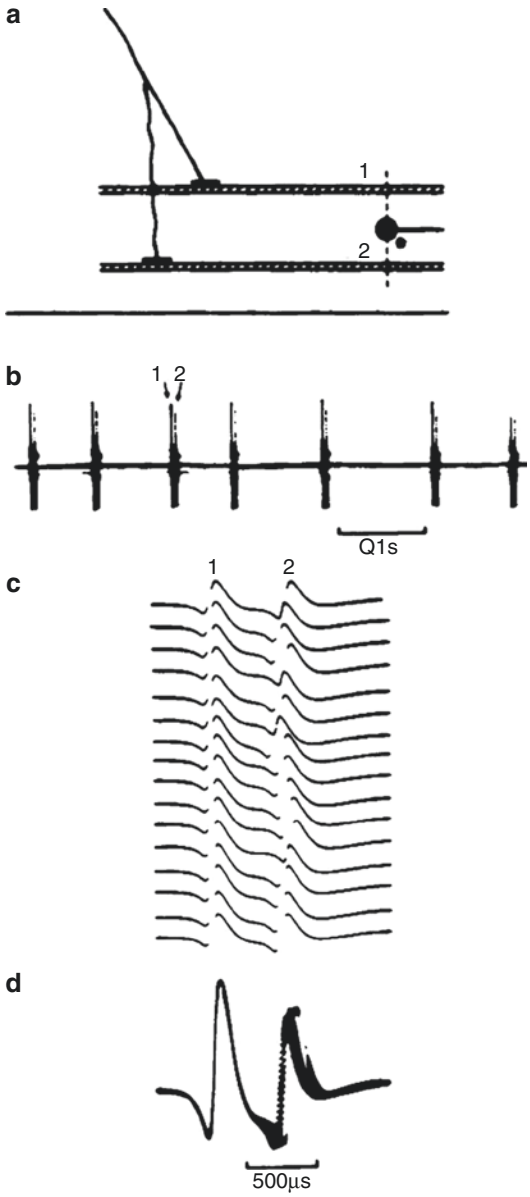


Fig. 9.5 Principle of voluntary single-fiber jitter recording. (**a**) The single-fiber EMG electrode is positioned by the electromyographer until it is possible to record from muscle fiber pair (one and two) innervated by the same motor axon. (**b**) Muscle fiber action potentials firing at a low degree of voluntary activation. (**c**) As in (**b**), but with a faster sweep speed, a sweep triggered by the first potential (the triggering potential) and showing successive discharges of the pair in a raster mode. (**d**) As in (**c**), but shown in a superimposed mode illustrating the variability in the interpotential intervals (IPIs) which reflect the neuromuscular jitter (Reproduced with permission from Stålberg E, Trontelj JV, Single-Fiber Electromyography, Studies in Healthy and Diseased Muscle, 2nd ed., Wolters Kluwer, 1994)

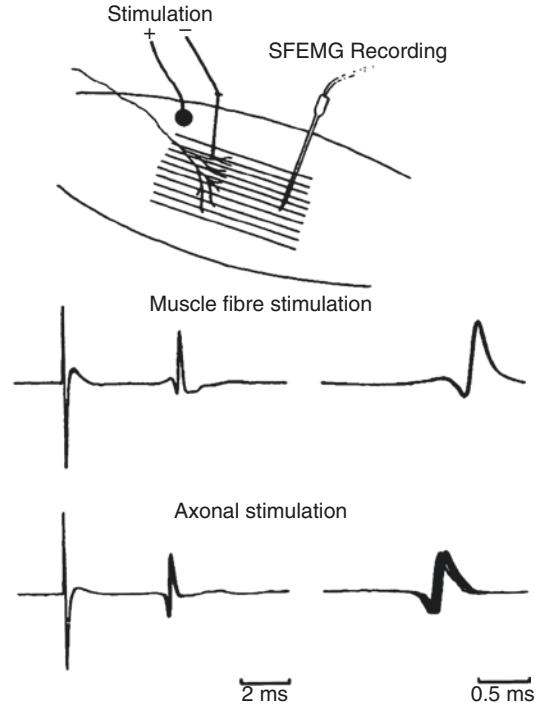


Fig. 9.6 Principle of stimulation single-fiber jitter recording. A monopolar needle stimulating cathode is inserted into the muscle near the motor point, while the anode is a surface electrode. Lower tracing discloses normal jitter with axonal stimulation, while the upper tracing discloses a very low jitter (<4 µs) resulting from direct muscle stimulation (Reproduced with permission from Stålberg E, Trontelj JV, Single-Fiber Electromyography, Studies in Healthy and Diseased Muscle, 2nd ed., Wolters Kluwer, 1994)

in NMJ disorders and the ability of most patients to control and sustain their voluntary activity to a minimum as required for the test.

Stimulation (axonal stimulation) SFEMG is an alternative method of motor unit activation and is performed by inserting an additional monopolar needle electrode near the intramuscular nerve twigs and stimulating at a low current and constant rate. The recording needle electrode is then moved slightly until one or more MFAPs are recorded (Fig. 9.6). This technique requires that the electromyographer manipulates two electrodes, a stimulating and recording electrode. Percutaneous (surface) stimulation could substitute for the needle electrode stimulations in anatomic areas where the motor branch is superficial and easily accessible, such as stimulating the zygomatic branch

of the facial nerve while recording the frontalis muscle [17]. Stimulation SFEMG has the advantage of not requiring patient participation and, thus, may be performed on the aforementioned patient populations whom may not be able to fully cooperate with a volitional study. Another advantage of this technique is that the rate of stimulation may be adjusted from slow rate (2–3 Hz) to a rapid rate (20–50 Hz). This is helpful in differentiating presynaptic from postsynaptic disorders since the jitter improves significantly with rapid RNS in LEMS, while it does not change or worsens in MG (see below) [18, 19].

In volitional SFEMG, the jitter is calculated as the variation in IPIs between two MFAPs; one potential is time-locked by the trigger, and all the variation of both end plates is expressed by the jitter of the slave potential. In contrast, the IPI in stimulation SFEMG is measured as the latency between the stimulus artifact and a single MFAP, and, thus, only one end plate is involved in the analysis. Hence, stimulation SFEMG jitter normal values are smaller than their volitional counterparts.

Measurements

The jitter is best expressed as the mean consecutive difference (MCD) of all interpotential intervals (IPI) recorded of the muscle pair [12, 20]. It is calculated as follows:

$$\mathrm{MCD} = \frac{(\mathrm{IPI1} - \mathrm{IPI2}) + (\mathrm{IPI2} - \mathrm{IPI3}) + \cdots + (\mathrm{IPI}(N-1) - \mathrm{IPI}N)}{N-1}$$

where IPI1 is the interpotential interval of the first discharge, IPI2 is of the second discharge, etc., and N is the number of discharges recorded. The mean jitter of the muscle sampled is reported after analyzing 20 muscle fiber pairs. Blocking is measured as the percentage of discharges of a motor unit in which a single-fiber potential does not fire. For example, in 100 discharges of the pair, if a single potential is missing 30 times, the blocking is 30%. In general, blocking occurs when the jitter values are significantly abnormal.

Normal values for jitter may be affected by a number of different variables including the specific muscle being examined, type of recording electrode used, mode of muscle fiber activation, and age of the subject [13, 21]. Reference values for jitter measurements with volitional muscle activation, utilizing a traditional single-fiber EMG needle, were published (see Table 9.1). As jitter values obtained by stimulation SFEMG are calculated on the basis of one end plate, the normal values are lower than those obtained by volitional activation. To calculate the normal stimulation jitter value, using a single-fiber EMG needle, it is recommended that the reference data for volitional SFEMG is multiplied by 0.80.

Given the growing use of standard concentric EMG needles, for the aforementioned reasons, a group recently completed a multicenter study to determine reference jitter values in healthy control subjects [16]. Normative data was established for three commonly studied muscles (e.g., orbicularis oculi, frontalis, extensor digitorum) using voluntary activation (Table 9.2). In this study, age did not appear to significantly affect jitter and was therefore omitted as a qualifying

Table 9.2 Baseline CMAP and repetitive stimulation findings in common neuromuscular junction disorders

NMJ disorder	NMJ defect	CMAP amplitude	Slow RNS	Rapid RNS or post-exercise facilitation
Myasthenia gravis	Postsynaptic	Normal	Decrement	Normal or decrement
Lambert-Eaton myasthenic syndrome	Presynaptic	Low in all muscles	Decrement	Marked increment: >100% in at least one muscle and >60% in all muscles
Botulism	Presynaptic	Low in proximal and weak muscles	Decrement	Modest increment: In weak muscles (50–100%)

NMJ neuromuscular junction, *CMAP* compound muscle action potential, *RNS* repetitive nerve stimulation

factor. In addition, rather than extrapolating data for stimulation values, approximately 200 stimulated studies were analyzed to formulate dedicated normative values for this modality as well.

The final results of SFEMG jitter study are expressed by (1) the mean jitter of all muscle pairs studied, (2) the percentage of pairs with impulse blocking, and (3) the percentage of pairs with abnormal jitter [4, 12, 22]. The study is considered abnormal when one or more of the following criteria are met:

1. Mean consecutive difference (MCD) exceeds an upper limit (normal mean + 2 SD for a given muscle).
2. More than 2 (10%) pairs have jitter considered to be in an outlier range when 20 individual muscle fiber pairs are analyzed. This second criterion should be augmented to 20% in patients over the age of 60 years, when using a traditional single-fiber EMG needle electrode.
3. Impulse blocking is frequently seen in the majority of fiber pairs in a muscle.

Findings in Neuromuscular Junction Disorders

Myasthenia Gravis

Myasthenia gravis (MG) is the best understood and thoroughly studied of all human organ-specific autoimmune diseases. MG is caused by an antibody-mediated attack on the postsynaptic nicotinic ACh receptors of the neuromuscular junction. It is characterized by a reduction of skeletal muscle postsynaptic ACh receptors resulting in a decrease in the EPP amplitude. Up to 85% of patients with MG have an elevated ACh receptor antibody (seropositive MG). Antibodies to muscle-specific tyrosine kinase (MuSK) are present in sera of 25–50% of MG patients who are negative for the ACh receptor antibody. Some patients with MG have antibodies against low protein LRP4, another end plate protein required for AChR clustering, and normal NMJ formation. The term "seronegative MG" should hence be reserved to patients who have negative autoantibodies to ACh receptor, MuSK and LRP4.

The EDX study is an essential component of the available armament in the diagnosis of MG. The EDX studies are most useful in seronegative patients with suspected MG, and in patients with a negative or equivocal edrophonium tests and few objective neurological findings. Individual EDX studies of patients with suspected MG should be tailored to the patient's symptomatology.

Baseline Motor Nerve Conduction Studies

Routine motor nerve conduction studies (NCSs), including CMAP amplitudes, are normal in MG. A single supramaximal stimulus to a motor nerve results in ACh release and postsynaptic EPPs which reach threshold despite ACh receptor blockade because of the presence of the safety factor. Hence, MFAPs are generated in all muscle fibers resulting in a normal CMAP. Occasionally, such as in the midst of a myasthenic crisis with profound muscle weakness, the CMAPs may be borderline or slightly diminished due to severe postsynaptic neuromuscular blockade (Fig. 9.7). Also, in patients

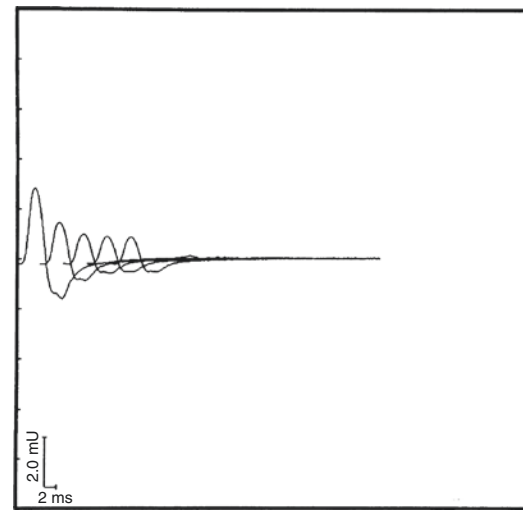


Fig. 9.7 Slow repetitive nerve stimulation (3 Hz) of the median nerve (recording thenar muscles) in a patient with myasthenic crisis. The largest decrement is between the first and the second CMAPs (45%), while the maximal decrement is between the first and third CMAP (65%). Note that the CMAP amplitude plateaus after the third response and that the baseline (first) CMAP amplitude is slightly diminished during myasthenic crisis (see text)

taking large quantities of cholinesterase inhibitors, such as pyridostigmine, there may be a tendency to record multiple CMAPs after a single stimulus applied to the nerve.

Slow Repetitive Nerve Stimulation

Slow RNS, usually at a rate of 2–3 Hz, results in a decrease in quantal release due to the depletion of the immediate (primary) ACh stores. In postsynaptic disorders such as MG, this stresses the NMJ to a point where many EPPs fail to reach the threshold needed to generate an action potential (Fig. 9.8a). The loss of many MFAPs is reflected as a decremental CMAP on slow RNS [23, 24]. Although the greatest drop in CMAP amplitude and area in MG occurs between the first and the second responses, the decrement usually continues and is usually maximal when comparing the first and the third or fourth CMAPs [2–4, 23, 24]. Often, after the fifth or sixth stimulus, the secondary stores are mobilized resulting in stabilization, or sometimes slight improvement of the CMAP and giving the characteristic "U-shaped" decrement.

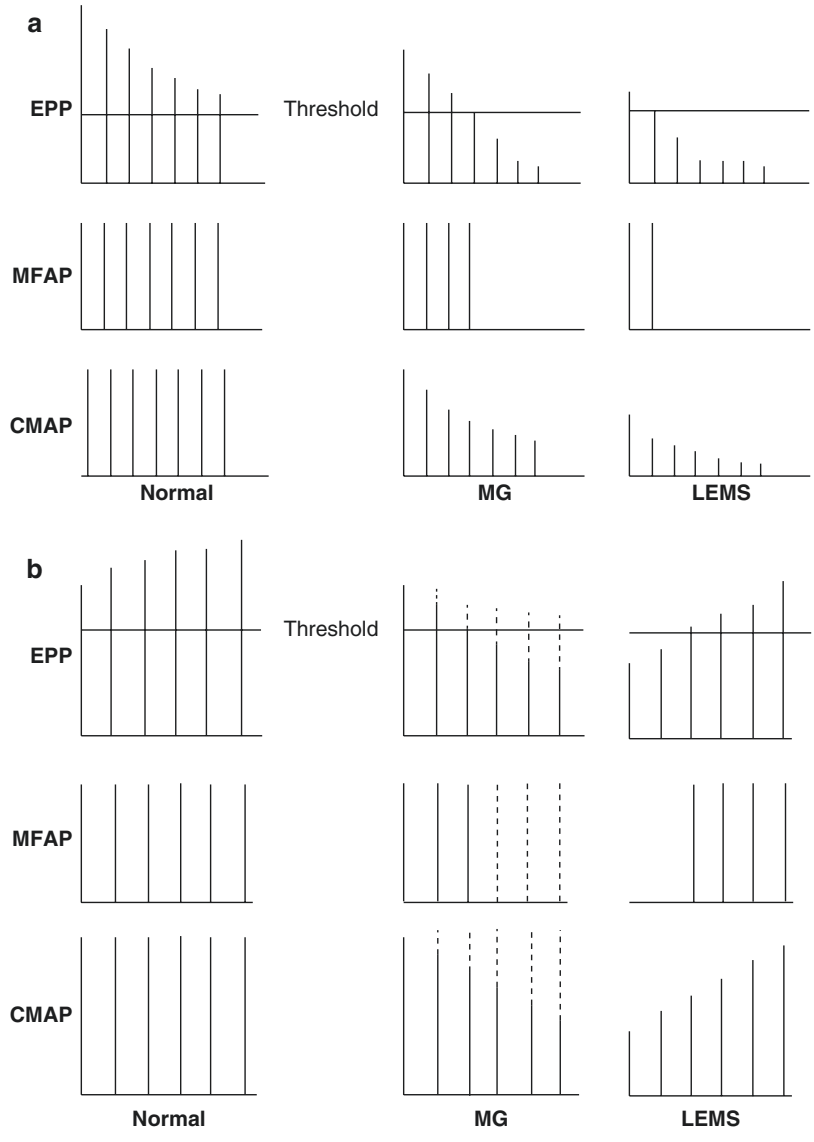


Fig. 9.8 Effects of slow repetitive nerve stimulation (**a**) and rapid repetitive nerve stimulation (**b**) on end plate potential (EPP), muscle fiber action potential (MFAP), and compound muscle action potential (CMAP) in normal subjects and in patients with MG and LEMS (Reproduced with permission from Oh S, Clinical Electromyography, Neuromuscular Transmission Studies, Wolters Kluwer, 1988)

A reproducible CMAP decrement of >10% is abnormal and eliminates false positives; CMAP decrement of 5–10% is considered equivocal. The diagnostic yield of slow RNS in MG is increased by:

1. *Recording from clinically weakened muscles such as proximal and facial muscles.* This often means recording from the trapezius in generalized MG and from the orbicularis oris or nasalis in ocular or bulbar MG (Fig. 9.9). This strategy increases the diagnostic sensitivity of slow RNS in the diagnosis of MG [25]. Compared to recording distal muscles (such as the abductor digiti minimi), slow RNS of the facial and spinal accessory nerves increases the diagnostic sensitivity of the study by about 5–20% [26]. Though often technically challenging, a decremental response on slow RNS of the facial nerve is highly specific for the diagnosis of MG and is common in MuSK-positive MG patients [27, 28].
2. *Obtaining slow RNS following exercise* looking for post-exercise exhaustion. After per-

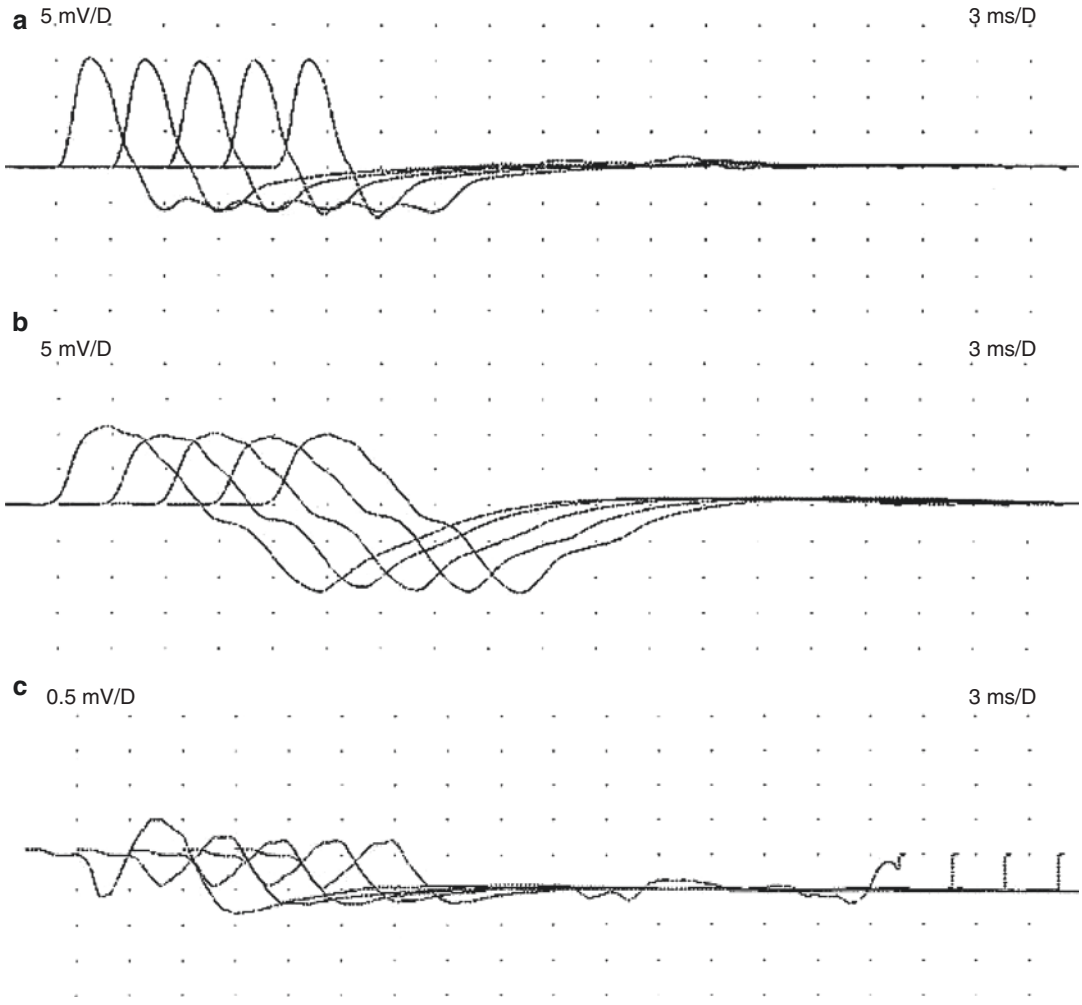


Fig. 9.9 Slow repetitive stimulation in a 35-year-old man with binocular diplopia and a history of ptosis, revealing (**a**) no decrement simulating median nerve (recording thenar muscles), (**b**) mild and equivocal decrement (10%) stimulating spinal accessory nerve (recording upper trapezius muscle), and (**c**) significant decrement (40%) simulating the facial nerve (recording orbicularis oculi muscle)

forming slow RNS at rest, the patient is asked to exercise the tested muscle for 1 min. Then, slow RNSs are repeated every 30–60 s for 4–5 min. Post-exercise exhaustion usually occurs 4–6 min after exercise and is particularly useful in patients with suspected MG and equivocal CMAP decrement at rest. However, post-exercise studies only increase the yield of RNS by about 5–7% [29].

Rapid Repetitive Nerve Stimulation and Post-exercise Facilitation

Rapid RNS in healthy subjects, usually at 20–50 Hz, increases EPP amplitude. Ultimately, this has no effect on the number of MFAPs or the size of CMAP since all EPPs are and remain above threshold (see Fig. 9.8b). In patients with postsynaptic disorders, the depleted ACh stores are usually compensated by increase ACh release due to the accumulation of presynaptic calcium resulting in no change of CMAP amplitude. For this reason, rapid RNS is not useful or necessary in the diagnosis of MG. It should only be considered when a presynaptic NMJ disorder (such as LEMS or botulism) is clinically suspected and needs to be excluded, or if the baseline CMAPs are borderline or low in amplitudes.

Single-Fiber EMG

Evaluation of neuromuscular transmission in patients with suspected MG is the most common indication for performing SFEMG. Commonly tested muscles in patients with suspected MG are the extensor digitorum, orbicularis oculi, and frontalis.

In patients with MG, abnormal jitter values are common (Fig. 9.10). This is frequently accompanied by neuromuscular impulse blocking, which reflects the failure of a muscle fiber to transmit an action potential due to the inability of the EPP to reach threshold. SFEMG is extremely sensitive in detecting MG; a normal SFEMG jitter study in a weak muscle virtually excludes the diagnosis of MG. The diagnostic sensitivity of SFEMG ranges from 90 to 99% (Fig. 9.11) [12, 22, 25, 30]. This makes SFEMG the most sensitive diagnostic study in MG [25]. However, abnormal jitter is nonspecific since it is often seen in a number of neuromuscular conditions including various neuropathies, myopathies, and anterior horn cell disorders. Thus, a diagnosis of a NMJ disorder, such as myasthenia gravis, by jitter analysis should always be interpreted only in the contest of the patient's clinical manifestations, nerve conduction studies, and needle EMG findings.

SFEMG sensitivity and specificity is equal in seropositive and seronegative MG patients. However, SFEMG of limb muscles, such as the extensor digitorum, are often normal in MuSK-positive MG patients who frequently have prominent facial and bulbar muscle weakness [31]. By comparison, SFEMG of facial muscles, such as the orbicularis oculi, has a similar sensitivity in MuSK-positive patients compared to ACh receptor-positive patients [31].

In addition to the diagnostic utility of SFEMG, SFEMG has a value as prognostication tool in MG. Patients with higher mean jitter values, a greater percentage of fibers with increased jitter, and/or impulse blocking have a higher risk of a severe disease exacerbation [32]. These findings also appear to correlate with the frequency of symptom exacerbation [33]. The degree of jitter change in patients with myasthenia gravis also predicts clinical course, with a potential use as a biomarker of disease control when performed longitudinally [34].

Conventional Needle EMG

The needle EMG examination in evaluation of MG serves mostly to exclude other neuromuscular causes of weakness, such as motor neuron disease, neuropathies, or myopathies. Though the needle EMG is usually normal in patients with MG, certain findings are occasionally present and not inconsistent with the diagnosis. These findings usually occur in patients with moderate or severe MG and are most evident in proximal muscles. Findings on needle EMG that are compatible with MG include (1) moment-to-moment variation of MUAP morphology (MUAP instability); (2) short-duration, low-amplitude, and polyphasic MUAPs; and (3) fibrillation poten-

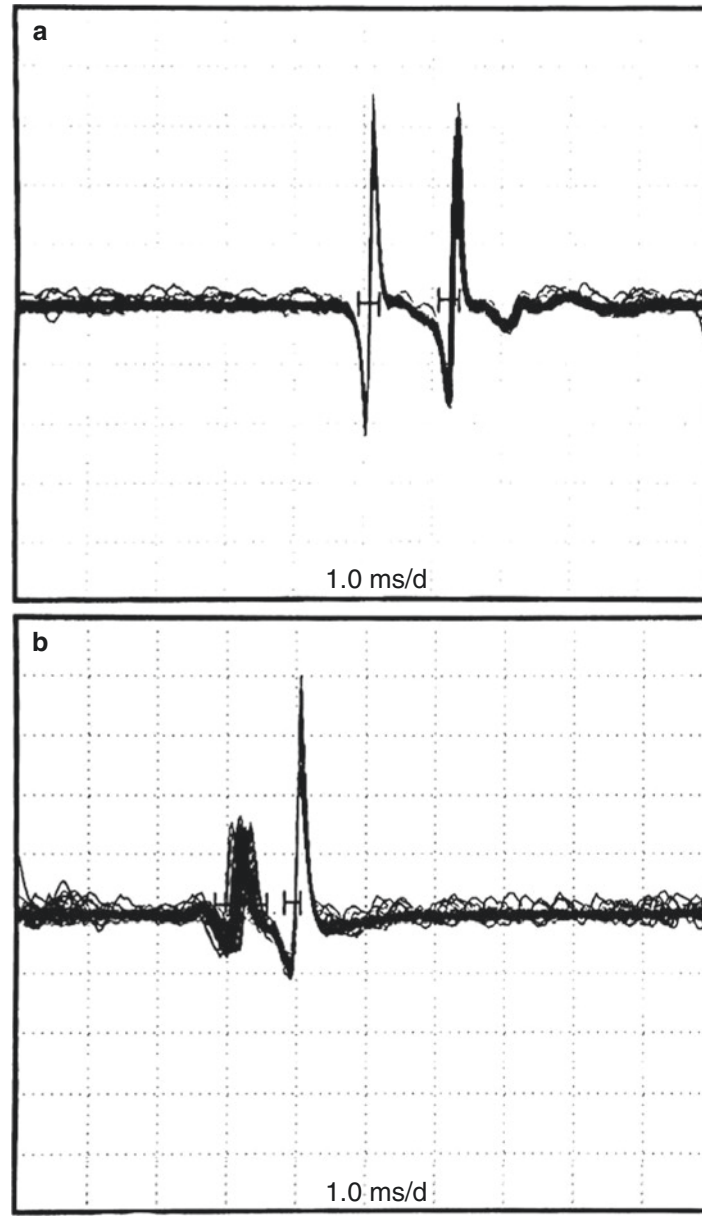


Fig. 9.10 Neuromuscular jitter recording frontalis muscle with voluntary activation in a 22-year-old patient with ptosis (shown in a superimposed mode). (**a**) Normal jitter in a pair (mean consecutive discharge (MCD) = 18.5 μs). (**b**) Abnormal jitter in another pair (MCD = 65 μs)

tials and fasciculation potentials. *MUAP instability* occurs when individual muscle fibers are either blocked or come to action potential at varying intervals, leading to an MUAP that change in configuration (amplitude and/or number of phases) from impulse to impulse. *Short-duration, low-amplitude, and polyphasic MUAPs* are seen when blocking is severe, resulting in drop out of muscle fibers, thereby reducing the number of muscles fibers per motor unit. *Fibrillation potentials*, rarely seen in MG, are due to effective denervation of muscle fibers due to chronic neuromuscular blockade or loss of end plates [5]. *Fasciculation potentials* may be encountered in patients treated with large doses of cholinesterase inhibitors. However, all these findings are not specific and more often seen in myopathies or denervating disorders.

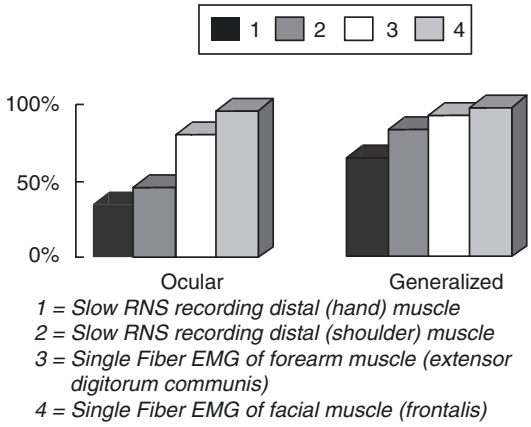


Fig. 9.11 The diagnostic sensitivity of slow repetitive nerve stimulation and single-fiber EMG in the diagnosis of myasthenia gravis (Data compiled from OH SJ, Kim DE, Kuruoglu R, Bradley RJ, Dwyer D. Diagnostic sensitivity of the laboratory tests in myasthenia gravis. Muscle Nerve. 1992;15:720–724 and from Howard, JF, Sanders DB, and Massey JM. The electrodiagnosis of myasthenia gravis and the Lambert-Eaton syndrome. Neurol Clin 1994;12:305–330)

Lambert-Eaton Myasthenic Syndrome

Lambert-Eaton myasthenic syndrome (LEMS) is a rare autoimmune disorder of the neuromuscular junction, associated with small cell lung cancer in approximately 40–50% of patients (Chap. 14) [10, 35, 36]. The cancer is commonly detected soon after the onset of LEMS symptoms and is rarely diagnosed beyond 5 years. Common risk factors for paraneoplastic LEMS include smoking history, weight loss, bulbar weakness, and fatigue (reduced Karnofsky performance status) [37]. LEMS is caused by antibodies against the presynaptic P-/Q-type voltage-gated calcium channels (VGCC), detected in 3/4 of patients with LEMS [38]. The block of VGCCs results in a decrease in calcium influx during depolarization of the presynaptic membrane and interferes with the calcium-dependent release of ACh from its stores in vesicles into the synaptic cleft. The EDX abnormalities encountered in LEMS constitute the mainstay of diagnosis as described originally by Lambert and Eaton [10, 35, 36].

Baseline Motor Nerve Conduction Studies

The CMAPs, obtained during routine motor NCSs, are usually low in amplitudes in patients with LEMS, since many EPPs do not reach threshold after a single stimulus due to inadequate release of synaptic vesicles. Thus, a low number of MFAPs are generated leading to low-amplitude CMAPs. These findings are usually present in all muscles. Low amplitude CMAPs may be the first clue that a patient with weakness may have LEMS, and the electromyographer may be the first to diagnose LEMS in the EMG laboratory by evaluating post-exercise CMAP in patients with universally low CMAP amplitude referred for a variety of reasons [4].

Slow Repetitive Nerve Stimulation

Slow RNS (usually 2–3 Hz) results in >10% CMAP decrement in all patients with LEMS [38]. With slow RNS, ACh release is reduced further because of the depletion of the immediately available stores, and, at this slow rate of stimulation, calcium does not accumulate in the presynaptic terminal. The end result is further loss of many MFAPs and a decrement of CMAP amplitude (see Fig. 9.8a). However, slow RNS is not useful in LEMS diagnosis since it cannot distinguish it from the decrement seen in postsynaptic disorders, such as MG.

Rapid Repetitive Nerve Stimulation and Post-exercise Facilitation

Rapid RNS (usually 20–50 Hz) or brief (10 s) isometric exercise results in a CMAP amplitude increment (Fig. 9.12). This rate of stimulation or type of exercise enhance calcium influx into the presynaptic terminal, which results in larger releases of quanta and larger EPPs. While many EPPs do not reach threshold after the first stimulus, many muscle fibers achieve threshold required for the generation of MFAPs with the subsequent stimuli (see Fig. 9.8b). The detected posttetanic facilitation exceeds 60% in all muscles and 100% in at least one muscle in 80% of LEMS patients [38, 39]. It is also common to

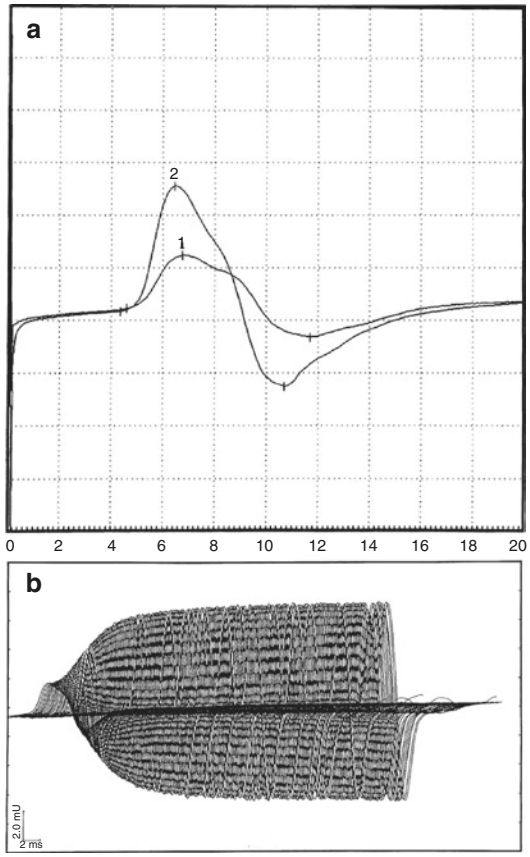


Fig. 9.12 Posttetanic compound muscle action potentials (CMAP) evaluation in a patient with LEMS stimulating the ulnar nerve (recording hypothenar muscles). (**a**) Baseline low-amplitude CMAP at 2.0 mV (waveform 1). Following 10 s of voluntary maximal isometric exercise, there is a significant CMAP increment (waveform 2) to 4.8 mV (increment = 140%) (sensitivity 2 mV/division). (**b**) Rapid (50 Hz) stimulation revealing marked increment of CMAP amplitude (250%)

have robust increments exceeding 200% in many LEMS patients, [10, 11, 35, 36, 40, 41] particularly those with positive VGCC serology [42].

Single-Fiber EMG

SFEMG jitter is abnormal in the majority of patients with LEMS, with findings similar to those seen in MG [4, 12, 18, 22, 43]. However, stimulation SFEMG technique may help distinguish the disorders. With this method, the jitter improves significantly with rapid rate stimulation (20–50 Hz) in LEMS, due to enhancement of calcium influx which results in larger releases of quanta. In contrast, rapid rate of axonal stimulation does not change or worsens the jitter in MG.

Conventional Needle EMG

The findings on needle EMG in LEMS are very similar to MG, although the abnormalities are often more conspicuous and only present in severe cases.

Botulism

Botulism is a rare but serious and potentially fatal illness. It is caused by the toxicity of botulinum toxin, which is produced by the anaerobic bacterium, *Clostridium botulinum.* The toxin has a significant affinity to both muscarinic and nicotinic cholinergic nerve terminals resulting in autonomic failure and skeletal muscle paralysis. Botulinum toxin results in failure of ACh release from the presynaptic terminal and ultimately leads to the destruction of the presynaptic terminal. Botulinum toxin first attaches irreversibly to the axonal terminal and interferes with the calcium-dependent intracellular cascade by cleaving proteins essential for docking and fusion of the presynaptic vesicles at the presynaptic active zones. Depending on the mode of entry of the toxin into the bloodstream, botulism is classified into four clinically distinct forms: food-borne (classical) botulism, infant botulism, wound botulism, and iatrogenic (inadvertent) botulism.

The EDX studies in botulism are a mainstay in diagnosis. The study is a rapid and readily available method of diagnosis for patients with suspected botulism, while definitive bioassays for botulinum toxin or stool cultures for *Clostridium botulinum* are usually delayed and may be negative. The EDX findings in botulism are compatible with a presynaptic defect of neuromuscular transmission and are somewhat similar to the findings in LEMS [6, 7, 44–46]. However, the results

of EDX studies are more variable and dependent on the amount of toxic exposure, degree and distribution of muscle weakness, and the timing of the study. During the early course of the disease, the EDX abnormalities may be limited and often change significantly from day to day, particularly as the muscle weakness worsens.

Baseline Motor Nerve Conduction Studies

Low CMAP amplitudes, obtained during routine motor NCSs, are the most consistent finding, present in 85% of patients, particularly when recording from weak muscles (usually proximal) [6, 46]. Diminished CMAP amplitudes are the result of the inadequate release of synaptic vesicles, which in turn render many EPPs unable to reach threshold after a single stimulus. Motor distal latencies and conduction velocities are normal. Rarely, the CMAPs are diffusely absent in severe cases of botulism, consistent with total blockade of ACh release [47].

Slow Repetitive Nerve Stimulation

Slow RNS in patients with botulism may cause a decrement of CMAP amplitude. However, this is infrequent and mild not exceeding 8–10% of baseline. It is caused by the progressive depletion of the immediately available ACh stores without accumulation of Ca^2 in the presynaptic terminals.

Rapid Repetitive Nerve Stimulation and Post-exercise Facilitation

Rapid RNS, or CMAP following brief (10 s) of isometric exercise, in patients with botulism results in CMAP increment. With tetanic stimulation, Ca^2 influx is greatly enhanced resulting in larger releases of quanta. This leads to increasing number of muscle fibers reaching the threshold required for the generation of muscle action potentials. The increment in botulism is modest, ranging between 50 and 100% (see Fig. 9.2), when compared to the increment in LES, which often surpasses 200% (Table 9.3) [6, 7, 46]. The increment may occasionally be absent, especially in severe cases such as those caused by type A toxin, presumably due to the degree of presynaptic blockade [47].

Table 9.3 Reference values for jitter measurements during volitional and stimulated muscle activation when recorded with a disposable, concentric EMG needle[a]

	Orbicularis oculi	Frontalis	Extensor digitorum
Volitional			
Mean MCD (μs) [mean (SD)]	22.9 (3.9)	20.6 (3.6)	23.4 (3.0)
Upper limit	31	28	30
18th MCD (μs) [mean (SD)][b]	29.0 (7.6)	25.8 (5.6)	30.0 (6.1)
Upper limit	45	38	43
Stimulated			
Mean MCD (μs) [mean (SD)]	19.1 (3.8)	14.5 (2.8)	18.2 (2.9)
Upper limit	27	21	24
27th MCD (μs) [mean (SD)][b]	25.9 (4.9)	19.3 (3.9)	18.2 (2.9)
Upper limit	36	28	35

[a]Adapted from [16]

[b]18th MCD (volitional) and 27th MCD (stimulated) were determined to be the cutoff jitter values for any particular muscle fiber pair in a given muscle, a reading above which is considered an "outlier"

Single-Fiber EMG

Increased jitter with blocking may be observed by single-fiber EMG [44, 45]. As is the case in LEMS, stimulation jitter usually improves with increasing stimulation frequency.

Conventional Needle EMG

The needle EMG in botulism is variable and depends on the amount of toxic exposure and timing of the study. The needle EMG may be normal or sometimes reveals increase in the number of short-duration, low-amplitude, and polyphasic MUAPs, occasional fibrillation potentials, and MUAP instability. In severe cases, rapid recruitment of only few MUAP may be apparent, and fibrillation potentials may be abundant. Extensive chemodenervation may result in electrically silent muscles, manifesting no spontaneous activity, end plate spikes, or noise [47].

Congenital Myasthenic Syndromes

Congenital myasthenic syndromes (CMS) are caused by genetic defects of the presynaptic or postsynaptic apparatus (Chap. 16). Most cases of CMS, regardless of primary etiology, demonstrate a degeneration of the postsynaptic region and simplification of junctional folds often with a concomitant reduction of ACh receptor density [48].

Given the similar anatomic pathology of CMSs, slow RNS often produces a decremental response, which may be absent during rest and only elicited after several minutes of exercise. In cholinesterase deficiency and slow-channel syndrome, a single electrical stimulation leads to repetitive CMAPs, similar to the findings in organophosphate poisoning and in patients taking large doses of cholinesterase inhibitors (Fig. 9.13).

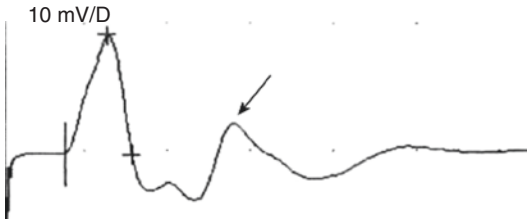


Fig. 9.13 Repetitive CMAP after single supramaximal stimulation of the ulnar nerve in a 20-year-old patient with the slow-channel syndrome. Note the second small second CMAP (arrow) that appears after the normal CMAP

EDX Strategy in a Patient with a Suspected Neuromuscular Junction Disorder

The EDX study of a patient with a suspected NMJ disorder should start with a detailed history and comprehensive neurological examination. Sensory and motor NCSs in two limbs (preferably an upper and a lower extremity) should be the initial studies. The clinical situation and baseline CMAP amplitudes dictate the next steps (Fig. 9.14) [2, 3].

- If the CMAP amplitudes are low, a presynaptic defect should be suspected and ruled out.
- If the diagnosis of LEMS is clinically suspected, baseline and post-exercise CMAPs of at least two distal motor nerves (such as the median and ulnar nerves) are a sufficient screening test. In LEMS, CMAP increment is usually robust and may surpass 200%. A rapid RNS of one distal nerve for 3–5 s, which is extremely painful, is sufficient for confirmation if there are definite CMAP increments after brief exercise.
- If the diagnosis of botulism is considered, the choice of muscle should include clinically weak muscles. In botulism, the CMAP increment is usually 50–100%. Also, the study may be repeated in 1–2 days, particularly if the ini-

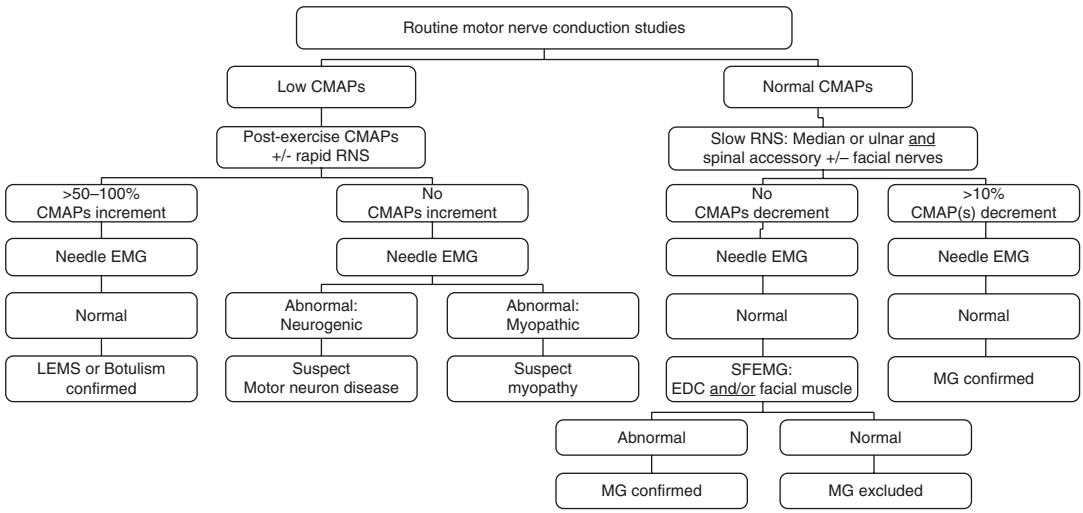


Fig. 9.14 Electrodiagnostic strategy in patients with suspected neuromuscular junction disorder

tial evaluation was done during the early phase of the illness.

- If the diagnosis of MG is clinically suspected, slow RNS at rest and for 4–5 min following a 1-min exercise should be performed on at least two motor nerves. The selection of nerves and muscles is dependent on the clinical manifestations with the goal to record from clinically weak muscles. One should start by performing slow RNS on a distal hand muscle (such as the abductor digiti minimi or abductor pollicis brevis) and move on to a proximal muscle such as the upper trapezius. Facial RNS should be reserved for patients with oculobulbar manifestations and normal slow RNS recording from distal and proximal muscles. SFEMG of one or two muscles (such as the frontalis, orbicularis oculi, or extensor digitorum communis) should be considered if the diagnosis of MG is still considered despite normal RNS studies and absent serum ACh receptor antibodies.
- If the CMAPs obtained on motor NCSs in a patient with suspected MG are low or borderline, post-exercise CMAP screening should always be done to exclude LEMS and botulism. A misdiagnosis of MG is often made if post-exercise CMAP evaluation is not done and a slow RNS shows a CMAP decrement.
- Post-exercise CMAP screening is recommended for patients with weakness associated with a malignancy (particularly a small cell lung cancer) or if the clinical situation does not clearly differentiate between LEMS and MG.

Electrodiagnostic Findings in Other Neuromuscular Disorders

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a relentlessly progressive, fatal disease caused by degeneration of both upper and lower motor neurons. Slow rates of RNS result in a decrementing response in at least 50% of ALS patients, suggesting functional alterations of the neuromuscular junction that accompany this disease [49, 50]. The decrement is usually inversely proportional to CMAP amplitude. Although many experts favor a presynaptic origin to the neuromuscular junction defect in ALS patients, none of these patients exhibit any significant post-exercise facilitation of the baseline low CMAP amplitude. Single-fiber EMG jitter, which is abnormal in the majority of patients with ALS, does not differentiate presynaptic from postsynaptic neuromuscular junction defects. The neuromuscular transmission defect is best explained by reinnervation of partial or completely denervated motor end plates with reduction of ACh release parameters.

Miller-Fisher Syndrome

The Miller-Fisher syndrome, a variant of Guillain-Barré syndrome, consists of a clinical triad of ataxia, ophthalmoplegia, and areflexia. In more than 90% of patients, serum antibodies to GQ1b ganglioside are detected. Single-fiber EMG studies in many patients with Miller-Fisher syndrome show abnormal neuromuscular transmission in cranial innervated as well as limb muscles [51–53]. These changes are more common in GQ1b-positive patients and are consistent with a terminal motor axon dysfunction. Sera from GQ1b-positive patients bind to the presynaptic terminal causing an increase in miniature end plate potential frequency. In vitro studies found both presynaptic and postsynaptic reversible blocking effects to GQ1b-positive sera [54]. Other studies found that anti-GQ1b antibodies induced irreversible quantal release of ACh from nerve terminals, eventually causing neuromuscular blockade [55].

Guillain-Barré Syndrome

Early on, it was recognized that increased jitter occurs in patients with early Guillain-Barré syndrome (GBS). This was felt to be due to unstable peripheral nerve conduction during the demyelination phase. Reversible blocking at the NMJ

was detected in vitro by sera from GBS patients who are seropositive or seronegative to anti-GM1 antibodies [56]. Dysfunction at the NMJ was documented by distinctly abnormal jitter values. However, intermittent genuine impulse blocking without correspondingly increased jitter seen in GBS patients is a likely sign of axolemmal dysfunction [57].

References

1. Boonyapisit K, Kaminski HJ, Ruff RL. The molecular basis of neuromuscular transmission disorders. Am J Med. 1999;6:97–113.
2. Katirji B. Electromyography in clinical practice. A case study approach. 2nd ed. St Louis: Mosby; 2007.
3. Katirji B, Kaminski HJ. An electrodiagnostic approach to the patient with neuromuscular junction disorder. Neurol Clin. 2003;20:557–86.
4. Oh SJ. Electromyography: neuromuscular transmission studies. Baltimore: Williams and Wilkins; 1988. p. 87–110. 243–253
5. Barberi S, Weiss GM, Daube JR. Fibrillation potentials in myasthenia gravis. Muscle Nerve. 1982;5:S50.
6. Keezer M, Benini M, Chalk C. Botulism. In: Katirji B, Kaminski HJ, Ruff 2nd RL, editors. Neuromuscular disorders in clinical practice. Boston: Butterworth-Heinemann; 2014. p. 1101–10.
7. Cornblath DR, Sladky JT, Sumner AJ. Clinical electrophysiology of infantile botulism. Muscle Nerve. 1983;6:448–652.
8. Lambert EH, Eaton LM, Rooke ED. Defect of neuromuscular conduction associated with malignant neoplasms. Am J Phys. 1956;187:612–3.
9. Hatanka Y, Oh SJ. Ten-second exercise is superior to 30-second exercise for post-exercise facilitation in diagnosing Lambert-Eaton myasthenic syndrome. Muscle Nerve. 2008;37:572–5.
10. Eaton LM, Lambert EH. Electromyography and electric stimulation of nerves in diseases of motor unit: observations on myasthenic syndrome associated with malignant tumors. JAMA. 1957;163:1117–24.
11. Tim RW, Sanders DB. Repetitive nerve stimulation studies in the Lambert-Eaton myasthenic syndrome. Muscle Nerve. 1994;17:995–1001.
12. Stålberg E, Trontelj JV, Sanders DB. Single fiber electromyography. Studies in healthy and diseased muscle. 3rd ed. Fiskebackdkil: Edshagen Publishing; 2010.
13. Gilchrist JM. Ad hoc committee of the AAEM special interest group on SFEMG. Single fiber EMG reference values: a collaborative effort. Muscle Nerve. 1992;15:151–61.
14. Sarrigiannis PG, Kennett RP, Read S, Farrugia ME. Single-fiber EMG with concentric needle electrode: validation in myasthenia gravis. Muscle Nerve. 2006;33:61–5.
15. Benatar M, Hammad M, Doss-Riney H. Concentric-needle single-fiber electromyography for the diagnosis of myasthenia gravis. Muscle Nerve. 2006;34:163–8.
16. Stalberg E, Sanders DB, Sajjad A, Cooray G, Leonardis L, Löseth S, et al. Reference values for jitter recorded by concentric needle electrodes in healthy controls: a multicenter study. Muscle Nerve. 2016;53:351–62.
17. Basio MB, Yildiz N, Ertas M. Surface stimulation single-fiber electromyography in myasthenia gravis. J Clin Neurophysiol. 2002;19(1):73–6.
18. Trontelj JV, Stålberg E. Single motor endplates in myasthenia gravis and Lambert Eaton myasthenic syndrome at different firing rates. Muscle Nerve. 1990;14:226–32.
19. Sanders DB. The effect of firing rate on neuromuscular jitter in Lambert-Eaton myasthenic syndrome. Muscle Nerve. 1992;15:256–8.
20. Ekstedt J, Nilsson G, Stålberg E. Calculation of the electromyographic jitter. J Neurol Neurosurg Psychiatry. 1974;37:526–39.
21. Gilchrist JM. Single fiber EMG. In: Katirji B, Kaminski HJ, Preston DC, Ruff RL, Shapiro EB, editors. Neuromuscular disorders in clinical practice. Boston: Butterworth-Heinemann; 2002. p. 141–50.
22. Sanders DB, Stålberg EV. Single fiber electromyography. Muscle Nerve. 1996;19:1069–83.
23. Desmedt JE, Borenstein S. Diagnosis of myasthenia gravis by nerve stimulation. Ann N Y Acad Sci. 1976;74:174–88.
24. Ozdemir C, Young RR. The results to be expected from electrical testing in the diagnosis of myasthenia gravis. Ann N Y Acad Sci. 1976;74:203–22.
25. Oh SH, Kim DE, Kuruoglu R, Bradley RJ, Dwyer D. Diagnostic sensitivity of the laboratory tests in myasthenia gravis. Muscle Nerve. 1992;5:720–4.
26. Costa J, Evangelista T, Conceicao I, de Carvalho M. Repetitive nerve stimulation in myasthenia gravis—relative sensitivity of different muscles. Clin Neurophysiol. 2004;115(12):2776–82.
27. Zinman LH, O'connor PW, Dadson KE, Leung RC, Ngo M, Bril V. Sensitivity of repetitive facial-nerve stimulation in patients with myasthenia gravis. Muscle Nerve. 2006;33:694–6.
28. Oh SJ, Hatanaka Y, Hemmi S, Young AM, Scheufele ML, Nations SP, et al. Repetitive nerve stimulation of facial muscles in MuSK antibody-positive myasthenia gravis. Muscle Nerve. 2006;33:500–4.
29. Rubin DI, Hentschel K. Is exercise necessary with repetitive nerve stimulation in evaluating patients with suspected myasthenia gravis? Muscle Nerve. 2007;35:103–6.
30. Sanders DB, Howard JF. Single fiber EMG in myasthenia gravis. Muscle Nerve. 1986;9:809–19.
31. Farrugia ME, Kennett RP, Newsom-Davis J, Hilton-Jones D, Vincent A. Single-fiber electromyography in limb and facial muscles in muscle-specific kinase

antibody and acetylcholine receptor antibody myasthenia gravis. Muscle Nerve. 2006;33:568–70.
32. Baruca M, Leonardis L, Podnar S, Hojs-Fabjan T, Grad A, Jerin A, et al. Single fiber EMG as a prognostic tool in myasthenia gravis. Muscle Nerve. 2016;54:1034–40.
33. Abraham A, Breiner A, Barnett C, Katzberg HD, Lovblom LE, Rt MN, et al. Electrophysiological testing is correlated with myasthenia gravis severity. Muscle Nerve. 2017;56(3):445–8. https://doi.org/10.1002/mus.25539.
34. Sanders DB, Massey JM. Does change in neuromuscular jitter predict or correlate with clinical change in MG? Muscle Nerve. 2017;56(1):45–50.
35. Lambert EH, Rooke ED, Eaton LM, et al. Myasthenic syndrome occasionally associated with bronchial neoplasm: neurophysiologic studies. In: Viets HR, editor. Myasthenia gravis: the second international symposium proceedings 1959. Springfield: Charles C. Thomas; 1961. p. 362–410.
36. O'Neil JH, Murray NMF, Newsom-Davis J. The Lambert-Eaton myasthenic syndrome: a review of 50 cases. Brain. 1988;111:577–96.
37. Titulaer MJ, Maddison P, Sont JK, Wirtz PW, Hilton-Jones D, Klooster R, et al. Clinical Dutch-English Lambert-Eaton myasthenic syndrome (LEMS) tumor association prediction score accurately predicts small-cell lung cancer in LEMS. J Clin Oncol. 2011;29(7):902–8.
38. Juel VC, Massey JM, Sanders DB. Lambert-Eaton myasthenic syndrome. Findings in 97 patients. Muscle Nerve. 2006;34:543.
39. Oh SJ, Kurokawa K, Claussen GC, Ryan HF Jr. Electrophysiological diagnostic criteria of Lambert-Eaton myasthenic syndrome. Muscle Nerve. 2005;32:515–20.
40. Maddison P, Newsom-Davis J, Mills KR. Distribution of electrophysiological abnormality in Lambert-Eaton myasthenic syndrome. J Neurol Neurosurg Psychiatry. 1998;5:213–7.
41. Maddison P, Newsom-Davis J. The Lambert-Eaton myasthenic syndrome. In: Katirji B, Kaminski HJ, Preston DC, Ruff RL, Shapiro EB, editors. Neuromuscular disorders in clinical practice. Boston: Butterworth-Heinemann; 2002. p. 931–41.
42. Oh SJ, Hatanaka Y, Claussen GC, Sher E. Electrophysiological differences in seropositive and seronegative Lambert-Eaton myasthenic syndrome. Muscle Nerve. 2007;35(2):178–83.
43. Guan Y, Ding Q, Liu M, Niu J, Cui L. Single-fiber EMG with concentric electrodes in Lambert-Eaton myasthenia. Muscle Nerve. 2017;56(2):253–7.
44. Schiller HH, Stålberg E. Human botulism studied with single-fiber electromyography. Arch Neurol. 1978;35:346–9.
45. Padua L, Aprile I, Monaco ML, Fenicia L, Anniballi F, Pauri F, et al. Neurophysiological assessment in the diagnosis of botulism: usefulness of single-fiber EMG. Muscle Nerve. 1999;22:1388–92.
46. Cherrington M. Electrophysiologic methods as an aid in diagnosis of botulism: a review. Muscle Nerve. 1982;5:528–9.
47. Souayah N, Karim H, Kamin SS, McArdle J, Marcus S. Severe botulism after focal injection of botulinum toxin. Neurology. 2006;67:1855–6.
48. Engel AG. Congenital myasthenic syndromes. In: Katirji B, Kaminski HJ, Preston DC, Ruff RL, Shapiro EB, editors. Neuromuscular disorders in clinical practice. Boston: Butterworth-Heinemann; 2002. p. 953–63.
49. Maselli RA, Wollman RB, Leung C, Distad B, Palombi S, Richman DP, et al. Neuromuscular transmission in amyotrophic lateral sclerosis. Muscle Nerve. 1993;16:1193–203.
50. Killian JK, Wilfong AA, Burnett L, Appel SH, Boland D. Decremental motor responses to repetitive nerve stimulation in ALS. Muscle Nerve. 1994;17:747–54.
51. Lo YL, Chan LL, Pan A, Ratnagopal P. Acute ophthalmoparesis in the anti-GQ1b antibody syndrome: electrophysiological evidence of neuromuscular transmission defect in the orbicularis oculi. J Neurol Neurosurg Psychiatry. 2004;75:436–40.
52. Sartucci F, Caffario G, Borghetti D, Domenici L, Orlandi G, Murri L. Electrophysiological evidence by single fiber electromyography of neuromuscular transmission impairment in a case of Miller Fisher syndrome. Neurol Sci. 2005;26:125–8.
53. Lange DJ, Deangelis T, Sivak MA. Single-fiver electromyography shows terminal axon dysfunction in Miller Fisher syndrome. Muscle Nerve. 2006;34:232–4.
54. Buchwald B, Bufler J, Carpo M, Heidenreich F, Pitz R, Dudel J, et al. Combined pre- and postsynaptic action of IgG antibodies in Miller Fisher syndrome. Neurology. 2001;56:67–74.
55. Plomp JJ, Molenaar PC, O'Hanlon GM, Jacobs BC, Veitch J, Daha MR, et al. Miller Fisher anti-GQ1b antibodies: alpha-latrotoxin–like effects on motor endplates. Ann Neurol. 1999;45:189–99.
56. Buchwald B, Toyka KV, Zielasek J, Weishaupt A, Schweiger S, Dudel J. Neuromuscular blockade by IgG antibodies from patients with Guillain–Barre syndrome: a macropatch-clamp study. Ann Neurol. 1998;44:913–22.
57. Spaans F, Vredeveld JW, Morre HH, Jacobs BC, De Baets MH. Dysfunction at the motor end-plate and axon membrane in Guillain–Barré syndrome: a single-fiber EMG study. Muscle Nerve. 2003;27:426–34.

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10 Autoantibody Testing in the Diagnosis and Management of Autoimmune Disorders of Neuromuscular Transmission and Related Diseases

Michelangelo Cao and Angela Vincent

Introduction

The neuromuscular junction (NMJ), the synapse between the motor nerve and the muscle fiber, includes the motor nerve terminal, the synaptic cleft, and the "endplate" region of the postsynaptic muscle fiber. A number of molecules, which include ion channels and other proteins at the NMJ, may be targeted by the immune system resulting in impaired neuromuscular transmission. Antibodies to acetylcholine receptors (AChR), associated with the clinical picture of acquired myasthenia gravis (MG), were the first to be identified and later shown to be pathogenic. Subsequently, additional targets were found for neuromuscular transmission disorders, including calcium and potassium ion channels in the motor nerve terminal and muscle-specific kinase (MuSK) on the postsynaptic membrane. Each of the antibodies binds to a membrane protein, and determination of the antibodies allows for specific therapies and, in certain situations, may suggest modifications to current treatment. There are also antibodies to intracellular proteins which are unlikely to be pathogenic but may be useful diagnostic biomarkers for subtypes of the disease.

One of the most satisfying aspects of current understanding of the role of antibodies in MG and other diseases of the NMJ is the ability to measure these antibodies effectively in the laboratory. This was previously due to the availability of radioactively-labeled neurotoxins that bind specifically and with high affinity to the ion channels that are the targets for these antibodies; for MuSK, direct radiolabeling of purified protein provided a sensitive assay. Alternative approaches, particularly new cell-based assays (CBAs), have been developed for autoantibody detection particularly antibodies to clustered AChRs, muscle-specific kinase (MuSK), and low-density lipoprotein receptor-related protein 4 (LRP4). Here we start by discussing some general principles and then go on to describe the clinical syndromes and the methods that can be used to investigate the pathogenic role of autoantibodies.

Spectrum of Antibodies to Targets at the NMJ and Associated Molecules

Most of the proteins targeted in autoimmune NMJ disorders are located in the cell membrane of the motor nerve or muscle endplate with an extracellular domain that is accessible to circulating substances. The lack of a functional

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© Springer International Publishing AG, part of Springer Nature 2018
H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*, Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_10

blood-nerve barrier, composed of endothelial cells and gap junctions, exposes the NMJ to the circulation. Whereas a pathogenic role for autoimmunity against NMJ proteins with an extracellular domain is well characterized, it is still unclear as to whether an immune attack against intracellular components is significant in the pathophysiology of these conditions.

The molecules identified to be targets in the NMJ are illustrated in Fig. 10.1. These include calcium channels and potassium channel complexes in the motor nerve terminal; AChRs,

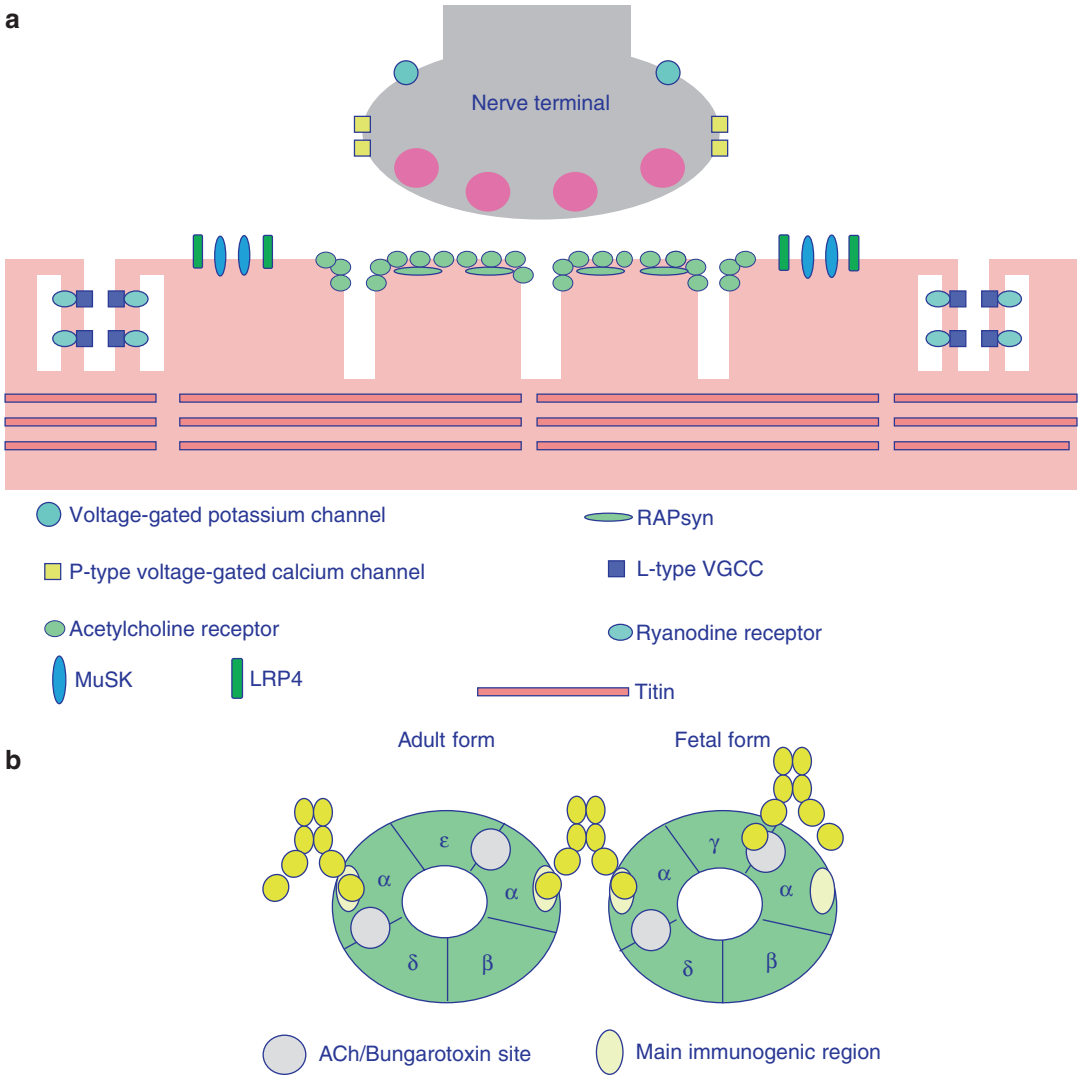

Fig. 10.1 (**a**) Diagram of the neuromuscular junction with proteins that are targeted for autoimmune attack. (**b**) The adult and fetal subtypes of the AChR. The AChR exists in two forms differing only in the ε or γ subunits. The adult form is normally present at the NMJ. During development, and following denervation or in the absence of functional adult AchR, e.g., genetic mutations in the ε subunit, the fetal form is expressed. Maternal antibodies in women with MG, and occasionally without symptoms of MG, can be biased toward the fetal AChRs; when they cross the placenta, they may impair NMJ function resulting in developmental and neonatal disorders

MuSK, and LRP4 in the postsynaptic muscle endplate; and rapsyn, titin, and the ryanodine receptor intracellularly in the muscle cell.

Identifying a Pathogenic Role for Antibodies in Antibody-Mediated Disorders

The classic studies that demonstrate a role for autoantibodies in the disorders discussed in this volume are described elsewhere. In MG, five observations in the early 1970s were crucial in leading to the identification of the autoimmune basis of the condition. First, active immunization of animals with purified AChR led to the development of experimental autoimmune myasthenia gravis (EAMG) [1]. Second, there was a reduction in AChRs at the motor endplates of patients [2]. Third, patients with MG had antibodies to AChR [3]. Fourth, it was possible to transfer the signs of the disease by injecting IgG of MG patients into mice [4], and subsequently, plasmapheresis was shown to be highly effective as a treatment [5]. Many years later similar approaches demonstrated the pathogenicity of MuSK antibodies [6].

By contrast, active immunization has still not been carried out satisfactorily for the Lambert-Eaton myasthenic syndrome (LEMS) and neuromyotonia (NMT). However, the combination of clinical response to plasma exchange and passive transfer of immunoglobulins to mice [7–9], or occasionally other species, showed that these conditions are due to autoantibodies, and the identification of the target antigens (or some of them) followed later.

Disease Phenotypes in Autoimmune Myasthenic Disorders

In immune-mediated diseases, the clinical syndrome is related to the target antigen and its role in the function of the NMJ and the muscle fiber. At the same time, similar phenotypes can occur as a consequence of immune attacks directed at distinct molecular targets. For instance, both AChR and MuSK antibodies are associated with MG, but the antibody subclasses and clinical presentations are partly different, and the neuromuscular transmission defects are caused by distinct mechanisms (see below). Conversely, patients with the same antibody can have different forms of the disease. This is most evident in AChR antibody MG (AChR-MG). Generalized acquired MG with AChR antibodies is the most common myasthenia subtype (80% of all cases), but the patients are usually classified into three main distinct groups according to the age of onset and to the associated thymic pathology: early-onset MG (EOMG) without thymoma, late-onset MG (LOMG) without thymoma, and thymoma-associated MG (Table 10.1) [10]. The late-onset group is becoming more common and may include at least two subpopulations of patients, those with and without titin or other muscle antibodies. In addition purely ocular MG is clearly part of the spectrum of AChR-MG with positive titers in around 50% of the patients, but other patients are either persistently negative or show binding to LRP4.

MuSK-MG represents an important subgroup, counting for up to 10% of all autoimmune myasthenic patients, and differs from the more common AChR-MG in terms of epidemiology, muscle involvement, and response to treatment. MuSK-MG usually has an early-onset and female predominance, while older patients and children are less affected [11–14]. Ocular muscles and limbs are often mildly involved compared to the bulbar muscles that are usually severely impaired with high frequency of respiratory crises [11, 15]. No association with thymic pathology has been reported so far [16].

Antibodies against LRP4 have been detected in a variable proportion (2–27%) of patients without AChR or MuSK-Abs [17–19]. Patients with detectable levels of LRP4-Abs are usually young women with ocular symptoms or mild generalized involvement, while more severe cases often have double positivity with MuSK-Abs [17]. Thymic hyperplasia has been reported in about 30% of LRP4 patients (see Table 10.1 [20]).

Table 10.1 Autoantigens in myasthenia gravis subtypes

	Antibodies to				Thymic pathology	Age of onset (years)
	AChR	MuSK	LRP4	Titin		
Early-onset MG	+++[a]	–	–	–	Germinal centers	<50 years
Thymoma MG	+	–	–	++	Thymoma	Any usually 30–60 years
Late-onset MG without titin antibodies	+	–	–	–		>50 years
Late-onset MG with titin antibodies	+	–	–	+	Atrophy	>60 years
CBA clustered AChR positive MG	–	–	–	–	±	Not clear, usually less severe
MuSK-MG	–	++	–	–	–	Mostly <50
LRP4-MG	±	–	++	–	–	Not clear, usually less severe; thymic hyperplasia may be present
Seronegative MG—none of the above antibodies	+[b]	–	–	–	Some germinal centers	Any, usually less severe

[a]+++, high serum antibody concentration
[b]+, low serum antibody concentration

Heterogeneity and Pathophysiological Effects of Antibodies in the Different Forms of Autoimmune Myasthenia Gravis

AChR-MG

Serum AChR antibodies provide a specific marker for MG. When present in humans, they mediate AChR loss, simplification of the postsynaptic membrane, dysfunction of NMJ transmission, and clinical disease. In patients with MG, plasma exchange-induced reduction in serum AChR antibody levels is generally associated with marked clinical improvement [5, 21, 22]. The duration of benefit, as in other antibody-mediated diseases, may last from 1 to 3 months. Within individual patients there is often a good correlation between severity of disease and the concentration of serum AChR antibodies [21, 23].

In order to understand the effects of antibodies on NMJ function, it is necessary to refer to the animal model, experimental autoimmune myasthenia gravis (EAMG), that results from active immunization against purified AChR. The majority of anti-AChR antibodies in EAMG and many AChR antibodies in MG bind to a conformational determinant, known as the main immunogenic region (MIR), on the extracellular aspect of the AChR alpha subunit. This region includes the alpha subunit 67–76 amino acid sequence [24] although other extracellular sequences contribute to the conformation of the MIR in the native structure. Complement activation, leading to the deposition of membrane attack complex, by these antibodies is pivotal to the development of AChR loss in EAMG. C4-deficient guinea pigs do not develop EAMG, and prevention of complement activation prevents EAMG [25]. The antibodies cross-link the AChRs in the membrane, and their Fc regions mediate C1q binding and macrophage activation. However, since efficient complement activation requires binding of C1q by cross-linked adjacent immunoglobulin molecules, this may be reduced or prevented by decreased AChR density or distortion of the postsynaptic membrane which results from the initial immune attack [26].

These findings can be applied to the NMJ in the human disease, with the caveat that MG is a chronic disease, the antigenic epitopes recognized may be partly different and likely are more diverse, the safety factor for neuromuscular transmission may be lower than in other species, and there are long-term compensatory changes that help to offset the chronic antibody-induced changes. Thus the effect of different antibodies in

reducing the number of functional AChRs and the extent to which this causes weakness is potentially highly variable.

Indeed, although antibodies against the MIR are found in the majority of MG patients, many have additional antibodies that bind to other conformational epitopes on the AChR and some that only bind to the fetal isoform of the AChR (Fig of AChR isoforms). Nevertheless, in human MG the NMJ pathology is similar to that in the rat, characterized by postsynaptic membrane simplification and reduced AChR content, mediated largely by complement activation [26]. Macrophages can be found at the postsynaptic membrane but in relatively few numbers [27]. AChR antibodies may have additional effects on the target molecules by blocking ion channel function [28], but an important additional mechanism is the increased internalization of AChR molecules by antibodies binding to the alpha subunits which are represented twice in each AChR [28, 29]. Passive transfer of these antibodies to rats or mice causes AChR loss even in the absence of complement, and in humans AChR internalization likely contributes to the NMJ dysfunction.

The lack of a precise correlation between serum AChR antibody concentrations and clinical weakness in patients with MG, therefore, is likely to be dependent on several confounding factors. These include variables related to the structure and function of the antibodies themselves, including the nature of the variable region forming the binding site which, consequently, determines the AChR site to which it binds. Effector functions of the constant regions in the different subclasses also likely influence the pathophysiological mechanisms. Most AChR antibodies in human MG belong to the IgG1 and IgG3 subclasses and are strong activators of complement. Nevertheless, the polyclonal B cell response means that changes in subpopulations of pathogenic antibodies may result in changes in the clinical manifestations, independent of the total level of the antibody. The relationship between the serum antibody concentration and pathology also involves equilibration between serum and tissue concentrations. The density and organization of the target epitopes is an additional factor. Lastly the safety factor for transmission and the compensatory changes may differ between muscles and between individual NMJs.

MuSK-MG

Muscle-specific kinase (MuSK) is a receptor tyrosine kinase expressed on the muscle sarcolemma at the NMJ (see Fig. 10.1). MuSK plays a fundamental role in the initiation of a phosphorylation cascade that leads to the clustering of AChRs. Therefore, it is clearly closely associated with AChR at the mature as well as the developing neuromuscular junction [30, 31].

Initial evidence indicating MuSK as a potential antigen in myasthenia gravis came from in vitro experiments where both AChR antibody-positive and AChR antibody-negative sera were found to bind to TE671 cells, which are derived from a rhabdomyosarcoma and express many muscle antigens in addition to AChR [32]. This suggested that, if not binding to the AChR itself, the serum antibodies must be binding to other muscle proteins. MuSK was the most attractive candidate. Seronegative myasthenia (SNMG) IgG was found to bind strongly to COS cells expressing recombinant MuSK [33]. To provide a screening assay for MuSK antibodies, Hoch et al. [33] used the soluble extracellular domain of recombinant MuSK in an ELISA. Antibodies from SNMG patients with well-established disease bound specifically to MuSK, and positive results were found in about 70% of SNMG; these patients were at the more severe end of the SNMG spectrum.

As mentioned above, MuSK-MG differs in several ways from the more common AChR-MG. MuSK-Abs are mainly of the IgG4 subclass [34], and, unlike IgG1-3 subclass antibodies, they do not activate the complement cascade. This is because IgG4 are polyclonal and functionally monovalent as they exchange their antigen-binding fragment (Fab) with other IgG4 antibody Fabs, independent of antigen specificity [35]. For these reasons, the main pathogenic effect of MuSK-Abs is thought to be interference

with the physiological function of MuSK rather than complement-mediated damage or internalization of the protein.

Evidence of the pathogenicity of MuSK-Abs come from different animal models created by both passive and active immunization. All these models showed clinical myasthenic features and/or impaired neuromuscular transmission, with reductions in endplate AChR and in EPP amplitudes [6, 36–38]. In vitro experiments on C2C12 myotubes provided further insights on the effects and role of MuSK-Abs showing their ability to inhibit MuSK phosphorylation and disperse AChR clustering [33, 39]. The first MuSK Ig-like domain, located in its extracellular part, is the main epitope recognized by the antibodies. This particular segment mediates the interaction between LRP4 and MuSK, and, therefore, the binding of the antibodies prevents agrin-induced LRP4-MuSK dimerization [40]. MuSK is not activated and, consequently, the whole pathway that leads to AChR clustering is inhibited. At the NMJ there is a balance between agrin-induced AChR clustering and acetylcholine-induced dispersal of AChRs. It is thought that, in the absence of MuSK activity, the effect of acetylcholine dominates with loss of AChRs and impairment of neuromuscular signaling.

The role of the relatively small proportion of IgG1-3 MuSK antibodies is still controversial. Like MuSK IgG4, IgG1-3 antibodies also inhibit agrin-induced AChR clustering in in vitro experiments on myotubes generated from mouse myoblasts, indicating that all four subclasses have pathogenic potential [39]. Moreover, a mouse model knockout of murine IgG1 (equivalent to human IgG4) developed myasthenic symptomatology when immunized against MuSK, and the immune response was sustained by the murine equivalent of human IgG1-3 subclasses [41]. Finally, all MuSK IgG subclasses were able to disperse the clusters in Dok7 overexpressing myotubes that form spontaneous AChR clusters independently of agrin [39]. This suggests that MuSK-Abs have different mechanisms of action, and their pathogenic effects are not confined to inhibition of LRP4-MuSK interaction.

It is worth noting that the in vivo models of MuSK myasthenia lack the presynaptic adaptive increase of ACh release observed in the AChR-Ab models [6, 42, 43]. An effective explanation of this observation has not been proposed yet, but it is likely that MuSK-Abs are able to disrupt retrograde signaling that compensates for the loss of AChRs in the AChR-Ab disease. The absence of this adaptive mechanism could partially explain the higher severity and resistance to treatment observed in MuSK patients.

LPR4-MG

LRP4 antibodies are mainly IgG1 and IgG2 and showed pathogenic potential by reducing agrin-dependent AChR clustering in vitro [18–20]. Moreover, a passive immunization model demonstrated clinical and neurophysiological myasthenic features [44]. However, passive transfer with human LRP4 antibodies has not been demonstrated, and the frequency and pathogenicity of LRP4 antibodies is still a matter of debate due to the high variability in detection of LRP4 antibodies among seronegative patients. Moreover, cases with severe phenotype often have double positivity with MuSK-Abs [17].

Other Autoantibodies

Apart from antibodies to molecular targets located at the NMJ, patients with MG may possess other autoimmune antibodies. These include antibodies directed against thyroglobulin, thyroid microsomes, parietal cells and intrinsic factor, and nuclear antigens, and their measurement is outside the scope of this chapter.

Detection of Antibodies in Autoimmune Myasthenia Gravis

Radioimmunoprecipitation Assay

The radioimmunoprecipitation assay (RIA) represents the most available and specific tests for

detection of AChR and MuSK-Abs. This assay is used widely in the USA, Europe, and Japan in institutions where radioactivity is permitted. A commercial preparation is available although in-house assays can also be used. In AChR-MG, the assay involves human AChR extracted from cell lines such as the TE671 line which expresses fetal AChR, and the CN21 subline which expressed mainly adult AChR. The presence of fetal AChR slightly increases the sensitivity, (as many patients have antibodies to fetal-specific epitopes as well as the shared alpha subunits), and makes the assay suitable for detection of low levels of antibodies, when adult specific antibodies may have been almost entirely absorbed by the NMJs, or in mothers of babies with various fetal AChR inactivation syndromes (see below) which can be due to a high proportion of fetal-specific antibodies.

The AChR is labeled with radio-iodinated alpha-bungarotoxin (α-BuTx) (Table 10.2). It detects AChR antibodies in 85% of patients with generalized MG and 50–75% of patients with purely ocular MG [45]. The patients with early-onset MG with generalized weakness tend to have the highest concentrations of serum AChR antibody levels when compared with other patients with MG (see Table 10.1). The AChR antibody-positive patients with ocular MG tend to have lower serum AChR antibody concentrations than patients with generalized disease. This suggests that the clinical manifestations may, at least in part, be a function of the absolute AChR antibody concentration. A particular susceptibility of ocular muscle NMJs for clinical manifestations of NMJ dysfunction is also suggested by the occurrence of ocular muscle involvement early in the course of disease in many patients with botulism [46] or with other neurotoxins. It is also possible that the antigenic targets in the NMJs of extraocular muscles and levator palpebrae provide distinct epitopes for the autoimmune process [46]. The patients with generalized MG, who are consistently negative for AChR antibodies, appear to manifest partly different disease mechanisms (see below).

A similar assay that uses the same principles of AChR RIA is available for the detection of MuSK-Abs, where the whole extracellular domain of MuSK is labeled with ^{125}I and used as radioactive antigen [34, 47] (Fig. 10.2). The specificity of MuSK RIA reaches 100%, but its sensitivity is hard to estimate as the proportion of MuSK patients varies among the different populations studied, apparently correlating with geographical latitude. In Northern Europe, for instance, MuSK-MG is less common than in Southern Europe; genetic predisposition (HLA DR4 DW15) may play a role [48].

Assay to Detect Blocking AChR Antibodies

Blocking antibodies that inhibit the binding of radiolabeled α-BuTx to the AChR probably compete for binding to the ACh binding site

Table 10.2 Neurotoxins used for labeling proteins to detect antibodies[a]

Antigen	Source	Neurotoxin	Source	Use
AChR	Muscle or muscle cell lines (TE671 cells)	α-bungarotoxin	Bungarus multicinctus	Diagnosis of MG
VGCC	Human or rabbit cerebellum or neuronal or small-cell lung cancer lines	ω-conotoxin MVIIC	Conus Magus	Diagnosis of LEMS and investigation of some CNS disorders
VGKC	Human or rabbit cortex	Dendrotoxins	Dendroaspis species	Diagnosis of NMT and investigation of some CNS disorders

[a]The original diagnostic assays were based on radioimmunoprecipitation assays using 125I-neurotoxins to label the antigens extracted from human muscle or muscle cell lines. The VGKC antibodies, however, are mainly directed against two proteins that are complexed with the VGKC, and cell-based assays are now used to detect the specific antibodies (see Chap. 15)

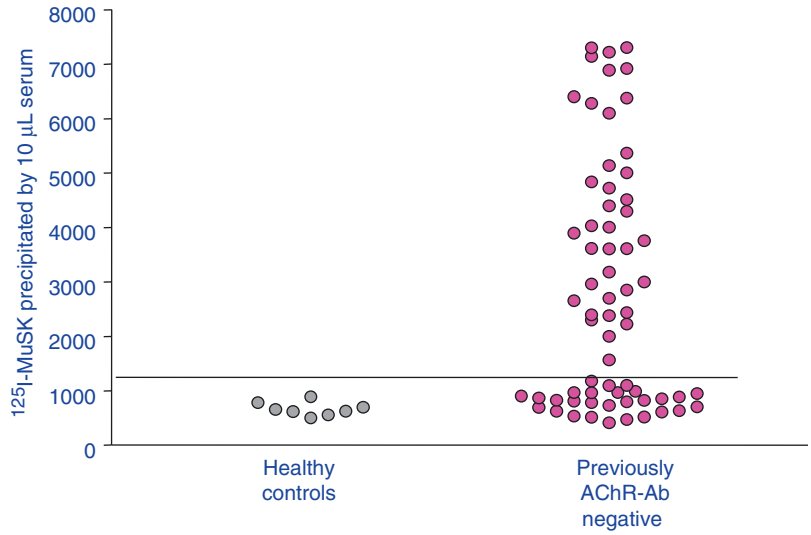

Fig. 10.2 Radioimmunoprecipitation assay for MuSK antibodies. Results from early studies comparing healthy control sera with sera from MG patients without AChR antibodies. This assay is available commercially, but cell-based assays are also used in specialist centers

[49], although in theory they may be noncompetitive and result in allosteric inhibition. While α-ButTx blocking antibodies may be detected in many patients with MG, they appear to represent a minority of the AChR antibodies and usually occur in association with AChR binding antibodies, and do not necessarily interfere with AChR function [45]. Antibodies that do inhibit function can be measured using ion flux through the AChR or ACh-induced currents [49, 50]. These antibodies are important in maternally mediated neuromuscular defects (see below) but may not be important in the majority of patient with typical MG.

Assays to Detect Modulating AChR Antibodies

The application of serum from patients with MG to muscle cell lines can interfere not only in function but also with AChR expression, as a result of antibody cross-linking, internalization, and degradation of the AChR as mentioned above. The AChR are measured by binding of α-ButTx after exposure to the serum for 16 h. It is a relatively non-specific test and the results should be compared with suitable control sera [51]. This approach is not used by many laboratories, and more direct identification of antibodies binding to AChR or other NMJ antigens expressed in cells by CBAs is now preferred.

Cell-Based Assay

Although the radioimmunoprecipitation assay for AChR antibodies is highly sensitive, it does miss some patients who have typical MG. One theoretical possibility was that some antibodies might not bind to the AChR in detergent extracts, where the AChRs are individually dispersed among many other proteins, but could bind at the NMJ where the density of AChRs is very high. A cell-based assay (CBA) for clustered AChR was established and demonstrated positive binding in a proportion of patients (up to 66%) previously negative in the conventional RIA, including 50% of the ocular forms; this test has proved particularly useful in differentiating seronegative MG from genetic myasthenic syndromes in children [52–54]. Patients positive for these clustered AChR antibodies usually have less severe generalized, or ocular, MG, but some of them do have

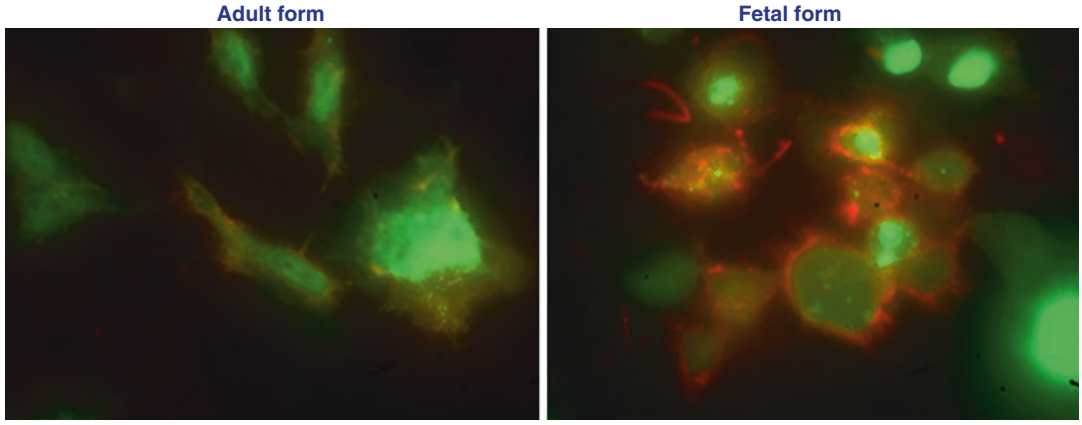

Fig. 10.3 Cell-based assay for clustered AChR antibodies. Human embryonic kidney cells are transiently transfected with DNAs encoding the protein(s) of interest, e.g., AChR subunits, adult or fetal, with the intracellular protein rapsyn to cluster the AChRs. Diluted serum is added to the live cells at room temperature, followed by washing and incubation in a fluorescence-labeled anti-human IgG secondary antibody. After washing and fixation, the fluorescence can be detected either by using the FACS on the dissociated cells or by visual observation as recorded here. The figure shows the results of a maternal serum that binds very poorly to adult AChR but strongly to the fetal AChR. Similar assays can be used for MuSK and LRP4 antibody detection

the thymic abnormalities found in typical AChR positive MG [52]. Serum from an unusual patient whose antibodies bind mainly to fetal AChRs are shown in Fig. 10.3.

In the case of MuSK-MG, a CBA has modestly increased the detection of antibodies in a further 8% of previously seronegative patients. The MuSK patients who were positive only in the CBA, presented with a milder phenotype, and their antibodies were less effective at inhibiting AChR clustering in vitro compared to the patients positive in the RIA [55].

CBA and enzyme-linked immunosorbent assay (ELISA) are the main tests used for LRP4 antibody detection [18–20], but their comparability, sensitivity, and specificity have not been determined yet. A commercial and reliable assay to detect LRP4 antibodies is needed in order to better understand their real incidence and significance.

Autoimmune MG in Association with Thymoma

Essentially 100% of patients with thymoma and MG have detectable serum AChR antibodies [56–58]. In addition, patients with thymoma without clinically detectable MG may sometimes have AChR antibodies suggesting the presence of subclinical disease [45]. The initial serum concentrations of AChR antibodies in thymoma cases tend to be lower than in those with generalized disease without thymoma, but they are likely to rise following thymectomy if immunotherapies are not used. The antibodies and their epitopes may differ in their fine specificities with those with early-onset autoimmune MG without thymoma [57].

Complement-activating, striated muscle antibodies were initially described in 1960 by immunofluorescence in patients with MG. The majority of the striational antibodies are directed against the large structural protein, titin [59], but the sarcolmnal protein ryanodine receptor (RyR) is another major antigen [60]. Ninety-five percent of patients with thymoma and MG also have titin antibodies, whereas 70% of patients with thymoma and MG possess anti-RyR antibodies [60]. The clinical pattern of weakness and fatigability of patients with MG and thymoma is usually indistinguishable from those with MG without thymoma, but some patients with thymoma, titin, and RyR antibodies have a myocarditis or a focal myositis [56, 60]. In addition, myocarditis in MG patients has been

reported to be associated with antibodies against a voltage-gated K^+ channel (Kv1.4) [61]. The roles of these antibodies are intriguing but there are few studies on their pathogenicity.

Patients with thymoma occasionally have antibodies to other autoimmune targets. These include the Kv1 voltage-gated K+ and voltage-gated calcium channels, as well as rapsyn [60–63] and other proteins including glutamic acid decarboxylase [64]. Titin and RyR antibodies, and antibodies to the cytokines interferon-alpha and interleukin 12, are also present in a high proportion of the subgroup of patients with late-onset MG who do not have a thymoma ([65], see below). Despite these interesting phenomena, in many cases, the presence of titin, ryanodine receptor, or other antibodies is not required for diagnosis or management.

Late-Onset MG with or Without Associated Thymoma

The mean AChR antibody concentration in these patients tends to be lower than the early-onset MG group without thymoma, but they represent a group of patients who can have severe disease and, of course, comorbidities. The use of antibody profiles utilizing AChR, titin, and RyR antibodies has characterized two subpopulations of patients in this group. Patients who do not have detectable anti-striated muscle antibodies may resemble the early-onset MG group and usually present before 60 years of age [65], whereas the patients with titin antibodies present later.

Detection of Muscle Antibody Assays

Titin and RyR antibodies may be detected by enzyme-linked immunosorbent assay (ELISA) or immunoblot. A single epitope of titin, MGT30, reacts with 90% of titin antibodies. This epitope has been employed for the titin assays, and a commercial version is available. Striated muscle antibody assays detected by immunofluorescence are also commercially available and may be used in lieu of specific titin and RyR antibody assays. Although titin antibodies are often indicative of a thymoma, LOMG patients >60 years of old may be positive, and using more sensitive assays titin antibodies have been reported in some patients with otherwise antibody negative MG [66].

Role of Antibodies in Diagnosis and Treatment in Patients with MG

Patients with AChR serum antibodies almost always have MG. These antibodies provide a valuable diagnostic tool and indicate a mechanism of disease pathogenesis that is more precise than can be obtained by electrophysiological studies. At this time, the usefulness of following serum concentrations of AChR antibodies in order to monitor treatment in individual patients is questioned. Identification of subsets of pathogenically important antibodies, including antibodies to additional targets, and measuring their concentration, could prove to be useful.

The presence of antibodies to targets other than the AChR may also influence treatment decisions. Given the observations summarized above, the presence of titin antibodies either in patients with thymoma or in LOMG without thymoma appears to suggest a role for more aggressive management early in the course of the disease, but there are not confirmatory studies yet. A useful observation is that acetylcholinesterase inhibitors are usually less effective and tolerated in MuSK myasthenia [11, 12]. An explanation for this phenomenon could be related to dynamics of the factors that influence AChR clustering at the NMJ. A mouse model of MuSK myasthenia, induced by injection of a highly active MuSK antibody, was treated with pyridostigmine. Investigations showed increased endplate AChR loss, probably due to the increased dispersal of AChRs by the prolonged action of acetylcholine mentioned above [67].

Seronegative (SN) MG

Seronegative myasthenia (SNMG) is a term used to define those patients who are not positive with the currently available antibody assays; therefore it now applies to patients without antibodies to

AChR, MuSK, or LRP4. Seronegative cases still count for up to 15% of all MG patients and for up to 50% of the pure ocular forms [68]. It should be appreciated, however, that some MG patients, who are seronegative for AChR antibodies at initial presentation, develop detectable serum anti-AChR antibodies over time [45]. It is possible that at presentation these patients have serum concentrations of AChR antibodies that are below the assay sensitivity because of the sequestration of the antibodies at the NMJs. They may be of lower affinity early in the disease and be only present when tested by CBAs for clustered AChRs, developing typical radioimmunoassay positive AChR antibodies later on.

Subsequent to the definition of AChR, MuSK, and LRP4 as relevant antigens, several other proteins of the neuromuscular junction, such as agrin, cortactin, or collagen Q, have been proposed as potential targets for antibodies in seronegative patients, and low titers of antibodies against agrin have been reported, but often coexisting with AChR or MuSK-Abs [69–73]. Despite the increasing number of reports, the specificities of these antibodies are unclear and their potential pathogenic effects unexplored.

Lambert-Eaton Myasthenic Syndrome and Cerebellar Ataxia

Plasma Exchange and Passive Transfer

Early studies showed that LEMS was associated with certain autoantibodies and other autoimmune diseases [74] and that plasma exchange was an effective treatment [75]. Particularly important was the demonstration that injection of patient IgG into mice resulted in a reduction in the quantal release of ACh and an altered number and distribution of active zone particles. The results mirrored the findings in patients [76, 77].

Functional and Binding Studies

In order to begin to define the nature of the antigen, functional studies on cell lines were used. These began by looking for a candidate antigen, voltage-gated calcium channel (VGCC), on small-cell lung cancer cells, and by showing that the LEMS IgG, when applied for several days in culture, were able to downregulate the VGCC numbers [78]. A number of small-cell lung cancer cells have been used subsequently, and in all cases VGCCs have been demonstrated and the effects of LEMS antibodies confirmed [79, 80]. The main target of the LEMS antibodies appears to be the P/Q-type VGCC, which contains the alpha 1a subunit. These VGCCs are expressed at the motor nerve terminal and also on the Purkinje cells in the cerebellum [81].

Radioimmunoprecipitation Assays

The radioimmunoprecipitation assay for VGCC antibodies was made possible by the discovery, in the late 1980s of the cone snail toxins (see Table 10.2) [82]. The various species of cone snails live on the seabed and produce a rich variety of neurotoxins that are specific for both pre- and postsynaptic ion channels. The first snail toxin to be used to identify VGCCs in LEMS sera was from C*onus geographus*. This neurotoxin binds to the N-type VGCC, which are expressed widely in the nervous and neuroendocrine systems. Using VGCCs extracted from human cerebellum or cortex labeled by ^{125}I-omega-conotoxin GVIA, about 40% of LEMS patients are positive [83]. However, using the P/Q-type selective omega-conotoxin MVIIC under very similar conditions, up to 90% of LEMS patients are positive [84, 85]. These results emphasize the exquisite specificity of these toxins. By contrast, the source of tissue is probably not so important. Similar results are found with rabbit or human cerebellum, indicating that the VGCCs are highly conserved in evolution and antigenically similar between species [86].

The radioimmunoassay has been used extensively as a diagnostic test, and some studies report serial estimations during treatment. For instance, VGCC antibodies are found to fall by 30% on average in patients treated with intravenous immunoglobulin [87]. The fall does not start until 2 weeks after the treatment, suggesting

that it is not a direct result of "blocking" antibodies. The VGCC levels return to pretreatment levels by about 8 weeks, roughly correlating with clinical changes.

Although principally in use as a diagnostic test for LEMS, there are other applications for measuring VGCC antibodies. It has been established that a proportion of patients with small-cell lung cancer have LEMS and that VGCC antibodies are present in such patients even without clear clinical evidence of LEMS [88–90]. Moreover, some patients with cerebellar ataxia with and without clinical evidence of LEMS have VGCC antibodies [90]. Thus serum from patients who are at risk of SCLC and who present with cerebellar or a myasthenic syndrome should be tested for VGCC antibodies.

Acquired Neuromyotonia

Plasma Exchange and Passive Transfer

Like LEMS, acquired NMT (see Chap. 15) was not thought to be autoimmune until clinical studies showed a response of some patients to plasma exchange or intravenous immunoglobulin therapies. In the few reports of passive transfer, an enhancement of neuromuscular transmission with resistance to the action of curare was induced in the mouse nerve-muscle preparations after passive transfer of NMT IgG [91].

Functional and Binding Assays

A functional effect of NMT IgG on neuronal cell lines was successfully demonstrated by using dorsal root ganglion cells, which developed repetitive action potentials, very similar to those found in the presence of 4-diamino-pyridine, a potassium channel blocker [8]. In the PC-12 and NB-1 neuroblastoma cell lines, incubating the cells overnight in the NMT IgG led to a reduction in the voltage-gated potassium channel (VGKC) currents without any observable change in the current/voltage density. These alterations, which were not seen with briefer incubation times, suggested that the antibodies are not blocking channel function directly but rather reducing the number of functional VGKC [92–94] and causing an increase in the quantal content of release [8].

Radioimmunoprecipitation Assays

There are numerous subtypes of VGKC, particularly of the shaker type. A radioimmunoprecipitation assay was established using high-specific activity ^{125}I-dendrotoxin to label VGKCs extracted from human or rabbit cortex. About 40% of NMT patients were positive by this approach [8, 95], and the antibodies did not appear to inhibit binding of dendrotoxin to the VGKC. Unexpectedly, VGKC antibodies and a clinical response to plasma exchange were found in a patient with classical Morvan's syndrome [96], which includes neuromyotonia with sleep disorder, limbic manifestations, and autonomic dysfunction. A few serial studies of VGKC antibodies were informative and then helped to move the field forward. The most striking early report was that of a woman with MG and thymoma who, following a thymoma recurrence, developed an episode of "limbic encephalitis". This responded strikingly to plasma exchange, strongly implying an antibody-mediated basis for the central disease. In a retrospective analysis of VGKC antibodies in over 80 sera collected from this patient over 13 years, a single peak of VGKC antibodies was found, corresponding closely to the time of appearance of her limbic manifestations [97]. Subsequently many patients with VGKC antibodies have been recognized. Most have either peripheral symptoms or limbic symptoms, but a few have a broad combination of peripheral hyperexcitability, autonomic dysfunction, and cognitive and sleep disturbance.

To understand better the relationship between antibody specificity and clinical phenotype, human embryonic kidney cells were used to express the different dendrotoxin binding Kv1 subtypes (Kv1.1, Kv1.2, and Kv1.6). Antibody binding to these cells could not be demonstrated,

and VGKC-antibody-positive sera did not affect the Kv1 channel currents [98]. Conversely, it appeared that the VGKC extracted from brain tissue, and used for the antibody tests, was complexed with other proteins, forming a VGKC-complex (see Chap. 15). When leucine-rich glioma inactivated protein 1 (LGI1) or contactin-associated protein 2 (CASPR2) were expressed in the HEK cell line, antibody binding could be demonstrated in the majority of patients with "VGKC-complex" antibodies [98]. Subsequently, these and similar cell-based assays have been used extensively for detection of antibodies to LGI1, CASPR2, and a growing number of other antigens in the central and peripheral nervous systems. In neuromyotonia, the antibodies are most often directed at CASPR2, but LGI1 and contactin-2 antibodies are also found in some patients (Vincent, Kiernan, and Watanabe unpublished data).

Conclusion

Acquired MG may be considered the prototypic autoimmune disorder. Observations pertaining to MG are also pertinent to other autoimmune disorders of the NMJ and related disorders. Thus in MG, phenotypic variations are at least in part a function of different mechanisms of disease. The antibody target, the concentration of antibodies, and the fraction of antibodies that are complement activating are all factors likely to influence the clinical presentation and the course. Serological testing is of value in the diagnosis as well as in the treatment of individual patients. AChR is the main autoantigen in most patients with acquired MG, but patients with MuSK antibodies are often more challenging to treat. Tests for newer antigens are still not commonly available and the significance of positive results less clear. While in most instances the diseases do not overlap with each other, e.g., MG and LEMS or MG and neuromyotonia, the antibody syndromes occur together as in some MG patients with thymoma. These relatively rare cases are often of clinical interest and may point the way to new recognition of disease.

References

1. Patrick J, Lindstrom J. Autoimmune response to acetylcholine receptor. Science. 1973;180:871–2.
2. Fambrough DM, Drachman DB, Satyamurti S. Neuromuscular junction in myasthenia gravis: decreased acetylcholine receptors. Science. 1973;182:293–5.
3. Lindstrom JM, Seybold ME, Lennon VA, Whittingham S, Duane DD. Antibody to acetylcholine receptor in myasthenia gravis: prevalence, clinical correlates, and diagnostic value. Neurology. 1976;26:1054–9.
4. Toyka KV, Drachman DB, Griffin DE, Pestronk A, Winkelstein JA, Fishbeck KH, et al. Myasthenia gravis: study of humoral immune mechanisms by passive transfer to mice. N Engl J Med. 1977;296:125–31.
5. Pinching AJ, Peters DK, Newsom-Davis JN. Remission of myasthenia gravis following plasma exchange. Lancet. 1976;2:1373–6.
6. Viegas S, Jacobson L, Waters P, Cossins J, Jacob S, Leite MI, et al. Passive and active immunization models of MuSK-Ab positive myasthenia: electrophysiological evidence for pre and postsynaptic defects. Exp Neurol. 2012;234(2):506–12.
7. Lang B, Newsom-Davis J, Prior C, Wray D. Antibodies to motor nerve terminals: an electrophysiological study of a human myasthenic syndrome transferred to a mouse. J Physiol Lond. 1983;344:335–45.
8. Shillito P, Molenaar PC, Vincent A, Leys K, Zheng W, van den Berg RJ, et al. Acquired neuromyotonia: evidence for vie autoantibodies directed against K+ channels of peripheral nerves. Ann Neurol. 1995;38(5):714–22.
9. Mossman S, Vincent A, Newsom-Davis J. Passive transfer of myasthenia gravis by immunoglobulins: lack of correlation between antibody bound, acetylcholine receptor loss and transmission defect. J Neurol Sci. 1988;84:15–28.
10. Compston DA, Vincent A, Newsom-Davis J, Batchelor JR. Clinical, pathological, HLA antigen and immunological evidence for disease heterogeneity in myasthenia gravis. Brain. 1980;103:579–601.
11. Guptill JT, Sanders DB, Evoli A. Anti-MuSK-Ab myasthenia gravis: clinical findings and response to treatment in two large cohorts. Muscle Nerve. 2011;44:36–40.
12. Evoli A, Tonali PA, Padua L, Monaco ML, Scuderi F, Batocchi AP, et al. Clinical correlates with anti-MuSK-Abs in generalized seronegative myasthenia gravis. Brain. 2003;126:2304–11.
13. Pasnoor M, Wolfe GI, Nations S, Trivedi J, Barohn RJ, Herbelin L, et al. Clinical findings in MuSK-antibody positive myasthenia gravis: a U.S. experience. Muscle Nerve. 2010;41:370–4.
14. Skjei KL, Lennon VA, Kuntz NL. Muscle specific kinase autoimmune myasthenia gravis in children: a case series. Neuromuscul Disord. 2013;23:874–82.

15. Evoli A. Clinical aspects of neuromuscular transmission disorders. Acta Neurol Scand Suppl. 2006;183:8–11. Review.
16. Marx A, Pfister F, Schalke B, Saruhan-Direskeneli G, Melms A, Ströbel P. The different roles of the thymus in the pathogenesis of the various myasthenia gravis subtypes. Autoimmun Rev. 2013;12:875–84.
17. Higuchi O, Hamuro J, Motomura M, Yamanashi Y. Autoantibodies to low-density lipoprotein receptor-related protein 4 in myasthenia gravis. Ann Neurol. 2011;69:418–22.
18. Zhang B, Tzartos JS, Belimezi M, Ragheb S, Bealmear B, Lewis RA, et al. Autoantibodies to lipoprotein-related protein 4 in patients with double-seronegative myasthenia gravis. Arch Neurol. 2012;69:445–51.
19. Pevzner A, Schoser B, Peters K, Cosma NC, Karakatsani A, Schalke B, et al. Anti-LRP4 autoantibodies in AChR- and MuSK-antibody-negative myasthenia gravis. J Neurol. 2012;259:427–35.
20. Zisimopoulou P, Evangelakou P, Tzartos J, Lazaridis K, Zouvelou V, Mantegazza R, et al. A comprehensive analysis of the epidemiology and clinical characteristics of anti-LRP4 in myasthenia gravis. J Autoimmun. 2014;52:139–45.
21. Newsom-Davis J, Pinching AJ, Vincent A, Wilson SG. Function of circulating antibody to acetylcholine receptor in myasthenia gravis: investigated by plasma exchange. Neurology. 1978;28:266–72.
22. Dau PC. Plasmpheresis therapy in myasthenia gravis. Muscle Nerve. 1980;3:468–82.
23. Oosterhuis HJGH, Limburg PC, Hummel-Tappel E. Anti-acetylcholine receptor antibodies in myasthenia gravis. Part 2. Clinical and serological follow-up of individual patients. J Neurol Sci. 1983;58:371–85.
24. Tzartos SJ, Loutrari HV, Tang F, Kokla A, Walgrave SL, Milius RP, et al. Main immunogenic region of torpedo electroplax and human muscle acetylcholine receptor: localization and microheterogeneity revealed by the use of synthetic peptides. J Neurochem. 1990;54:51–61.
25. Lennon VA, Seybold ME, Lindstrom JM, Cochrane C, Ulevitch R. Role of complement in the pathogenesis of experimental autoimmune myasthenia gravis. J Exp Med. 1978;147:973–83.
26. Gomez CM, Richman DP. Chronic experimental autoimmune myasthenia gravis induced by monoclonal antibody to acetylcholine receptor: biochemical and electrophysiological criteria. J Immunol. 1987;139:73–6.
27. Maselli RA, Richman DP, Wollmann RL. Inflammation at the neuromuscular junction in myasthenia gravis. Neurology. 1991;41:1497–504.
28. Drachman DB, Adams RN, Josifek LF, Self SG. Functional activities of autoantibodies to acetylcholine receptors and the clinical severity of myasthenia gravis. N Engl J Med. 1982;307:769–75.
29. Howard FM, Lennon VA, Finley J, Matsumoto J, Elveback LR. Clinical correlations of antibodies that bind, block or modulate human acetylcholine receptors in myasthenia gravis. Ann N Y Acad Sci. 1987;505:526–38.
30. Hubbard SR, Gnanasambandan K. Structure and activation of MuSK, a receptor tyrosine kinase central to neuromuscular junction formation. Biochim Biophys Acta. 2013;1834:2166–9.
31. Darabid H, Perez-Gonzalez AP, Robitaille R. Neuromuscular synaptogenesis: coordinating partners with multiple functions. Nat Rev Neurosci. 2014;15:703–18. Review.
32. Blaes F, Beeson D, Plested P, Lang B, Vincent A. IgG from "seronegative" myasthenia gravis patients binds to a muscle cell line, TE671, but not to human acetylcholine receptor. Ann Neurol. 2000;47:504–10.
33. Hoch W, McConville J, Helms S, Newsom-Davis J, Melms A, Vincent A. Autoantibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. Nat Med. 2001;7:365–8.
34. McConville J, Farrugia ME, Beeson D, Kishore U, Metcalfe R, Newsom-Davis J, et al. Detection and characterization of MuSK-Abs in seronegative myasthenia gravis. Ann Neurol. 2004;55(4):580–4.
35. Koneczny I, Stevens JA, De Rosa A, Huda S, Huijbers MG, Saxena A, et al. IgG4 autoantibodies against muscle-specific kinase undergo Fab-arm exchange in myasthenia gravis patients. J Autoimmun. 2017;77:104–15.
36. Shigemoto K, Kubo S, Maruyama N, Hato N, Yamada H, Jie C, et al. Induction of myasthenia by immunization against muscle-specific kinase. J Clin Invest. 2006;116:1016–24.
37. Cole RN, Ghazanfari N, Ngo ST, Gervásio OL, Reddel SW, Phillips WD. Patient autoantibodies deplete postsynaptic muscles-pecific kinase leading to disassembly of the ACh receptor scaffold and myasthenia gravis in mice. J Physiol. 2010;588:3217–29.
38. Klooster R, Plomp JJ, Huijbers MG, Niks EH, Straasheijm KR, Detmers FJ, et al. Muscle-specific kinase myasthenia gravis IgG4 autoantibodies cause severe neuromuscular junction dysfunction in mice. Brain. 2012;135:1081–101.
39. Koneczny I, Cossins J, Waters P, Beeson D, Vincent A. MuSK myasthenia gravis IgG4 disrupts the interaction of LRP4 with MuSK but both IgG4 and IgG1-3 can disperse preformed agrin-independent AChR clusters. PLoS One. 2013;8:e80695.
40. Huijbers MG, Zhang W, Klooster R, et al. MuSK IgG4 autoantibodies cause myasthenia gravis by inhibiting binding between MuSK and LRP4. Proc Natl Acad Sci U S A. 2013;110:20783–8.
41. Küçükerden M, Huda R, Tüzün E, Yılmaz A, Skriapa L, Trakas N, et al. MuSK induced experimental autoimmune myasthenia gravis does not require IgG1 antibody to MuSK. J Neuroimmunol. 2016;295–296:84–92.
42. Mori S, Kubo S, Akiyoshi T, Yamada S, Miyazaki T, Hotta H, et al. Antibodies against muscle specific kinase impair both presynaptic and postsynaptic func-

tions in a murine model of myasthenia gravis. Am J Pathol. 2012;180:798–810.
43. Patel V, Oh A, Voit A, Sultatos LG, Babu GJ, Wilson BA, et al. Altered active zones, vesicle pools, nerve terminal conductivity,and morphology during experimental MuSK myasthenia gravis. PLoS One. 2014;9:e110571.
44. Shen C, Lu Y, Zhang B, Figueiredo D, Figueiredo D, Bean J, Jung J, et al. Antibodies against low-density lipoprotein receptor-related protein 4 induce myasthenia gravis. J Clin Invest. 2013;123:5190–202.
45. Vincent A, Newsom-Davis J. Acetylcholine receptor antibody as a diagnostic test for myasthenia gravis: results in 153 validated cases and 2967 diagnostic assays. J Neurol Neurosurg Psychiatry. 1985;48:1246–52.
46. Kaminski HJ. Acetylcholine receptor epitopes in ocular myasthenia. Ann N Y Acad Sci. 1998;841:309–19.
47. Matthews I, Chen S, Hewer R, McGrath V, Furmaniak J, Rees Smith B. Muscle-specific receptor tyrosine kinase autoantibodies—a new immunoprecipitation assay. Clin Chim Acta. 2004;348:95–9.
48. Niks EH, Kuks JB, Roep BO, Haasnoot GW, Verduijn W, Ballieux BE, et al. Strong association of MuSK antibody-positive myasthenia gravis and HLA-DR14-DQ5. Neurology. 2006;66:1772–4.
49. Lang B, Richardson G, Rees J, Vincent A, Newsom-Davis J. Plasma from myasthenia gravis patients reduces acetylcholine receptor agonist-induced Na+ flux into TE671 cell line. J Neuroimmunol. 1988;19:141–8.
50. Bufler J, Pitz R, Czep M, Wick M, Franke C. Purified IgG from seropositive and seronegative patients with myasthenia gravis reversibly blocks currents through nicotinic acetylcholine receptor channels. Ann Neurol. 1998;43:458–64.
51. Lennon VA. Serological diagnosis of myasthenia gravis and the Lambert Eaton Myasthenic syndrome. In: Lisak RP, editor. Handbook of myasthenia gravis and myasthenic syndromes. New York: Marcel Dekker; 1994. p. 149–64.
52. Leite MI, Jacob S, Viegas S, Cossins J, Clover L, Morgan BP, et al. IgG1 antibodies to acetylcholine receptors in 'seronegative' myasthenia gravis. Brain. 2008;131:1940–52.
53. Jacob S, Viegas S, Leite MI, Webster R, Cossins J, Kennett R, et al. Presence and pathogenic relevance of antibodies to clustered acetylcholine receptor in ocular and generalized myasthenia gravis. Arch Neurol. 2012;69:994–1001.
54. Rodríguez Cruz PM, Al-Hajjar M, Jacobson L, Woodhall M, Jayawant S, et al. Clinical features and diagnostic usefulness of antibodies to clustered acetylcholine receptors in the diagnosis of seronegative myasthenia gravis. JAMA Neurol. 2015;72:642–9.
55. Huda S, Waters P, Woodhall M, Leite MI, Jacobson L, De Rosa A, et al. IgG-specific cell-based assay detects potentially pathogenic MuSK-Abs in seronegative MG. Neurol Neuroimmunol Neuroinflamm. 2017;4:e357.
56. Aarli JA, Stefansson K, Marton LSG, Wollmann RL. Patients with myasthenia gravis and thymoma have in their sera IgG autoantibodies against titin. Clin Exp Immunol. 1990;82:284–8.
57. Skeie GO, Lunde PK, Sejersted OM, Mygland A, Aarli JA, Gilhus NE. Autoimmunity against the ryanodine receptor in myasthenia gravis. Acta Physiol Scand. 2001;171:379–84.
58. Romi F, Skeie GO, Aarli JA, Gilhus NE. Muscle autoantibodies in subgroups of myasthenia gravis patients. J Neurol. 2000;247:369–75.
59. Romi F, Skeie GO, Aarli JA, Gilhus NE. The severity of myasthenia gravis correlates with the serum concentration of titin and ryanodine receptor antibodies. Arch Neurol. 2000;57:1596–600.
60. Mygland A, Vincent A, Newsom-Davis J, Kaminski H, Zorzato F, Agius M, et al. Autoantibodies in thymoma-associated myasthenia gravis with myositis or neuromyotonia. Arch Neurol. 2000;57:527–31.
61. Suzuki S, Baba A, Kaida K, Utsugisawa K, Kita Y, Tsugawa J, et al. Cardiac involvements in myasthenia gravis associated with anti-Kv1.4 antibodies. Eur J Neurol. 2014;21:223–30.
62. Lee E-K, Maselli RA, Ellis WG, Agius MA. Morvan's fibrillary chorea: a paraneoplastic manifestation of thymoma. J Neurol Neurosurg Psychiatry. 1998;65:857–62.
63. Agius MA, Zhu S, Kirvan CA, Schafer AL, Lin MY, Fairclough RH, et al. Rapsyn antibodies in myasthenia gravis. Ann N Y Acad Sci. 1998;841:516–24.
64. Hagiwara H, Enomoto-Nakatani S, Sakai K, Ugawa Y, Kusunoki S, Kanazawa I. Stiff-person syndrome associated with invasive thymoma: a case report. Neurol Sci. 2001;193:59–62.
65. Buckley C, Newsom-Davis J, Willcox N, Vincent A. Do titin and cytokine antibodies in MG patients predict thymoma or thymoma recurrence? Neurology. 2001;57:1579–82.
66. Cordts I, Bodart N, Hartmann K, Karagiorgou K, Tzartos JS, Mei L, et al. Screening for lipoprotein receptor-related protein 4-, agrin-, and titin-antibodies and exploring the autoimmune spectrum in myasthenia gravis. J Neurol. 2017;264:1193–203.
67. Morsch M, Reddel SW, Ghazanfari N, Toyka KV, Phillips WD. Pyridostigmine but not 3,4-diaminopyridine exacerbates ACh receptor loss and myasthenia induced in mice by muscle-specific kinase autoantibody. J Physiol. 2013;591:2747–62.
68. Kerty E, Elsais A, Argov Z, Evoli A, Gilhus NE. EFNS/ENS guidelines for the treatment of ocular myasthenia gravis. Eur J Neurol. 2014;21:687–93.
69. Cossins J, Belaya K, Zoltowska K, Koneczny I, Maxwell S, Jacobson L, et al. The search for new antigenic targets in myasthenia gravis. Ann N Y Acad Sci. 2012;1275:123–8. Review.

70. Gasperi C, Melms A, Schoser B, Zhang Y, Meltoranta J, Risson V, et al. Anti-agrin autoantibodies in myasthenia gravis. Neurology. 2014;82:1976–83.
71. Gallardo E, Martínez-Hernández E, Titulaer MJ, Huijbers MG, Martínez MA, Ramos A, et al. Cortactin autoantibodies in myasthenia gravis. Autoimmun Rev. 2014;13:1003–7.
72. Zoltowska KM, Belaya K, Leite M, Patrick W, Vincent A, Beeson D. Collagen Q—a potential target for autoantibodies in myasthenia gravis. J Neurol Sci. 2015;348:241–4.
73. Zhang B, Shen C, Bealmear B, Ragheb S, Xiong WC, Lewis RA, et al. Autoantibodies to agrin in myasthenia gravis patients. PLoS One. 2014;9:e91816.
74. Lennon VA, Lambert EH, Whittingham S, Fairbanks V. Autoimmunity in the Lambert-Eaton myasthenic syndrome. Muscle Nerve. 1982;5:S21–5.
75. Newsom-Davis J, Murray NM. Plasma exchange and immunosuppressive drug treatment in the Lambert-Eaton myasthenic syndrome. Neurology. 1984;34:480–5.
76. Fukunaga H, Engel AG, Osame M, Lambert EH. Paucity and disorganization of presynaptic membrane active zones in the Lambert-Eaton myasthenic syndrome. Muscle Nerve. 1982;5:686–97.
77. Fukuoka T, Engel AG, Lang B, Newsom-Davis J, Prior C, Wray DW. Lambert-Eaton myasthenic syndrome: I. Early morphological effects of IgG on the presynaptic membrane active zones. Ann Neurol. 1987;22:139–99.
78. Roberts A, Perera S, Lang B, Vincent A, Newsom-Davis J. Paraneoplastic myasthenic syndrome IgG inhibits 45Ca2+ flux in a human small cell carcinoma line. Nature. 1985;317:737–9.
79. Viglione MP, O'Shaughnessy TJ, Kim Y. Inhibition of calcium currents and exocytosis by Lambert-Eaton myasthenic syndrome antibodies in human lung cancer cells. J Physiol Lond. 1995;488:303–17.
80. Protti DA, Reisen R, MacKinley TA, Uchitel OD. Calcium channel blockers and transmitter release at the normal human neuromuscular junction. Neurology. 1996;46:1391–6.
81. Pinto A, Gillard S, Moss F, Whyte K, Brust P, Williams M, et al. Human autoantibodies specific for α_{1A} calcium channel subunit reduce both P-type and Q-type calcium currents in cerebellar neurones. Proc Natl Acad Sci. 1998;95:8328–33.
82. Espiritu DJ, Watkins M, Dia-Monje V, Cartier GE, Cruz LJ, Olivera BM. Venomous cone snails: molecular phylogeny and the generation of toxin diversity. Toxicon. 2001;39:1899–916.
83. Sher E, Comola M, Nemni R, Canal N, Clementi F. Calcium channel autoantibody and non-small-cell lung cancer in patients with Lambert-Eaton syndrome. Lancet. 1990;335:413.
84. Motomura M, Johnston I, Lang B, Vincent A, Newsom-Davis J. An improved diagnostic assay for Lambert-Eaton myasthenic syndrome. J Neurol Neurosurg Psychiatry. 1995;58:85–7.
85. Lennon VA, Kryzer TJ, Greismann GE, O'Suilleabhain PE, Windebank AJ, Woppmann A, et al. Calcium channel antibodies in the Lambert-Eaton myasthenic syndrome and other paraneoplastic syndromes. N Engl J Med. 1995;332(22):1467–74.
86. Motomura M, Lang B, Johnston I, Palace J, Vincent A, Newsom-Davis J. Incidence of serum anti-P/O--type and anti-N-type calcium channel autoantibodies in the Lambert-Eaton myasthenic syndrome. J Neurol Sci. 1997;147:35–42.
87. Bain PG, Motomura M, Newsom-Davis J, Misbah SA, Chapel HM, Lee ML, et al. Effects of intravenous immunoglobulin on muscle weakness and calcium channel antibodies in the Lambert-Eaton myasthenic syndrome. Neurology. 1996;47:678–83.
88. Mason WP, Graus F, Lang B, Honnorat J, Delattre JY, Valldeoriola F, et al. Paraneoplastic cerebellar degeneration and small-cell carcinoma. Brain. 1997;120:1279–300.
89. Trivedi R, Mundanthanan G, Amyes L, Lang B, Vincent A. Which antibodies are worth testing in subacute cerebellar ataxia? Lancet. 2000;356:565–6.
90. Graus F, Lang B, Pozo-Rosich P, Saiz A, Casamitjana R, Vincent A. P/Q type calcium-channel antibodies in paraneoplastic cerebellar degeneration with lung cancer. Neurology. 2002;59:764–6.
91. Sinha S, Newsom-Davis J, Mills K, Byrne N, Lang B, Vincent A. Autoimmune aetiology for acquired neuromyotonia (Isaacs' syndrome). Lancet. 1991;338(8759):75–7.
92. Sonoda Y, Arimura K, Kurono A, Suehara M, Kameyama M, Minato S, et al. Serum of Isaacs' syndrome suppresses potassium channels in PC-12 cell lines. Muscle Nerve. 1996;19:1439–46.
93. Arimura K, Watanabe O, Kitajima I, Suehara M, Minato S, Sonoda Y, et al. Antibodies to potassium channels of PC12 in serum of Isaacs' syndrome: Western blot and immunohistochemical studies. Muscle Nerve. 1997;20:299–305.
94. Nagado T, Arimura K, Sonoda Y, Kurono A, Horikiri Y, Kameyama A, et al. Potassium current suppression in patients with peripheral nerve hyperexcitability. Brain. 1999;122:2057–66.
95. Hart IK, Waters C, Vincent A, Newland C, Beeson D, Pongs O, et al. Autoantibodies detected to expressed K^+ channels are implicated in neuromyotonia. Ann Neurol. 1997;41:238–46.
96. Liguori R, Vincent A, Clover L, Neudorfer C, Poggenborg J, Goßmann A, et al. Morvan's syndrome: peripheral and central nervous system and cardiac involvement with antibodies to voltage-gated potassium channels. Brain. 2001;124:2417–26.
97. Buckley C, Oger J, Clover L, Tüzün E, Carpenter K, Jackson M, et al. Potassium channel antibodies in two patients with reversible limbic encephalitis. Ann Neurol. 2001;50:73–8.
98. Irani SR, Alexander S, Waters P, Kleopa KA, Pettingill P, Zuliani L, et al. Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactivated 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan's syndrome and acquired neuromyotonia. Brain. 2010;133:2734–48.

Treatment of Myasthenia Gravis

11

Henry J. Kaminski

Introduction

Modern therapy for myasthenia gravis (MG) begins with the 1934 report of Walker's observation that the cholinesterase inhibitor, physostigmine, produced a prompt improvement of ptosis and facial weakness in a patient with MG [1, 2]. Remen had published a report [3] 2 years prior to Walker's work demonstrating the beneficial effects of neostigmine in a patient with MG, but his discovery was unnoticed by the medical community [4]. In the late 1800s, it had been appreciated that patients with MG often had pathology of the thymus [5]. Blalock encountered a 19-year-old woman who had suffered for years with relapsing and remitting weakness [6, 7]. A chest radiograph demonstrated a mediastinal mass. Blalock removed a thymic cyst, and, postoperatively, the patient improved significantly. Later, he reported observations from six patients who improved or stabilized [8, 9]. The efficacy of thymectomy was debated since these original reports [10, 11] but has been verified for acetylcholine receptor (AChR) antibody-positive patients (see Chap. 13) [12].

Therapy advanced in the 1970s. Widespread use of prednisone occurred after the earlier unimpressive studies of previous decades were dispelled [10], and azathioprine began to be used. In 1976, plasma exchange was identified as an effective acute treatment for severe MG and also served to support the existence of circulating factors as a mediator of MG [13]. Current common treatments of intravenous immunoglobulin (IVIg), tacrolimus, and mycophenolate mofetil came into use in the final two decades of the last century. Since the last edition of this book, the MG community has made significant strides to enhance the rigor of clinical trials [14], several treatment guidelines produced by expert panels have been developed [15–17], and major therapeutic evaluations have been completed.

Patient Education and Initial Consideration

Education is among the most important factors in achieving optimal health for patients with MG. Care of the patient with MG should begin with a thorough discussion of natural history, treatment options, and disease pathogenesis. The patient, family, and the other interested parties should all participate in this discussion and all benefit from the appreciation of the chronic nature of a sickness with a variable prognosis, which often requires treatments with significant

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H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*, Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_11

complications. Psychological adjustment is difficult for an illness in which definite predictions of outcome cannot be made, and fear exists regarding the development of incapacitating weakness. Poor psychological adjustment may limit functional improvement despite significant resolution of myasthenic weakness (see Chap. 18). The neurologist should attempt to strike a balance between the difficulties in MG treatment and the hope of remission. Treatment decisions must be made with consideration of cost and insurance coverage, which vary by nation across the globe. Patient advocacy organizations provide support through educational materials, newsletters, and support groups. The MG Foundation of America maintains an Internet site (www.myasthenia.org), which provides excellent resources on various aspects of the disease. Patients have turned to social media to develop discussion groups. Often persons with only treatment-resistant disease present their experiences on-line or at support group meetings. The role of the physician is to work with patients to refine understanding of information from non-reviewed sites that is anecdotal or, worse, false and potentially dangerous and to provide a balanced view of prognosis.

A list of medications, which may exacerbate myasthenic weakness, should be given to each patient (see Chap. 17). The MG Foundation of America (myasthenia.org) maintains a listing on their website and supports the MyMG smartphone application, which contains the same information. Presently, the application is available in English and Japanese. A survey of cholinesterase prescriptions suggests a large minority of patients with MG are prescribed contraindicated medications, supporting the need for education of each patient about these contraindications [18]. An emerging challenge is the use of the cancer immunotherapies that target PD-1 or CTLA-4. Such agents can de novo activate or exacerbate MG by breakdown of immune tolerance checkpoints but are also potent treatments of several cancers with a poor prognosis [19].

Each patient should undergo computed tomography (CT) of the chest, which is the standard imaging procedure to evaluate for thymoma [20]. If a tumor is discovered, surgical removal is necessary. False positives and negatives occur. An investigation of 34 CT scans indicated a negative predictive value of 91% and positive predictive value of only 39% [21]. Since nearly all patients with thymoma have AChR antibodies, they are likely to undergo thymectomy, and non-radiologically identified thymomas will be removed. MRI has not been found to be superior to CT scanning [20]. Although striational antibodies are associated with thymoma, they are not of adequate sensitivity or specificity to eliminate the need for imaging [22, 23]. Patients with positive striational antibodies who do not undergo thymectomy or are beyond the age typically treated with thymectomy may require serial imaging for thymoma assessment, in particular if they are treatment-resistant. The presence or absence of thymic hyperplasia by imaging does not assist in determination of whether an individual should undergo a thymemctomy.

Because of the high frequency of coexistent thyroid disease and MG, thyroid function testing is reasonable to perform at presentation and intermittently during patient follow-up. At times achieving a euthyroid state will lead to significant improvement in symptoms ascribed to MG. Systemic lupus erythematosus, rheumatoid arthritis, and vitamin B12 deficiency are associated with MG with laboratory evaluation being performed, if clinically indicated. In anticipation of IVIg treatment, consideration should be given for assessment of IgA deficiency [24]. Screening for tuberculosis in high-risk populations should be considered in anticipation of future corticosteroid treatment because of the concern for activation of dormant tuberculosis.

Treatments

Figure 11.1 provides a general treatment approach used by the author for the newly diagnosed patient with generalized MG. I start with pyridostigmine and assess tolerance and efficacy over the course of the first month of treatment. Of course, if the patient has life-threatening weakness or symptoms that significantly compromise activities of daily living, I admit patients for

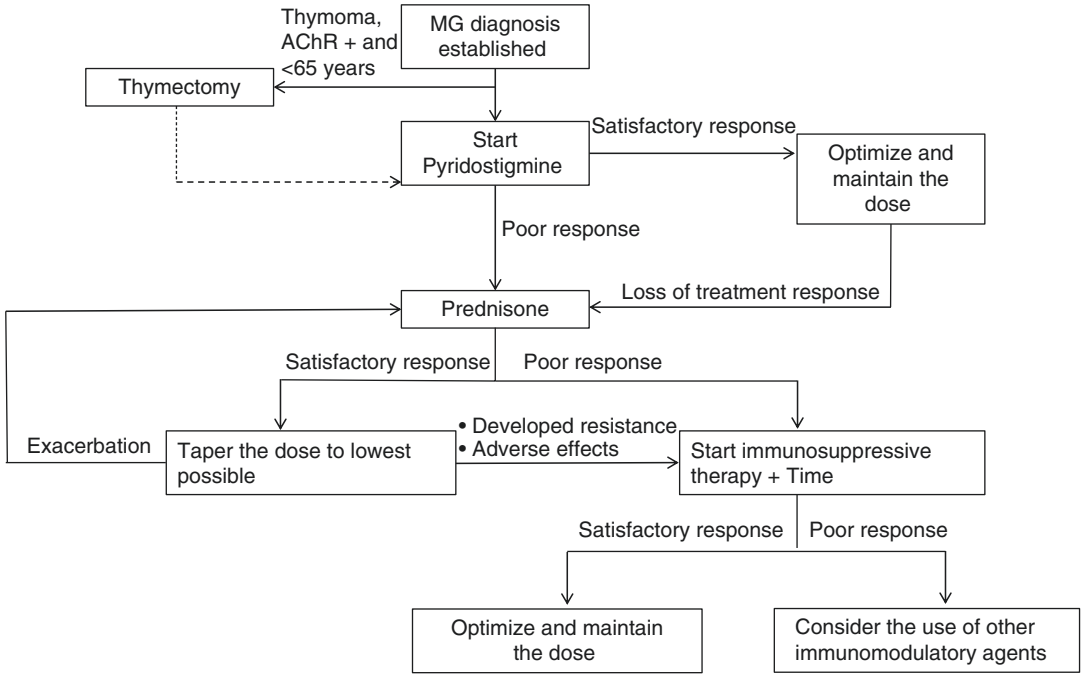

Fig. 11.1 Flow chart summary of treatment for generalized myasthenia gravis

institution of corticosteroids along with administration of IVIg. If a patient has severe weakness with the likelihood of impending crisis, I prefer plasma exchange over IVIg. For patients who are younger than 65 and have AChR antibodies, I recommend a thymectomy (see Chap. 13) performed by a surgeon who I know performs an extensive removal of thymic tissue. Chronic management is highly variable. Many patients will do well with corticosteroid treatment gradually tapered to a low level of 10 mg per day [25]. However, if a patient develops significant corticosteroid complications or remains with significant weakness, I start mycophenolate. For patients who do not improve with mycophenolate after 1 year, I move to tacrolimus. For patients with seronegative MG or muscle-specific kinase (MuSK) MG and significant general weakness, I tend to use rituximab earlier in the course. Moderate exacerbations are treated with IVIg. As with many rare diseases with a limited evidence base, the specifics of treatment approaches differ, as one can see comparing my summary to the recently published guidelines, which also vary among each other [15–17].

Treatment choices should be individualized based on the severity of the disease, patient's lifestyle and career, associated medical conditions, as well as the patient's assessment of risk and benefit of various therapies. For example, a young woman with an active career who is recommended immunosuppressive treatment needs to be aware of reproductive concerns and the potential for increased risk of cancer with long-term use. An older individual with a sedentary lifestyle and mild general weakness may do well with cholinesterase inhibitors. Generalized MG is no longer the grave disease it once was based on observational studies of mortality rates [26], with life spans of patients approaching normal [27]. An excellently performed study, but now dated investigation, indicated that MG patients do have a more limited life expectancy compared to the general population [28].

Despite improvements in mortality, quality of life remains compromised, which is impacted by the disease itself and adverse reactions of medications (Chap. 18). The complications of treatment need thorough explanation, and the patient and physician must work together to optimize the

care plan. Insurance coverage in the United States is a major challenge for some therapies, in particular mycophenolate mofetil [29], IVIg, and rituximab in the experience of the author. Nearly all pharmaceutical corporations have established programs to provide medications at reduced cost for patients that document financial need, which can be challenging to do. The website www.needymeds.com maintains contact information for such programs.

Symptomatic Treatment

Cholinesterase Inhibitors

Acetylcholinesterase (AChE) inhibitors, which retard the hydrolysis of acetylcholine at the neuromuscular junction (Chap. 1), are usually the initial treatments for MG [30, 31]. For some patients cholinesterase inhibitor therapy is all that is necessary [30]. Pyridostigmine bromide (Mestinon®) is the most commonly used agent. Initial therapy begins at doses of 30–60 mg with dosing intervals varying from 3 to 6 h depending on symptom response and complications related to the medication. Individual doses of greater than 180 mg rarely are effective. Because of variability in absorption, patient activity levels, severity of disease, and degrees of muscarinic effects, strict dosing guidelines are difficult to recommend. Once a patient becomes aware of the effects of pyridostigmine on their symptoms and the adverse reactions to the medication, they should be able to modify drug administration within limits set by the physician. Timed-release 180-mg tablets are available but tend to have erratic absorption; however, rare patients demonstrate a preference for these, particularly when given at bedtime to limit symptoms on awakening. Pyridostigmine elixir is available, which may be helpful for some patients with difficulty swallowing and is easier to administer for patients. Pyridostigmine for intramuscular and intravenous injection is available. Intravenous administration has potential for significant morbidity with rapid onset of action leading to bradycardia. Prostigmin and mytelase chloride are shorter-acting cholinesterase inhibitors with greater adverse effects and are no longer available in the United States.

Patients and physicians may appreciate a diminution of beneficial effect of cholinesterase inhibition over time especially with effective immunosuppressive therapy. AChE inhibitor treatment may be tapered when clinical benefit is lost, and patients may restart the medication, if symptoms develop during the taper. Some patients perceive a benefit from AChE inhibitors and prefer to maintain some level of treatment. In experimental MG produced in animals and with cholinesterase inhibition, a splice variant of the AChE is produced, which is soluble and more effectively eliminates acetylcholine from the synapse [32, 33]. This may contribute the reduction of efficacy of AChE inhibitors over time.

AChE inhibitor treatment is generally safe, but complications may occur. If a patient has prominent bulbar muscle weakness, administration of AChE inhibitors will often not reverse weakness to an extent that a patient will be safe to swallow. Swallowing difficulties may be further worsened by excess and thickened saliva, especially if concomitant treatment with antimuscarinic medications is used. Such apparent paradoxical responses should be appreciated, and a reduction of AChE inhibitors may improve symptoms. In addition, MuSK antibody patients often have a poor response to AChE inhibitors [34]. AChE inhibitors do not reverse respiratory muscle weakness reliably. Respiratory secretions may be increased, which complicates treatment of patients with pulmonary diseases and may actually worsen breathing. Reductions or discontinuation of AChE inhibitors may improve respiratory symptoms [35]. Rare patients develop significant bradycardia [36, 37].

Gastrointestinal complaints of nausea, vomiting, diarrhea, and abdominal cramps are common but may be improved with administration of atropine or glycopyrrolate. Muscle twitching, fasciculation, and cramps may be particularly bothersome to patients. Stretching exercises help cramps, while reassurance may limit concerns regarding abnormal muscle movements. Rarely, patients may develop confusion from AChE inhibitor therapy.

AChE inhibitor-induced weakness ("cholinergic crisis") is frequently discussed but is rarely encountered. The use of intravenous edrophonium to determine if muscle weakness is secondary to MG or AChE treatment is unreliable [10, 38, 39]. If cholinergic weakness is seriously considered, then AChE therapy should be temporarily discontinued and the patient monitored for improvement. In patients with myasthenic crisis, discontinuation of AChE inhibitors is recommended to limit secretions while on artificial ventilation [40].

Other Neuromuscular Transmission Enhancers

Ephedrine was first used for MG in the 1930s [41, 42] and variably used since with "*n* of 1" studies supporting efficacy [43]. Ephedrine does enhance neuromuscular transmission and has been shown to be beneficial agent for some congenital myasthenias, but the author does not think the agent adds a significant treatment option for autoimmune MG. 3,4-Diaminopyridine, commonly used for Lambert-Eaton myasthenic syndrome, may be considered for MuSK-related MG, although extensive experience has not been reported [44].

Immune System Targeted Therapeutics for Chronic Use

Corticosteroids

Corticosteroids are the least expensive, most reliable, and rapid acting of therapies for MG, and this statement is based on overwhelming clinical experience rather than controlled clinical trials [45, 46]. Their use is complicated by a myriad of adverse effects (Table 11.1). Corticosteroids are recommended for patients who are compromised by generalized weakness that limits their ability to function and cannot be adequately improved by AChE inhibitors and moderation of activity [47, 48]. Patients need to be made aware of expected complications and agree to treatment. Some patients may not accept steroid therapy or have significant contraindications, and treatment with the slower-acting agents (see below) often coupled with IVIg or plasma exchange may be considered as alternatives.

Table 11.1 Immunomodulating drugs for myasthenia gravis and common adverse effects

Treatment	Major adverse effects
Prednisone	Osteoporosis, weight gain with central obesity, glaucoma, cataracts, hypertension, peripheral edema, psychiatric changes (depression, mania, personality alterations), sleep disturbance, easy bruising, glucose intolerance
Azathioprine	Idiosyncratic flu-like reaction, leukopenia, hepatotoxicity, alopecia, teratogen, possible risk of neoplasia
Cyclosporine	Renal insufficiency, hypertension, gingival hyperplasia, drug interactions
Methotrexate	Diarrhea, anemia, abdominal pain, hepatotoxicity, hair loss
Mycophenolate	Anemia, leukopenia, gastrointestinal discomfort, diarrhea
Tacrolimus	Tremor, headache, diarrhea, hypertension, nausea, renal insufficiency, hyperkalemia, hypomagnesemia, drug interactions

The optimal dosing of corticosteroids is not known; expert opinion varies considerably and ultimately subject to prescriber personal preference [49]. Individuals vary in treatment response with possibly up to 30% being poorly responsive based on lack of improvement in weakness or intolerance of adverse effects [50–52]. With initiation of 60–80 mg per day, up to 50% of patients develop a worsening of strength in the first month, usually within the first few days, of treatment [10, 53–57]. Hospitalization may be considered for patients initiating corticosteroid treatment. Sustained improvement usually begins within 2 weeks and in the majority, within a month, but rare patients have a delay of 2 months. Gradual initiation of corticosteroids beginning with 20 mg and increasing 5 mg every 3 days until a dose of 60–80 mg is reached decreases the frequency of exacerbation but delays the onset of improvement [56, 58, 59]. Such a regimen may be considered in patients

with moderate weakness and allows outpatient therapy. Regardless of treatment initiation, ultimately converting to an every-other-day dosing schedule is desired to limit steroid complications [54]. A trial of thymectomy plus prednisone used every-other-day dosing as a consensus decision of investigators for safety [60, 61]. Regardless of regimen, the corticosteroid should be given as a single morning dose in an attempt to mimic the morning peak of cortisol production. Some patients with mild to moderate weakness respond well to low doses (prednisone 20 mg) [62]. High-dose solumedrol treatments are under investigation and recommended by Japanese treatment guideline [17].

Monitoring for the broad spectrum of complications is necessary [63]. Calcium, vitamin D supplement, and bisphosphonates are indicated for most patients [64]. Ophthalmological evaluation for cataracts and glaucoma should be performed on a yearly basis. Treatments for gastric ulcer prophylaxis need not be instituted unless patients have a previous history ulcer disease or develop symptoms of gastric irritation [65]. The emotional effects of corticosteroids are common and for some patients often the most immediate and deleterious [66, 67]. Laboratory monitoring for development of hyperglycemia and hypokalemia is necessary for certain patients. In some patients prolonged treatment leads to permanent adrenal insufficiency, which will require lifelong corticosteroid replacement. The symptoms of adrenal insufficiency may be a point of confusion with the fatigue of MG.

Mechanism of Action. Corticosteroids have numerous effects on the immune system leading to general immunosuppression [68]. The immunosuppressive actions of glucocorticoids appear to be mediated primarily by interference with signaling by the key inflammatory transcriptional regulators: NF-κB and AP-1 leading to reductions of lymphocyte differentiation and proliferation, redistribution of lymphocytes from the circulation into tissues that remove them from sites of immunoreactivity, alterations of cytokine activity, and alteration of antigen processing and presentation. Treatment resistance of patients is likely to be more a function of individual differences in corticosteroid responsiveness than severity of disease per se [51, 52, 69].

Azathioprine

Azathioprine is used alone or in combination with corticosteroids, and its efficacy has been confirmed in randomized trials [70, 71]. Although azathioprine is often utilized for its "corticosteroid-sparing" effect, there are suggestions that it may be used effectively alone [72–74]. Both the physician and patient need to be cognizant that improvement requires at least 1 year at a dose based on total body weight (1–3 mg/kg/day in divided doses). Limited improvement in less than 1 year should not provide a false impression of a lack of efficacy. Clinical benefit appears to correlate with elevations of mean red blood cell volume [75] and reduction of white blood cell count. If either of these is not observed, then the dose may be increased to its maximum.

Azathioprine has the potential for significant hepatotoxicity and myelosuppression [76]. If white blood cell counts fall below 3–3500 white blood cells/mm^3, the dose needs to be reduced and adjusted to maintain counts above 3500. Levels below 1000 white blood cells/mm^3 require discontinuation of the drug [40, 77]. Mild hepatoxicity is likely underestimated and may only manifest with elevations of serum transaminase, but without clinically evident liver disease. Azathioprine needs to be discontinued if transaminase levels double [76]. Monitoring of liver function tests and complete blood counts is necessary throughout the treatment course. Pancreatitis rarely occurs with azathioprine treatment. Allopurinol reduces metabolism of azathioprine necessitating reduction of dose by one-third to avoid complications. There is an increased risk of neoplasm, primarily lymphoma, among patients taking azathioprine [78, 79]. The agent also has small teratogenic potential. For women and men planning a pregnancy [80], consideration needs to be given to discontinue its use depending on the specific clinic situation. Azathioprine is a substrate for thiopurine *S*-methyltransferase. Deficiency in enzyme activity may lead to severe idiosyncratic drug

reaction and myelosuppression when exposed to azathioprine. Assessment for thiopurine *S*-methyltransferase enzyme activity prior to initiation of azathioprine therapy is advisable [81].

Mechanism of Action. Azathioprine is a purine analogue, which inhibits synthesis of nucleic acids. The drug is converted to 6-mercaptopurine and metabolized to 6-thioguanine nucleotides, which are the cytotoxic [82]. The primary effect on the immune system is interference with T- and B-cell proliferation and has been presumed to be related to its direct effect on nucleic acid synthesis. Thioguanosine triphosphate is an azathioprine metabolite, which interacts with Rac1 and blocks interaction with Bcl-x1, which ultimately leads to apoptosis of T cells [83]. Each of these actions would lead to suppression of cellular responses driving the autoimmune pathology of MG.

Mycophenolate Mofetil

Mycophenolate mofetil has become an increasingly popular agent for treatment of MG in the United States. Small, retrospective studies suggest that mycophenolate reduces corticosteroid dose, improves strength, and lowers AChR antibody levels [84–86] for generalized MG and improved purely ocular myasthenia [87]. However, two randomized, placebo-controlled studies did not demonstrate efficacy [62, 88], but each was compromised by their short duration given the expected slow onset of biological action of mycophenolate [89]. Despite these negative studies, mycophenolate continues to be used and recommended by many neurologists [15, 29]. A three-institution, retrospective study of almost one hundred patients found that mycophenolate may be tapered safely for those patients with long periods of remission and recommended a slow taper of 500 mg per year [90].

Mycophenolate is given at a standard dose of 1 g twice daily, but no data exist regarding whether higher doses are more effective. The author typically raises the dose to 3 g per day, if improvement has not occurred within 9 months, and in particular among patients over 90 kg. Mycophenolate is well tolerated, and most patients have limited complaints when initiating treatment, other than gastrointestinal upset, which rarely is so severe as to require discontinuation. Leukopenia may occur, and monitoring complete blood counts is indicated. Mycophenolate has teratogenic potential, and women of childbearing age should be educated about this and appropriate counseling provided [91]. Experience from studies of transplant patients indicates that there may be an increased risk of cancer with mycophenolate [92, 93], and case reports have appeared of mycophenolate treatment associated with lymphoma [94] and severe infection, including progressive multifocal leukoencephalopathy [95].

Mechanism of Action. Mycophenolate is hydrolyzed to mycophenolic acid, which inhibits inosine monophosphate dehydrogenase, a key enzyme in the de novo pathway of purine synthesis [96]. Lymphocytes exclusively use the de novo pathway, and thereby mycophenolate selectively inhibits proliferation of T and B lymphocytes. Additional mechanisms of immunosuppression include (1) apoptosis of activated T lymphocytes, (2) alterations in cell adhesion molecule expression decreasing recruitment of lymphocytes to sites of inflammation, and (3) reduction of inducible nitric oxide synthase activity.

Tacrolimus

Tacrolimus is the primary immunosuppressive agent used in Japan and has been used throughout the world based on retrospective or open-label investigations [17, 97–99]. A randomized, controlled trial evaluated whether addition of tacrolimus over 28 weeks to low-dose prednisolone treatment among patients with stable symptoms would lead to a reduction of prednisolone dose [100]. Although the mean prednisolone dose was diverging between groups at the time of the primary endpoint, there was no statistical difference. Several methodological criticisms were raised regarding the study, which could have led to an inability to identify efficacy [101]. A retrospective study supports use of tacrolimus for ocular myasthenia [102]. The recommended dosage of tacrolimus is 0.035 mg/kg twice daily with adjustments made to achieve a trough level of

6–9 ng/mL [103]. Common side effects are gastrointestinal discomfort, diarrhea, tremor, and paresthesias. Tacrolimus is nephrotoxic and may worsen hypertension and diabetes. Several drugs raise or lower tacrolimus levels.

Mechanism of Action. Tacrolimus is a calcineurin inhibitor and thereby modulates the activity of T cells, which support antibody-producing B cells [104, 105]. It may also enhance T regulatory cells [106]. Tacrolimus enhances muscle contraction by modulation of intracellular calcium-release channels, which would enhance muscle contraction rapidly. The direct effect on muscle may provide an explanation for the impression of a rapid onset of improvement in some patients, which would not be explained by the immunosuppressive effect [107].

Methotrexate

Efficacy of methotrexate was suggested by uncontrolled trials, but a randomized, controlled trial did not find a steroid-sparing effect after 1 year of treatment [108]. A single-blind comparison study against azathioprine of 24 subjects found both drugs to have similar steroid-sparing effects [109]. A motivation by both these groups to evaluate methotrexate for MG was its relatively inexpensive generic preparation compared to other immunosuppressives. Therefore, for some regions of the world, methotrexate may still be an option for treatment. Typical dosing is a gradual increase to a maximum of 20 mg of methotrexate weekly with the provision of folate or leucovorin. Methotrexate has significant adverse effects of hepatoxicity and anemia. Most patients develop hair loss, which is reversible.

Mechanism of Action. The mechanism of action on the immune response by methotrexate is not fully elucidated. Methotrexate does inhibit de novo pyrimidine and purine syntheses necessary for DNA and RNA syntheses, which would inhibit cellular proliferation of lymphocytes supporting MG [110].

Cyclosporine

Cyclosporine has been used as a steroid-sparing agent since the 1980s for patients with treatment-resistant disease [111–114] but has generally fallen out of favor because of renal toxicity. Five milligram per kilogram per day in two divided doses are recommended. In a retrospective review, 55 of 57 patients who took cyclosporine for an average of 3.5 years, clinical improvement usually occurred in less than 7 months. Corticosteroids were discontinued or decreased in nearly all the patients taking them [112]. Five percent of individuals could not afford or tolerate the drug [112]. Cyclosporine may be used alone and this approach does have the advantage of avoiding corticosteroid complications [48], although it should be appreciated that cyclosporine does promote osteoporosis [115]. Monitoring trough levels is important to limit toxicity and supports compliance. The primary adverse effects of cyclosporine are renal insufficiency and hypertension. Creatinine measures need to be monitored throughout treatment with cyclosporine. In a small study, nearly a third of patients needed to stop treatment because of renal toxicity, which in some occurred several years after treatment initiation [112]. Cyclosporine has many drug interactions, and it is critical to check updated drug databases for interactions that may lower or elevate cyclosporine levels or lead to specific toxicities.

Mechanism of Action. Cyclosporine forms a complex with cyclophilin, which inhibits the phosphatase activity of calcineurin, and thereby modulates nuclear translocation and subsequent activation of nuclear factor of activated T cell transcription factor. Cyclosporin also blocks the activation of JNK and p38 signaling pathways, which are triggered by antigen recognition. These mechanisms make cyclosporin a highly specific inhibitor of T cell activation [104, 116].

Cyclophosphamide

Intravenous and oral cyclophosphamide has been used for treatment-resistant patients, and one study found half of patients were asymptomatic after 1 year [114, 117]. Additional complications include diarrhea, nausea, vomiting, and hemorrhagic cystitis, which may be severe. The drug has carcinogenic and teratogenic potential as well as a likelihood of producing infertility. Rarely, interstitial pneumonitis and hepatic injury occur. Nearly, all patients develop alopecia.

High-dose cyclophosphamide alone is not myeloablative, which allows a patient's endogenous stem cells to repopulate the hematopoietic/immune systems. Such an approach has been applied to patients with treatment-resistant autoimmune diseases, including MG [118]. Autologous hematopoietic stem cell transplantation has been used in combination with cyclophosphamide for treatment-resistant patients [119]. Despite the hope that such therapy would place patients in permanent remission, there have been recurrences.

Mechanism of Action. Through the cytochrome P-450 oxidase system of the liver, cyclophosphamide is converted into phosphoramide mustard, which introduces alkyl radicals into DNA and compromise cell replication. This activity is likely cytotoxic to lymphocytes. However, cyclophosphamide is being appreciated to have more widespread immunomodulatory effects, which include modulation of Th2/Th1 ratios, altered cytokine production, and enhanced proliferation and survival of certain lymphocyte populations and modulation of dendritic cell activity [120]. Therefore, the effects of cyclophosphamide on MG are likely to be more complex than previously considered.

Rituximab

Rituximab is a monoclonal antibody directed against CD20 cells. Retrospective evaluations have led to great enthusiasm for application of the agent for treatment-resistant MG [121–124]. A blinded, prospective review for MuSK MG supports rituximab improves disease severity and limits use of other MG treatments [125]. A phase II trial for ACHR and MuSK-positive MG is ongoing at the time of this writing. Dosing of rituxan is typically a once a week regimen for 4 weeks at a dose of 375 mg/m^2 of body surface. Pretreatment with acetaminophen, diphenhydramine, corticosteroid, or a combination is given by some investigators. Repeat dosing may be given in 6 months as is being performed in the phase II trial, or patients have been monitored for recurrent weakness and then repeat treatment given. Infusion reactions of varying severity consisting of fever, nausea, headache, pruritus, headache, and angioedema occur during infusion and may improve with pretreatment and slowing the infusion rate. Severe infections, including progressive multifocal leukoencephalopathy, may occur. Activation of latent hepatitis B infection may occur.

Mechanism of Action. Rituxan removes CD20-expressing cells, which include activated B cells and pre-B cells. These cells serve to produce pro-inflammatory cytokines, present antigen, and provide T cell support. The reduction of CD20 cells impacts these properties, and the reduction of antibody-producing cells is likely a mechanism that reduces MG severity [126, 127].

Eculizumab

The complement system has been speculated to be a potential therapeutic target for MG for decades; however, only recently has an agent been evaluated for human use [128]. Eculizumab, an antibody directed against the C5 component of complement, in phase 2 testing of treatment-resistant MG, demonstrated an efficacy signal [129]. A phase 3 trial has confirmed efficacy for AChR antibody-positive patients who had failed immunosuppressive treatment [130], and the Food and Drug Administration of the United States has approved its use for generalized MG. The author expects eculizumab will be used for treatment-resistant patients and possibly as acute management of severe disease. Despite the potential risk of Neisseria infections, eculizumab thus far has good safety and tolerability [131, 132]. A deficiency of eculizumab is its inactivity among patients with a genetic variant of the C5 epitope to which it binds [133, 134] and its general complement inhibition in an immunosuppressed population. Also, eculizumab is among the most expensive drugs in use at cost of approximately $400,000 per year.

Acute Immunotherapies

Plasma Exchange

Plasma exchange (plasmapheresis) produces rapid improvement in weakness [34, 135–137]. It is used in myasthenic crisis as well as to opti-

mize muscle function prior to surgery, including thymectomy. Typically, exchanges are done to remove one to two plasma volumes three times per week for up to six exchanges. In my experience, more than 6 exchanges during a single course are not beneficial, but others describe benefits after 14 exchanges [77]. Patients may show improvement within 48 h of the first or second exchange and continue to improve during the course of exchanges. Treatment reduces immunoglobulin levels rapidly; however, rebound will occur in weeks, leading to clinical worsening if concomitant immunosuppressive treatment is not used. In rare patients, plasma exchange is used as a chronic therapy, but this has not been formally studied [138]. The exchange is usually performed with resins that remove proteins at certain molecular weights, and studies of immunoabsorbent resins do not demonstrate any superiority to standard resins thus far [139, 140]. Plasma exchange may be more beneficial for MuSK MG then IVIg.

The usefulness of plasma exchange is limited by its restriction to major medical centers and the frequent need for large-bore intravenous catheters [135]. During the infusion patients may complain of paresthesias from citrate-induced hypocalcemia, and hypotension may occur at initiation of the exchange [141]. Some patients have nausea and vomiting related to fluid shifts and electrolyte alterations during the exchange. Infectious and thrombotic complications related to venous access occur. The exchange process reduces coagulation factors, and concomitant use of heparin in the care of venous catheters may reduce platelet levels leading to bleeding tendencies. Thrombotic complications may occur [141]. Whenever possible peripheral venous catheters should be used for exchanges [135].

Mechanism of Action. Removal of circulating pathogenic antibody, and in all likelihood other factors, such as complement proteins, produces the clinical benefits of plasma exchange [142]. The removal of antibodies that block AChR function may be primary in mediating the improvement that is sometimes observed within hours of plasma exchange.

Intravenous Immunoglobulin

IVIg is used in similar circumstances as plasma exchange, and a comparative study to plasma exchange indicated similar clinical efficacy [143]. A comparative effectiveness study of a large hospital database also indicated equivalent efficacy, but IVIg was found to be a less costly treatment regimen [144]. A randomized study demonstrated that IVIg is superior to placebo in treatment of MG exacerbation [145]. The standard treatment regimen is a 4- to 6-h infusion of 400 mg/kg/day dose for 5 days, but some advocate a 1 g/kg/day dose for 2 days [24]. The latter can be administered without a higher rate of complications; however, in older patients with cardiac disease, the lower dose regimen may be prudent. IVIg therapy improves strength rapidly, often within 5 days of initiation but on average 3 weeks [145], but the response is short-lived and not uniformly observed. A retrospective study comparing IVIg and plasma exchange for treatment of myasthenic crisis showed patients had a better respiratory and functional outcome with plasma exchange, but IVIg had fewer complications [146]. A randomized trial compared three exchanges to either 3 or 5 days of IVIg (400 mg/kg/day) and showed a similar efficacy with again IVIg having less adverse effects [147]. IVIg may also be used as a maintenance therapy to limit corticosteroid dosage, while other agents, such as mycophenolate or azathioprine, are taking effect.

Adverse effects with IVIg occur commonly but usually are relatively minor [24, 148]. Headache occurs frequently during infusion, which if mild, headaches may be treated with acetaminophen or nonsteroidal anti-inflammatory drugs (since MG patients are frequently receiving corticosteroids, nonsteroidals should be used with caution to limit gastrointestinal irritation). More severe migraine headaches occur, particularly in patients with a history of migraine. In appropriate individuals, triptan agents are effective. Aseptic meningitis may occur and recur with subsequent treatments. Chills, myalgia, or chest discomfort may occur early during the infusion and usually resolves with stopping the infusion for 30 min and resuming at a slower rate. Flu-like symptoms may develop in the days

following treatment. Urticaria, lichenoid cutaneous lesions, pruritus of the palms, and petechiae may occur within days or weeks of treatment, which resolve over weeks to months. Pretreatment with diphenhydramine or acetaminophen may limit adverse effects.

Rarely, significant complications occur with IVIg therapy. Anaphylactic reactions occur in patients with immunoglobulin A deficiency, which may be present in 1 in a 1000 people [24]. This deficiency should be screened for prior to infusion and consideration given to provide an IVIg preparation depleted of immunoglobulin A [149]. Acute renal tubular necrosis, which is usually reversible, occurs in patients who have renal insufficiency. Age over 65, diabetes, and dehydration increase the risk of renal injury. Renal failure is associated with the high concentration of sucrose in one proprietary IVIg product but occurs with other preparations [148]. Monitoring of creatinine and blood urea nitrogen is essential, if the agent needs to be given to patients with renal insufficiency. Deep vein thrombosis, pulmonary embolism, cerebral infarction, and myocardial infarction rarely occur [150, 151]. For the immobilized MG patient, physicians should be vigilant for the signs of deep vein thrombosis and appropriate prophylactic treatments administered.

Mechanism of Action. IVIg impacts on the autoimmune process by several mechanisms including inhibition of cytokines, competition with autoantibodies, inhibition of complement deposition, interference with Fc receptor binding on macrophages or the immunoglobulins on B cells, and interference with antigen recognition by sensitized T cells [152]. Some reports suggest anti-idiotype mechanism appear to be the most critical in clinical efficacy in MG [153, 154].

Specific Clinical Situations

Myasthenia Gravis and Pregnancy

MG preferentially affects women of childbearing years leading to particular therapeutic issues [155]. The effect of pregnancy on the clinical course of MG is highly variable among women, and each pregnancy may affect an individual woman differently [156]. A patient appears to have an equal probability of worsening, staying the same, or improving during the pregnancy and the postpartum period [156–158], despite the consideration that the state of pregnancy is a naturally "immunosuppressed" state and would moderate disease severity. Labor is an exhausting event and should be expected to weaken the symptomatic MG patient; however, the majority of women are able to deliver vaginally without complications [156, 159, 160]. If preeclampsia or eclampsia is present, then the obstetrician should be made aware that intravenous magnesium sulfate may lead to worsening weakness [161]. MG is not a contraindication to pregnancy [162].

MG treatments may affect the fetus [159]. The potential for birth defects with immunosuppressants, in particular mycophenolate and methotrexate, requires discussion, and every attempt should be made to discontinue the medications prior to conception [155]. Mestinon and prednisone are safe. There are no large studies of the effect of plasma exchange or IVIg in pregnant women but they appear to be safe. Thymectomy should be delayed until after delivery, since there is no reason to believe the delay would be deleterious compared to the potential complications of performing a major surgery during pregnancy.

Approximately a third of infants of MG patients have neonatal myasthenia [156, 159] which may occur with MuSK and seronegative MG [163–165]. This is a transient disorder manifest by general weakness, difficulty feeding, and respiratory insufficiency in some. Such infants require supportive care and cholinesterase treatment. Weakness resolves over weeks without any permanent weakness or risk of development of MG later in life. Development of neonatal MG is independent of the clinical status of the mother, and the mother may not be known to have MG. AChR antibody levels of the mother also do not predict occurrence of MG. An increased ratio of antibodies against the fetal AChR compared to the antibodies directed against the adult AChR isoform correlates with the development of neonatal myasthenia [166]. Arthrogryposis multiplex

and myopathy may occur due to passage of antibodies against the fetal AChR, which severely compromise fetal muscle development [167–169]. If a woman with MG has a history of children with arthrogryposis multiplex or recurrent fetal loss, consideration should be given to more intensive therapy of MG during pregnancy [170–172].

Thymoma-Associated Myasthenia Gravis

A significant minority of patients with MG have a thymoma (see Chap. 8). Thymoma is more likely to occur among men over the age of 40, but certainly occurs in women and rarely among children [173]. The treatment of patients with thymoma does not differ from those patients without thymoma but is complicated by the necessity to treat the neoplasm. Most, but not all, studies suggest that thymoma-associated MG has a more severe clinical picture [174–176]. Once a thymoma is detected, it should be removed along with removal of the remaining thymus, although the patient should be aware that the surgery is not a cure for MG. If the tumor has broken through its capsule to involve adjacent tissue, local irradiation is necessary. Some thymomas are aggressive and produce widespread metastases. Patients require lifelong monitoring for recurrence of tumor [177]. In the elderly patient with a contraindication to surgery, consideration may be given to monitor tumor growth by computed tomography, since many tumors are slow growing. Clinical trials have eliminated patients with thymoma from investigation leading to questions on whether there is a differential susceptibility of patients to certain treatments.

Pediatric Myasthenia Gravis

The pathophysiology of children presenting with MG younger than 18 years is not known to differ from the adult-onset disorder [178–180]. Differential diagnosis and diagnostic methods are identical to adults, although congenital myasthenic syndromes are more likely. MuSK MG occurs in children [181]. Treatment considerations are more difficult [180]. Prepubertal patients appear to have higher rates of spontaneous remission, which prompts some to consider a delay in thymectomy. Corticosteroids retard growth and increase the severity of osteoporosis later in life. Treatment with nonsteroidal immunosuppressive drugs has the potential total dose-dependent risk of neoplasia. As in adults, treatment must be individualized, and the parents and patient need to appreciate the complications of therapy.

The Treatment-Resistant Patient

Patients referred to the author for a poor treatment response come in four varieties, those who (1) never had MG, (2) have complications related to treatment, (3) have developed a new disorder, and (4) have severe MG not responding to treatment.

When faced with a patient who continues to complain of weakness, it is imperative to confirm that the diagnosis of MG is based on unequivocal clinical manifestations and supported by serological or electrophysiological studies. Care is further complicated, if the patients have been treated for MG with immunosuppressive treatments and developed complications, which produce weakness or fatigue. If on review of previous evaluations, the physician concludes MG is not present, then the patient must be informed of the determination using the utmost sensitivity. Medications for MG will need to be tapered. Even if an alternate medical diagnosis is identified as a cause of symptoms, patients will have been traumatized and require expert psychological counseling to assist them in recovering from misdiagnosis.

Corticosteroids produce a legion of adverse effects, which may lead patients to complaints of “fatigue,” “tiredness,” and “weakness,” which they often do not distinguish from their presenting complaints of MG. A detailed history and examination is required to clearly distinguish what is, and is not, MG related. I have seen

several patients with MG who have developed steroid myopathy after several months of daily, high-dose therapy, who were also treated aggressively with plasma exchange and other immunosuppressive treatments. These patients have proximal muscle weakness and obvious systemic complications of steroids without MG manifestations [182]. Taper of steroids, in particular to an every-other-day regimen, in concert with physical therapy, leads to functional improvement after several months.

The most common disorders that I see complicating treatment of MG are sleep and thyroid disorders as well as deconditioning. Sleep apnea may complicate MG and be exacerbated by weight gain related to steroid therapy producing excessive somnolence, misinterpreted as muscle fatigue [183–185]. Thyroid disorders occur in 10% of patients, and the slow development of hypothyroidism during the course of MG treatment may be difficult to appreciate clinically. Therefore, there should be a low threshold for obtaining thyroid function tests for patients complaining of fatigue. The combination of hospitalization, corticosteroid treatment, and inactivity forced upon a patient from MG makes general deconditioning likely. Despite decades of considering energy budgeting strategies for MG patients, it is clear that exercise programs have greater benefits than risks [186, 187].

Upward of 30% of MG patients may be treatment-resistant with persistent weakness despite prolonged therapy (usually this means thymectomy, corticosteroids, and failure of at least one additional therapy). In these situations it is critical that immunosuppressants have been used at the appropriate dose and for expected time to take an effect, which is at least a year for all of the immunosuppressives. If these are true, then a switch to another immunosuppressive should occur. If a patient has moderate to severe weakness, then use of chronic IVIg or plasma exchange needs to be considered. Rituximab also should be an option for individuals who have failed other immunosuppressives and high-dose corticosteroids. New immunotherapeutics are in early phase testing and preclinical evaluation (Chap. 21) with the hope of improving treatment for patients.

References

1. Walker MB. Treatment of myasthenia gravis with physostigmine. Lancet. 1934;1:1200–1.
2. Keesey JC. Contemporary opinions about Mary Walker: a shy pioneer of therapeutic neurology. Neurology. 1998;51(5):1433–9.
3. Remen L. Zur Pathogenese und Therapie der Myasthenia gravis pseudoparalytica. Dtsch Ztschr f Nervenheilkunde. 1932;128:66–78.
4. Viets HR. Introductory remarks. In: Ossermann KE, editor. Myasthenia gravis, vol. 135. New York: New York Academy of Sciences; 1966. p. 5–7.
5. Bell ET. Tumors of the thymus in myasthenia gravis. J Nerv Ment Dis. 1917;45:130–43.
6. Blalock A, Mason MF, Morgan HJ, Riven SS. Myasthenia gravis and tumors of the thymic region. Report of a case in which the tumor was removed. Ann Surg. 1939;110:544–61.
7. Kirschner PA. The history of surgery of the thymus gland. Chest Surg Clin N Am. 2000;10(1):153–65.
8. Blalock A. Thymectomy in treatment of myasthenia gravis. J Thorac Surg. 1944;13:316.
9. Blalock A, Harvey AM, Ford FF, Lilienthal JL. The treatment of myasthenia gravis by removal of the thymus gland: preliminary report. JAMA. 1941;117:1529.
10. Rowland LP. Controversies about the treatment of myasthenia gravis. J Neurol Neurosurg Psychiatry. 1980;43:644–59.
11. Gronseth GS, Barohn RJ. Practice parameter: thymectomy for autoimmune myasthenia gravis (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology [see comments]. Neurology. 2000;55(1):7–15.
12. Aban IB, Wolfe GI, Cutter GR, Kaminski HJ, Jaretzki A 3rd, Minisman G, et al. The MGTX experience: challenges in planning and executing an international, multicenter clinical trial. J Neuroimmunol. 2008;201–202:80–4.
13. Pinching AJ, Peters DK, Newsom-Davis J. Remission of myasthenia gravis following plasma exchange. Lancet. 1976;2:1373–6.
14. Benatar M, Sanders DB, Burns TM, Cutter GR, Guptill JT, Baggi F, et al. Recommendations for myasthenia gravis clinical trials. Muscle Nerve. 2012;45(6):909–17.
15. Sanders DB, Wolfe GI, Benatar M, Evoli A, Gilhus NE, Illa I, et al. International consensus guidance for management of myasthenia gravis: executive summary. Neurology. 2016;87(4):419–25.
16. Fuhr P, Gold R, Hohlfeld R, Melms A, Melzer N, Tackenberg B, et al. Diagnostik und therapie der myasthenia gravis und des Lambert-Eaton syndroms. In: Diener H, Weimer C, editors. Leitlinien für Diagnostik und Therapie in der Neurologie. 5th ed. Stuttgart: Thieme; 2012. p. 830–56.

17. Murai H. Japanese clinical guidelines for myasthenia gravis: putting into practice. Clin Exp Neuroimmunol. 2015;2015:21–31.
18. Machado-Alba JE, Calvo-Torres LF, Gaviria-Mendoza A, Augusto Meji AVC. Prescription profile of pyridostigmine use in a population of patients with myasthenia gravis. Muscle Nerve. 2017;56(6):1041–6.
19. Makarious D, Horwood K, Coward JIG. Myasthenia gravis: an emerging toxicity of immune checkpoint inhibitors. Eur J Cancer. 2017;82:128–36.
20. Priola AM, Priola SM. Imaging of thymus in myasthenia gravis: from thymic hyperplasia to thymic tumor. Clin Radiol. 2014;69(5):e230–45.
21. de Kraker M, Kluin J, Renken N, Maat AP, Bogers AJ. CT and myasthenia gravis: correlation between mediastinal imaging and histopathological findings. Interact Cardiovasc Thorac Surg. 2005;4(3):267–71.
22. Kaminski HJ, Santillan C, Wolfe GI. Autoantibody testing in neuromuscular disorders. Neuromuscular junction and muscle disorders. J Clin Neuromuscul Dis. 2000;2:96–105.
23. Meriggioli MN, Sanders DB. Muscle autoantibodies in myasthenia gravis: beyond diagnosis? Expert Rev Clin Immunol. 2012;8(5):427–38.
24. Dalakas MC. Immunotherapies in the treatment of neuromuscular disorders. In: Katirji B, Kaminski H, Preston D, Ruff R, Shapiro B, editors. Neuromuscular disorders in clinical practice. Boston: Butterworth Heinemann; 2002. p. 364–83.
25. Utsugisawa K, Suzuki S, Nagane Y, Masuda M, Murai H, Imai T, et al. Health-related quality-of-life and treatment targets in myasthenia gravis. Muscle Nerve. 2014;50(4):493–500.
26. Grob D, Brunner N, Namba T, Pagala M. Lifetime course of myasthenia gravis. Muscle Nerve. 2008;37(2):141–9.
27. Phillips LH, Torner JC. Epidemiologic evidence for a changing natural history of myasthenia gravis. Neurology. 1996;47:1233–8.
28. Somnier F, Keiding N, Paulson O. Epidemiology of myasthenia gravis in Denmark: a longitudinal and comprehensive study. Arch Neurol. 1991;48:733–9.
29. Burns TM, Sanders DB, Kaminski HJ, Wolfe GI, Narayanaswami P, Venitz J. Two steps forward, one step back: mycophenolate mofetil treatment for myasthenia gravis in the United States. Muscle Nerve. 2015;51(5):635–7.
30. Maggi L, Mantegazza R. Treatment of myasthenia gravis: focus on pyridostigmine. Clin Drug Investig. 2011;31(10):691–701.
31. Mehndiratta MM, Pandey S, Kuntzer T. Acetylcholinesterase inhibitor treatment for myasthenia gravis. Cochrane Database Syst Rev. 2014;(10):CD006986.
32. Brenner T, Hamra-Amitay Y, Evron T, Boneva N, Seidman S, Soreq H. The role of readthrough acetylcholinesterase in the pathophysiology of myasthenia gravis. FASEB J. 2003;17(2):214–22.
33. Brenner T, Nizri E, Irony-Tur-Sinai M, Hamra-Amitay Y, Wirguin I. Acetylcholinesterase inhibitors and cholinergic modulation in myasthenia gravis and neuroinflammation. J Neuroimmunol. 2008;201–202:121–7.
34. Guptill JT, Sanders DB, Evoli A. Anti-MuSK antibody myasthenia gravis: clinical findings and response to treatment in two large cohorts. Muscle Nerve. 2011;44(1):36–40.
35. Echaniz-Laguna A, Pistea C, Philippi N, Enache I, Oswald-Mammosser M, De Seze J, et al. Asthma as a cause of persistent dyspnea in treated myasthenia gravis patients. Eur Neurol. 2012;68(5):300–3.
36. Arsura EL, Brunner NG, Namba T, Grob D. Adverse cardiovascular effects of anticholinesterase medications. Am J Med Sci. 1987;293(1):18–23.
37. Said S, Cooper CJ, Alkhateeb H, Elhanafi S, Bizet J, Gosavi S, et al. Pyridostigmine-induced high grade SA-block in a patient with myasthenia gravis. Am J Case Rep. 2013;14:359–61.
38. Daroff RB. Ocular myasthenia: diagnosis and therapy. In: Glaser J, editor. Neruo-opthalmology. St. Louis: C.V. Mosby; 1980. p. 62–71.
39. Daroff RB. The office tensilon test for ocular myasthenia gravis. Arch Neurol. 1986;43:843–4.
40. Juel VC, Massey JM. Autoimmune myasthenia gravis: recommendations for treatment and immunologic modulation. Curr Treat Options Neurol. 2005;7(1):3–14.
41. Edgeworth H. A report of progress on the use of ephedrine in a case of myasthenia gravi. JAMA. 1930;94:1136.
42. Edgeworth H. The effect of ephedrine in the treatment of myasthenia gravis, second report. JAMA. 1933;100:1401.
43. Lipka AF, Vrinten C, van Zwet EW, Schimmel KJ, Cornel MC, Kuijpers MR, et al. Ephedrine treatment for autoimmune myasthenia gravis. Neuromuscul Disord. 2017;27(3):259–65.
44. Evoli A, Alboini PE, Damato V, Iorio R. 3,4-Diaminopyridine may improve myasthenia gravis with MuSK antibodies. Neurology. 2016;86(11):1070–1.
45. Schneider-Gold C, Gajdos P, Toyka KV, Hohlfeld RR. Corticosteroids for myasthenia gravis. Cochrane Database Syst Rev. 2005;(2):CD002828.
46. Hoffmann S, Kohler S, Ziegler A, Meisel A. Glucocorticoids in myasthenia gravis—if, when, how, and how much? Acta Neurol Scand. 2014;130(4):211–21.
47. Bedlack RS, Sanders DB. Steroids have an important role. Muscle Nerve. 2002;25(1):117–21.
48. Rivner MH. Steroids are overutilized. Muscle Nerve. 2002;25(1):115–7.
49. Gilhus NE. Myasthenia gravis. N Engl J Med. 2016;375(26):2570–81.
50. Evoli A, Batocchi AP, Palmisani MT, Lo Monaco M, Tonali P. Long-term results of corticosteroid therapy in patients with myasthenia gravis. Eur Neurol. 1992;32(1):37–43.

51. Xie Y, Li H-F, Sun L, Kusner LL, Wang S, Meng Y, et al. The role of osteopontin and its gene on glucorticoid response in myasthenia gravis. Front Neurol. 2017;8:230.
52. Xie Y, Meng Y, Li HF, Hong Y, Sun L, Zhu X, et al. GR gene polymorphism is associated with intersubject variability in response to glucocorticoids in patients with myasthenia gravis. Eur J Neurol. 2016;23(8):1372–9.
53. Bae JS, Go SM, Kim BJ. Clinical predictors of steroid-induced exacerbation in myasthenia gravis. J Clin Neurosci. 2006;13(10):1006–10.
54. Pascuzzi RM, Coslett HB, Johns TR. Long-term corticosteroid treatment of myasthenia gravis: report of 116 patients. Ann Neurol. 1984;15:291–8.
55. Beghi E, Antozzi C, Batocchi AP, Cornelio F, Cosi V, Evoli A, et al. Prognosis of myasthenia gravis: a multicenter follow-up study. J Neurol Sci. 1991;106:213–20.
56. Seybold M, Drachman D. Gradually increasing doses of prednisone in myasthenia gravis: reducing the hazards of treatment. N Engl J Med. 1974;290:81–4.
57. Grob D, Arsura EL, Brunner NG, Namba T. The course of myasthenia gravis and therapies affecting outcome. Ann N Y Acad Sci. 1987;505:472–99.
58. Durelli L, Maggi G, Casadio C, Ferri R, Rendine S, Bergamini L. Actuarial analysis of the occurrence of remissions following thymectomy for myasthenia gravis in 400 patients. J Neurol Neurosurg Psychiatry. 1991;54:406–11.
59. Drachman DB. Myasthenia gravis. N Engl J Med. 1994;330:1797–810.
60. Wolfe GI, Kaminski HJ, Aban IB, Minisman G, Kuo HC, Marx A, et al. Randomized trial of thymectomy in myasthenia gravis. N Engl J Med. 2016;375(6):511–22.
61. Wolfe GI, Kaminski HJ, Jaretzki A 3rd, Swan A, Newsom-Davis J. Development of a thymectomy trial in nonthymomatous myasthenia gravis patients receiving immunosuppressive therapy. Ann N Y Acad Sci. 2003;998:473–80.
62. Group MS. A trial of mycophenolate mofetil with prednisone as initial immunotherapy in myasthenia gravis. Neurology. 2008;71(6):394–9.
63. Liu D, Ahmet A, Ward L, Krishnamoorthy P, Mandelcorn E, Leigh R, et al. A practical guide to the monitoring and management of the complications of systemic corticosteroid therapy. Allergy Asthma Clin Immunol. 2013;9(1):30.
64. Lewis SJ, Smith PE. Osteoporosis prevention in myasthenia gravis: a reminder. Acta Neurol Scand. 2001;103(5):320–2.
65. Narum S, Westergren T, Klemp M. Corticosteroids and risk of gastrointestinal bleeding: a systematic review and meta-analysis. BMJ Open. 2014;4(5):e004587.
66. Brown ES, Vera E, Frol AB, Woolston DJ, Johnson B. Effects of chronic prednisone therapy on mood and memory. J Affect Disord. 2007;99(1–3):279–83.
67. Perantie DC, Brown ES. Corticosteroids, immune suppression, and psychosis. Curr Psychiatry Rep. 2002;4(3):171–6.
68. Coutinho AE, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. Mol Cell Endocrinol. 2011;335(1):2–13.
69. Quax RA, Manenschijn L, Koper JW, Hazes JM, Lamberts SW, van Rossum EF, et al. Glucocorticoid sensitivity in health and disease. Nat Rev Endocrinol. 2013;9(11):670–86.
70. Palace J, Newsom-Davis J, Lecky B. A randomized double-blind trial of prednisolone alone or with azathioprine in myasthenia gravis. Myasthenia Gravis Study Group. Neurology. 1998;50(6):1778–83.
71. Heckmann JM, LeePan EB, Eastman RW. High-dose immunosuppressive therapy in generalised myasthenia gravis—a 2-year follow-up study. S Afr Med J. 2001;91(9):765–70.
72. Cosi V, Lombardi M, Erbetta A, Piccolo G. Azathioprine as a single immunosuppressive drug in the treatment of myasthenia gravis. Acta Neurol (Napoli). 1993;15(2):123–31.
73. Mantegazza R, Antozzi C, Peluchetti D, Sghirlanzoni A, Cornelio F. Azathioprine as a single drug or in combination with steroids in the treatment of myasthenia gravis. J Neurol. 1988;235:449–53.
74. Gupta A, Goyal V, Srivastava AK, Shukla G, Behari M. Remission and relapse of myasthenia gravis on long-term azathioprine: an ambispective study. Muscle Nerve. 2016;54(3):405–12.
75. Witte AS, Cornblath DR, Parry GJ, Lisak RP, Schatz NJ. Azathioprine in the treatment of myasthenia gravis. Ann Neurol. 1984;15:602–5.
76. Jack KL, Koopman WJ, Hulley D, Nicolle MW. A review of azathioprine-associated hepatotoxicity and myelosuppression in myasthenia gravis. J Clin Neuromuscul Dis. 2016;18(1):12–20.
77. Sanders D, Howard F Jr. Disorders of neuromuscular transmission. In: Bradley W, Daroff R, Fenichel G, Marsden C, editors. Neurology in clinical practice. Boston: Butterworth Heinemann; 2000. p. 2167–85.
78. Pedersen EG, Pottegard A, Hallas J, Friis S, Hansen K, Jensen PE, et al. Use of azathioprine for non-thymoma myasthenia and risk of cancer: a nationwide case-control study in Denmark. Eur J Neurol. 2013;20(6):942–8.
79. Machkhas H, Harati Y, Rolak LA. Clinical pharmacology of immunosuppressants: guidelines for neuroimmunotherapy. In: Rolak LA, Harati Y, editors. Neuroimmunology for the clinician. Boston: Butterworth Heinemann; 1997. p. 77–104.
80. Cleary BJ, Kallen B. Early pregnancy azathioprine use and pregnancy outcomes. Birth Defects Res A Clin Mol Teratol. 2009;85(7):647–54.
81. Lennard L. Implementation of TPMT testing. Br J Clin Pharmacol. 2014;77(4):704–14.

82. Maltzman JS, Koretzky GA. Azathioprine: old drug, new actions. J Clin Invest. 2003;111(8):1122–4.
83. Karran P, Attard N. Thiopurines in current medical practice: molecular mechanisms and contributions to therapy-related cancer. Nat Rev Cancer. 2008;8(1):24–36.
84. Ciafaloni E, Massey JM, Tucker-Lipscomb B, Sanders DB. Mycophenolate mofetil for myasthenia gravis: an open-label pilot study. Neurology. 2001;56(1):97–9.
85. Chaudhry V, Cornblath DR, Griffin JW, O'Brien R, Drachman DB. Mycophenolate mofetil: a safe and promising immunosuppressant in neuromuscular diseases. Neurology. 2001;56(1):94–6.
86. Meriggioli MN, Rowin J, Richman JG, Leurgans S. Mycophenolate mofetil for myasthenia gravis: a double-blind, placebo-controlled pilot study. Ann N Y Acad Sci. 2003;998:494–9.
87. Chan JW. Mycophenolate mofetil for ocular myasthenia. J Neurol. 2008;255(4):510–3.
88. Sanders DB, Hart IK, Mantegazza R, Shukla SS, Siddiqi ZA, De Baets MH, et al. An international, phase III, randomized trial of mycophenolate mofetil in myasthenia gravis. Neurology. 2008;71(6):400–6.
89. Benatar M, Rowland LP. The muddle of mycophenolate mofetil in myasthenia. Neurology. 2008;71(6):390–1.
90. Hobson-Webb LD, Hehir M, Crum B, Visser A, Sanders D, Burns TM. Can mycophenolate mofetil be tapered safely in myasthenia gravis? A retrospective, multicenter analysis. Muscle Nerve. 2015;52(2):211–5.
91. Coscia LA, Armenti DP, King RW, Sifontis NM, Constantinescu S, Moritz MJ. Update on the teratogenicity of maternal mycophenolate mofetil. J Pediatr Genet. 2015;4(2):42–55.
92. Buell C, Koo J. Long-term safety of mycophenolate mofetil and cyclosporine: a review. J Drugs Dermatol. 2008;7(8):741–8.
93. O'Neill JO, Edwards LB, Taylor DO. Mycophenolate mofetil and risk of developing malignancy after orthotopic heart transplantation: analysis of the transplant registry of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant. 2006;25(10):1186–91.
94. Vernino S, Salomao DR, Habermann TM, O'Neill BP. Primary CNS lymphoma complicating treatment of myasthenia gravis with mycophenolate mofetil. Neurology. 2005;65(4):639–41.
95. Termsarasab P, Katirji B. Opportunistic infections in myasthenia gravis treated with mycophenolate mofetil. J Neuroimmunol. 2012;249(1–2):83–5.
96. Villarroel MC, Hidalgo M, Jimeno A. Mycophenolate mofetil: an update. Drugs Today (Barc). 2009;45(7):521–32.
97. Minami N, Fujiki N, Doi S, Shima K, Niino M, Kikuchi S, et al. Five-year follow-up with low-dose tacrolimus in patients with myasthenia gravis. J Neurol Sci. 2011;300(1–2):59–62.
98. Zhao CB, Zhang X, Zhang H, Hu XQ, Lu JH, Lu CZ, et al. Clinical efficacy and immunological impact of tacrolimus in Chinese patients with generalized myasthenia gravis. Int Immunopharmacol. 2011;11(4):519–24.
99. Cruz JL, Wolff ML, Vanderman AJ, Brown JN. The emerging role of tacrolimus in myasthenia gravis. Ther Adv Neurol Disord. 2015;8(2):92–103.
100. Yoshikawa H, Kiuchi T, Saida T, Takamori M. Randomised, double-blind, placebo-controlled study of tacrolimus in myasthenia gravis. J Neurol Neurosurg Psychiatry. 2011;82(9):970–7.
101. Benatar M, Sanders D. The importance of studying history: lessons learnt from a trial of tacrolimus in myasthenia gravis. J Neurol Neurosurg Psychiatry. 2011;82(9):945.
102. Yagi Y, Sanjo N, Yokota T, Mizusawa H. Tacrolimus monotherapy: a promising option for ocular myasthenia gravis. Eur Neurol. 2013;69(6):344–5.
103. Kanai T, Uzawa A, Kawaguchi N, Himuro K, Oda F, Ozawa Y, et al. Adequate tacrolimus concentration for myasthenia gravis treatment. Eur J Neurol. 2017;24(2):270–5.
104. Azzi JR, Sayegh MH, Mallat SG. Calcineurin inhibitors: 40 years later, can't live without. J Immunol. 2013;191(12):5785–91.
105. MacMillan D. FK506 binding proteins: cellular regulators of intracellular Ca2+ signalling. Eur J Pharmacol. 2013;700(1–3):181–93.
106. Furukawa Y, Yoshikawa H, Iwasa K, Yamada M. Clinical efficacy and cytokine network-modulating effects of tacrolimus in myasthenia gravis. J Neuroimmunol. 2008;195(1–2):108–15.
107. Imai T, Tsuda E, Hozuki T, Yamauchi R, Saitoh M, Hisahara S, et al. Early effect of tacrolimus in improving excitation-contraction coupling in myasthenia gravis. Clin Neurophysiol. 2012;123(9):1886–90.
108. Pasnoor M, He J, Herbelin L, Burns TM, Nations S, Bril V, et al. A randomized controlled trial of methotrexate for patients with generalized myasthenia gravis. Neurology. 2016;87(1):57–64.
109. Heckmann JM, Rawoot A, Bateman K, Renison R, Badri M. A single-blinded trial of methotrexate versus azathioprine as steroid-sparing agents in generalized myasthenia gravis. BMC Neurol. 2011;11:97.
110. Wessels JA, Huizinga TW, Guchelaar HJ. Recent insights in the pharmacological actions of methotrexate in the treatment of rheumatoid arthritis. Rheumatology (Oxford). 2008;47(3):249–55.
111. Tindall RSA, Phillips JT, Rollins JA, Wells L, Hall K. A clinical therapeutic trial of cyclosporine in myasthenia gravis. Ann N Y Acad Sci. 1993;681:539–51.
112. Ciafaloni E, Nikhar NK, Massey JM, Sanders DB. Retrospective analysis of the use of cyclosporine in myasthenia gravis. Neurology. 2000;55(3):448–50.
113. Lavrnic D, Vujic A, Rakocevic-Stojanovic V, Stevic Z, Basta I, Pavlovic S, et al. Cyclosporine in the

treatment of myasthenia gravis. Acta Neurol Scand. 2005;111(4):247–52.
114. Hart IK, Sathasivam S, Sharshar T. Immunosuppressive agents for myasthenia gravis. Cochrane Database Syst Rev. 2007;(4):CD005224.
115. Tamler R, Epstein S. Nonsteroid immune modulators and bone disease. Ann N Y Acad Sci. 2006;1068:284–96.
116. Marder W, McCune WJ. Advances in immunosuppressive therapy. Semin Respir Crit Care Med. 2007;28(4):398–417.
117. Nagappa M, Netravathi M, Taly AB, Sinha S, Bindu PS, Mahadevan A. Long-term efficacy and limitations of cyclophosphamide in myasthenia gravis. J Clin Neurosci. 2014;21(11):1909–14.
118. Drachman DB, Brodsky RA. High-dose therapy for autoimmune neurologic diseases. Curr Opin Oncol. 2005;17(2):83–8.
119. Bryant A, Atkins H, Pringle CE, Allan D, Anstee G, Bence-Bruckler I, et al. Myasthenia gravis treated with autologous hematopoietic stem cell transplantation. JAMA Neurol. 2016;73(6):652–8.
120. Sistigu A, Viaud S, Chaput N, Bracci L, Proietti E, Zitvogel L. Immunomodulatory effects of cyclophosphamide and implementations for vaccine design. Semin Immunopathol. 2011;33(4):369–83.
121. Stieglbauer K, Pichler R, Topakian R. 10-year-outcomes after rituximab for myasthenia gravis: efficacy, safety, costs of inhospital care, and impact on childbearing potential. J Neurol Sci. 2017;375:241–4.
122. Tandan R, Hehir MK 2nd, Waheed W, Howard DB. Rituximab treatment of myasthenia gravis: a systematic review. Muscle Nerve. 2017;56(2):185–96.
123. Anderson D, Phan C, Johnston WS, Siddiqi ZA. Rituximab in refractory myasthenia gravis: a prospective, open-label study with long-term follow-up. Ann Clin Transl Neurol. 2016;3(7):552–5.
124. Robeson KR, Kumar A, Keung B, DiCapua DB, Grodinsky E, Patwa HS, et al. Durability of the rituximab response in acetylcholine receptor autoantibody-positive myasthenia gravis. JAMA Neurol. 2017;74(1):60–6.
125. Hehir M, Hobson-Webb L, Benatar M, Barnett C, Silvestri N, Howard J, et al. Rituximab is an effective treatment for anti-MuSK myasthenia gravis. Neurology. 2017;89(10):1069–77.
126. Chambers SA, Isenberg D. Anti-B cell therapy (rituximab) in the treatment of autoimmune diseases. Lupus. 2005;14(3):210–4.
127. Luu VP, Vazquez MI, Zlotnik A. B cells participate in tolerance and autoimmunity through cytokine production. Autoimmunity. 2014;47(1):1–12.
128. Kusner LL, Kaminski HJ. The role of complement in experimental autoimmune myasthenia gravis. Ann N Y Acad Sci. 2012;1274:127–32.
129. Howard JF Jr, Barohn RJ, Cutter GR, Freimer M, Juel VC, Mozaffar T, et al. A randomized, double-blind, placebo-controlled phase II study of eculizumab in patients with refractory generalized myasthenia gravis. Muscle Nerve. 2013;48(1):76–84.
130. Howard JF Jr, Utsugisawa K, Benatar M, Murai M, Barohn RJ, Illa I, Jacob S, Vissing J, Burns TM, Kissel JT, Muppidi S, Nowak RJ, O'Brien F, Wang JJ, Mantegazza R, Regain Study Group. Safety and efficacy of eculizumab in anti-acetylcholine receptor antibody-positive refractory generalised myasthenia gravis (REGAIN): a phase 3, randomised, double-blind, placebo-controlled, multicentre study. Lancet Neurol. 2017;16(12):976–86.
131. Licht C, Greenbaum LA, Muus P, Babu S, Bedrosian CL, Cohen DJ, et al. Efficacy and safety of eculizumab in atypical hemolytic uremic syndrome from 2-year extensions of phase 2 studies. Kidney Int. 2015;87(5):1061–73.
132. Ninomiya H, Obara N, Chiba S, Usuki K, Nishiwaki K, Matsumura I, et al. Interim analysis of post-marketing surveillance of eculizumab for paroxysmal nocturnal hemoglobinuria in Japan. Int J Hematol. 2016;104(5):548–58.
133. Nishimura J, Yamamoto M, Hayashi S, Ohyashiki K, Ando K, Brodsky AL, et al. Genetic variants in C5 and poor response to eculizumab. N Engl J Med. 2014;370(7):632–9.
134. Razzak M. Anaemia: mutations in C5 explain eculizumab resistance. Nat Rev Nephrol. 2014;10(4):182.
135. Guptill JT, Oakley D, Kuchibhatla M, Guidon AC, Hobson-Webb LD, Massey JM, et al. A retrospective study of complications of therapeutic plasma exchange in myasthenia. Muscle Nerve. 2013;47(2):170–6.
136. Mandawat A, Kaminski HJ, Shaker ZA, Alawi AA, Alshekhlee A. Outcome of plasmapheresis in myasthenia gravis: delayed therapy is not favorable. Muscle Nerve. 2011;43(4):578–84.
137. Kohler W, Bucka C, Klingel R. A randomized and controlled study comparing immunoadsorption and plasma exchange in myasthenic crisis. J Clin Apher. 2011;26(6):347–55.
138. Gajdos P, Chevret S, Toyka K. Plasma exchange for myasthenia gravis. Cochrane Database Syst Rev. 2002;(4):CD002275.
139. Haupt WF, Rosenow F, van der Ven C, Birkmann C. Immunoadsorption in Guillain-Barre syndrome and myasthenia gravis. Ther Apher. 2000;4(3):195–7.
140. Grob D, Simpson D, Mitsumoto H, Hoch B, Mokhtarian F, Bender A, et al. Treatment of myasthenia gravis by immunoadsorption of plasma. Neurology. 1995;45(2):338–44.
141. Shemin D, Briggs D, Greenan M. Complications of therapeutic plasma exchange: a prospective study of 1,727 procedures. J Clin Apher. 2007;22(5):270–6.
142. Vincent A. John Newsom-Davis: clinician-scientist and so much more. Brain. 2011;134(Pt 12):3755–74.
143. Barth D, Nabavi Nouri M, Ng E, Nwe P, Bril V. Comparison of IVIg and PLEX in patients with

myasthenia gravis. Neurology. 2011;76(23): 2017–23.
144. Mandawat A, Kaminski HJ, Cutter G, Katirji B, Alshekhlee A. Comparative analysis of therapeutic options used for myasthenia gravis. Ann Neurol. 2010;68(6):797–805.
145. Zinman L, Ng E, Bril V. IV immunoglobulin in patients with myasthenia gravis: a randomized controlled trial. Neurology. 2007;68(11):837–41.
146. Qureshi AI, Choudhry MA, Akbar MS, Mohammad Y, Chua HC, Yahia AM, et al. Plasma exchange versus intravenous immunoglobulin treatment in myasthenic crisis. Neurology. 1999;52(3):629–32.
147. Gajdos P, Chevret S, Toyka K. Intravenous immunoglobulin for myasthenia gravis. Cochrane Database Syst Rev. 2008;(1):CD002277.
148. Kazatchkine MD, Kaveri SV. Immunomodulation of autoimmune and inflammatory diseases with intravenous immune globulin. N Engl J Med. 2001;345:747–55.
149. Cunningham-Rundles C, Zhou Z, Mankarious S, Courter S. Long-term use of IgA-depleted intravenous immunoglobulin in immunodeficient subjects with anti-IgA antibodies. J Clin Immunol. 1993; 13(4):272–8.
150. Paran D, Herishanu Y, Elkayam O, Shopin L, Ben-Ami R. Venous and arterial thrombosis following administration of intravenous immunoglobulins. Blood Coagul Fibrinolysis. 2005;16(5):313–8.
151. Vucic S, Chong PS, Dawson KT, Cudkowicz M, Cros D. Thromboembolic complications of intravenous immunoglobulin treatment. Eur Neurol. 2004;52(3):141–4.
152. Luzi G, Bongiorno F, Paparo Barbaro S, Bruno G. Intravenous IgG: biological modulating molecules. J Biol Regul Homeost Agents. 2009;23(1): 1–9.
153. Zhu KY, Feferman T, Maiti PK, Souroujon MC, Fuchs S. Intravenous immunoglobulin suppresses experimental myasthenia gravis: immunological mechanisms. J Neuroimmunol. 2006;176(1–2):187–97.
154. Fuchs S, Feferman T, Meidler R, Margalit R, Sicsic C, Wang N, et al. A disease-specific fraction isolated from IVIG is essential for the immunosuppressive effect of IVIG in experimental autoimmune myasthenia gravis. J Neuroimmunol. 2008;194(1–2):89–96.
155. Norwood F, Dhanjal M, Hill M, James N, Jungbluth H, Kyle P, et al. Myasthenia in pregnancy: best practice guidelines from a U.K. multispecialty working group. J Neurol Neurosurg Psychiatry. 2014;85(5):538–43.
156. Batocchi AP, Majolini L, Evoli A, Lino MM, Minisci C, Tonali P. Course and treatment of myasthenia gravis during pregnancy. Neurology. 1999;52(3):447–52.
157. Plauché WC. Myasthenia gravis in mothers and their newborns. Clin Obstet Gynecol. 1991;34:82–99.
158. Ducci RD, Lorenzoni PJ, Kay CS, Werneck LC, Scola RH. Clinical follow-up of pregnancy in myasthenia gravis patients. Neuromuscul Disord. 2017;27(4):352–7.
159. Hoff JM, Daltveit AK, Gilhus NE. Myasthenia gravis in pregnancy and birth: identifying risk factors, optimising care. Eur J Neurol. 2007;14(1):38–43.
160. Ciafaloni E, Massey JM. The management of myasthenia gravis in pregnancy. Semin Neurol. 2004;24(1):95–100.
161. Kalidindi M, Ganpot S, Tahmesebi F, Govind A, Okolo S, Yoong W. Myasthenia gravis and pregnancy. J Obstet Gynaecol. 2007;27(1):30–2.
162. Wen JC, Liu TC, Chen YH, Chen SF, Lin HC, Tsai WC. No increased risk of adverse pregnancy outcomes for women with myasthenia gravis: a nationwide population-based study. Eur J Neurol. 2009;16(8):889–94.
163. O'Carroll P, Bertorini TE, Jacob G, Mitchell CW, Graff J. Transient neonatal myasthenia gravis in a baby born to a mother with new-onset anti-MuSK-mediated myasthenia gravis. J Clin Neuromuscul Dis. 2009;11(2):69–71.
164. Lee JY, Min JH, Han SH, Han J. Transient neonatal myasthenia gravis due to a mother with ocular onset of anti-muscle specific kinase myasthenia gravis. Neuromuscul Disord. 2017;27(7):655–7.
165. Townsel C, Keller R, Johnson K, Hussain N, Campbell WA. Seronegative maternal ocular myasthenia gravis and delayed transient neonatal myasthenia gravis. AJP Rep. 2016;6(1):e133–6.
166. Gardnerova M, Eymard B, Morel E, Faltin M, Zajac J, Sadovsky O, et al. The fetal/adult acetylcholine receptor antibody ratio in mothers with myasthenia gravis as a marker for transfer of the disease to the newborn. Neurology. 1997;48:50–4.
167. Hoff JM, Daltveit AK, Gilhus NE. Artrogryposis multiplex congenita—a rare fetal condition caused by maternal myasthenia gravis. Acta Neurol Scand Suppl. 2006;183:26–7.
168. Hacohen Y, Jacobson LW, Byrne S, Norwood F, Lall A, Robb S, et al. Fetal acetylcholine receptor inactivation syndrome: a myopathy due to maternal antibodies. Neurol Neuroimmunol Neuroinflamm. 2015;2(1):e57.
169. Midelfart Hoff J, Midelfart A. Maternal myasthenia gravis: a cause for arthrogryposis multiplex congenita. J Child Orthop. 2015;9(6):433–5.
170. Riemersma S, Vincent A, Beeson D, Newland C, Hawke S, Vernet-der Garabedian B, et al. Association of arthrogryposis multiplex congenita with maternal antibodies inhibiting fetal acetylcholine receptor function. J Clin Invest. 1996;98(10):2358–63.
171. Polizzi A, Huson SM, Vincent A. Teratogen update: maternal myasthenia gravis as a cause of congenital arthrogryposis. Teratology. 2000;62(5):332–41.
172. Brueton LA, Huson SM, Cox PM, Shirley I, Thompson EM, Barnes PR, et al. Asymptomatic maternal myasthenia as a cause of the Pena-Shokeir phenotype. Am J Med Genet. 2000;92(1):1–6.
173. Mao ZF, Mo XA, Qin C, Lai YR, Hackett ML. Incidence of thymoma in myasthenia gravis: a systematic review. J Clin Neurol. 2012;8(3):161–9.

174. Zielinski M. Management of myasthenic patients with thymoma. Thorac Surg Clin. 2011;21(1):47–57, vi.
175. Romi F, Gilhus NE, Aarli JA. Myasthenia gravis: disease severity and prognosis. Acta Neurol Scand Suppl. 2006;183:24–5.
176. Bril V, Kojic J, Dhanani A. The long-term clinical outcome of myasthenia gravis in patients with thymoma. Neurology. 1998;51(4):1198–200.
177. Evoli A, Minisci C, Di Schino C, Marsili F, Punzi C, Batocchi AP, et al. Thymoma in patients with MG: characteristics and long-term outcome. Neurology. 2002;59(12):1844–50.
178. Parr JR, Jayawant S. Childhood myasthenia: clinical subtypes and practical management. Dev Med Child Neurol. 2007;49(8):629–35.
179. Andrews PI. Autoimmune myasthenia gravis in childhood. Semin Neurol. 2004;24(1):101–10.
180. Della Marina A, Trippe H, Lutz S, Schara U. Juvenile myasthenia gravis: recommendations for diagnostic approaches and treatment. Neuropediatrics. 2014;45(2):75–83.
181. Matthews HJ, Thambundit A, Allen BR. Anti-MuSK-positive myasthenic crisis in a 7-year-old female. Case Rep Emerg Med. 2017;2017:8762302.
182. Al-Shekhlee A, Kaminski HJ, Ruff RL. Endocrine myopathies and muscle disorders related to electrolyte disturbance. In: Katirji B, Kaminski HJ, Preston D, Shapiro B, Ruff RL, editors. Neuromuscular disorders in clinical practice. Boston: Butterworth Heinemann; 2002. p. 1187–204.
183. Prudlo J, Koenig J, Ermert S, Juhasz J. Sleep disordered breathing in medically stable patients with myasthenia gravis. Eur J Neurol. 2007;14(3):321–6.
184. Nicolle MW, Rask S, Koopman WJ, George CF, Adams J, Wiebe S. Sleep apnea in patients with myasthenia gravis. Neurology. 2006;67(1):140–2.
185. Fernandes Oliveira E, Nacif SR, Alves Pereira N, Fonseca NT, Urbano JJ, Perez EA, et al. Sleep disorders in patients with myasthenia gravis: a systematic review. J Phys Ther Sci. 2015;27(6):2013–8.
186. Ambrogi V, Mineo TC. Benefits of comprehensive rehabilitation therapy in thymectomy for myasthenia gravis: a propensity score matching analysis. Am J Phys Med Rehabil. 2017;96(2):77–83.
187. Rahbek MA, Mikkelsen EE, Overgaard K, Vinge L, Andersen H, Dalgas U. Exercise in myasthenia gravis: a feasibility study of aerobic and resistance training. Muscle Nerve. 2017;56(4):700–9.

Neurocritical Care of Myasthenic Crisis

12

Jose Americo M. Fernandes and Jose I. Suarez

Introduction

Myasthenic crisis (MC) is defined as an exacerbation of myasthenia gravis (MG) weakness provoking an acute episode of respiratory failure that leads to institution of mechanical ventilation (MV). Weakness may involve respiratory, altering respiratory mechanics, or bulbar muscles, which compromise airway protection and patency. MC is the most dangerous complication of MG and is a life-threatening condition that requires immediate recognition and patient care in an intensive care unit setting for its optimal management [1, 2].

MC usually occurs within the first 2 years after disease onset (74% of patients) [3], and 15–20% of patients with MG will experience at least one episode of crisis. Its mortality has declined from 40% in the early 1960s to 5% in the 1970s, which most likely reflects improvement of ventilatory and general medical management of these patients in intensive care units. However, despite the decrease in mortality, the duration of MC has not changed significantly and continues to average 2 weeks [3]. This chapter reviews the most important aspects of MC including its pathophysiology, etiology, and management.

Pathophysiology

Precipitants

MC is most commonly precipitated by infections (30–40% of cases) [4, 5]. The most frequent are respiratory tract infections caused by viral or bacterial agents. Aspiration pneumonia occurs in about 10% of MC patients [4, 5] and may be more prevalent in patients with oropharyngeal weakness as manifested by difficulty in swallowing and chewing, altered facial expression, and dysarthria [6]. Certain medications exacerbate MG and lead to MC (Table 12.1) [7, 8]. It is crucial to obtain a reliable history of current medication use including specific inquiry of the patient, family, and friends of nonprescription drugs, including so-called alternative medicines. Telithromycin, an antibiotic of the ketolide class, has recently been reported to cause myasthenia exacerbation or crisis or unmasking of MG shortly after initial intake (minutes to a few days) which may be confused with an anaphylactic

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H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*, Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_12

Table 12.1 Drugs that exacerbate myasthenia gravis

Definite association	Corticosteroids
Probable association	*Antibiotics*: Aminoglycosides, ciprofloxacin, clindamycin, telithromycin *Antiarrhythmics*: Procainamide, propranolol, timolol *Neuropsychiatric*: Phenytoin, trimethadione, lithium
Possible association	*Antibiotics*: Ampicillin, imipenem/cilastatin, erythromycin *Antiarrhythmics*: Propafenone, verapamil, quinidine, *Miscellaneous*: Trihexyphenidyl, chloroquine, neuromuscular-blocking drugs, carbamazepine, oral contraceptives, transdermal nicotine

reaction. Extreme caution should be used when prescribing this medication to myasthenic patients [9, 10]. When treatment of MG was limited to anticholinesterase medications, overdose could lead to MC, but now is rare, and its importance previously may have been overstated [4, 11]. Other factors, which may lead to MC, include botulinum toxin injection [12], recent surgical procedures (including thymectomy), and trauma. In 30–40% of the patients, no trigger can be identified [5]. The presence of thymoma seems to be a risk factor for MC. MG patients with thymoma may have a more severe disease course (see Chap. 8), and thymoma patients are identified twice as often among patients in crisis compared to patients without thymoma (30% vs. 15%) [4]. Two relevant issues that have been highlighted recently are the presence of hypoalbuminemia upon hospital admission and that of post-thymectomy MC [13, 14]. Hypoalbuminemia upon hospital admission has been associated with more severe MG and MC [13]. It is likely that hypoalbuminemia is just a marker of overall burden of illness. However, until further evidence becomes available, it seems prudent to monitor serum albumin levels in all hospitalized MG patients, and if hypoalbuminemia is confirmed, closer monitoring is warranted. The most significant challenge in the management of MG patients undergoing thymectomy is the development of postoperative MC [14]. Several risk factors have been described for postoperative MC including the following: alcohol consumption prior to the surgical procedure, more severe MG symptoms, and postoperative complications such as atrial fibrillation, pneumothorax, and acute respiratory distress syndrome (ARDS). We would recommend screening for these factors and either avoiding or treating them aggressively to avoid MC or decrease hospital stay.

Respiratory Abnormalities

As weakness of respiratory and oropharyngeal muscles progresses, there is a decrease in lung expansion along with the efficiency of cough reflex to clear the airway. Patients' forced vital capacity (FVC) progressively decreases, making them prone to developing atelectasis and, eventually, to respiratory failure [4]. It is proposed that patients follow a stereotyped sequence of events leading to MC directly related to FVC [15]:

1. Normal respiratory function is present with FVC of 65 mL/kg.
2. Poor cough is evident with accumulation of secretions with FVC of 30 mL/kg.
3. With FVC of 20 mL/kg, the sigh mechanism is impaired with development of atelectasis and hypoxia.
4. Sigh is lost with FVC of 15 mL/kg and atelectasis and shunting appear.
5. Hypoventilation starts with FVC of 10 mL/kg.
6. Hypercapnia develops with FVC between 5 and 10 mL/kg.

Oropharyngeal Dysfunction

The oropharyngeal muscles maintain the patency of the upper airway by regulation of its cross-sectional area, and their dysfunction increases resistance to airflow [16]. Weakness of laryngeal muscles causes the vocal cords to remain adducted during inspiration, instead of the normal abduction [16, 17]. This creates the so-called

sail phenomenon. Paralyzed vocal cords, because of its position and curvature, catch the airflow during inspiration being pulled inward to midline like the sail of a boat [16]. Weakness of the tongue obstructs the oropharyngeal cavity. In addition to the mechanical obstruction of the airway, oropharyngeal dysfunction leads to an inability to protect from aspiration [6, 16].

Clinical Presentation and Evaluation

MC may occur in patients previously diagnosed but also may be the presenting event of MG. Patients with the definite diagnosis of MG that complain of worsening weakness or shortness of breath need to be assessed for dysphagia, stridor, and adequacy of ventilation. During the examination, the respiratory pattern requires assessment. Rapid, shallow breathing indicates respiratory muscle fatigue, which should not be confused with psychogenic hyperventilation [4]. Diaphragmatic function is assessed by observation of abdominal movements during the respiratory cycle. With severe weakness, a paradoxical respiratory pattern develops with inward movement of the abdomen during inspiration. The strength of neck muscles correlates with diaphragm strength, and weakness of these muscles should alert the clinician to possible respiratory compromise. A simple way to evaluate ventilatory reserve is by asking the patient to count from 1 to 25 in a single breath [4]. Patients with significant limitation should undergo measures of respiratory parameters.

Assessment of oropharyngeal muscles begins with inquiries of difficulty swallowing, episodes of choking, or coughing while eating. A wet, gurgling voice or stridor may indicate the need for intubation for airway protection [4]. Swallowing may be tested at bedside by observing the patient swallow three ounces of water and monitoring for coughing or choking [18]. However, caution should be taken in performing this assessment, and it need not be done in patients with clear signs of oropharyngeal weakness.

Patients may present with worsening of respiratory status because of vocal cord paralysis. In these patients, flexible laryngoscopy or flow volume loops should be performed [16, 17]. The finding of vocal cords in the adducted position should alert the physician for the possible need of a cricothomy in the case that conventional endotracheal intubation is not feasible [17].

In one series of 63 MC, initial manifestations were generalized weakness in 76% of patients, focal bulbar weakness in 19%, and isolated weakness of respiratory muscles in 5%. The majority of the patients (68%) required MV between 1 and 3 days after initial myasthenic exacerbation [1]. Some patients may present with respiratory failure without any evidence of generalized weakness [3, 16].

The respiratory status of patients at risk for MC should be monitored closely. Bedside measurements of forced vital capacity (FVC, normal ≥60 mL/kg), negative inspiratory force (NIF, normal >70 cm H_2O), and positive expiratory force (PEF, normal >100 cm H_2O) should be done serially. Arterial blood gases are also important, and pulse oximetry is not a substitute for this measurement. Blood gas assessments are abnormal (showing hypoxia and hypercarbia) with advanced respiratory dysfunction, but patients may have normal oxygenation by pulse oximetry, while arterial blood gases show developing hypercarbia, a sign of impending respiratory arrest.

Values of FVC ≤1.0 L (less than 15 mL/kg body weight), NIF < 20 cm H_2O, and PEF <40 cm H_2O are indications for institution of MV [4, 5, 19]. However, each patient should be considered individually, with assessment of comfort level, heart rate, respiratory rate, arterial blood gas values, and the ability to protect and maintain their airway. Respiratory dysfunction may progress rapidly and should be promptly recognized. Some independent predictors identified for the need to institute MV include abnormal chest roentgenogram on hospital admission (pneumonia or atelectasis, Fig. 12.1) and complications during hospitalization such as atelectasis, cardiac arrhythmia, and anemia requiring

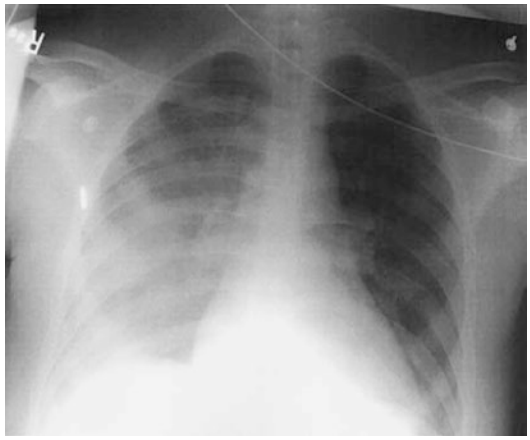

Fig. 12.1 Anteroposterior chest x-ray of a 33-year-old man with known MG for 1 year, who presented with worsening of generalized weakness, and dysphagia, followed by tachypnea and fever. The x-ray on admission revealed right lower lung pneumonia and left lower lobe atelectasis. The patient required mechanical ventilation within 12 h of admission

transfusion [20]. Early MV is preferable to prevent worsening atelectasis. Patients with low FVC but not to the level to warrant prompt intubation and those with independent predictors of MV should be admitted to an intensive care unit for serial measurements of the spirometry parameters. Cardiac arrhythmias are common among patients with MC (14–17% of patients) and are an additional reason for intensive care monitoring of patients with MC and evidence of impending MC [1, 21].

Differential Diagnosis

In any patient who is difficult to wean from a ventilator, MG should be considered; other possible disorders are Lambert-Eaton myasthenic syndrome, botulism, Guillain-Barré syndrome, polymyositis, motor neuron disease, critical illness myopathy/polyneuropathy, and organophosphate poisoning, among others (Table 12.2) [22–26]. Electrodiagnostic studies (Chap. 9) are instrumental in differentiating among these conditions and may guide additional specific evaluation as well as provide prognostic information [22].

Table 12.2 Neuromuscular disorders in critical care patients

Condition	Key presenting features
Acute intermittent porphyria	Asymmetric limb weakness progressing to quadriplegia after several attacks
Botulism	Nausea and vomiting preceding muscle weakness. Blurred vision, dysphagia, dysarthria, descending muscle paralysis, dilated pupils, dry mouth, constipation, and urinary retention
ICU-acquired weakness: Critical illness myopathy type	Patient with COPD or asthma requiring mechanical ventilation and use of neuromuscular blockers and corticosteroids; patients with sepsis, "critical" systemic illness, transplant patients
ICU-acquired weakness: Critical illness polyneuropathy type	Patient with sepsis and difficulty weaning from the ventilator, diminished or absent reflexes
Electrolyte imbalance	Generalized muscle weakness, cardiac arrhythmias with or without rhabdomyolysis
Guillain-Barré syndrome	Preceded by upper respiratory or gastrointestinal infection; ascending paralysis, areflexia
Lambert-Eaton myasthenic syndrome	Symmetric proximal muscle weakness, hypoactive or absent deep tendon reflexes, dry mouth, blurred vision, orthostatic hypotension
Lead poisoning	Pure motor weakness, initially of extensors muscles, fasciculations, abdominal pain, constipation, anemia, renal failure
Motor neuron disease	Weakness, wasting, fasciculations
Organophosphate poisoning	Exposure to insecticides, petroleum additives, and modifiers of plastics followed by acute cholinergic crisis (muscle weakness, myosis, abdominal cramping)
Polymyositis	Proximal, symmetrical muscle weakness, elevated creatine kinase
Prolonged neuromuscular blocking	Patient with impaired renal or hepatic failure who had been on continuous neuromuscular-blocking agents

Abbreviations: *COPD* Chronic obstructive pulmonary disease, *ICU* Intensive care unit

Management

General

To assure recovery of patients from MC, their overall medical condition must be optimized. Identified triggers should be removed or corrected. All patients should undergo extensive evaluation for infection including cultures of sputum, urine, and blood as well as other sites as clinically indicated. Empiric use of antibiotics needs to be carefully tailored to the clinical situation because of the potential for some antibiotics to further impair neuromuscular transmission further as well as the development of bacterial resistance. Avoidance of the unnecessary use of antibiotics is further emphasized by the observation that *Clostridium difficile* colitis complicates prolonged crisis [3, 4]. Nutritional assessment needs to be made early in the course of MC. A determination is made for the necessity of short-term nasogastric tube feeding, peripheral hyperalimentation, or a gastrostomy tube with the expectation that weakness may not resolve quickly. Electrolyte imbalance will compromise neuromuscular function, and close monitoring with appropriate correction required. Intermittent positive pressure breathing may be useful in preventing development of atelectasis, and if the patient is already intubated or with respiratory tract infection, aggressive pulmonary toilet is necessary. Deep venous thrombosis prophylaxis measures should be undertaken with the use of compression stockings, sequential compression devices, and subcutaneous heparin. Coagulation status may be deranged from heparin use when plasma exchange is performed, and intravenous immunoglobulin therapy may predispose to hypercoagulation. Gastrointestinal prophylaxis is given either with histamine receptor blockers or proton pump inhibitors to prevent stress ulcers and gastrointestinal bleeding. Psychological support for the patient and family is also important with emphasis on the ultimate ability to return most patients to an excellent functional level.

Ventilatory Management

Noninvasive bilevel positive pressure ventilation (BiPAP) is used for patients with chronic ventilatory insufficiency caused by neuromuscular disorders like muscular dystrophies, amyotrophic lateral sclerosis, and spinal muscular atrophy. It has also been shown to be useful in patients with acute respiratory insufficiency caused by disorders like MG. In one series, endotracheal intubation during episode of myasthenia crisis was successfully avoided in 70% of trials. In this series, the presence of hypercapnia ($PaCO_2 > 50$ mmHg) predicted BiPAP failure and need for intubation. The Emergency Neurological Life Support (ENLS) module for acute nontraumatic weakness recommends a trial of noninvasive positive ventilation as a temporizing measure in patients with MG exacerbation who are expected to recover quickly and in whom immunotherapy (see below) can be instituted promptly [27]. However, patients with severe ventilatory impairment and bulbar dysfunction should be excluded from a trial of noninvasive ventilation [28, 29].

Once the decision for MV is made, rapid sequence intubation should be performed [19, 27]. This entails bag-masking the patient to obtain arterial oxygen saturation of greater than 97%, administration of free running intravenous normal saline, continuous blood pressure monitoring, and bolus infusion of sedative medications (usually etomidate at 0.2–0.3 mg/kg). If short-acting muscle relaxants are needed (preferably they are to be avoided), non-depolarizing agents like vecuronium should be used. Oral intubation should be undertaken whenever possible.

Once the patient is intubated, a mode of ventilation needs to be chosen, but there is no perfect selection. The assist-control (AC) and synchronized intermittent mandatory ventilation (SIMV) are commonly used, each one with its advantages and disadvantages. Muscle fatigability with weakness and poor lung expansibility are the main determinants in the development of MC. Thus, the initial objectives of mechanical

ventilation should be to promote rest and expand the lungs. It was thought that, as long as the patient does not have primary lung disease compromising lung compliance, large tidal volumes (15 mL/kg) combined with lower rates to maintain a normal minute ventilation, with positive end-expiratory pressure at levels of 5–15, could be used, provided that peak airway pressures are maintained within acceptable limits (<40 cm H_2O) (3). However, the literature available in the past 10 years suggests that smaller tidal volumes (6–8 mL/kg of ideal body weight) with faster respiratory rates (12–16 breaths/min) should be used to avoid lung injury adding intermittent sighs (1.5 × tidal volume, three to four times every hour) to avoid atelectasis [30]. Such strategy has been called lung-protective ventilation which should achieve lung plateau pressures less than 30 cm H_2O allowing some permissive hypercarbia. Patients require continuous monitoring of end-tidal CO_2 to adjust respiratory rates to levels as close to normal as possible to avoid significant acid-base derangements.

Ventilator Weaning

Several parameters may be helpful in when to initiate weaning from MV: FVC >15 mL/kg, NIF < −30, PEF ≥40 cm H_2O, and minute ventilation <15 L/min [4, 19, 31]. However, these parameters have limited predictive power, and other general conditions should be satisfied before weaning is attempted [19]: (1) The patient needs to be adequately oxygenating with a PO_2 > 60 mmHg with FiO_2 40% and PEEP <5 cm H_2O. (2) The patient also needs to have intact respiratory drive (in patients with MG, respiratory drive is found not to be impaired [32]), to be able to protect their airway, and have an adequate cough reflex. (3) The hemodynamic status should be stable, electrolyte levels normal, and nutritional status adequate. (4) The patient should be free of infection or other significant medical complications. (5) The need for respiratory suctioning should be less than every 2–3 h.

For MG patients, one major and initial indicator of timing for MV weaning is the improvement of general muscle strength by objective physical examination. The rapid shallow breathing index is thought to be the best predictor of successful weaning [19] and is calculated by division of the tidal volume by the respiratory rate of the patient while temporarily off the ventilator. Patients with index values greater than 105 have a 95% likelihood of failing a weaning trial [31].

Once patients meet criteria for extubation, the method chosen for a weaning trial varies according to an individual physician's clinical experience. One method is a daily trial of continuous positive airway pressure (CPAP) and pressure support levels of 5–15 cm H_2O. If the patient remains comfortable after 1–4 h, the level of pressure support can be decreased by 1–3 cm H_2O each day. Patients should be returned to the original ventilatory mode overnight or when signs of fatigue appear [4].

The SIMV mode is another method of weaning. It is accomplished by decreasing the ventilatory rate by 2–3 breaths once or more times a day based on patient comfort level [19]. An increase in respiratory rate, fall in tidal volumes, agitation, and tachycardia may all be indicators of fatigue [4]. Once a patient has demonstrated good endurance (the number of hours a patient tolerates the weaning mode with minimal support, usually more than 2 h), and general conditions are adequate, the patient may be extubated.

Extubation should be performed early in the day. Stridor may occur immediately to 1 h after extubation, and aerosolized racemic epinephrine may reverse the condition, but reintubation might be necessary. Laryngospasm is less common but is a life-threatening condition.

Treatment of Neuromuscular Dysfunction

Therapies may be divided into those that improve strength rapidly with a short duration of action and those that improve strength slowly with a more permanent response. In the first category, there are acetylcholinesterase inhibitors (AchI), plasmapheresis (PLEX), and intravenous immunoglobulin (IVIg) (Chap. 11). The second cate-

gory comprises immunosuppressive agents such as corticosteroids, azathioprine, cyclophosphamide, cyclosporine, mycophenolate, and tacrolimus [30]. The latter are not used in rescuing patients from MC but in the long-term management. Corticosteroid treatment during MC is debated. Some advocate their use during crisis in patients only with refractory weakness, and others suggest that they be started while the patient is treated with IVIg or PLEX [4, 5, 33–38]. The Myasthenia Gravis Foundation of America appointed a Task Force to develop treatment guidance for MG, including MC, and a panel of 15 international experts was convened [39]. These published guidelines should help streamline management of MC patients.

Cholinesterase inhibitors provide symptomatic improvement by means of decreasing the degradation of acetylcholine at the neuromuscular junction. The continuous intravenous infusion of pyridostigmine is reported as an effective treatment in MC compared to plasmapheresis as measured by mortality, duration of ventilation, and outcome. Its use has been defended especially when MC is triggered by an infection [1]. However, it is common practice to discontinue these agents in mechanically ventilated patients due to the propensity for promoting excess respiratory secretions and mucous plugging [4]. Another concern is the reported occurrence of cardiac arrhythmias in MC patients, including those receiving intravenous pyridostigmine [1, 21]. Cholinesterase inhibitors could increase cholinergic activity at cardiac muscarinic synapses leading to arrhythmias.

PLEX has traditionally been the preferred treatment in managing MC, with a reported efficacy of 75% [4]. Its mechanism of action is related to the removal of circulating factors, in addition to acetylcholine receptor (AChR) antibodies. Even though AChR antibody titers do not correlate well with MG severity, the decrease of AChR antibodies titers correlates with clinical improvement. There is no standard protocol for PLEX. The usual regimen is exchange of 1–1.5 plasma volumes every day or every other day for five to six treatments. Clinical improvement may be seen as early as 24 h, but in most patients, the first effect appears after two to three sessions of PLEX. Some patients may have an initial worsening thought to be due to reduction in plasma concentration of cholinesterase. The duration of effect is usually less than 10 weeks if the other immunosuppressive treatment is not instituted [35–38].

The most frequent complications of PLEX are hypotension, electrolyte imbalance (reduction of calcium, potassium, and magnesium), depletion of clotting factors, and thrombocytopenia. Obtaining vascular access may at times be difficult and may be complicated pneumothorax, thrombosis, and infection [4, 35–39]. When a large volume of plasma is removed, intravascular volume should be replaced with albumin in saline solution. Any electrolyte imbalance should be corrected to prevent exacerbating weakness. The coagulopathy is usually mild but should be kept in mind when utilizing subcutaneous heparin for deep venous thrombosis prophylaxis. Reducing anti-hypertensive drug dosages and administering intravenous fluids before the procedure may avoid hypotension.

Another option for specific treatment of MC is IVIg. A standard course consists of 400 mg/kg/day of IVIg for 5 days [33, 38]. Response to IVIg usually is observed 5 days after treatment initiation. There is a general consensus that IVIg may be as effective as PLEX. However, IVIg may be a better choice in patients with hemodynamic instability, vascular access problems, or with a poor response to PLEX. Adverse effects occur in fewer than 10% of patients and most commonly include headache, chills and fever, fluid overload, and rarely renal failure. Anaphylaxis may occur to IgA components in patients with IgA deficiency. IgA levels and baseline renal function should be obtained before treatment is initiated.

The Myasthenia Gravis Foundation Task Force agreed that although clinical trials suggest that IVIg and PLEX are equally effective in the treatment of impending or manifest MC, the panel of expert's consensus was that PLEX is more effective and works more quickly. They also indicated that the choice between the two therapies depends on patient comorbidities (e.g., PLEX cannot be used in sepsis and IVIg is con-

traindicated in hypercoagulable states, renal failure, or hypersensitivity to immunoglobulin) and other factors, including availability [39].

The use of preoperative PLEX is advocated in patients with generalized MG and preoperative symptoms of oropharyngeal or respiratory weakness, or in patients with symptoms controlled by use of cholinesterase inhibitors, in order to prevent delayed extubation and reintubation. IVIg has also been used preoperatively, but the variability in reaching maximal response (3–19 days) should be kept in mind [2].

Outcome of Myasthenic Crisis

One study identified three main independent predictors of prolonged MV: age > 50, pre-intubation serum bicarbonate ≥30, and peak vital capacity <25 mL/kg at day 1–6 post-intubation. The proportion of patients intubated for more than 2 weeks was 88% in patients with three risk factors, 46% with two, 21% with one, and zero for patients with no risk factors. The same study also revealed that atelectasis, anemia requiring transfusion, *Clostridium difficile* infection, and congestive heart failure were complications associated with prolonged intubation [3]. Tracheostomy is usually performed after 2 weeks of intubation, but early tracheostomy is recommended for patients expected to require prolonged MV. Tracheostomy is more comfortable for the patient, reduces the risk of tracheolaryngeal stenosis, permits more effective suctioning of tracheal secretions, and facilitates weaning from MV due to reduction in dead space and in resistance to airflow from endotracheal tube [4]. In general, 25% of patients will be extubated by 1 week, 50% by 2 weeks, and 75% by 1 month. Intubation and MV for more than 2 weeks is associated with a threefold increase in hospital stay (median 63 days) and a twofold increase in likelihood of functional dependency at discharge [3]. One-third of patients who survive the first crisis will have a second episode [4].

Although crisis is potentially a lethal event, and is always devastating for patient and loved ones, optimizing critical care and long-term treatment of MG has prevented the event from being "grave," as it once was.

References

1. Berrouschot J, Baumann I, Kalischewski P, Sterker M, Schneider D. Therapy of myasthenic crisis. Crit Care Med. 1997;25:1228–35.
2. Vern JC. Myasthenia gravis: management of myasthenic crisis and preoperative care. Semin Neurol. 2004;24:75–81.
3. Thomas CE, Mayer SA, Gungor Y, Swarup R, Webster EA, Chang I, et al. Myasthenic crisis: clinical features, mortality, complications, and risk factors for prolonged intubation. Neurology. 1997;48:1253–60.
4. Mayer SA. Intensive care of the myasthenic patient. Neurology. 1997;48(Suppl 5):S70–5.
5. Bedlack RS, Sanders DB. How to handle myasthenic crisis. Postgrad Med. 2000;107(4):211–4, 220–2.
6. Weijnen FG, Van Der Bilt A, Wokke JHJ, Wassenberg MWM, Oudenaarde I. Oral functions of patients with myasthenia gravis. Ann N Y Acad Sci. 1998;841:773–6.
7. Wittbrodt ET. Drugs and myasthenia gravis. Arch Intern Med. 1997;157:399–408.
8. Pascuzzi RM. Medications and myasthenia gravis: a reference for health care professionals. http://www.myathenia.org/drugs/reference.htm.
9. Perrot X, Bernard N, Vial C, Antoine JC, Laurent H, Vial T, et al. Myasthenia gravis exacerbation or unmasking associated with telithromycin treatment. Neurology. 2006;67:1–3.
10. Jennet AM, Bali D, Jasti P, Shah B, Browning LA. Telithromycin and myasthenia crisis. Clin Infect Dis. 2006;43:1621–2.
11. Grob D. Natural history of myasthenia gravis. In: Engel AG, editor. Myathenia gravis and myasthenic disorders. New York: Oxford University Press; 1999. p. 131–45.
12. Borodic G. Myasthenic crisis after botulinum toxin. Lancet. 1998;352:1832.
13. Weng YY, Yang DH, Qian MZ, Wei MM, Yin F, Li J, et al. Low serum albumin concentrations are associated with disease severity in patients with myasthenia gravis. Medicine. 2016;95(39):e5000.
14. Li KK, Qian K, Feng YG, Guo W, Tan QY, Deng B. Predictive factors of prolonged mechanical ventilation, overall survival, and quality of life in patients with post-thymectomy myasthenic crisis. World J Surg Oncol. 2017;15:150. https://doi.org/10.1186/s12957-017-1209-1.
15. Ropper AH. The Guillain-Barré syndrome. N Engl J Med. 1992;326:1130–6.
16. Putman MT, Wise RA. Myasthenia gravis and upper airway obstruction. Chest. 1996;109:400–4.
17. Cridge PB, Allegra J, Gerhard H. Myasthenic crisis presenting as isolated vocal cord paralysis. Am J Emerg Med. 2000;18:232–3.

18. DePippo KL, Holas MA, Reding MJ. Validation of the 3 oz water swallow test for aspiration after stroke. Arch Neurol. 1992;49:1259–61.
19. McDonagh DL, Borel CO. Ventilatory management in the neurosciences critical care unit. In: Suarez JI, editor. Critical care neurology and neurosurgery. Totowa: Humana Press; 2004. p. 151–66.
20. Suarez JI, Boonyapisit K, Zaidat OO, Kaminski HJ, Ruff RL. Predictors of mechanical ventilation in patients with myasthenia gravis exacerbation [abstract]. J Neurol Sci. 2001;187:S464.
21. Mayer SA, Thomas CE. Therapy of myasthenic crisis. Crit Care Med. 1998;26:1136.
22. Maher J, Rutledge F, Remtulla H, Parkes A, Bernardi L, Bolton CF. Neuromuscular disorders associated with failure to wean from the ventilator. Intensive Care Med. 1995;21:737–43.
23. Vita G, Girlanda P, Puglisi RM, Marabello L, Messina C. Cardiovascular-reflex testing and single fiber eletromyography in botulism. Arch Neurol. 1987;44:202–6.
24. Suarez JI, Cohen ML, Larkins J, Kernich CA, Hricick DE, Daroff RB. Acute intermittent porphyria. Clinicopathologic correlation. Report of a case and review of the literature. Neurology. 1997;48:1678–83.
25. Torbey MT, Suarez JI, Geocadin R. Less common causes of quadriparesis and respiratory failure. In: Suarez JI, editor. Critical care neurology and neurosurgery. Totowa: Humana Press; 2004. p. 493–513.
26. Bosch EP, Smith BE. Disorders of peripheral nerves. In: Bradley WG, Daroff RB, Fenichel GM, Marsden CD, editors. Neurology in clinical practice, vol. 2. 3rd ed. Boston: Butterworth-Heineman; 2000. p. 2045–130.
27. Flower O, Wainwright MS, Caulfield AS. Emergency neurological life support: acute non-traumatic weakness. Neurocrit Care. 2015;23(Suppl 2):S23–47. https://doi.org/10.1007/s12028-015-0160-7.
28. Rabinstein A, Wijdicks FM. BiPAP in acute respiratory failure due to myasthenic crisis may prevent intubation. Neurology. 2002;59:1647–9.
29. Agarwal R, Reddy C, Gupta D. Noninvasive ventilation in acute neuromuscular respiratory failure due to myasthenic crisis: case report and review of literature. Emerg Med J. 2006;23:e6.
30. The Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. N Engl J Med. 2000;342:1301–8.
31. Wijdicks FM. Management of airway and mechanical ventilation. In: Wijdicks FM, editor. The clinical practice of critical care neurology. Philadelphia: Lippincott-Raven; 1997. p. 25–45.
32. Borel CO, Teitelbaum JS, Hanley DF. Ventilatory drive and carbon dioxide response in ventilatory failure due to myasthenia gravis and Guillain-Barré syndrome. Crit Care Med. 1993;21:1717–26.
33. Kokontis L, Gutmann L. Current treatment of neuromuscular diseases. Arch Neurol. 2000;57:939–43.
34. Seybold ME. Treatment of myasthenia gravis. In: Engel AG, editor. Myasthenia gravis and myasthenic disorders. New York: Oxford University Press; 1999. p. 167–91.
35. Batocchi AP, Evoli A, Di Schino C, Tonali P. Therapeutic apheresis in myasthenia gravis. Ther Apher. 2000;4:275–9.
36. Gajdos P, Chevret S, Clair B, Tranchant C, Chastang C. Clinical trial of plasma exchange and high-dose intravenous immunoglobulin in myasthenia gravis. Ann Neurol. 1997;41:789–96.
37. Qureshi AI, Choudhry MA, Akbar MS, Mohammad Y, Chua HC, Yahia AM, et al. Plasma exchange versus intravenous immunoglobulin treatment in myasthenic crisis. Neurology. 1999;52:629–32.
38. Stricker RB, Kwiatkowska BJ, Habis JA, Kiprov DD. Myasthenic crisis. Response to plasmapheresis following failure of intravenous γ-globulin. Arch Neurol. 1993;50:837–40.
39. Sanders DB, Wolfe GI, Benatar M, Evoli A, Gilhus NE, Illa I, et al. International consensus guidance for management of myasthenia gravis. Neurology. 2016;87:419–25.

Thymectomy for Non-thymomatous Myasthenia Gravis

13

Joshua R. Sonett, Bianca Bromberger, and Alfred Jaretzki III

Introduction

Despite having been employed for over 75 years in the treatment of myasthenia gravis (MG), evidence for the effectiveness of thymectomy has until only recently been limited to observational data. The publication of the first randomized prospective study of thymectomy in non-thymomatous myasthenia gravis unequivocally demonstrates the benefit of surgical removal of thymic tissue in the treatment of this disease [1]. However, many critical issues remain, including patient selection, timing of surgery, perioperative management, appropriate surgical approach, and role of thymectomy in the various subtypes of MG. While many studies that address such issues exist in the current literature, current practice recommendations are drawn from retrospective data rather than controlled prospective studies.

In addition to reviewing the surgical anatomy of the thymus, various thymectomy techniques, and their outcomes, this chapter will attempt to summarize remaining controversies as well as make recommendations based on available evidence regarding surgical management of non-thymomatous MG.

Total Thymectomy Is Indicated

Even before the role of the thymus in MG was understood, "total" thymectomy was considered the goal of surgery. In 1941 Blalock wrote "complete removal of all thymic tissue offers the best chance of altering the course of the disease" [2], and this has been reiterated by most leaders in the field [3–16]. Pathologic and immunologic studies have since clearly demonstrated that the thymus plays a central role in the autoimmune pathogenesis of MG [17–19]. However, its pathologic function may vary between the different antibody subtypes (anti-AChR, anti-MuSK, or seronegative) [20, 21], with important clinical implications regarding which patients with MG benefit from thymectomy.

When thymectomy is undertaken in the treatment of non-thymomatous MG, the concept that the entire thymus should be removed is supported by animal models and by clinical experience. In an animal model of myasthenia gravis, complete neonatal thymectomy in rabbits

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© Springer International Publishing AG, part of Springer Nature 2018
H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*, Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_13

prevents experimental autoimmune myasthenia gravis, whereas incomplete removal does not [22]. In humans, both incomplete transcervical and incomplete transsternal resections have been followed by persistent symptoms that were later relieved by a more extensive reoperation with the finding of residual thymus [23–29]. And removal of as little as 2 g of residual thymus has been therapeutic [27]. In addition, several studies comparing aggressive thymic resections with limited resections support the premise that the entire thymus should be removed [30, 31].

Surgical Anatomy of the Thymus

Since complete removal of the thymus appears indicated when a thymectomy is performed in the treatment of non-thymomatous MG, its anatomy should be understood by all those involved in the treatment of these patients and in the analysis of the results of the surgery. Importantly, the thymus is not "two well-defined lobes that appear almost as distinct as do the two lobes of the thyroid" as described by Blalock [2].

Detailed surgical-anatomical studies (Fig. 13.1) have demonstrated that the thymus

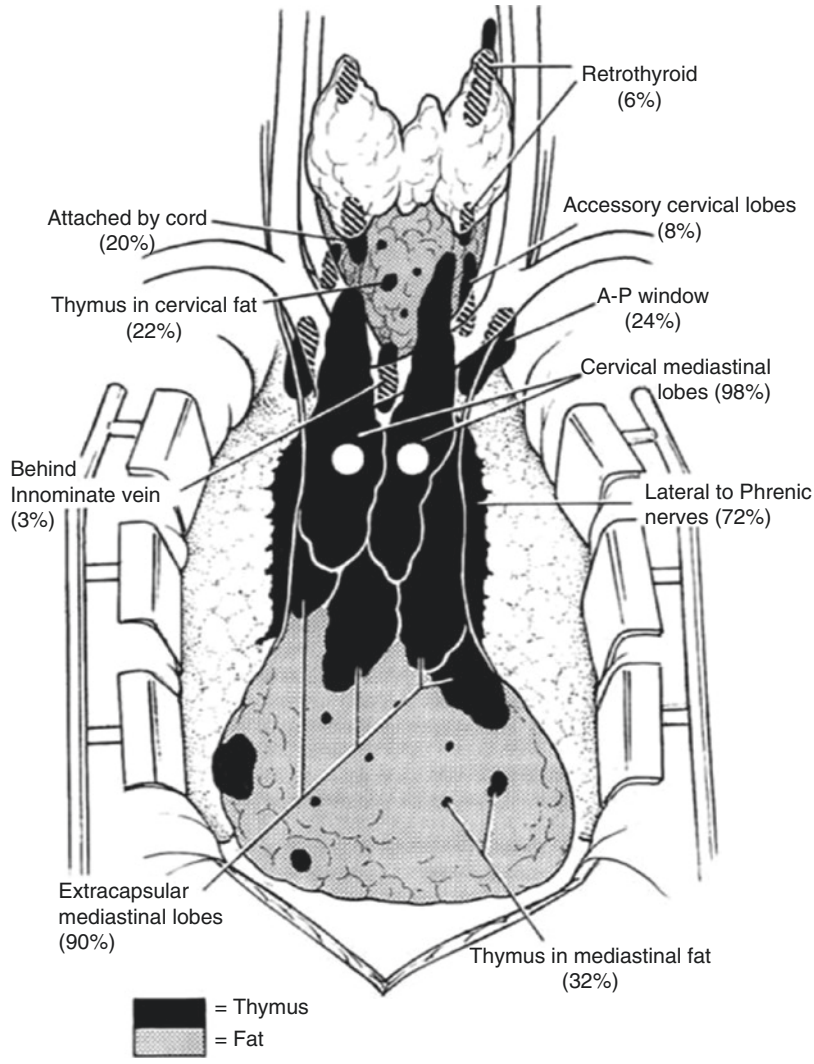

Fig. 13.1 Composite anatomy of the thymus. This illustration represents what is now accepted as the surgical anatomy of the thymus. The frequencies (percentages of occurrence) of the variations are noted. Thymus was found outside the confines of the two classical cervical-mediastinal lobes (A and B) in the neck in 32% of the specimens, in the mediastinum in 98% (black, thymus; gray, fat, which may contain islands of thymus and microscopic thymus) [37]. Reprinted with permission from Jaretzki A, Thymectomy for myasthenia gravis: Analysis of the controversies regarding technique and results, Neurology, 48, Suppl 5, S52–S63, http://www.neurology.org/content/48/Suppl_5/52S.extract

frequently consists of multiple lobes in the neck and mediastinum, often separately encapsulated, and these lobes may not be contiguous. In addition, unencapsulated lobules of thymus and microscopic foci of thymus may be widely and invisibly distributed in the pretracheal and anterior mediastinal fat from the level of the thyroid, and occasionally above, to the diaphragm and bilaterally from beyond each phrenic nerve [32, 33]. Occasionally, microscopic foci of thymic tissue have been found in the subcarinal fat [34].

Surgical Techniques and Their Resectional Potential

The use of a standardized thymectomy classification system is necessary in order to compare outcomes between different techniques. Accordingly, we rely on the system developed by the Myasthenia Gravis Foundation of America (MGFA), with the understanding that the classification system will require continual modification as new more minimally invasive techniques are introduced, including novel approaches that are in essence a combination of those previously described (Table 13.1) [35, 36]. These hybrid operations, utilizing a combination of cervical, thoracoscopic, and infrasternal approaches with or without the use of robotic technology, are beginning to defy easy categorization as set out by the MGFA guidelines. Rather than becoming mired in the semantics of classification, the focus should remain on studies clearly describing operative techniques, so that as long-term, high-quality outcomes data become available, the various approaches can be accurately compared and the benefit of additional exposure or technology evaluated.

If total thymectomy should remain the goal in the surgical treatment of MG, an understanding of how much gross and microscopic thymus each resectional technique is capable of removing is necessary. The following presents a basic description of the resection technique and estimates of what each type of resection can accomplish. These estimates are based on published reports, drawings, photographs of resected specimens, videos of the procedures when available, and our personal experience. The videos taken at operation and photographs of the resected specimens are frequently the most revealing. A review of what appears to be the potential of each thymectomy technique strongly suggests that all resections are not equal in extent [37].

Regardless of the selected technique, however, when complete removal of all thymic tissue is the goal of the thymectomy technique employed, the actual extent of each individual resection is determined in part by the operating surgeon's conviction that as much thymus that can be removed safely should be removed, the surgeon's commitment to take the time to do so meticulously, and the surgeon's experience with the technique employed. In that respect, while a classification system is necessary in order to compare outcomes, the ultimate question is not which resectional technique is best, but whether or not a particular approach allows for total thymectomy *when properly performed*.

Table 13.1 MGFA thymectomy classification (italics signifying modification from the original classification [35])

T-1 transcervical thymectomy
(a) Basic
(b) Extended
(c) Extended with partial sternal split
(d) Extended with videoscopic technology
T-2 videoscopic thymectomy
(a) Classic VATS (unilateral)
(b) VATET (bilateral with cervical incision)
(c) Bilateral VATS (no cervical incision)
(d) Videoscopic with Robotic Technology (unilateral or bilateral)
T-3 transsternal thymectomy
(a) Standard
(b) Extended
T-4 Transcervical and transsternal thymectomy
T-5 Infrasternal thymectomy
(a) Combined transcervical-subxiphoid
(b) Videoscopic subxiphoid (uni- or multiportal)
(c) Videoscopic subxiphoid with robotic technology

Combined Transcervical and Transsternal Thymectomy (T-4)

Combined transcervical–transsternal maximal thymectomy [33], also known as extended cervico-mediastinal thymectomy [38] or "maximal" thymectomy, is considered the benchmark operation against which other resectional procedures should be measured [11]. Its design is based on the surgical anatomy of the thymus and employs both a complete median sternotomy and a cervical incision to achieve wide exposure in the neck [39]. An en bloc resection is used, removing in a single specimen all gross thymus, suspected cervical-mediastinal thymus, and anterior cervical-mediastinal fat. Sharp dissection is utilized in the removal of the specimen with both sheets of mediastinal pleura and on the pericardium, as blunt dissection may leave microscopic foci of the thymus on these structures [40]. Extreme care is taken to protect the recurrent laryngeal, left vagus, and phrenic nerves.

These resections are exenteration in extent and are "performed as if it were an en bloc dissection for a malignant tumor" so as ensure that islands of thymus are not left behind and to guard against the potential of seeding of thymus in the wound [41]. It predictably removes all surgically available thymus in the neck and mediastinum, including the unencapsulated lobes and the lobules of the thymus and microscopic thymus in the pre-cervical and anterior mediastinal fat (an estimated 98–100% of thymic tissue).

Transsternal Thymectomies (T-3)

Standard Transsternal Thymectomy (T-3a)

The standard transsternal thymectomy used by the pioneers Blalock, Keynes, and Clagett [2, 3, 42, 43] was originally limited to removal of the well-defined cervical-mediastinal lobes that were thought to be the entire gland [44]. Currently, although a complete or partial [45–48] sternotomy may be performed, the resection is more extensive than originally described and includes removal of all visible mediastinal thymic lobes. Mediastinal fat, varying in extent, may or may not be removed. The cervical extensions of the thymus are removed from below, with or without some adjacent cervical fat.

This technique appears to fall short of total thymectomy as the residual thymus has been found in the neck and in the mediastinum at reoperation following a standard transsternal thymectomy [26–28]. Accordingly, many consider the standard transsternal thymectomy incomplete and no longer use it for the treatment of MG [49, 50].

Extended Transsternal Thymectomy (T-3b)

The extended transsternal thymectomy [51, 52] is also known as aggressive transsternal thymectomy [53] or transsternal radical thymectomy [54]. The extent of these resections varies in the mediastinum, but is more extensive than the standard transsternal thymectomy. Ideally, the extended technique is identical to the mediastinal dissection of the "maximal" T-4 procedure [51]. However, similar to the standard transsternal technique, cervical thymic extensions are removed from below, with or without some additional cervical tissue, but without a formal neck dissection.

While the extended transsternal technique may approximate the mediastinal resection achieved with a "maximal" T-4 approach, they remove less tissue in the neck (where a small amount of thymic tissue is present in approximately 30% of specimens) [33]. Mulder has expressed the view, however, that the risk to the recurrent laryngeal nerves in performing the extensive neck dissection of the combined transcervical–transsternal maximal thymectomy is not "justified by the small potential gain" [55].

Transcervical Thymectomies (T-1)

Basic Transcervical Thymectomy (T-1a)

The basic transcervical thymectomy employs an intracapsular extraction of the mediastinal thymus via a small cervical incision and is limited to the removal of the intracapsular portion of the central cervical-mediastinal lobes. No other tissue is removed in the neck or mediastinum [5, 56,

57]. Although originally considered to be a "total thymectomy" [5], the basic transcervical thymectomy is unequivocally an incomplete resection in both the neck and the mediastinum as evidenced by the findings of residual thymus during reoperations [23–29].

Extended Transcervical Thymectomy (T-1b)

The extended transcervical thymectomy [58–60] employs a special manubrial retractor for improved exposure of the mediastinum via a cervical incision. The mediastinal dissection is extracapsular and includes resection of the visible mediastinal thymus and mediastinal fat. Sharp dissection may or may not be performed on the pericardium. The mediastinal pleural sheets may be included in the resection but usually are not. The neck exploration and dissections vary in extent and may or may not be limited to exploration and removal of the cervical-mediastinal extensions of the thymus.

The extended transcervical thymectomy resection, as warned by Cooper [58], when performed by others may be less extensive than the procedure described by him.

Extended Transcervical Thymectomy Variations (T-1c-d)

The extended transcervical thymectomy has been modified to include a partial median sternotomy (T-1c) [61] and use of videoscopic technology (T-1d) to aid in visualization and dissection of the mediastinum. Video-assisted variations include addition of transcervical thoracoscopy [62, 63] or a subxiphoid videoscopic inferior approach (described below in Infrasternal Thymectomies) [64].

Videoscopic-Assisted Thymectomies (T-2)

Classic VATS (Unilateral) (T-2a)

The video-assisted thoracic surgery (VATS) thymectomy employs unilateral videoscopic exposure of the mediastinum (right or left) with removal of the grossly identifiable thymus and variable amounts of anterior mediastinal fat. Sharp dissection may or may not be used on the pericardium, and the mediastinal pleural sheets are not routinely removed with the specimen. Complete removal of the mediastinal and diaphragmatic fat on the operative side is routinely performed. The cervical extensions of the thymus are usually removed from below [16, 64–69].

The VATS resections, based on the published reports, vary in extent in the mediastinum. They appear to be more extensive than the standard transsternal resections. Since it is unilateral, left or right, the contralateral side of the mediastinum does not appear to be as well visualized as the operative side.

Video-Assisted Thoracoscopic Extended Thymectomy (Bilateral with Cervical Incision) (T-2b)

The video-assisted thoracoscopic extended thymectomy (VATET) uses bilateral thoracoscopic exposure for improved visualization of both sides of the mediastinum as well as a possible cervical incision for exposure of the recurrent laryngeal nerves and removal of the cervical thymic lobes and pretracheal fat under direct vision [70, 71]. Extensive removal of the mediastinal thymus and peri-thymic fat is described, the thymus and fat being removed separately. Sharp dissection may or may not be used on the pericardium. The mediastinal pleural sheets are usually not removed. Modifications to VATET include addition of an anterior chest wall-lifting method [72]. The VATET operation is conceptually more complete than the unilateral VATS since it offers excellent visualization of both sides of the mediastinum and includes a neck dissection as well.

Bilateral VATS (No Cervical Incision) (T-2c)

Bilateral VATS thymectomy without a cervical incision has also been described [73, 74], with reportedly similar resection capability to extended transsternal thymectomies (T-3b) [73]. Notably, bilateral VATS thymectomy without a cervical incision is occasionally referred to as VATET in the literature, though the two procedures may differ in their resectional extent.

A study designed to evaluate bilateral VATS thymectomy with and without transcervical neck dissection observed that between 2 and 3% of thymic tissue may be left in the absence of additional cervical approach [75]. As such, we believe bilateral VATS without cervical incision should be treated as a separate entity from VATET when comparing outcomes and deserves a distinct classification.

Videoscopic with Robotic Technology (Unilateral or Bilateral) (T-2d)

Robotic-aided video-assisted thoracoscopic thymectomy, unilateral or bilateral, is becoming increasingly common since its introduction in the early 2000s [76–80, 163]. The instrumentation offers enhanced optics with three-dimensional visualization and ×12 magnification; the surgical arms allow precise tissue dissection. Therefore the technique has the potential for extensive, yet safe, resection of the thymus and anterior mediastinal fat as well as exploration of the neck. However, robotic technology certainly adds significant cost and time to the procedure.

Infrasternal Thymectomies (T-5)

The infrasternal thymectomies make use of a subxiphoid incision that reportedly allows improved visualization and dissection of bilateral mediastinal spaces that would otherwise be difficult from a unilateral thoracoscopic approach [81]. The operation may be performed solely through a subxiphoid incision [82, 83] or supplemented with bilateral thoracoscopic ports [84] and may include a cervical incision to facilitate neck dissection [82]. A combined transcervical-subxiphoid thymectomy (T-5a) utilizes both an open cervical dissection and subxiphoid video-assisted inferior approach and has been reported to achieve comparable resection to the "maximal" T-4 approach [64]. While the single incision subxiphoid approaches may result in aesthetically more appealing results, decreased maneuverability may increase operative time or hamper adequate dissection needed to achieve total thymectomy.

Robotic technology has also been employed in subxiphoid thymectomy, through single or multiple ports [85, 86]. While robotic technology has the aforementioned benefits and may overcome the limitations in dexterity encountered in single port operations, such procedures are still in their infancy, and the benefits in terms of long-term clinical outcomes versus cost and time remain to be demonstrated.

The Results of Thymectomy

Introduction

The publication of the first randomized prospective trial investigating thymectomy versus medical management marks a great step forward in the evidence supporting the benefit of thymectomy in non-thymomatous MG [1]. The following review summarizes the evidence supporting thymectomy as a treatment modality in non-thymomatous MG, the problems in the analysis of thymectomy for MG, as well as best available information on the outcomes of different thymectomy techniques, relying on studies employing uniform definitions and reliable statistical methods of analysis and acknowledging when no such information exists.

We firmly support the clinical research standards set out by the MGFA in 2000 and revised in 2012, which have added much needed consistency and clarity to the design, implementation, and interpretation of MG clinical research [35, 87]. Adherence to a unified system of classification of MG clinical subtypes, disease severity, therapy status, morbidity and mortality information, and measure of response is essential for accurate assessment of treatment impact. Despite wide adoption, not all data included in this review adhere to this classification system (due in part to its relatively recent introduction).

Problems in the Analysis of Thymectomy for MG

Inappropriate statistical analysis, including the comparison of unrelated statistical techniques,

has led in many instances to incorrect conclusions concerning the relative merits of the thymectomy techniques. The following is a brief review of material previously reviewed and analyzed in detail [37, 88].

Remission has been considered the measurement of choice in defining results following thymectomy and the most desirable outcome from the patient standpoint [89–91]. However, not all studies adhere to its strictest definition as delineated in the MGFA guidelines, arguing that it represents a relatively rare outcome. Additionally, other studies argue that outcomes such as minimal manifestation status (MMS) or pharmacologic remission are more attainable and equally acceptable post-intervention statuses compared to complete stable remission.

Life table analysis, using the Kaplan–Meier method, is considered the preferred statistical technique for the analysis of remissions following thymectomy [92]. It provides a comparative analysis using all follow-up information accumulated to the date of assessment, including information on patients subsequently lost to follow-up and on those who have not yet reached the date of assessment [93, 94]. This analysis should be supplemented by multivariable analysis to identify and correct for significant variables. Hazard rates (remissions per 1000 patient-months) correct for length of follow-up and censor patients lost to follow-up. However, these rates depend on the risk (remissions per unit of time) being constant [95], which may not be the case in MG.

Uncorrected crude rates, the number of remissions divided by the number of patients operated upon or sometimes divided by only the number of patients followed (potentially a very different denominator), have been the primary form of analysis in the comparative evaluation of remissions and improvement following thymectomy. This is unfortunate since this form of analysis does not include in the evaluation all the follow-up information accumulated to the date of assessment. In addition, patients evaluated many years after surgery may appear to do as well or better than patients with a shorter follow-up. And even the differing denominators in the two subsets (patients operated upon versus patients followed) are not comparable but have frequently been compared without comment. Accordingly, uncorrected crude data should have no place in the comparative analysis of results of thymectomy and for this reason has been omitted from this review. Although correcting crude data for mean length of follow-up enables a rough comparison of the uncorrected crude data remission rates and confirms the fallacy of comparing uncorrected crude data, it should not be used as a substitute for life table analysis and therefore is also not included in this presentation.

In comparing results of different thymectomy techniques, other confounding factors are also frequently ignored and may conceal the disadvantages of the procedure being touted [37]. These include (1) failure to assess or define the length of illness preoperatively, (2) failure to account for the length of postoperative follow-up, (3) inclusion of multiple surgical techniques and combining two or more series with differing definitions and standards, (4) combining patients with and without thymoma in a composite analysis, (5) including reoperations in the primary analysis when most patients at the time of the reoperation had severe symptoms of long duration and may have failed earlier thymectomy for undetermined reasons, (6) use of meta-analysis based on mixed and uncontrolled data, (7) failure to report relapses, and (8) failure to consider the rate of spontaneous remissions.

Additionally, when immunosuppression is included in the preoperative or postoperative thymectomy regimen, the patients are not always compared to a control group and do not follow a predefined schedule of medications and dose reduction that is required in assessing the additive benefit from thymectomy and immunosuppression. Thus, under these circumstances, it is not possible to infer retrospectively the effects of thymectomy itself [96].

Thymectomy Versus Medical Management

The results of the Thymectomy Trial in Non-thymomatous Myasthenia Gravis Patients

Receiving Prednisone Therapy (MGTX) provide the first class I evidence in support of thymectomy for non-thymomatous MG [1]. The study, published in 2016, was a multicenter, prospective, randomized, and rater-blinded trial designed to evaluate the effect of thymectomy plus prednisone versus treatment with prednisone alone on improvement of MG symptoms, MG exacerbations, total prednisone and alternative immunosuppression requirements, and treatment-related complications.

The trial enrolled 126 patients between the ages of 16 and 65 with acetylcholine receptor antibody-positive MG, not associated with a thymoma, with disease duration of less than 5 years. Patients with ocular symptoms only or severe disease requiring intubation (class I and class V, respectively) were excluded, as were those who had undergone immunotherapy with anything other than prednisone. MGFA classification guidelines were utilized with regard to disease severity (Quantitative Myasthenia Gravis score, QMG) and post-intervention status. Participants were randomized to either undergo extended transsternal thymectomy (T-3b) in addition to a standardized prednisone protocol or the prednisone protocol alone and were followed for three years. Surgeons were required to adhere to a prescribed approach and underwent mandatory training to eliminate potential variability introduced by different thymectomy techniques.

Participants undergoing thymectomy in comparison to those treated with prednisone alone were observed to have significantly lower QMG scores (6.15 vs. 8.99, $p < 0.001$) indicating a clinically significant reduction in disease severity. Additionally, patients undergoing thymectomy required lower doses of prednisone to achieve minimal manifestation status (MMS) (44 mg vs. 60 mg, $p < 0.001$). Alternative immunosuppression requirements and MG exacerbations were significantly lower in the thymectomy group. Patients who underwent thymectomy were hospitalized with MG exacerbations less frequently (9% vs. 37%, $p < 0.001$) than those treated with prednisone alone. Treatment-related complications were equivalent.

While the MGTX trial demonstrates the benefit of thymectomy for patients with non-thymomatous MG, it is limited to patients with generalized MG associated with AChR antibodies and of relatively recent disease onset. Additionally, subgroup analysis of the trial failed to demonstrate the benefit of thymectomy for patients who had not previously been taking prednisone on QMG score or prednisone requirement, or for men with regard to QMG score, though this may be related to a relative small number of patients in such groups. Additionally, the primary outcome studied was MMS, rather than remission. The authors stated that remission, when strictly defined, is a relatively rare occurrence and that MMS is considered a desirable and achievable outcome [97].

Additional data that lend support for thymectomy in the treatment of non-thymomatous MG is limited to nonrandomized observational studies. A 2014 best-practice review that included papers evaluating thymectomy in non-thymomatous MG summarized that patients undergoing thymectomy are "more likely to achieve medication-free remission, become asymptomatic and clinically improve, particularly [those] with severe and generalized symptoms" compared to those undergoing medical management only [98]. A 2016 review of such studies demonstrated an odds ratio of 2.44 for remission in patients undergoing thymectomy to those treated nonoperatively [99]. However, such systematic reviews are limited by high heterogeneity among studies with regard to how patients are selected to undergo surgical versus nonsurgical treatment, operative approaches, and definition of remission.

Of note, the benefit of thymectomy is not immediate; remission rates more than 30% are rarely reported at 2 to 3 years post-thymectomy, though they typically continue to increase over time, and benefit may be seen as late as 15 years after surgery (Table 13.2).

Table 13.2 Comparative Kaplan–Meier remission rates of thymectomy techniques

	n	Pre-op duration					Years post-thymectomy						
		Class/severity (%)[a]					Symptom (% of patients achieving CSR)						
		Ocular	Mild	Mod	Severe	(Med years)	2–3	5	6	7.5	9–10	15	
No thymectomy[b]													
	149	9	16	73	2	–	10	15	–	18	20	–	[118]
Transcervical thymectomy													
T-1a	651	0.46	–	–	–	–	14	23	–	33	40	–	[119]
T-1b	151	21	39	28	12	–	34	–	34	–	36	–	[120]
T-1c	215	16	52	22	10	0.75	–	29	–	–	36	40	[121]
T-1d	120	11	32	37	20	0.8	–	30	–	–	91	–	[62]
Videoscopic thymectomy													
T-2a	36	22	42	14	22	–	–	13	20	–	75	–	[122]
	240	0	49	35	16	1.17	–	60	–	–	88	–	[123]
	79	4	33	43	20	–	–	20	–	–	–	–	[127]
	59	5	30	33	32	–	15	28	–	–	–	–	[160]
T-2b	159	12	–	76	12	–	35	51	51	–	–	–	[124]
T-2c	31	29	32	39	–	–	–	52	–	–	–	–	[161]
T-2d	100	–	–	–	–	0.92	–	29	–	–	–	–	[79]
	74	5	34	47	14	–	–	39	–	–	–	–	[127]
Transsternal thymectomy													
T-3a	60	–	–	–	–	1.95	12	15	–	–	–	–	[126]
T-3b	47	0	0	89	11	–	19	45	49	–	–	–	[124]
	98	18	41	23	18	–	–	30	–	–	45	–	[125]
	75	–	–	–	–	2.9	39	49	–	–	–	–	[126]
Transsternal-transcervical thymectomy													
T-4	72	0	18	52	30	–	30	50	–	81	–	–	[27]
	51	18	43	12	27	–	–	8.4	–	–	51	87	[162]
Infrasternal thymectomy													
T-5	292	–	–	–	–	2.3	39	48	–	–	–	–	[126]

[a]Osserman Classification or MGFA Clinical Classification

[b]Since we did not find for comparison spontaneous life table analysis remission rates for adults, we included life table analysis remission rates in children [118]. However, not included in this table is a study of standard transsternal thymectomies limited to children under the age of 17 years. It had a Kaplan–Meier remission rate at 7.5 years of 44% [118]

Indications for Thymectomy

In light of the MGTX trial, thymectomy appears indicated for adults less than 65 years with generalized, AChR antibody-positive non-thymomatous MG [1]. Expert consensus guidelines recommend thymectomy for non-thymomatous MG patients in order to improve symptoms, increase the chance of remission, and decrease need for immunosuppressants, though with caveats regarding (1) MG subtype, (2) patient age, (3) timing during disease course, and 4) disease severity [100].

Thymectomy is recommended for patients with AChR antibodies and generalized MG [1, 100, 101]. However, the benefit of thymectomy for patients with ocular MG is controversial; though some studies have shown that ocular MG patients undergoing thymectomy were more likely to achieve remission and less likely to progress to generalized MG [102, 103], others argue against it and believe that the data in favor of it is still lacking [104]. Expert guidelines do not recommend thymectomy as a first line treatment for ocular MG, but it may be considered if medical management fails [105].

The role for thymectomy is again unclear in patients with generalized MG but who are AChR antibody negative. Some argue that a percentage of seronegative MG patients (no detectable AChR and MuSK antibodies) may represent "false negatives": patients with undetectable but present anti-AChR antibody, as studies have shown similar benefit to thymectomy compared to AChR-antibody-positive patients [106, 107]. As such, thymectomy is generally recommended in seronegative patients [100, 101]. However, patients who are MuSK or LRP4 antibody positive have not consistently been demonstrated to benefit from thymectomy [108, 109]; consensus guidelines recommend against its use in these cases [100, 101].

It is suggested that thymectomy provides greater benefit when performed early during the course of the disease and should be performed within 3 to 5 years of onset [110, 111], the rationale being that early surgery provides the highest chance at achieving remission while minimizing the total exposure to immunosuppressives. Additionally, as the thymus naturally involutes over time, older patients with atrophic thymuses may derive less benefit from thymectomy [112, 113].

Furthermore, surgical risk increases with age. Given limited data in this age group, thymectomy is not routinely recommended in patients over 60 [113]. However, age has not been demonstrated to be an absolute contraindication to thymectomy (as evidenced by the inclusion of patients up to 65 years of age in the MGTX trial) and the decision to proceed with thymectomy in elderly patient should be decided on an individualized basis. The indication for thymectomy in children is a separate issue addressed by others [114–117].

Given that the benefits of thymectomy are not immediate and the increased risk of perioperative complications with poorly controlled symptoms, thymectomy should be performed on an elective basis when patients are as symptom-free as possible [118, 119]. While evidence indicates that patients with less severe illness who are operated upon early in their course do better, a thymectomy should be considered for most adult patients with more than very mild generalized non-thymomatous MG, regardless of the duration of symptoms or age of the patient, unless a specific contraindication exists.

Comparative Results Following Thymectomy

The available evidence continues to suggest that the less thymus left behind, including extra-lobar lobules of the thymus and microscopic thymus in the peri-thymic fat, the better the long-term results. However, while there are no well-designed prospective studies that conclusively indicate which resectional technique is the thymectomy of choice, as previously mentioned, there may be multiple approaches that allow for total thymectomy. The question then becomes which techniques allow for as total a resection as achieved by the T-4 "maximal" thymectomy. While certainly the size and weight of specimens may on average vary between the techniques, the most meaningful

measure is clinical outcome or, more specifically, rates of complete sustained remission.

We believe that the most reliable data for a comparative analysis of thymectomy techniques are reports that employ Kaplan–Meier life table analysis to report rates of remission. Table 13.2 represents a summary of such reports. We have omitted a presentation of crude data and adjusted crude data because, as previously discussed, the use of this form of analysis is not a valid statistical method to compare the results of the various thymectomy techniques. We recognize, however, that even though these studies have employed Kaplan–Meier analysis, they are not truly comparable because of significant variations in the severity and duration of the preoperative illness, variable use of pre- and postoperative corticosteroids and other immunosuppressive therapies, and various definitions of remission. However, this represents what we consider to be the best available evidence at this time.

Upon review of the table, it is immediately apparent that the patient populations widely vary between studies with regard to disease severity and preoperative duration, two factors with important implications for post-intervention outcomes. Additionally, while the MGFA defines complete stable remission as "no symptoms or signs of MG for at least 1 year and has received no therapy for MG during that time," a number of studies have defined remission as patients asymptomatic for at least 6 months off all medication or while only taking low-dose non-cholinesterase inhibitors [120, 121]. With this in mind, in addition to the variability in definitions of remission between studies, it is tempting to dismiss any attempt at comparison as futile.

However, a number of interesting observations can be made, the first being that the benefit of thymectomy is indeed not immediate and the superiority of one technique versus another may not be apparent until 5 or more years after surgery. Additionally, additional outcome data reported since our initial review continue to reinforce that all resections are not equal.

The transcervical resections appear to plateau at approximately 40% remission rate, with the exception of 91% remission rate at 10 years for the T-1d approach [62], which certainly deserves further consideration as a minimally invasive means to achieve good long-term results.

Of the videoscopic-assisted (T-2) techniques, the majority of data is limited to results within 6 years of operation. The most data exist on the unilateral VATS thymectomy (T-2a), with marked variability in rates of remission at 5 years between the studies, ranging from 13 to 60%. The 75% and 88% remission rates of the T-2a technique reported by Manulu et al. (2005) and Tomelescu et al. (2011) at 10 years are certainly intriguing and cannot be explained by more favorable patient populations or generous interpretations of "remission" [122, 123]. Long-term follow-up of the other videoscopic approaches is much needed.

Of the transsternal resections, while relatively few studies of short follow-up duration exist, the limited T-3 standard approach does not appear to achieve acceptably comparable results to other techniques. Notably, the T-3b extended transsternal thymectomy while demonstrating 30–49% remission rates at 5 years that are comparable to those of other approaches that demonstrate continued benefit at 10–15 years is lacking long-term data that it does similarly [124, 125].

The results of the two maximal T-4 thymectomy studies demonstrate its ability to achieve a relatively high rate of remission, but notably at a time point relatively remote from surgery. Of the one paper reporting life table analysis of an infrasternal–transcervical approach, while it appears to have relatively good 5-year remission rates, as with many of the other approaches, long-term prospective data is needed [126].

Selecting the Thymectomy Technique

While numerous studies utilizing MGFA research standards aimed at investigating both established and novel thymectomy techniques have been added to the literature since our initial review, in the absence of controlled prospective studies, it is

still not possible to state with certainty which technique should be the procedure of choice. Accordingly, it is necessary for the referring neurologist to have an understanding of the debate herein presented in determining what procedure to recommend. However, regardless of the surgical approach, surgical expertise and experience are required. The surgeon should be convinced of the importance of complete removal of the thymus and be willing to commit the necessary time and care to achieve this goal safely. Since the need for complete removal of all gross and microscopic thymus has not been definitively confirmed, it is generally considered preferable to leave behind small amounts of suspected thymus, or even likely thymus, than injure the recurrent laryngeal, the left vagus, or the phrenic nerves. Injury to these nerves can be devastating to a patient with MG.

Based on an analysis of the available data, it appears that the more thorough the removal of all tissue that may contain the thymus, the better the long-term results. Therefore, historically and conceptually, the combined transcervical–transsternal thymectomy best fulfills these criteria. However it is now clear that varying minimally invasive thechniqes and approaches can encompass and replicate the resectional specimens that had been performed in open procedures.

We continue to support the extended transsternal thymectomy since in the hands of experienced surgeons, it predictably removes all but a possible small amount of the thymic variations in the neck, has less risk of injuring the recurrent laryngeal nerves, and has produced good 5-year results. However, it is now clear that minimally invasive approaches, again in experienced hands, can reliably remove the same tissue as a properly performed extended transsternal thymectomy. The most data exists for T-2a unilateral VATS thymectomy, and the two studies reporting outcomes at 10 years suggested it may provide an acceptable "minimally invasive" alternative to the T-4 approach. The VATET thymectomy (T-2b) is conceptually appealing based on the surgical anatomy and the 51% 6-year remission rate reported in the one available study is promising, though longer-term results are needed [124]. Bilateral approach overall appears to best afford maximal dissection with protection of the phrenic nerves, although the unilateral robotic technique described by Ruckert has been uniquely able to use the robotic approach to remove bilateral disease that others have not been able to achieve unilaterally [127]. The variability of 5-year remission rates of the video-assisted thymectomies underscores the need for well-designed, prospective trials comparing surgical techniques.

Increasing expertise and facility with minimally invasive techniques in combination with the conviction that total thymectomy is necessary may yield a number of surgical approaches to thymectomy that achieve comparable results to the "maximal" T-4 method. Thus, the debate is shifted from which thymectomy technique is best to whether a not a particular technique can allow for adequate exposure for safe removal of all thymic tissue. Based on the handful of studies that exist with long-term outcomes data, it appears likely that a number of approaches, including those relying solely on thoracoscopic or videoscopic techniques, may achieve outcomes comparable to the "maximal" thymectomy. If one of the minimally invasive techniques can consistently be demonstrated to be as effective in the treatment of MG as the procedures that employ a median sternotomy, it would be our choice for most patients.

Reoperation

A repeat thymectomy may be appropriate and highly desirable for patients who have had an unsatisfactory result after one of the more limited thymic resections [128]. This recommendation appears to be straightforward when applied to a patient who still has, or progresses to, an incapacitating and poorly controlled illness, especially if repeated hospitalizations and ICU stays

have been required 3–5 or more years following a basic transcervical (T-1a) or a standard transsternal thymectomy (T-3a).

It is more difficult to recommend reoperation for less severe symptoms or after more extensive original operations, but it probably is appropriate in selected instances. Unfortunately at this time, it is not possible to determine the location, or even presence, of even moderate amounts of residual thymus prior to surgery or even by gross inspection at the time of surgery. Negative CT scans or MRI examinations do not exclude the presence of the residual thymus, and antibody studies are usually not helpful [29]. However, a review of the operative note and pathological report of the original surgery, which is mandatory in any case, usually makes it clear that an incomplete resection had been performed. Hopefully the day will come when techniques are developed that can determine the location of even small amounts of thymic tissue. The timing of reoperations is also a difficult decision. Although a 3- to 5-year wait frequently seems prudent, earlier reoperation may be more appropriate and many years later should not disqualify a patient from reoperation.

If reoperation is undertaken for persistent or recurrent symptoms, it should be as extensive as the combined transcervical–transsternal T-4 resections in both the neck and mediastinum, regardless of the thymectomy technique employed in the initial operation. This recommendation is supported by the findings following reoperations using the "maximal" technique [27]. Obviously, however, a reoperation is more difficult and time consuming than a primary resection, and the risk to the nerves and the thoracic duct is greater. Therefore, wide exposure in the neck and mediastinum, with the use of a "T" incision rather than separate neck and sternal incisions, appears highly desirable [33]. If the surgeon is not experienced in neck surgery, the assistance of a surgeon experienced in this area is suggested.

Reoperation thymectomies via extended transsternal [23, 28] and VATS [129] approaches have been described with reported improvement in the majority of patients, though studies are limited to retrospective examinations of relatively small, heterogeneous patient groups [130]. Incomplete resection and failure to remove residual thymus must account for at least some of those who fail to benefit from reoperation; we therefore cannot recommend any approach less extensive than the maximal T-4 approach without high-quality data that suggests otherwise.

Perioperative Patient Management

Patients undergoing thymectomy in the treatment of MG require the care of a team of neurologists, pulmonologists, respiratory therapists, intensive care specialists, anesthesiologists, and surgeons who have had experience in the care of these patients. Such teamwork has been demonstrated to improve outcome following thymectomy for MG [131], as has operative volume in other cardiothoracic operations [132–134]. And regardless of the surgical technique employed, the surgeons should be totally conversant with the special problems of patients with MG and committed to the frequently difficult postoperative care. To accomplish these goals, these operations should be performed at centers where such teams exist. It is, of course, important that the diagnosis of MG be unequivocally established prior to thymectomy and, as emphasized by Kaminski [135], the patient and the patient's family should have a thorough understanding of the illness, nonsurgical and surgical treatment options, anticipated postoperative course and morbidity, and, of course, the potential results, or lack thereof, of the surgery.

The special problems associated with the perioperative care of these patients are directly related to the severity of the MG manifestations at the time of surgery. The major risk is the presence of oropharyngeal and respiratory muscle weakness with the potential for aspiration of oral secretions, inability to cough effectively postoperatively, and respiratory failure. A number of variables are associated with increased risk of postoperative myasthenic crisis and respiratory failure, including a history of myasthenic crisis and longer duration of disease; some, however,

such as preoperative bulbar symptoms and lower vital capacity, are potentially modifiable [136, 137].

Accordingly, the preoperative preparation of these patients is central to the success of surgery [138]. The patient should be as symptom-free as possible at the time of surgery, especially free of signs and symptoms of oropharyngeal and respiratory weakness. Cholinesterase inhibitors are the mainstay of symptomatic treatment of MG, though their use in the perioperative period is complicated by interactions with neuromuscular blocking agents (NMBAs), medications often essential in anesthesia and critical care (see below). Most recommend continuing anticholinesterase medications up to the day of surgery to prevent worsening of respiratory symptoms preoperatively [138, 139]. However, anticholinesterase medications should not be the sole means used to achieve adequate control of symptoms as these inhibitors only temporarily mask MG-related weakness, which may then flare up in the early postoperative period and result in a high rate of postoperative respiratory complications [140, 141]. Steroids are essential in the perioperative management of MG symptoms [142]. The concern about wound healing and infection associated with steroids is likely overemphasized and much less of a concern than performing an operation on an inadequately prepared patient [143]; clinicians should be cognizant of the potential for need for stress-dose steroids perioperatively. Plasmapheresis and/or IVIG administration is routinely used to improve the preoperative status of patients with history of severe respiratory compromise due to their MG [144–146].

The preoperative evaluation should include a detailed evaluation of the pulmonary function status. Vital capacity (VC) and respiratory muscle force measurements are recommended, both before and after cholinergic inhibitors, if the patient is receiving this medication and can tolerate its withdrawal for the 6–8 h interval necessary for this type of testing. The dual before and after measurements gives an indication of the deficits that may be masked by the cholinesterase inhibitors and the potential need for preoperative plasmapheresis or immunosuppression. Some studies suggest that vital capacity <2.0–2.9 L is predictive of increased risk of postoperative respiratory failure [147, 148]. The measurement of maximum expiratory force (MEF) is easy to perform, is an excellent measure of cough effectiveness (an important determination), and is more sensitive and reliable than the VC in the evaluation of these patients, both preoperatively and in the early postoperative period [149]. An MEF of less than 40–50 cm H_2O indicates a potential for postoperative respiratory complications and respiratory failure. These determinations are also helpful in assessing the timing of extubation in those patients that require postoperative ventilatory support [138, 149].

Due to the effects of antibodies directed toward acetylcholine receptors, patients with MG have a variable response to NMBAs. They may be resistant to depolarizing NMBAs while demonstrating acute sensitive to the effects of nondepolarizing NMBAs. The concomitant use of anticholinesterase medications (often used to reverse such medications during anesthesia) may exacerbate this effect and place the patient at risk for a cholinergic crisis [150]. Therefore, many believe that NMBAs should be avoided if possible. However, it is preferable to face the potential need for prolonged postoperative ventilation than have the patient suffer the consequences of hypoxia. Accordingly, should intubation be difficult, whether during anesthesia induction or at anytime intubation is required, immediate muscle paralysis may be mandatory to achieve intubation rapidly and successfully.

If the patient is well-prepared preoperatively and the preoperative MEF off cholinergic medication is satisfactory, regardless of the surgical technique employed and including transsternal incisions, extubation may be acceptable immediately postoperatively. However, emergency reintubation and respiratory support should be instituted immediately at any time for early signs of fatigue, progressive weakness, or impending respiratory failure. The use of cholinergic medication at such a time is usually ineffective and may delay needed intubation.

Postoperatively, these patients should be closely monitored in an intensive care setting by experienced personnel [151, 152]. The use of epidural anesthesia for control of pain following a median sternotomy is extremely helpful. An institutional protocol for the management of the postoperative MG patient is helpful and can include details of the ventilatory support management, defining the role of MEF measurements in deciding when to extubate, the role of physical therapy and bronchoscopy in maintaining a clear airway, pain control techniques, immunosuppression if indicated, the role of cholinergic medication, and timing and technique of a tracheostomy if necessary.

Surgical Management of Thymoma

Because approximately 10% of patients with MG will have a thymoma [153], a chest CT scan is indicated prior to thymectomy in all these patients. It should identify thymomas in most instances [154]. The presence of antibodies to striated muscle antigens also predicts the existence of a thymoma [15]. However, small thymomas may first be discovered at surgery. To avoid tumor seeding and late recurrences, the well-encapsulated and invasive tumors require wide local resection with as good tumor margins as practical, including the removal of adherent pericardium or wedges of adherent lung if necessary. Although a phrenic nerve may also appear to be involved, although always a difficult and individual decision, in most instances it can and perhaps should be preserved in patients with MG. In addition, a total thymectomy should be performed unless a specific contraindication exists.

Median sternotomy is the current standard of care approach [155]. While more minimally invasive techniques are becoming increasingly common in the resection of smaller tumors [156], they are not currently recommended by expert consensus guidelines [155] and should only be considered at centers with significant experience.

Outcomes Research

Well-designed and well-controlled prospective studies are required to begin to resolve the many conflicting statements and unanswered questions that exist concerning thymectomy in the treatment of MG. This goal must be achieved if patient protocols and operative techniques are to be properly evaluated. In this era of evidenced-based therapy, these steps are not only desirable but mandatory.

The ideal method of such evaluation is to undertake a prospective randomized clinical trial, class I evidence in the American Academy of Neurology (AAN) nomenclature [157]. However, since the development of such a study comparing thymectomy techniques is not only unlikely but probably unnecessary, a prospective risk-adjusted outcome analysis of nonrandomly assigned treatment [158], class II evidence in the AAN nomenclature, is an acceptable and achievable method of study that if properly controlled and carefully monitored should resolve many of the unresolved issues. To eliminate bias, the use of two or more surgical centers, comparing their respective preferences, is preferable to a single team performing and comparing two types of thymectomies. We strongly recommended that one or more such class II studies be undertaken.

In addition to a prospective study, the use of clinical research standards, which include definitions of clinical classification, quantitative assessment of disease severity, grading systems of post-intervention status, and approved methods of analysis are required. The “data bank” concept, appropriately developed and rigorously monitored [35], should be particularly useful and practical for multiple institutions to compare the relative value of the various thymectomy techniques. The primary focus of comparative analysis of thymectomy for MG should remain complete stable remission. Complete stable remission, while rare, is not only the most reliable measure of success but the most desirable result from the patient standpoint. “Survival” instruments, which are used in the analysis of remissions, are the most reliable determinant.

The Kaplan–Meier life table analysis is the technique of choice. The use of uncorrected crude data has no place in these analyses. Quality of life instruments, such as the Myasthenia Gravis Composite (MGC) score, should also be employed because therapy for MG is usually not innocuous and frequently does not produce a completely stable remission [87, 159]. They should not, however, replace "survival" instrument evaluation. Experts in the field of biostatistics and outcomes analysis should be included in the design of all studies, in the collection of the information, and in the evaluation of the data.

Conclusions

The results of the MGTX trial provide the first class I evidence validating the use of thymectomy in the treatment of non-thymomatous MG. But rather than marking the end of a decade-long debate, it should herald the beginning of a new era of high-quality research, utilizing standardized definitions, appropriate statistical methods, and prospective studies to address lingering questions.

Although arguments can undoubtedly be submitted to refute some of the statements herein, we hope that this presentation will lead to a better understanding of the thymectomy controversies and improved results following thymic resection for MG. We also hope, and expect, the day will come when some form of targeted immunosuppression or other nonsurgical therapy, with no significant side effects, produces long-term remissions in patients with autoimmune non-thymomatous myasthenia gravis. At that time, thymectomy in any form, especially the transsternal procedures, will be considered barbaric. Until such time, however, a thymectomy, properly performed, should be considered an integral part of the therapy of MG.

References

1. Wolfe GI, Kaminski HJ, Aban IB, Minisman G, Kuo H-C, Marx A, et al. Randomized trial of thymectomy in myasthenia gravis. N Engl J Med. 2016;375:511–22.
2. Blalock A, Harvey AM, Ford FF, Lilienthal J Jr. The treatment of myasthenia gravis by removal of the thymus gland. JAMA. 1941;117:1529–33.
3. Keynes G. The surgery of the thymus gland. Br J Surg. 1946;32:201–14.
4. Kirschner PA, Osserman KE, Kark AE. Studies in myasthenia gravis: transcervical total thymectomy. JAMA. 1969;209:906–10.
5. Kark AE, Kirschner PA. Total thymectomy by the transcervical approach. Br J Surg. 1971;56:321–6.
6. Faulkner SL, Ehyai AS, Fisher RD, Fenichel GM, Bender HW. Contemporary management of myasthenia gravis: the clinical role of thymectomy. Ann Thorac Surg. 1977;23:348–52.
7. Olanow CW, Wechsler AS, Roses AD. A prospective study of thymectomy and serum acetylcholine receptor antibodies in myasthenia gravis. Ann Surg. 1982;196:113–21.
8. Vincent A, Newsom-Davis J, Newton P, Beck N. Acetylcholine receptor antibody and clinical response to thymectomy in myasthenia gravis. Neurology. 1983;33:1276–82.
9. Hankins JR, Mayer RF, Satterfield JR, Turney SZ, Attar A, Sequeira AJ, et al. Thymectomy for myasthenia gravis: 14 year experience. Ann Surg. 1985;201:618–25.
10. Otto TJ, Strugalska H. Surgical treatment for myasthenia gravis. Thorax. 1987;42:199–204.
11. Cooper J, Jaretzki A III, Mulder DG, Papatestas AS. Symposium: thymectomy for myasthenia gravis. Contemp Surg. 1989;34:65–86.
12. Papatestas AE, Cooper J, Jaretzki A III, Mulder DG. Symposium: thymectomy for myasthenia gravis. Contemp Surg. 1989;34:65–85.
13. Verma P, Oger J. Treatment of acquired autoimmune myasthenia gravis: a topic review. Can J Neurol Sci. 1992;19:360–75.
14. Drachman DB. Myasthenia gravis. N Engl J Med. 1994;330:1797–810.
15. Penn AS. Thymectomy for myasthenia gravis. In: Lisak RP, editor. Handbook of myasthenia gravis and myasthenic syndromes. New York: Marcel Dekker, Inc; 1994. p. 21–339.
16. Mack MJ, Landreneau RD, Yim AP, Hazelrigg SR, Scruggs GR. Results of video-assisted thymectomy in patients with myasthenia gravis. J Thor Cardiovasc Surg. 1996;112:1352–60.
17. Penn AS, Jaretzki A III, Wolff M, Chang HW, Tennyson V. Thymic abnormalities: antigen or antibody? Response to thymectomy in myasthenia gravis. Ann NY Acad Sci. 1981;377:786–803.
18. Wekerle H, Muller-Hermelink HK. The thymus in myasthenia gravis. Curr Top Pathol. 1986;75:179–206.
19. Younger DS, Worrall BB, Penn AS. Myasthenia gravis: historical perspective and overview. Neurology. 1997;48(Suppl 5):1–7.
20. Hohlfeld R, Wekerle H. Reflections on the "intrathymic pathogenesis" of myasthenia gravis. J Neuroimmunol. 2008;201–202:21–7.

21. Lauriola L, Ranelletti F, Maggiano N, Guerriero M, Punzi C, Marsili F, et al. Thymus changes in anti-MuSK-positive and -negative myasthenia gravis. Neurology. 2005;64:536–8.
22. Penn AS, Lovelace RE, Lange DJ, Toufexis G, Brockbank K. Experimental myasthenia gravis in neonatally thymectomized rabbits. Neurology. 1977;27:365.
23. Masaoka A, Monden Y, Seike Y, Tanioka T, Kagotani K. Reoperation after transcervical thymectomy for myasthenia gravis. Neurology. 1982;32:83–5.
24. Rosenberg M, Jauregui WO, Vega MED, Herrera MR, Roncoroni AJ. Recurrence of thymic hyperplasia after thymectomy in myasthenia gravis: its importance as a cause of failure of surgical treatment. Am J Med. 1983;74:78–82.
25. Henze A, Biberfeld P, Christensson B, Matell G, Pirskanen R. Failing transcervical thymectomy in myasthenia gravis: an evaluation of transsternal re-exploration. Scand J Thor Cardiovasc Surg. 1984;18:235–8.
26. Rosenberg M, Jauregui WO, Herrera MR, Roncoroni A, Rojas OR, Olmedo GSM. Recurrence of thymic hyperplasia after trans-sternal thymectomy in myasthenia gravis. Chest. 1986;89:888–91.
27. Jaretzki A III, Penn AS, Younger DS, Wolff M, Olarte MR, Lovelace RE, et al. "Maximal" thymectomy for myasthenia gravis: results. J Thorac Cardiovasc Surg. 1988;95:747–57.
28. Miller RG, Filler-Katz A, Kiprov D, Roan R. Repeat thymectomy in chronic refractory myasthenia gravis. Neurology. 1991;41:923–4.
29. Kornfeld P, Merav A, Fox S, Maier K. How reliable are imaging procedures in detecting residual thymus after previous thymectomy. Ann NY Acad Sci. 1993;681:575–6.
30. Monden MAY. Comparison of the results of transsternal simple, transcervical simple and extended thymectomy. Ann NY Acad Sci. 1981;377:755–64.
31. Matell G, Lebram G, Osterman PO, Pirskanen R. Follow up comparison of suprasternal vs transsternal method for thymectomy in myasthenia gravis. Ann NY Acad Sci. 1981;377:844–5.
32. Masaoka A, Nagaoka Y, Kotake Y. Distribution of thymic tissue at the anterior mediastinum: current procedures in thymectomy. J Thorac Cardiovasc Surg. 1975;70:747–54.
33. Jaretzki A III, Wolff M. "Maximal" thymectomy for myasthenia gravis: surgical anatomy and operative technique. J Thorac Cardiovasc Surg. 1988;96:711–6.
34. Fukai I, Funato Y, Mizuno Y, Hashimoto T, Masaoka A. Distribution of thymic tissue in the mediastinal adipose tissue. J Thorac Cardiovasc Surg. 1991;101:1099–102.
35. Jaretzki A III, Barohn RJ, Ernstoff RM, Kaminski HJ, Keesey JC, Penn AS, et al. Myasthenia gravis: recommendations for clinical research standards. Neurology. 2000;55:16–23.
36. Sonett JR, Jaretzki A III. Thymectomy for nonthymomatous myasthenia gravis: a critical analysis. Ann N Y Acad Sci. 2008;1132:315–28.
37. Jaretzki A III. Thymectomy for myasthenia gravis: an analysis of the controversies regarding technique and results. Neurology. 1997;48:52–63.
38. Bulkley GB, Bass KN, Stephenson R, Diener-West M, George S, Reilly PA, et al. Extended cervicomediastinal thymectomy in the integrated management of myasthenia gravis. Ann Surg. 1997;226:324–35.
39. Anastasiadis K, Ratnatunga C. Thymectomy. In: Anastasiadis K, Ratnatunga C, editors. The thymus gland: diagnosis and surgical management. Berlin: Springer; 2007. p. 63–83.
40. Sonett JR, Jaretzki A III. Transcervical-transsternal maximal thymectomy for myasthenia gravis. In: Shields TW, LoCicero III J, Reed CE, Feins RH, editors. General thoracic surgery. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2009. p. 2306–11.
41. Clark RE, Marbarger JP, West PN, Sprat JA, Florence JM, Roper CL, Ferguson TB, et al. Thymectomy for myasthenia gravis in the young adult. Long-term results. J Thorac Cardiovasc Surg. 1980;80:696–701.
42. Blalock A, Mason MF, Morgan HJ, Riven SS. Myasthenia gravis and tumors of the thymus region: report of a case in which the tumor was removed. Am Surg. 1939;110:544–61.
43. Clagett OT, Eaton LM. Surgical treatment of myasthenia gravis. J Thorac Surg. 1947;16:62–80.
44. Blalock A. Thymectomy in the treatment of myasthenia gravis: report of 20 cases. J Thorac Surg. 1944;13:1291–308.
45. Nichols FC, Trastek VF. Standard thymectomy. In: Shields TW, LoCicero III J, Reed CE, Feins RH, editors. General thoracic surgery. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2009. p. 2278–82.
46. LoCicero J III. The combined cervical and partial sternotomy approach for thymectomy. Chest Surg Clin N Am. 1996;6:85–93.
47. Trastek VF. Thymectomy. In: Kaiser LR, Krin IL, Spray TL, editors. Mastery of cardiothoracic surgery. Philadelphia: Lippincott-Raven; 1998. p. 105–11.
48. deCampos JRM, Filomeno LTB, Marchiori PE, Jatene FB. Partial sternotomy approach to the thymus. In: Yim AP, Hazelrigg SR, Izzat B, Landreneau RJ, Mack MJ, Naunheim KS, editors. Minimally access cardiothoracic surgery. Philadelphia: WB Saunders; 1999. p. 205–8.
49. Masaoka A, Yamakawa Y, Niwa H, Fukai I, Condo S, Kobayashi M, et al. Extended thymectomy for myasthenia gravis: a 20 year review. Ann Thor Surg. 1996;62:853–9.
50. Zieliński M, Kuzdzal J, Szlubowski A, Soja J. Comparison of late results of basic transsternal and extended transsternal thymectomies in the treatment of myasthenia gravis. Ann Thorac Surg. 2004;78:253–8.
51. Mulder DG. Extended transsternal thymectomy. Chest Surg Clin N Am. 1996;6:95–105.

52. Masaoka A. Extended transsternal thymectomy. In: Shields TW, LoCicero III J, Reed CE, Feins RH, editors. General thoracic surgery. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2009. p. 2298–304.
53. Fischer JE, Grinvalski HT, Nussbaum MS, Sayers HK, Cole RE, Samaha FJ. Aggressive surgical approach for drug-free remission from myasthenia gravis. Ann Surg. 1987;205:496–503.
54. Hatton P, Diehl J, Daly BDT, Rheinlander HF, Johnson H, Shrader JB, et al. Transternal radical thymectomy for myasthenia gravis: a 15-year review. Ann Thorac Surg. 1989;47:838–40.
55. Mulder DM. Extended transsternal thymectomy. In: Shields TW, LoCicero III J, Reed CE, Feins RH, editors. General thoracic surgery. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2009. p. 2233–7.
56. Kark AE, Papatestas AE. Some anatomic features of the transcervical approach for thymectomy. Mt Sinai J Med. 1971;38:580–4.
57. Papatestas AE, Genkins G, Kornfeld P, Horowitz S, Kark AE. Transcervical thymectomy in myasthenia gravis. Surg Gynecol Obst. 1975;140:535–40.
58. Cooper J, Al-Jilaihawa A, Pearson F, Humphrey J, Humphrey HE. An improved technique to facilitate transcervical thymectomy for myasthenia gravis. Ann Thorac Surg. 1988;45:242–7.
59. Bril V, Kojic S, Ilse W, Cooper J. Long-term clinical outcome after transcervical thymectomy for myasthenia gravis. Ann Thorac Surg. 1998;65:1520–2.
60. Shrager JB, Deeb ME, Mick R, Brinster CJ, Childers HE, Marshall MB, et al. Transcervical thymectomy for myasthenia gravis achieves results comparable to thymectomy by sternotomy. Ann Thorac Surg. 2002;74:320–7.
61. Maggi G, Cristofori RC, Marzio PD, Pernazza F, Turello D, Ruffini E. Transcervical thymectomy with partial sternal split. Osp Ital Chir. 2004;10:53–60.
62. dePerrot M, Bril V, McRae K, Keshavjee S. Impact of minimally invasive trans-cervical thymectomy on outcome in patients with myasthenia gravis. Eur J Cardiothoracic Surg. 2003;24:677–83.
63. Bramis J, Diamantis T, Tsigris C, Pikoulis E, Papaconstaninou I, Nikolaou A, et al. Video-assisted transcervical thymectomy. Surg Endosc. 2004;18:1535–8.
64. Zielinski M, Kuzdzal J, Szlubowski A, Soja J. Transcervical-subxiphoid-video thoracoscopic "maximal" thymectomy—operative technique and early results. Ann Thorac Surg. 2004;78:399–403.
65. Sugarbaker DJ. Thoracoscopy in the management of anterior mediastinal masses. Ann Thor Surg. 1993;56:653–6.
66. Roviaro G, Rebuffat C, Varoli F, Vergani C, Maciocco M, Scalambra SM. Videothoracoscopic excision of mediastinal masses: indications and technique. Ann Thorac Surg. 1994;58:1679–84.
67. Sabbagh MN, Garza JJS, Patten B. Thoracoscopic thymectomy in patients with myasthenia gravis. Muscle Nerve. 1995;18:1475–7.
68. Yim APC, Kay RLC, Ho JKS. Video-assisted thoracoscopic thymectomy for myasthenia gravis. Chest. 1995;108:1440–3.
69. Magee MJ, Mack MJ. Video-assisted thymectomy. In: Shields TW, LoCicero III J, Reed CE, Feins RH, editors. General thoracic surgery. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2009. p. 2294–5.
70. Novellino L, Longoni M, Spinelli L, Andretta M, Cozzi M, Faillace G, et al. "Extended" thymectomy, without sternotomy, performed by cervicotomy and thoracoscopic technique in the treatment of myasthenia gravis. Int Surg. 1994;79:378–81.
71. Scelsi R, Ferro MT, Scelsi L, Novellino L, Mantegazza R, Cornelio F, et al. Detection and morphology of thymic remnants after video-assisted thoracoscopic thymectomy (VATET) in patients with myasthenia gravis. Int Surg. 1996;81:14–7.
72. Shiono H, Ohta M, Okumura M. Open video-assisted techniques: thoracoscopic extended thymectomy with bilateral approach and anterior chest wall lifting. In: Lavini C, Moran CA, Morandi U, Schoenhuber R, editors. Thymus gland pathology. Milan: Springer; 2008. p. 187–94.
73. Chang PC, Chou SH, Kao EL, Cheng YJ, Chuang HY, Liu CK, et al. Bilateral video-assisted thoracoscopic thymectomy vs. extended transsternal thymectomy in myasthenia gravis: a prospective study. Eur Surg Res. 2005;37:199–203.
74. Lee CY, Kim DJ, Lee JG, Park IK, Bae MK, Chung KY. Bilateral video-assisted thoracoscopic thymectomy has a surgical extent similar to that of transsternal extended thymectomy with more favorable early surgical outcomes for myasthenia gravis patients. Surg Endosc. 2011;25:849–54.
75. Shigemura N, Shiono H, Inour M, Minami M, Ochta M, Okumura M, et al. Inclusion of the transcervical approach in video-assisted thoracoscopic extended thymectomy (VATET) for myasthenia gravis: a prospective trial. Surg Endosc. 2006;20:1614–8.
76. Morgan JA, Ginsburg ME, Sonett JR, Morales DLS, Kohmoto T, Gorenstein LA, et al. Advanced thoracoscopic procedures are facilitated by computer aided robotic technology. Eur J Cardiothoracic Surg. 2003;23:883–7.
77. Ashton RC, McGinnis KM, Connery CP, Swistel DS, Ewing DR, DeRose JJ. Total endoscopic robotic thymectomy for myasthenia gravis (case report). Ann Thorac Surg. 2003;75:569–71.
78. Rea F, Marulli G, Bortolotti L, Feltracco P, Zuin A, Sartori F. Experience with the "Da Vinci" robotic system for thymectomy in patients with myasthenia gravis: report of 33 cases. Ann Thorac Surg. 2006;81:455–9.
79. Marulli G, Schiavon M, Perissinotto E, Bugana A, Di Chiara F, Rebusso A, et al. Surgical and neurologic outcomes after robotic thymectomy in 100 consecutive patients with myasthenia gravis. J Thorac Cardiovasc Surg. 2013;145:730–5.

80. Melfi FM, Fanucchi O, Mussi A. Minimally invasive mediastinal surgery. Ann Cardiothorac Surg. 2016;5:10–7.
81. Hsu CP, Chuang CY, Hsu NY, Chen CY. Comparison between the right side and subxiphoid bilateral approaches in performing video-assisted thoracoscopic extended thymectomy for myasthenia gravis. Surg Endosc. 2004;18:821–4.
82. Uchiyama A, Shimizu S, Murai H, Kuroki S, Okido M, Tanaka M. Infrasternal mediastinoscopy thymectomy in myasthenia gravis: surgical results in 23 patients. Ann Thor Surg. 2001;72:1902–5.
83. Suda T, Sugimura H, Tochii D, Kihara M, Hattori Y. Single-port thymectomy through an infrasternal approach. Ann Thor Surg. 2012;93:334–6.
84. Hsu CP, Chuang CY, Hsu NY, Shia SE. Subxiphoid approach for video-assisted thoracoscopic extended thymectomy in treating myasthenia gravis. Interact Cardiovasc Thorac Surg. 2002;1:4–8.
85. Suda T, Tochii D, Tochii S, Takagi Y. Trans-subxiphoid robotic thymectomy. Interact Cardiovasc Thorac Surg. 2015;20:669–71.
86. Suda T, Kaneda S, Hachimaru A, Tochii D, Maeda R, Tochii S, et al. Thymectomy via a subxiphoid approach: single-port and robot-assisted. J Thorac Dis. 2016;8(Suppl 3):265–71.
87. Benatar M, Sanders DB, Burns TM, Cutter GR, Guptill JT, Baggi F, et al.; Task Force on MG Study Design of the Medical Scientific Advisory Board of the Myasthenia Gravis Foundation of America. Recommendations for myasthenia gravis clinical trials. Muscle Nerve. 2012;45:909–17.
88. Weinberg A, Gelijns A, Moskowitz A, Jaretzki A III. Myasthenia gravis: outcomes analysis. 2000. www.neurology.org/content/suppl/2001/04/…/Myasth_Gravis-Outcomes_Analysis.doc.
89. McQuillen MP, Leone MG. A treatment carol: thymectomy revisited. Neurology. 1977;1277:1103–6.
90. Viet HR, Schwab RS. Thymectomy for myasthenia gravis. In: Veit HR, editor. Records of experience of Mass Gen Hosp. Springfield: Charles C Thomas; 1960. p. 597–607.
91. Oosterhuis HJ. Observations of the natural history of myasthenia gravis and the effect of thymectomy. Ann NY Acad Sci. 1981;377:678–90.
92. Lawless JF. Life tables, graphs, and related procedures. In: Lawless JF, editor. Statistical models and methods for life table data. New York: Wiley; 1982. p. 52–94.
93. Durelli L, Maggi G, Casadio C, Ferri R, Rendine S, Bergamini L. Actuarial analysis of the occurrence of remission following thymectomy for myasthenia gravis in 400 patients. J Neurol Neurosurg Psychiatry. 1991;54:406–11.
94. Olak J, Chiu RCJ. A surgeon's guide to biostatistical inferences. Part II. ACS Bull. 1993;78:27–31.
95. Blackstone EH. Outcome analysis using hazard function methodology. Ann Thorac Surg. 1996;61(Suppl 2):2–7.
96. Sanders DB, Kaminski HJ, Jaretzki A III, Phillips LH II. Thymectomy for myasthenia gravis in older patients. J Am Coll Surg. 2001;193:340–1.
97. Wolfe GI, Kaminski HJ, Sonett JR, Aban IB, Kuo H, Cutter GR. Randomized trial of thymectomy in myasthenia gravis. J Thorac Dis. 2016;8:1782–3.
98. Diaz A, Black E, Dunning J. Is thymectomy in non-thymomatous myasthenia gravis of any benefit? Interact Cardiovasc Thorac Surg. 2014;18:381–9.
99. Taioli E, Paschal PK, Liu B, Kaufman AJ, Flores RM. Comparison of conservative treatment and thymectomy on myasthenia gravis outcome. Ann Thorac Surg. 2016;102:1805–13.
100. Sanders DB, Wolfe GI, Benatar M, Evoli A, Gilhus NE, Illa I, et al. International consensus guidance for management of myasthenia gravis: executive summary. Neurology. 2016;87:419–25.
101. Skeie GO, Apostolski S, Evoli A, Gilhus NE, Illa I, Harms L, et al.; European Federation of Neurological Societies. Guidelines for treatment of autoimmune neuromuscular transmission disorders. Eur J Neurol. 2010;17:893–902.
102. Mineo TC, Ambrogi V. Outcomes after thymectomy in class I myasthenia gravis. J Thorac Cardiovasc Surg. 2013;145:1319–24.
103. Roberts PF, Venuta F, Rendina E, De Giacomo T, Coloni GF, Follette DM, et al. Thymectomy in the treatment of ocular myasthenia gravis. J Thorac Cardiovasc Surg. 2001;122:562–8.
104. Gilbert ME, De Sousa EA, Savino PJ. Ocular Myasthenia Gravis treatment: the case against prednisone therapy and thymectomy. Arch Neurol. 2007;64:1790–2.
105. Kerty E, Elsais A, Argov Z, Evoli A, Gilhus NE. EFNS/ENS Guidelines for the treatment of ocular myasthenia. Eur J Neurol. 2014;21:687–93.
106. Guillermo GR, Téllez-Zenteno JF, Weder-Cisneros N, Mimenza A, Estañol B, Remes-Troche JM, et al. Response of thymectomy: clinical and pathological characteristics among seronegative and seropositive myasthenia gravis patients. Acta Neurol Scand. 2004;109:217–21.
107. Yuan HK, Huang BS, Kung SY, Kao KP. The effectiveness of thymectomy on seronegative generalized myasthenia gravis: comparing with seropositive cases. Acta Neurol Scand. 2007;115:181–4.
108. Evoli A, Tonali PA, Padua L, Monaco ML, Scuderi F, Batocchi AP, et al. Clinical correlates with anti-MuSK antibodies in generalized seronegative myasthenia gravis. Brain. 2003;126:2304–11.
109. Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. Lancet Neurol. 2015;14:1023–36.
110. Monden Y, Nakahara K, Kagotani K, Fujii Y, Nanjo S, Masaoka A, et al. Effects of preoperative duration of symptoms on patients with myasthenia gravis. Ann Thorac Surg. 1984;38:287–91.
111. Kumar V, Kaminski HJ. Treatment of myasthenia gravis. Curr Neurol Neurosci Rep. 2011;11:89–96.

112. Keesey JC. A treatment algorithm for autoimmune myasthenia in adults. Ann NY Acad Sci. 1998;841:753–68.
113. Saperstein DS, Barohn RJ. Management of myasthenia gravis. Semin Neurol. 2004;24:41–8.
114. Adams C, Theodorescu D, Murphy E, Shandling B. Thymectomy in juvenile myasthenia gravis. J Child Neurol. 1990;5:215–8.
115. Andrews PIA. treatment algorithm for autoimmune myasthenia gravis in children. Ann NY Acad Sci. 1998;841:789–802.
116. Seybold ME. Thymectomy in childhood myasthenia gravis. Ann NY Acad Sci. 1998;841:731–41.
117. Della Marina A, Trippe H, Lutz S, Schara U. Juvenile myasthenia gravis: recommendations for diagnostic approaches and treatment. Neuropediatrics. 2014;45:75–83.
118. Rodriguez M, Gomez MR, Howard F, Taylor WF. Myasthenia gravis in children: long-term follow-up. Ann Neurol. 1983;13:504–10.
119. Papatestas AE, Genkins G, Kornfeld P, Eisenkraft JB, Fagerstrom RP, Pozner J, et al. Effects of thymectomy in myasthenia gravis. Ann Surg. 1987;206:79–88.
120. Shrager JB, Nathan D, Brinster CJ, Yousuf O, Spence A, Chen Z, et al. Outcomes after 151 extended transcervical thymectomies for myasthenia gravis. Ann Thorac Surg. 2006;82:1863–9.
121. Ruffini E, Guerrera F, Filosso PL, Bora G, Nex G, Gusmano S, et al. Extended transcervical thymectomy with partial upper sternotomy: results in non-thymomatous patients with myasthenia gravis. Eur J Cardiothorac Surg. 2015;48:448–54.
122. Manlulu A, Lee TW, Wan I, Law CY, Chang C, Garzon JC, et al. Video-assisted thoracic surgery thymectomy for nonthymomatous myasthenia gravis. Chest. 2005;128:3454–60.
123. Tomulescu V, Sgarbura O, Stanescu C, Valciu C, Campeanu A, Herlea V, et al. Ten-year results of thoracoscopic unilateral extended thymectomy performed in nonthymomatous myasthenia gravis. Ann Surg. 2011;254(5):761.
124. Mantegazza R, Baggi F, Bernasconi P, Antozzi C, Confalonieri P, Novellino L, et al. Video-assisted thoracoscopic extended thymectomy and extended transsternal thymectomy (T-3b) in non-thymomatous myasthenia gravis patients: remission after 6 years of follow-up. J Neurol Sci. 2003;212:31–6.
125. Park IK, Choi SS, Lee JG, Kim DJ, Chung KY. Complete stable remission after extended transsternal thymectomy in myasthenia gravis. Eur J Cardiothorac Surg. 2006;30:525–8.
126. Zielinski M, Hauer L, Hauer J, Pankowski J, Nabialek T, Szlubowski A. Comparison of complete remission rates after 5 year follow-up of three different techniques of thymectomy for myasthenia gravis. Eur J Cardiothorac Surg. 2010;37:1137–43.
127. Rückert JC, Swierzy M, Ismail M. Comparison of robotic and nonrobotic thoracoscopic thymectomy: a cohort study. J Thorac Cardiovasc Surg. 2011;141:673–7.
128. Kirschner PA. Reoperation on the thymus. A critique. Chest Surg Clin N Am. 2001;11:439–45.
129. Pompeo E, Nofroni I, Iavicoli N, Mineo TC. Thoracoscopic completion thymectomy in refractory nonthymomatous myasthenia. Ann Thorac Surg. 2000;70:918–23.
130. Ng JK, Ng CS, Underwood MJ, Lau KK. Does repeat thymectomy improve symptoms in patients with refractory myasthenia gravis? Interact Cardiovasc Thorac Surg. 2014;18:376–80.
131. Mussi A, Lucchi M, Murri L, Ricciardi R, Luchini L, Angeletti CA. Extended thymectomy in myasthenia gravis: a team-work of neurologist, thoracic surgeon, and anaesthetist may improve the outcome. Eur J Cardiothorac Surg. 2001;19(5):570.
132. Houghton A. Variation in outcome of surgical procedures. Br J Surg. 1994;81:653–60.
133. Crawford FA, Anderson RP, Clark RE, Grover FL, Kouchoukos NT, Waldhausen JA, et al. Volume requirements for cardiac surgery credentialing: a critical examination. Ann Thorac Surg. 1996;61:12–6.
134. Silvestri GA, Handy J, Lackland D, Corley E, Reed CE. Specialists achieve better outcomes than generalists for lung cancer surgery. Chest. 1998;114:675–80.
135. Kaminski HJ. Treatment of myasthenia gravis. In: Kaminski HJ, editor. Myasthenia gravis and related disorders. Totowa: Humana Press; 2003. p. 197–221.
136. Watanabe A, Watanabe T, Obama T, Mawatari T, Ohsawa H, Ichimiya Y, et al. Prognostic factors for myasthenic crisis after transsternal thymectomy in patients with myasthenia gravis. J Thorac Cardiovasc Surg. 2004;127(3):868–76.
137. Leventhal SR, Orkin FK, Hirsh RA. Prediction of the need for postoperative mechanical ventilation in myasthenia gravis. Anesthesiology. 1980;53:26–30.
138. Krucylak PE, Naunheim KS. Preoperative preparation and anesthetic management of patients with myasthenia gravis. Semin Thorac Cardiovasc Surg. 1999;11:47–53.
139. Tripathi M, Kaushik S, Dubey P. The effect of use of pyridostigmine and requirement of vecuronium in patients with myasthenia gravis. J Postgrad Med. 2003;49:311–4.
140. Kas J, Kiss D, Simon V, Svastics E, Major L, Szobor A. Decade-long experience with surgical therapy of myasthenia gravis: early complications of 324 transsternal thymectomies. Ann Thorac Surg. 2001;72:1691–7.
141. Jaretzki A III, Phillips L II, Aarli J, Kaminski HJ, Sanders DB. Preoperative preparation of patients with myasthenia gravis for stalls postoperative respiratory complications following thymectomy. Ann Thor Surg. 2003;75:1068–9.
142. Kadota Y, Horio H, Mori T, Sawabata N, Goto T, Yamashita S, et al. Perioperative management in myasthenia gravis: republication of a systematic

review and a proposal by the guideline committee of the Japanese Association for Chest Surgery 2014. Gen Thorac Cardiovasc Surg. 2015;63:201–15.
143. Zieliński M, Kuzdzał J, Staniec B, Harazda M, Nabiałek T, Pankowski J, et al. Safety for preoperative use of steroids for transsternal thymectomy in myasthenia gravis. Eur J Cardiothorac Surg. 2004;26:407–11.
144. d'Empaire G, Hoaglin DC, Perlo VP, Pontoppidan H. Effect of prethymectomy plasma exchange on postoperative respiratory function in myasthenia gravis. J Thorac Cardiovasc Surg. 1985;89:592–6.
145. Cumming WJK, Hudgson P. The role of plasmapheresis in preparing patients with myasthenia for thymectomy. Muscle Nerve. 1986;9:155–8.
146. Gotti P, Spinelli A, Marconi G, Duranti R, Gigliotti F, Pizzi A, et al. Comparative effects of plasma exchange and pyridostigmine on expiratory muscle strength and breathing pattern in patients with myasthenia gravis. Thorax. 1995;50:1080–6.
147. Loach AB, Young AC, Spalding JM, Smith AC. Postoperative management after thymectomy. Br Med J. 1975;1:309–12.
148. Lee HS, Lee HS, Lee HE, Bae MK, Chung KY, Shin HY, et al. Predictive factors for myasthenic crisis after videoscopic thymectomy in patients with myasthenia gravis. Muscle Nerve. 2015;52:216–20.
149. Younger DS, Braun NMT, Jaretzki A III, Penn AS, Lovelace AE. Myasthenia gravis: determinants for independent ventilation after transsternal thymectomy. Neurology. 1984;34:336–40.
150. Abel M, Eisenkraft JB. Anesthetic implications of myasthenia gravis. Mt Sinai J Med. 2002;69:31–7.
151. Mayer SA. Intensive care of the myasthenic patient. Neurology. 1997;48(Suppl 5):70–81.
152. Juel VC. Myasthenia gravis: management of myasthenic crisis and perioperative care. Semin Neurol. 2004;24:75–81.
153. Wekerle H, Muller-Hermelink HK. The thymus in myasthenia gravis. In: Muller-Hermelink HK, editor. Current topics in pathology. The human thymus. Histophysiology and pathology. New York: Springer; 1986. p. 179–206.
154. Ellis K, Austin JHM, Jaretzki A III. Radiologic detection of thymoma in patients with myasthenia gravis. Am J Radiol. 1988;151:873–81.
155. Falkson CB, Bezjak A, Darling G, Gregg R, Malthaner R, Maziak DE, et al.; Lung Cancer Disease Site Group of Cancer Care Ontario's Program in Evidence-Based Care. The management of thymoma: a systematic review and practice guideline. J Thorac Oncol. 2009;4:911–9.
156. Shields TW. Thymic tumors. In: Shields TW, LoCicero III J, Reed CE, Feins RH, editors. General thoracic surgery. 7th ed. Philadelphia: Lippincott Williams & Wilkins; 2009. p. 2323–62.
157. Miller RG, Rosenberg JA, Gelinas DF, Mitsumoto H, Newman D, Sufit R, et al. Practice parameter: the care of the patient with amyotrophic lateral sclerosis (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology: ALS Practice Parameters Task Force. Neurology. 1999;52:1311–23.
158. Kirklin JW, Blackstone EH. Clinical studies with non-randomly assigned treatment. In: Kirklin JW, Barrett-Boyes BG, editors. Cardiac surgery. 2nd ed. New York: Churchill-Livingstone; 1993. p. 269–70.
159. Burns TM, Conaway M, Sanders DB, MG Composite and MG-QOL15 Study Group. The MG composite: a valid and reliable outcome measure for myasthenia gravis. Neurology. 2010;74:1434–40.
160. Keating CP, Kong YX, Tay V, Knight SR, Clarke CP, Wright GM. VATS thymectomy for nonthymomatous myasthenia gravis: standardized outcome assessment using the myasthenia gravis foundation of America clinical classification. Innovations (Phila). 2011;6:104–9.
161. Liu Z, Yang J, Lin L, Huang J, Jiang G. Unilateral video-assisted thoracoscopic extended thymectomy offers long-term outcomes equivalent to that of the bilateral approach in the treatment of non-thymomatous myasthenia gravis. Interact Cardiovasc Thorac Surg. 2015;21(5):610.
162. Prokakis C, Koletsis E, Salakou S, Apostolakis E, Baltayiannis N, Chatzimichalis A, et al. Modified maximal thymectomy for myasthenia gravis: effect of maximal resection on late neurologic outcome and predictors of disease remission. Ann Thorac Surg. 2009;88:1638–45.
163. Yoshino I, Hashizume M, Shimada M, Tomikawa M, Tomiyasu M, Suemitsu R, et al. Thoracoscopic thymomectomy with the da Vinci computer-enhanced surgical system. J Thorac Cardiovasc Surg. 2001;122:783–5.

Lambert-Eaton Syndrome

14

C. Michel Harper and Vanda A. Lennon

Historical Background

The first reported association of a myasthenic syndrome with lung cancer is commonly attributed to Anderson, Churchill-Davidson, and Richardson who, in 1953, described a patient with bronchial carcinoma who presented with generalized proximally predominant weakness, diplopia, and dysphagia [1]. No electrophysiological abnormality was found. A neurological diagnosis of myasthenia was based on a positive edrophonium test and therapeutic benefit of neostigmine. The patient may indeed have had paraneoplastic myasthenia gravis, which does occur, albeit rarely, with lung carcinomas of small-cell and non-small-cell types [2]. Lambert was the first to describe the presynaptic myasthenic syndrome that later bore his name. Beginning in 1956, he and colleagues at the Mayo Clinic in Minnesota described in a series of papers the clinical and electrodiagnostic features of a unique "myasthenic syndrome associated with malignant tumors" [3–8]. Characteristic findings were facilitation of both muscle strength and the compound muscle action potential (CMAP) amplitude after exercise or high-frequency electrical stimulation. Elmqvist and Lambert demonstrated the presynaptic origin of LES by using microelectrodes to study biopsied intercostal nerve-muscle preparations [8, 9]. They documented a severe reduction in endplate potential (EPP) amplitude and quantal content, with preserved miniature endplate potential (MEPP) amplitude.

Progress in understanding the pathogenesis and improvement in treatment of LES has continued over the six decades since its discovery. Studies linking LES with autoimmune disorders and autoantibodies were first published in the 1970s and early 1980s [10, 11]. In 1982, the Mayo Clinic team of Fukunaga, Engel, Osame, and Lambert demonstrated ultrastructural disorganization of the active zones for vesicle release in LES patients' presynaptic nerve terminals [12]. This observation coincided with recognition that those transmembrane particles were voltage-gated calcium channels, critical for nerve-stimulated release of ACh [13]. The autoimmune nature of LES was reinforced by Lang, Newsom-Davis, and colleagues at Oxford University, who documented for the first

Dr. Vanda A. Lennon wishes to dedicate this chapter to the memory of Dr. Edward H. Lambert, M.D., Ph.D., 1915–2003.

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H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*, Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_14

time that IgG from serum of LES patients could transfer the microelectrode findings of LES to mice [14]. This observation was confirmed by Kim [15] and extended by the Oxford and Mayo groups who demonstrated loss of presynaptic membrane active zones [16] and development of muscle weakness and EMG findings characteristic of LES [17] in mice injected with LES-IgG. By demonstrating that LES serum antibodies interfered with depolarization-dependent influx of Ca^{2+} into cultured lung cancer cells, the Oxford group was able to link SCLC pathogenically to LES [18]. Development of Ca_v channel subtype-specific ligands and sensitive radioimmunoassays advanced understanding of the basic pathophysiology of LES and enabled its serological diagnosis [19–23]. Treatment of LES was improved by (1) advances in imaging and treatment of small-cell lung carcinoma [24], (2) immunomodulatory therapy [25–28], and (3) drugs that promote the quantal release of ACh [29–31].

Pathogenesis

Physiology of Neuromuscular Transmission

Normal neuromuscular transmission ensures the generation of a single muscle fiber action potential in response to each motor nerve action potential firing at rates of up to 50 Hz or greater. The safety margin of neuromuscular transmission is the "excess" voltage of the EPP above the level required to reach the threshold for muscle action potential activation. Postsynaptic factors that affect the size of the EPP (and therefore the size of the safety margin) are the number of functioning nicotinic acetylcholine receptors (AChRs) located on the crests of the folds of the muscle's postsynaptic membrane, the presence of acetylcholinesterase in the overlying basal lamina, and the three-dimensional configuration of the synapse [32]. Presynaptic factors that affect the EPP size are the number of ACh-containing vesicles in the active zones of the nerve terminal and the number of functional $Ca_v2.1$ channels in the nerve terminal membrane. In normal as well as diseased endplates, the EPP amplitude falls with slow rates of repetitive nerve stimulation (2–5 Hz) due to depletion of immediately available stores of ACh [33]. When the EPP amplitude drops below threshold, the muscle fiber action potential is blocked. The safety margin of neuromuscular transmission is large enough to prevent blocking at normal endplates [33–35]. The characteristic abnormality observed in neuromuscular junction disease of either presynaptic or postsynaptic origin is blocking of neurotransmission at multiple endplates. This produces fatigable weakness and correlates with increased jitter and blocking on single-fiber EMG studies, motor unit potential (MUP) amplitude variation on standard concentric needle EMG, and a decrement of the CMAP observed at slow rates of repetitive stimulation on nerve conduction studies [36–38].

During exercise or rapid rates of repetitive nerve stimulation, mobilization of ACh stores and influx of Ca^{2+} into the nerve terminal through the $Ca_v2.1$ channel temporarily improve the safety margin of neuromuscular transmission (post-activation facilitation). This is followed by several minutes of reduced safety margin below baseline levels (post-activation exhaustion). A decrement of the EPP or CMAP observed at slow rates of repetitive nerve stimulation is often repaired by brief exercise or by increasing the rate of repetitive stimulation above 10 Hz, but depending on severity of the underlying disorder, the decrement invariably returns and increases for several minutes. In patients with a severe disorder of neuromuscular transmission, the EPP fails to reach threshold in a majority of fibers even at rest or after a single nerve stimulus. This results in objective weakness and a reduction of the CMAP amplitude below the lower limit of normal at rest. In these cases brief exercise or repetitive stimulation at a rapid rate produces a transient increase in the EPP above threshold and temporary facilitation of the CMAP.

The mechanism by which Ca^{2+} facilitates the release of ACh from the motor nerve terminal has been partially elucidated [35, 37, 39–42]. The $Ca_v2.1$ channels are arranged within the terminal membrane in double parallel rows that lie above the crests of the postsynaptic folds. This brings the

ACh vesicle release point in close proximity to the area of postsynaptic membrane where AChR concentration is greatest. Entry of Ca^{2+} through the $Ca_v2.1$ channel is triggered by nerve terminal depolarization. Prolongation of the time constant of depolarization (e.g., by blocking voltage-dependent potassium channels using 3,4-diaminopyridine) increases the amount of Ca^{2+} influx. The abrupt local rise in Ca^{2+} concentration in the nerve terminal is "sensed" by active zone proteins exemplified by synaptotagmin, a vesicular membrane soluble N-ethylmaleimide-sensitive factor attachment protein receptor (vSNARE). The binding of synaptotagmin to the "synaptic core complex" (formed by association of vesicular synaptobrevin and nerve terminal proteins syntaxin-1 and SNAP-25) destabilizes the vesicular and nerve terminal membranes creating a "fusion pore" leading to exocytosis of ACh. The $Ca_v2.1$ channel itself, a nerve terminal target membrane (tSNARE) protein, interacts noncovalently with the "synaptic core complex" and with soluble cytoplasmic factors, including NSF and α-SNAP. Following exocytosis, vesicle membranes are retrieved from the plasma membrane by clathrin-dependent and clathrin-independent processes. Synaptotagmin plays a role in the former, which involves sequential interaction of dynamin and amphiphysin with other highly conserved cytoplasmic proteins [39–42].

Neuronal $Ca_v2.1$ channels consist of five subunits [39, 43–45]. The largest subunit, α1, contains the voltage sensor and cation pore. It has four nearly identical domains, each with six transmembrane segments. The M4 segment contains the voltage sensor, loops between M5 and M6 segments in each domain, forms the ion channel, and determines Ca^{2+}-selective permeability. The α1 subunit also bears high-affinity binding sites for channel antagonists (including ω conopeptides) and for regulatory G-proteins and SNARE proteins. Its sequence determines the family and subfamily of the Ca_v channel complex (Table 14.1). In high voltage-activated channels, the auxiliary subunits, β and α2-δ (and perhaps γ), influence the insertion and stability of α1 in the plasma membrane and modify the conductance and kinetics of the channel.

Table 14.1 Classification of voltage-gated calcium (Ca_v) channels[a]

Class	Type	α1 subunit	Antagonists	Effector function	Predominant cell expression
$Ca_v1.1$	L	S	Dihydropyridines	Contraction	Skeletal muscle
$Ca_v1.2$		C	Phenylalkylamines	Contraction	Heart
$Ca_v1.3$		D	Benzothiazepines	Secretion; gene transcription	Neuron soma; pancreatic islet; kidney; ovary; cochlea
$Ca_v1.4$		F		Gene transcription	Retina
$Ca_v2.1$	P/Q	A	ω-Conotoxin M_{VIIC}, ω-agatoxin $_{IIIA}$ and $_{IVA}$	Neurotransmitter release	Central, autonomic, and peripheral motor neurons
$Ca_v2.2$	N	B	ω-Conotoxin G_{VIA} and M_{VIIA}	Neurotransmitter release	Central sensory and motor and autonomic neurons
$Ca_v2.3$	R	E	SNX-482, ω-agatoxin $_{IIIA}$	Neurotransmitter release	Central neurons, cochlea, retina, heart, pituitary
$Ca_v3.1$	T	G	ω-Agatoxin $_{IIIA}$	Pacemaker activity	Central and peripheral neuron soma
$Ca_v3.2$		H	Succinimides	Neurotransmitter release	Central neurons, heart, kidney, liver
$Ca_v3.3$		I		Neurotransmitter release	Central neurons

[a]Channels of Ca_v1 and Ca_v2 classes are activated by high voltage (HVA); class Ca_v3 (T-type) channels are activated by low voltage (LVA). The α1 subunit by which the channels are genomically classified is complemented by α2/δ and γ subunits in HVA channels (Ca_v1 and Ca_v2) [139]

Pathophysiology of Neuromuscular Transmission in LES

LES was initially recognized as a neuromuscular transmission disorder because of its clinical similarities to myasthenia gravis, including weakness that is fatigable and is improved with cholinesterase inhibitors. The characteristic electrophysiological findings described by Lambert and colleagues were established by standard electrodiagnostic studies and by microelectrode studies [4, 9, 38, 46]. The microelectrode findings are miniature endplate potential (MEPP) of normal amplitude and frequency, with reduced EPP amplitude secondary to a low quantal content of the nerve action potential. The quantal content and the size of the EPP increase during high-frequency nerve stimulation and with addition of extracellular Ca^{2+}. Biochemical studies of nerve-muscle preparations from LES patients have revealed a reduction in the amount of ACh released and normal function of acetylcholinesterase [33, 44, 46].

The fully developed defect of neuromuscular transmission in LES is typically severe, reducing the EPP amplitude in resting muscle below threshold in the majority of fibers. The patient is weak at rest, and CMAP responses to initial supramaximal nerve stimuli are low in amplitude (Fig. 14.1). Brief exercise produces transient clinical improvements in strength and tendon reflex responses and facilitation of the CMAP amplitude. If exercise is impractical for the patient, repetitive stimulation at 20–50 Hz can be used to elicit facilitation of the CMAP amplitude. At this stage, standard concentric needle EMG shows normal insertional activity and low-amplitude MUPs. MUPs are unstable with amplitude variation, and stimulated single-fiber EMG shows increased jitter and blocking. The severity of abnormal jitter and blocking improves at higher rates of stimulation [46].

The patient with early LES may have a relatively mild defect of neuromuscular transmission, in which the safety margin is not reduced sufficiently to drop the EPP below threshold at rest [47]. This ensures relatively preserved strength at rest and a normal CMAP amplitude (Fig. 14.2). During repetitive stimulation at slow rates (2–5 Hz), there is decrement of the CMAP, which repairs with exercise or higher rates of repetitive stimulation. Thus, when LES is mild, the results of electrophysiological tests of neuromuscular transmission are very similar to those of myasthenia gravis or other postsynaptic disorders of neuromuscular transmission [46]. The abnormalities revealed by standard clinical electrophysiological testing (nerve conduction studies, repetitive stimulation, and needle EMG) reflect the severity more than the synaptic element responsible for the defect of neuromuscular transmission.

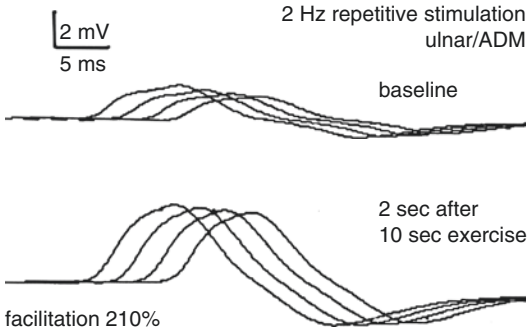

Fig. 14.1 Repetitive stimulation study in a patient with moderate-severe weakness caused by LES. *x* axis = time (milliseconds); *y* axis = CMAP amplitude (millivolts); *ADM* abductor digiti minimi; *sec* seconds

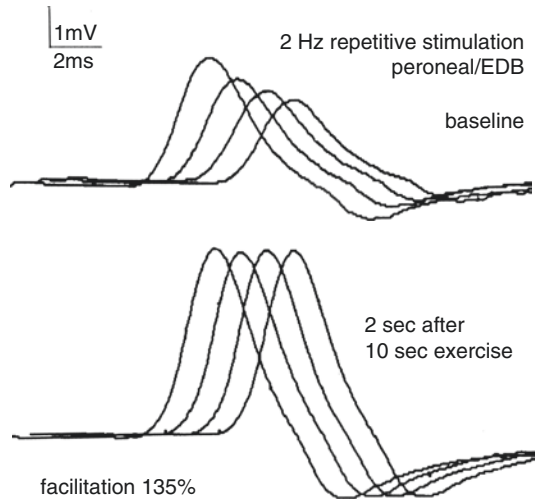

Fig. 14.2 Repetitive stimulation study in a patient with mild weakness caused by LES. *x* axis = time (milliseconds); *y* axis = CMAP amplitude (millivolts); *EDB* extensor digitorum brevis; *sec* seconds

Immunopathophysiology of LES

LES may occur as a paraneoplastic syndrome or as an idiopathic organ-specific autoimmune disease [11, 14, 48]. One or more of a thyrogastric cluster of autoimmune diseases, which includes thyroiditis, pernicious anemia, and type 1 diabetes mellitus, and their serological markers, frequently coexists with LES, particularly in patients who lack evidence of lung cancer [11, 28, 47, 49]. The P-/Q-type ($Ca_v2.1$) calcium channel in the terminal axonal membrane at the neuromuscular junction is strongly implicated as the target of pathogenic autoantibodies and also is implicated in some dysautonomias [50] and in some autoimmune cerebellar ataxias [51]. The finding of P-/Q-type calcium channel antibody in the serum of more than 90% of non-immunosuppressed LES patients [23] is compelling albeit circumstantial evidence for its being the effector of neuromuscular transmission impairment, as is the capacity of polyclonal IgG from serum of LES patients to transfer to mice clinical, electrophysiological, and ultrastructural nerve terminal lesions characteristic of LES [16, 17, 52]. There have been no reports of a monoclonal or affinity-purified antibody of P-/Q-type calcium channel specificity transferring the neuromuscular or autonomic transmission defect of LES to laboratory animals. This long-awaited evidence will definitively prove the pathogenicity of P-/Q-type calcium channel antibodies. A single report of electrophysiological abnormalities consistent with LES being induced in rats immunized with a peptide corresponding to an extracellular segment of the α_{1A} subunit of the $Ca_v2.1$ channel complex awaits confirmation [53]. Small-cell lung carcinoma (SCLC) is the most common neoplasm associated with paraneoplastic LES and is usually occult when neurological manifestations appear. The $Ca_v2.1$ channel at the neuromuscular junction is related antigenically and molecularly to the Ca_v channels in SCLC and other neuroendocrine cells [18–20, 54, 55]. Reports that prognosis for SCLC is better when associated with LES [54] and that successful treatment of SCLC leads to remission of LES in 70% of patients [24, 48, 56] support the hypothesis that paraneoplastic LES represents an immune response initiated by antigens expressed in the patient's neoplasm. The immunogen that initiates and perpetuates Ca_v channel autoimmunity in the idiopathic form of LES has not been identified.

The pathophysiology of LES is reproduced in mice injected with IgG isolated from LES patient serum. As we reviewed above, LES IgG confers electrophysiological and morphological characteristics of LES at the motor nerve terminal [15–17]. Mice receiving IgG from selected individual patients become profoundly weak, exhibit characteristic EMG findings of LES, and die from respiratory failure [17]. Patients whose IgG is exceptionally potent when transferred to mice presumably were producing IgG with extremely high affinity for $Ca_v2.1$ channel ectodomain epitopes common to human and mouse. Impairment of $Ca_v2.1$ channel function is independent of complement and is attributed to antigenic modulation, which is an IgG-mediated internalization of Ca_v channel through bivalent cross-linking of adjacent channels, resulting in accelerated degradation [17, 55–58]. LES-IgG is thought to bind directly to $Ca_v2.1$ channels [59], reducing Ca^{2+} influx through multiple Ca_v channel subtypes in cultured SCLC cells and motor neurons [55, 59–62] and reducing neurotransmitter release at the mouse neuromuscular junction [17, 63]. The benefit of immunotherapy in LES patients is explained most plausibly by clearance of circulating antibodies, effected by plasmapheresis or intravenous immune globulin therapy [64] or by reduced production of $Ca_v2.1$ channel autoantibodies (immunosuppressive medications). Effector T lymphocytes have not been implicated in the pathophysiology of LES, but the production of antigen-specific IgG by plasma cell progeny of activated B lymphocytes requires continuing helper T lymphocyte activity [65]. P-/Q-type calcium channel antibodies are detectable in approximately 90% of patients who have LES. The 10% of LES cases who appear to be seronegative for $Ca_v2.1$ channel autoantibodies do not differ clinically or electrophysiologically from seropositive patients [66, 67]. Negative assays for P-/Q-type calcium channel antibodies could result from very low antibody titers, immunosuppressive drugs, or alternative antigenic targets [68].

Epidemiology

The annual incidence of LES is approximately 0.5 per million with an estimated prevalence of 2.5 per million [69]. This makes LES significantly less common than myasthenia gravis, which itself is a rare disorder. Shorter life expectancy related to the associated cancer in paraneoplastic LES contributes to the low prevalence of LES. Idiopathic autoimmune LES exhibits a female sex bias (approximating 60%; V.A. Lennon, unpublished serological observations in Mayo Clinic's Neuroimmunology Laboratory since 1987), but life expectancy does not appear to be shortened. Its onset age ranges from the first decade to old age [48, 69]. Patients with idiopathic LES are frequently nonsmokers and often have a personal or family history or serological markers of organ-specific autoimmune diseases and a different profile of certain genetic markers than patients with paraneoplastic LES [70, 71].

The frequency of LES in patients with newly diagnosed SCLC is estimated to be 2–3% [72, 73]. In the earliest descriptions of the disease, paraneoplastic LES predominantly affected men. This sex bias was attributed to cigarette smoking habits. Despite the frequency of female tobacco smoking approximating if not exceeding that of males in the past three decades and there being a corresponding increase in the frequency of female small-cell lung cancer cases (now declining in both sexes due to societal smoking cessation [74]), paraneoplastic LES does not occur more frequently in women. This differs from cytotoxic T-cell-mediated autoimmune neurological disorders associated with small-cell lung cancer, such as encephalomyeloradiculoneuropathies related to ANNA-1 IgG ("anti-Hu") [75] or CRMP5 IgG [76], in which female sex predominates 58–67%. Unlike LES, neurological disorders associated with ANNA-1 autoantibody are characterized by inflammatory lesions affecting the peripheral and central nervous system. The cytokine milieu at the time of initial helper T lymphocyte activation determines whether or not the immunological outcome of an immune response is inflammatory. It is conceivable that the outcome is influenced both by sex hormones and by onconeural antigen dominance among proteins released to lymph nodes by necrosing tumor cells. A pro-inflammatory response would yield cytotoxic T-cell effectors, for which nuclear and cytoplasmic-directed neural autoantibodies serve as a surrogate marker [75–77]; perhaps a non-inflammatory response favors production of plasma membrane ion channel antibodies.

Cancer is diagnosed in about 45% of contemporary LES cases [72], and SCLC accounts for 90% of paraneoplastic cases [67, 69]. An 18% seropositivity rate for P-/Q-type calcium channel antibodies has been reported for Mayo Clinic patients who have SCLC without clinical evidence of LES or other overt paraneoplastic autoimmune neurological disorders [23], but only 4.2% in a UK study that examined patients neurologically [78]. The diagnosis of LES may be overlooked when multiple paraneoplastic disorders coexist, particularly in the setting of severe peripheral neuropathy or subacute cerebellar ataxia [79]. Other cancers have been reported with LES [48, 80–85], but in the setting of LES and significant smoking history or other lung cancer risk factors, the presence of a coexisting occult SCLC should be considered and extended serological and imaging studies performed [77, 86]. Anticipation of SCLC in these cases is supported by observations that LES diagnosis can antedate the detection of SCLC by 5–8 years [48, 68, 72]. Primary small-cell carcinoma in an extrapulmonary site, such as the skin (Merkel cell carcinoma), tongue, larynx, pancreas, breast, cervix, or prostate [87], also should be considered in patients presenting with LES, particularly in the absence of recognized lung cancer risk factors.

Clinical Presentation

Symptoms and Signs

Subacute progressive fatigue, weakness, and injurious falls are the most common presenting manifestations of LES. The weakness increases with exertion and improves transiently with rest.

Sometimes a history of transient clinical facilitation of strength following brief exercise is elicited, but complaints of weakness exacerbated by exercise are more common. Aching pain in the hip and posterior thigh region is a common complaint with paraneoplastic LES, but other sensory symptoms are rare unless LES is accompanied by paraneoplastic manifestations of sensory neuropathy or radiculoplexopathy. Muscle atrophy is rare, and fasciculations and cramps are not observed. The distribution of weakness is characteristic, with symptoms usually beginning in hip flexors and other proximal lower limb muscles. Patients commonly complain of difficulty arising from low chairs or a squatted position or trouble climbing stairs and inclines. In one series of 50 LES patients, weakness began in the lower limbs in 65% and was generalized in 12% of patients [48]. Even after weakness generalizes, most patients have disproportionate involvement of hip girdle muscles. Proximal muscles of the upper limb and neck, and interossei muscles of the hand, also are preferentially affected in LES. Cranial muscles are involved at some point in up to 25% of patients [88]. Ptosis, facial weakness, dysphagia, dysarthria, and difficulty chewing are milder than in myasthenia gravis and usually follow the onset of limb weakness [48, 89]. Respiratory involvement is usually mild and of restrictive functional pattern, but when complicated by emphysema related to smoking, respiratory failure can occur terminally. In rare cases respiratory failure is the presenting manifestation of LES [90]. Deep tendon reflexes are characteristically reduced or absent in LES, but it is important to note that reflexes may be preserved early in the course of the illness. Facilitation of the reflex after brief exercise is an important sign supporting the diagnosis of LES and is easier to demonstrate at the bedside than facilitation of muscle strength.

Symptoms of autonomic dysfunction occur in approximately 80% of LES patients, and in 6% of patients the presenting symptom is autonomic [50]. Male impotence and xerostomia (both sexes) are common symptoms. Other common dysautonomic findings are hypohidrosis, impaired cardiovagal reflexes, and reduced lacrimation [50]. Slow pupillary reflexes, gastrointestinal dysmotility, orthostatic hypotension, and urinary retention are sometimes noted, but these manifestations usually indicate a coexisting paraneoplastic autonomic neuropathy in the setting of SCLC and are often accompanied by one or more marker autoantibodies specific for neuronal nuclear or cytoplasmic antigens, particularly CRMP5, ANNA-1, ANNA-2, ANNA-3, PCA-2/MAP 1B, SOX1, or amphiphysin autoantibody [50, 75–78, 91–93].

The sensory system is spared in LES, but sensory manifestations may be present if there is an associated paraneoplastic sensory neuronopathy, peripheral neuropathy, myelopathy, or encephalopathy. Constitutional symptoms of anorexia and weight loss are usually attributed to an underlying malignancy. These symptoms may represent autoimmune gastroparesis for which autoantibodies of ANNA-1, CRMP5, voltage-gated N-type calcium channel, Kv1 potassium channel complex, and ganglionic AChR specificities are valuable serological markers [94–97]. Symptoms of lung cancer itself, such as hemoptysis and chest pain, are very uncommon. On the other hand, patients with idiopathic LES commonly have manifestations of coexisting organ-specific autoimmune disorders (e.g., pernicious anemia, type 1 diabetes, hyperthyroidism, or hypothyroidism).

Natural History

The course of LES is more variable among patients with lung cancer or at high risk for it than among those without lung cancer risk. Symptoms of LES frequently antecede the diagnosis of cancer by months or years. If chest x-ray and CT are negative in LES patients at risk for SCLC, the diagnostic yield is increased by performing MRI or CT/PET imaging with appropriate biopsy of areas suspicious for a neoplasm. A search for extrapulmonary small-cell carcinoma is warranted when chest studies are negative. These have been recorded in the skin, tongue, larynx, pancreas, cervix, prostate, breast, and ovary [87]. The clinical course of LES, with and without

cancer, is generally progressive in the first year, with less fluctuation and a lower spontaneous remission rate than autoimmune myasthenia gravis. Strength often improves following effective treatment of the underlying malignancy. It has been reported that SCLC in the context of LES may have a less aggressive natural history and a better response to therapy [56, 98]. The results of a prospective serological study of 238 newly diagnosed SCLC patients who lacked any neurological symptoms or signs at cancer diagnosis revealed that survival time was significantly longer for those who were seropositive for antineuronal nuclear autoantibodies (ANNA-1 or unclassified) than for seronegative patients or patients positive for nonneuronal antinuclear antibody or VGCC antibodies without ANNA-1 [99]. It seems likely therefore that the humoral immune response that causes LES (namely, P-/Q-type VGCC autoantibody) does not per se confer a protective immune response against SCLC. These findings accord with the conclusion drawn from a smaller Mayo Clinic serological study [100]. Weakness and fatigue can cause long-term disability in LES in the absence of cancer [28, 101]. Most patients benefit from continued therapy with an oral cholinesterase inhibitor and agents that increase the quantal release of ACh such as 3,4-diaminopyridine [29, 102–104], amifampridine phosphate [105], or low-dose guanidine [31]. Immunosuppressive therapy also is beneficial [26–28, 49, 66, 98, 106], but symptomatic response to immunosuppressive therapy tends to be less in LES than in myasthenia gravis.

Diagnosis

Clinical Manifestations

LES should be suspected in a patient who presents with generalized fatigue or weakness worsened by exertion, with a waddling gait, or symptoms of autonomic dysfunction (i.e., impotence or xerostomia). LES, like myasthenia gravis, should be considered in the differential diagnosis of prolonged postoperative apnea involving a neuromuscular blocking drug [107]. Factors favoring LES diagnosis rather than myasthenia gravis include early and prominent involvement of hip flexor muscles, xerostomia, impotence, reduced or absent reflexes (in lower limbs initially and more diffuse in the established disease), facilitation of tendon reflexes or strength on clinical examination, a personal history of smoking, asbestos, secondary tobacco smoke exposure or lung cancer, or a family history of lung cancer [49]. A personal or family history of autoimmunity is common in both idiopathic and paraneoplastic LES.

The diagnosis of LES should also be suspected when a patient presents with paraneoplastic sensorimotor or sensory neuropathy related to lung cancer and has prominent weakness, sicca symptoms, recent onset erectile dysfunction, respiratory failure of undetermined cause, P-/Q-type calcium channel antibody seropositivity, or a provisional diagnosis of seronegative myasthenia gravis. Note, however, that 10% of LES patients (both idiopathic and paraneoplastic) have muscle AChR antibodies and or striational autoantibody, without evidence of a postsynaptic neuromuscular transmission defect [23].

Electrodiagnostic Studies

Unambiguous electrodiagnosis of LES requires that the patient be well rested and warm, that medications affecting the neuromuscular junction be withheld for several hours before testing, and that clinically weak muscles be tested. When weakness is moderate to severe, electrodiagnostic studies reveal a pathognomonic pattern (Table 14.2) [46, 68, 108, 109]. The baseline amplitude of the CMAP is reduced. A decrement is observed with slow rates of repetitive nerve stimulation, and facilitation of more than 200% (doubling of the baseline amplitude) is observed following brief exercise or high-frequency repetitive nerve stimulation. As with myasthenia gravis, post-exercise exhaustion is typical and observed as worsening of the decrement 1–3 min following exercise [46, 110]. With longer trains of repetitive stimulation at low rates, the decrement worsens in LES, whereas it generally

Table 14.2 Electrodiagnostic characteristics of Lambert-Eaton syndrome

Severity of weakness	Nerve conduction studies CMAP[a] amplitude	Needle examination
Moderate-severe	1 Baseline (initial stimulus): low 2. During RS[b] at 2–3 Hz: decrement 3. After brief exercise or after RS at 2050 Hz: facilitation >200%	1. Insertional activity: normal 2. Motor unit potentials: small with amplitude variation 3. Single-fiber EMG: increased jitter and blocking
Mild	1. Baseline (initial stimulus): normal 2. During RS at 2–3 Hz: decrement 3. After brief exercise or after RS at 2050 Hz: facilitation <200%	1. Insertional activity: normal 2. Motor unit potentials: normal to small with amplitude variation 3. Single-fiber EMG: increased jitter and blocking

[a]CMAP = compound muscle action potential elicited by supramaximal stimulation of nerve in clinically weak muscle, with patient well rested and warm and with neuromuscular-active medication withheld
[b]RS = repetitive stimulation

improves in myasthenia gravis [110, 111]. Needle examination shows low-amplitude varying MUP. Fibrillation potentials are usually absent but may occur in severe cases. Single-fiber EMG shows increased jitter and blocking that transiently improve at higher firing rates. The electrodiagnostic findings are usually demonstrated most readily in small intrinsic hand muscles.

When weakness is mild, as in the early course of LES, the CMAP amplitude may be reduced only slightly or may be in the lower range of normal. In this setting the CMAP decrement is usually modest, and facilitation is often less than 200%. Needle EMG is normal or shows only mild MUP amplitude variation. Mild cases of LES are difficult to differentiate from myasthenia gravis [46]. The distribution of symptoms, the profile of serum autoantibodies, and the result of stimulated single-fiber EMG testing can help, but in some cases the correct diagnosis requires continued observation and retesting at a later date after the disease progresses (Table 14.3).

Serological Tests

P-/Q-type ($Ca_v2.1$) calcium channel antibodies are detectable in at least 90% of non-immunosuppressed patients with LES with or without cancer of any type [23]. It remains to be determined whether or not other motor nerve terminal antibodies may be defined as causative of LES. It has been reported that when IgG from "seronegative" LES patients was transferred to mice, microelectrophysiological findings consistent with LES were observed within 48 h of intravenous injection [15]. However, as reported for myasthenia gravis [112], we have encountered apparent seronegativity at disease onset with seroconversion when reevaluated at 12 months. N-type ($Ca_v2.2$) calcium channel antibodies are detectable in 75% of LES patients with lung cancer, and in 40% without cancer risk [23], but generally not in LES patients who have other types of cancer [19, 20]. Eighteen percent of SCLC patients without clinical evidence of LES have P-/Q-type calcium channel antibodies, and 22% have N-type calcium channel antibodies [23]. Antibodies against SOX1 strongly suggest SCLC because SOX1-IgG is detectable in 65% of SCLC-associated LES but in only 5% of idiopathic autoimmune LES [49, 92]. When used in conjunction with demographic and clinical variables as part of the Dutch-English LEMS Tumor Association Prediction (DELTA-P) score, the coexistence of SOX1 and P-/Q-type voltage-gated calcium channel antibodies predicted the presence of small-cell lung cancer with a high degree of accuracy early in the course of LES [113]. Nicotinic AChR antibodies (muscle-type or ganglionic-type) or striational antibodies are found in ~13% of patients with LES, with and without cancer [23, 114]. Calcium channel antibodies of N-type and P-/Q-type also serve as serological markers of paraneoplastic neurological disorders affecting the central and peripheral nervous system (i.e., other than LES) in the

Table 14.3 Serum profiles of organ-specific autoantibodies in non-immunosuppressed adult patients with Lambert-Eaton myasthenic syndrome and generalized myasthenia gravis and controls[a]

	Frequency (% seropositive)				
Autoantibody	LES without cancer	LES with lung cancer	MG without thymoma	MG with thymoma	Non-autoimmune neurological diseases
Calcium channel, P/Q	91	99	<2	<2	<2
Calcium channel, N	40	75	<2	<2	<5
Muscle AChR	7	7	90	100	<5
Striational	5	5	30	80	<2
Thyrogastric[b]	55	20	50[c]	30	20
Ganglionic AChR	10	10	<2	8	<2
CRMP5	<2	0[d]	<5[e]	18	<2
Anti-glial nuclear (SOX-1 transcription factor)[f]	<5	65	<2	<2	<2
One or more of above	96	100	95	100	20

[a]Based on refs. 22, 23, 49, 66, 77, 92 and authors' unpublished data
[b]Thyroperoxidase, thyroglobulin, gastric parietal cell, intrinsic factor blocking, or glutamic acid decarboxylase (GAD65) antibody
[c]Prevalence highest in ocular myasthenia
[d]Positive only if coexisting paraneoplastic neurological accompaniments
[e]Positive only with thymoma-predictive autoantibody profile [23]; negative chest imaging (usually elder-onset MG) does not exclude thymoma
[f]SOX-2 autoantibody has similar prevalence in patients with paraneoplastic neurological autoimmunity related to small-cell lung cancer [78]

context of small-cell lung carcinoma, ovarian carcinoma, and breast carcinoma [23, 115]. Calcium channel antibody values in those patients tend to be lower than in untreated LES patients. IgG autoantibody markers of small-cell lung carcinoma that are specific for neuronal nuclear and cytoplasmic antigens (e.g., ANNA-1, CRMP5, amphiphysin, PCA-2/MAP1B and ANNA-3, SOX1) [75–79, 91–93] are rarely found in patients with paraneoplastic LES in the absence of a coexisting encephalomyelopathy or neuropathy.

Differential Diagnosis

The differential diagnosis of LES includes autoimmune myasthenia gravis, congenital myasthenic syndromes, and defects of neuromuscular transmission induced by drugs or toxins. A congenital myasthenic syndrome that resembles LES is severe at birth [116, 117]. Autoimmune myasthenia gravis can usually be distinguished from LES by differences in clinical manifestations as well as electrodiagnostic tests and autoimmune serological tests [23, 46]. Cases of mild LES or severe myasthenia gravis can yield quite similar electrophysiological findings with baseline CMAP amplitudes in the low normal range, a decrement at low rates of repetitive nerve stimulation, and facilitation of less than 200% following exercise or high rates of repetitive stimulation [46]. The distribution of weakness and the autoantibody profile usually differentiate the disorders [23]. Sometimes additional time for observation and retesting is needed, as LES typically progresses in severity if not treated. If a patient complains of severe weakness, negative electromyographic findings are generally more common when the cause is myasthenia gravis than when it is LES. Although cases of an overlap or combination of LES and myasthenia gravis have been reported, no instance of a dual syndrome has been documented by in vitro microelectrode studies performed on biopsied intercostal or anconeus nerve-muscle preparations. Those reports may reflect misinterpretation of autoantibody profiles [118].

Botulism usually presents as an acute and severe weakness of cranial, axial, and limb muscles, requiring respiratory insufficiency and emergent hospitalization. LES usually presents more gradually and rarely presents with respiratory failure.

Electrodiagnostic studies in botulism reveal CMAPs of low amplitude with less prominent decrement and facilitation than LES. On needle EMG, fibrillation potentials are typically widespread in botulism but are rare in LES. The diagnosis of botulism is usually made on clinical grounds and confirmed by electrodiagnostic studies and demonstration of the toxin in the stool. Toxins delivered through the bite of venomous snakes and spiders may produce findings similar to botulism. These as well as acute intoxication with magnesium, neuromuscular blocking drugs, and organophosphates are unlikely to be mistaken for the diagnosis of LES.

Treatment

Symptomatic Treatment

Cholinesterase Inhibitors

Because the quantal content is usually severely reduced in LES, the beneficial effect of AChE inhibition is usually modest unless it is combined with medications that simultaneously increase the release of ACh (e.g., 3,4-diaminopyridine, amifampridine phosphate, or guanidine).

Guanidine

The beneficial effect of guanidine was initially described by Lambert and colleagues [6, 8]. Guanidine increases intracellular calcium within the nerve terminal by inhibiting calcium uptake by organelles [119]. Its clinical utility is limited by significant hemopoietic and renal toxicity [120, 121], but guanidine is reported to be effective with less risk of toxicity when the dose is strictly limited and the drug is used in combination with pyridostigmine [31, 122].

3,4-Diaminopyridine and Amifampridine Phosphate

The effectiveness of 3,4-diaminopyridine in LES has been documented in placebo-controlled prospective trials and in long-term follow-up studies [30, 101–104, 123–126]. This agent blocks K_v channels in the nerve terminal, thus prolonging the action potential and enhancing Ca^{2+} entry. 3,4-Diaminopyridine may also have a direct effect on the voltage-gated calcium channel [127]. Objective measures of strength are improved, including respiratory muscle function. Electromyographically there is an increase in amplitude of the CMAP and decreased CMAP decrement, and SFEMG reveals less jitter and blocking [123–125, 128]. The peak beneficial effect of a single oral dose of 3,4-diaminopyridine occurs in about 1 h and lasts for 25 h [123, 128]. The usual effective dose is 10–20 mg four to five times daily [123–128]. Adverse effects include perioral and acral paresthesia, lightheadedness, headache, insomnia, and seizures [129]. Seizures are rare if the baseline EEG is normal and the daily dose does not exceed 100 mg. Cardiac arrhythmia was described in a patient given an intentional overdose of 3,4-diaminopyridine [130]. There is a theoretical risk of cardiac arrhythmia in patients with the prolonged QT syndrome, but 3,4-diaminopyridine toxicity has not been reported in that setting. Because 3,4-diaminopyridine is not approved for routine use in the United States of America, its use requires application as an investigational drug on a compassionate use basis. The drug is contraindicated if preliminary screening by electrocardiogram and electroencephalogram reveals a prolonged QT interval or epileptiform discharges. It is Mayo Clinic practice to monitor complete blood count, liver function, and serum creatinine three times per year, but the authors have not yet encountered bone marrow, liver, or kidney toxicity, attributable to 3,4-diaminopyridine therapy.

Amifampridine, the phosphate salt of 3,4-diaminopyridine, has been licensed in Europe as an orphan drug for the treatment of LES, based on existing evidence of efficacy from unlicensed preparations. A recently completed multicenter phase 3 clinical trial demonstrated class I evidence for efficacy of amifampridine phosphate with a side effect profile similar to that of 3,4-diaminopyridine [105].

A modified form of (R)-roscovitine, GV-58, selectively activates P-/Q-type and N-type voltage-gated calcium channels and increases calcium-mediated quantal release of ACh in a mouse passive transfer model of LES [131]. When combined with 3,4-diaminopyridine, quantal release was returned to normal levels [132].

Treatment of the Associated Neoplasm

When cancer is found, its effective treatment may be followed by amelioration of LES manifestations or even remission [24, 56]. This has been documented particularly for SCLC treated by chemotherapy and radiation. Because neurological improvement is not universal, patients should be cautioned that their neuromuscular disorder may need continued symptomatic treatment or immunotherapy even if the underlying cancer is cured.

Immunotherapy

The general approach for LES is as for myasthenia gravis and other autoimmune neurological disorders. Immunotherapy is reserved for patients who fail to respond adequately to symptomatic therapy or, in the case of paraneoplastic LES, when tumor therapy is not accompanied by neurological improvement. Alleviation of symptoms in LES is generally less dramatic than in myasthenia gravis [28, 48, 66, 68, 69, 98, 101]. Nevertheless, benefit has been documented with plasmapheresis [133], intravenous immune globulin (IVIG) [134–136], and corticosteroids alone or in combination with azathioprine [26–28, 68] and rituximab [106, 137]. In patients who are considered at high risk for cancer, immunotherapy is customarily reserved for those who are severely disabled by weakness and failing to respond to symptomatic therapy. Reluctance to treat stems from fear of compromising the patient's survival by promoting evasion of the suspected tumor from an effective immune response. Parenthetically, however, we do not recall having observed noteworthy emergence of metastatic cancer in three decades of using prednisone and azathioprine to treat numerous LES patients at risk for lung cancer. This anecdotal observation implies that an inherently effective cancer immune response is not impaired by the standard immunosuppressant protocols used to treat neurological autoimmune disorders.

The type of immunotherapy chosen is tailored to the individual patient's need, depending on the magnitude and duration of benefit. Plasmapheresis is beneficial in LES, but because the duration of benefit is limited to several weeks, it is generally reserved for severely weak patients, particularly in the setting of respiratory insufficiency. Occasionally plasmapheresis is used as maintenance therapy at a frequency of 12 exchanges every 34 months. This usually requires placement of a central line or arteriovenous shunt, which increases the risk of complications from infection, coagulation disorders, and fluid/electrolyte imbalance.

Evidence for the benefit of IVIG therapy is less convincing than for plasmapheresis. A small controlled trial did show statistically significant improvement in muscle strength and a lowering of calcium channel antibodies in IVIG-treated LES patients [138]. The beneficial effects peaked at 2–4 weeks and disappeared by 8 weeks. The high cost of IVIG and relatively short duration of action have limited its use as a maintenance therapy in LES. Treatment protocols have utilized 400–500 mg/kg daily for 25 consecutive days or intermittent infusions at a frequency of 12 every 28 weeks.

Oral corticosteroid therapy benefits most LES patients, but improvement of strength is less dramatic than observed in myasthenia gravis, and weakness may be worsened if steroid myopathy ensues. In one reported study, long-term treatment with prednisolone at 20–30 mg daily or on alternate days produced modest symptomatic benefit in most patients [28]. Azathioprine also is effective either as a steroid-sparing agent or as a standalone immunosuppressant. Insufficient data are available concerning the efficacy of cyclosporine, cyclophosphamide, mycophenolate, or rituximab as alternative immunosuppressants in LES. In principle, experimental treatment strategies that are being investigated for myasthenia gravis should be applicable to the treatment of LES, including the deletion of antigen-specific B cells or T cells and interference with costimulatory molecules that perpetuate the autoimmunization process.

References

1. Anderson HJ, Churchill-Davidson HC, Richardson AT. Bronchial neoplasm with myasthenia; prolonged apnoea after administration of succinylcholine. Lancet. 1953;265(6799):1291–3.
2. Griesmann GE, Harper CM, Lennon VA. Paraneoplastic myasthenia gravis and lung carcinoma: distinction from Lambert-Eaton myasthenic syndrome and hypothesis of aberrant muscle acetylcholine receptor expression. Muscle Nerve. 1998;7(Suppl 7):S122.
3. Lambert EH, Eaton LM, Rooke ED. Defect of neuromuscular transmission associated with malignant neoplasm. Am J Phys. 1956;187:612.
4. Eaton LM, Lambert EH. Electromyography and electrical stimulation of nerves in diseases of the motor unit: observations on myasthenic syndrome associated with malignant tumors. JAMA. 1957;163:1117–24.
5. Lambert EH, Rooke ED, Eaton LM, Hodgson CH. Myasthenic syndrome occasionally associated with bronchial neoplasm. In: Thomas CC, editor. Myasthenia gravis; 1959. p. 362.
6. Lambert EH, Rooke ED, Eaton LM, Hodgson CH. Myasthenic syndrome occasionally associated with bronchial neoplasm: neurophysiologic studies. In: Viets HR, editor. Myasthenia gravis. Springfield: Charles C. Thomas; 1961. p. 362–410.
7. Lambert EH, Rooke ED. Myasthenic state and lung cancer. In: Brain WR, Norris FH, editors. The remote effects of cancer on the nervous system (contemporary neurology symposia), vol. 1. New York: Grune & Stratton; 1965. p. 67–80.
8. Elmqvist D, Lambert EH. Detailed analysis of neuromuscular transmission in a patient with the myasthenic syndrome sometimes associated with bronchogenic carcinoma. Mayo Clin Proc. 1968;43:689–713.
9. Lambert EH, Elmqvist D. Quantal components of end-plate potentials in the myasthenic syndrome. Ann N Y Acad Sci. 1971;183:183–99.
10. Gutmann L, Crosby TW, Takamori M, Martin JD. The Eaton-Lambert syndrome and autoimmune disorders. Am J Med. 1972;53:354–6.
11. Lennon VA, Lambert EH, Whittingham S, Fairbanks V. Autoimmunity in the Lambert-Eaton myasthenic syndrome. Muscle Nerve. 1982;5:S21–5.
12. Fukunaga H, Engel AG, Osame M, Lambert EH. Paucity and disorganization of presynaptic membrane active zones in the Lambert-Eaton myasthenic syndrome. Muscle Nerve. 1982;5:686–97.
13. Engel AG. Anatomy and molecular architecture of the neuromuscular junction. In: Engel AG, editor. Myasthenia gravis and myasthenic syndromes. New York: Oxford Press; 1999. p. 3–40.
14. Lang B, Newsom-Davis J, Wray D, Vincent A, Murray N. Autoimmune etiology for myasthenic (Eaton-Lambert) syndrome. Lancet. 1981;2:224–6.
15. Kim YI. Passively transferred Lambert-Eaton syndrome in mice receiving purified IgG. Muscle Nerve. 1986;9:523–30.
16. Fukunaga H, Engel AG, Lang B, Newsom-Davis J, Vincent A. Passive transfer of Lambert-Eaton myasthenic syndrome with IgG from man to mouse depletes the presynaptic membrane active zones. Proc Natl Acad Sci U S A. 1983;80:7636–40.
17. Lambert EH, Lennon VA. Selected IgG rapidly induces Lambert-Eaton myasthenic syndrome in mice: complement independence and EMG abnormalities. Muscle Nerve. 1988;11:1133–45.
18. Roberts A, Perera S, Lang B, Vincent A, Newsom-Davis J. Paraneoplastic myasthenic syndrome IgG inhibits ^{45}Ca flux in human small cell carcinoma line. Nature. 1985;317:737–9.
19. Lennon VA, Lambert EH. Autoantibodies bind solubilized calcium channel-omega-conotoxin complexes from small cell lung carcinoma: a diagnostic aid for Lambert-Eaton myasthenic syndrome. Mayo Clin Proc. 1989;64:1498–504.
20. Sher E, Canal N, Piccolo G, Gotti C, Scoppetta C, Evoli A, Clementi F. Specificity of calcium channel autoantibodies in Lambert-Eaton myasthenic syndrome. Lancet. 1989;ii:640–3.
21. Leys K, Lang B, Johnston I, Newsom-Davis J. Calcium channel autoantibodies in the Lambert-Eaton myasthenic syndrome. Ann Neurol. 1991;29:307–14.
22. Lennon VA, Kryzer TJ, Griesmann GE, O'Suilleabhain PE, Windebank AJ, Woppmann A, Miljanich GP, Lambert EH. Calcium-channel antibodies in the Lambert-Eaton syndrome and other paraneoplastic syndromes. N Engl J Med. 1995;332:1467–71.
23. Lennon VA. Serologic profile of myasthenia gravis and distinction from Lambert-Eaton myasthenic syndrome. Neurology. 1997;48(Suppl 5):S23–7.
24. Chalk CH, Murray NM, Newsom-Davis J, O'Neill JH, Spiro SG. Response of the Lambert-Eaton myasthenic syndrome to treatment of associated small-cell lung carcinoma. Neurology. 1990;40:1552–6.
25. Streib EW, Rothner AD. Lambert-Eaton myasthenic syndrome: long-term treatment of three patients with prednisone. Ann Neurol. 1981;10:448–53.
26. Newsom-Davis J, Murray NM. Plasma exchange and immunosuppressive drug treatment in the Lambert-Eaton myasthenic syndrome. Neurology. 1984;34:480–5.
27. Lundh H, Nilsson O, Rosen I. Current therapy of the Lambert-Eaton myasthenic syndrome. Prog Brain Res. 1990;84:163–70.
28. Maddison P, Lang B, Mills K, Newsom-Davis J. Long-term outcome in Lambert-Eaton myasthenic syndrome without lung cancer. J Neurol Neurosurg Psychiatry. 2001;70:212–7.
29. Lundh H, Nilsson O, Rosen I. Treatment of Lambert-Eaton syndrome: 3,4-diaminopyridine and pyridostigmine. Neurology. 1984;34:1324–30.

30. McEvoy KM, Windebank AJ, Daube JR, Low PA. 3,4-Diaminopyridine in the treatment of Lambert-Eaton myasthenic syndrome. N Engl J Med. 1989;321:1567–71.
31. Oh SJ, Kim DS, Head TC, Claussen GC. Low dose guanidine and pyridostigmine: relatively safe and effective long-term symptomatic therapy in Lambert-Eaton myasthenic syndrome. Muscle Nerve. 1997;20:1146–52.
32. Boonyapisit K, Kaminski HJ, Ruff RL. The molecular basis of neuromuscular transmission disorders. Am J Med. 1999;106:97–113.
33. Lambert EH, Okihiro M, Rooke ED. Clinical physiology of the neuromuscular junction. In: Paul WM, Daniel EE, Kay CM, Monckton G, editors. Muscle. Oxford: Pergamon Press; 1965. p. 487–97.
34. Hughes BW, Kusner LL, Kaminski HJ. Molecular architecture of the neuromuscular junction. Muscle Nerve. 2006;33:445–61.
35. Anderson CR, Stevens CF. Voltage clamp analysis of acetylcholine produced end-plate current fluctuation at frog neuromuscular junction. J Physiol. 1973;235:655–91.
36. Jablecki CK. Electrodiagnostic evaluation of patients with myasthenia gravis and related disorders. Neurol Clin. 1985;3:557–72.
37. Jones SW. Overview of voltage-dependent calcium channels. J Bioenerg Biomembr. 1998;30:299–312.
38. Tim RW, Massey JM, Sanders DB. Lambert-Eaton myasthenic syndrome: electrodiagnostic findings and response to treatment. Neurology. 2000;54:2176–8.
39. Slater CR. The functional organization of motor nerve terminals. Prog Neurobiol. 2015;134:55–103.
40. Sudhof TC. The synaptic vesicle cycle. Annu Rev Neurosci. 2004;27:509–47.
41. Striegel AR, Biela LM, Evans CS, Wang Z, Delehoy JB, Sutton RB, et al. Calcium binding by synaptotagmin's C2A domain is an essential element of the electrostatic switch that triggers synchronous synaptic transmission. J Neurosci. 2012;32:1253–60.
42. Takamura M. Structure of the neuromuscular junction: function and cooperative mechanisms in the synapse. Ann N Y Acad Sci. 2012;1274:14–23.
43. Black JL, Lennon VA. Identification and cloning of human neuronal high voltage-gated calcium channel γ-2 and γ-3 subunits: neurological implications. Mayo Clin Proc. 1999;74:357–61.
44. Molenaar PC, Newsom-Davis J, Polak RL, Vincent A. Eaton-Lambert syndrome: acetylcholine and choline acetyltransferase in skeletal muscle. Neurology. 1982;32:1061–5.
45. Simms BA, Zamponi GW. Neuronal voltage-gated calcium channels: structure, function, and dysfunction. Neuron. 2014;82:24–45.
46. Harper CM. Electrodiagnosis of endplate disease. In: Engel AG, editor. Myasthenia gravis and myasthenic syndromes. New York: Oxford Press; 1999. p. 65–86.
47. Denys E, Lennon VA. Asymptomatic Lambert-Eaton syndrome. Muscle Nerve. 2014;49:764–7.
48. O'Neill JH, Murray NM, Newsom-Davis J. The Lambert-Eaton myasthenic syndrome. A review of 50 cases. Brain. 1988;111:577–96.
49. Titulaer MJ, Lang B, Jan J, Verschuuren GM. Lambert–Eaton myasthenic syndrome: from clinical characteristics to therapeutic strategies. Lancet Neurol. 2011;10:1098–107.
50. O'Suilleabhain P, Low PA, Lennon VA. Autonomic dysfunction in the Lambert-Eaton myasthenic syndrome: serological and clinical correlates. Neurology. 1998;50:88–93.
51. Hillman D, Chen S, Aung TT, Cherksey B, Sugimori M, Llinás RR. Localization of P-type calcium channels in the central nervous system. Proc Natl Acad Sci U S A. 1991;88:7076–80.
52. Waterman SA, Lang B, Newsom-Davis J. Effect of Lambert-Eaton myasthenic syndrome antibodies on autonomic neurones in the mouse. Ann Neurol. 1997;42:147–56.
53. Komai K, Jwasa K, Takamori M. Calcium channel peptide can cause an autoimmune-mediated model of Lambert-Eaton myasthenic syndrome in rats. J Neurol Sci. 1999;166:126–30.
54. Oguro-Okano M, Griesmann GE, Wieben ED, Slaymaker S, Snutch TP, Lennon VA. Molecular diversity of neuronal type calcium channels identified in small cell lung carcinoma. Mayo Clin Proc. 1992;67:1150–9.
55. De Aizpurua HJ, Lambert EH, Griesmann GE, Olivera BM, Lennon VA. Antagonism of voltage gated calcium channels in small cell carcinomas of patients with and without Lambert Eaton myasthenic syndrome by autoantibodies, ω conotoxin and adenosine. Cancer Res. 1988;48:4719–24.
56. Maddison P, Newsom-Davis J, Mills KR, Souhami RL. Favorable prognosis in Lambert- Eaton myasthenic syndrome and small-cell lung carcinoma. Lancet. 1999;353:117–8.
57. Prior C, Lang B, Wray D, Newsom-Davis J. Action of Lambert-Eaton myasthenic syndrome IgG at mouse motor nerve terminals. Ann Neurol. 1985;17:587–92.
58. Fukuoka T, Engel AG, Lang B, Newsom-Davis J, Prior C, Wray DW. Lambert-Eaton myasthenic syndrome: I. Early morphological effects of IgG on the presynaptic membrane active zones. Ann Neurol. 1987;22:193–9.
59. Hajela RK, Huntoon KM, Atchison WD. Lambert-Eaton syndrome antibodies target multiple subunits of voltage-gated Ca2+ channels. Muscle Nerve. 2015;51:176–84.
60. Lang B, Vincent A, Murray NM, Newsom-Davis J. Lambert-Eaton myasthenic syndrome: immunoglobulin G inhibition of Ca2+ influx in tumor cells correlates with disease severity. Ann Neurol. 1989;25:265–71.

61. Meriney SD, Hulsizer SC, Lennon VA, Grinnell AD. Lambert-Eaton myasthenic syndrome immunoglobulins react with multiple types of calcium channels in small cell lung carcinoma. Ann Neurol. 1996;40:739–49.
62. Garcia KD, Beam KG. Reduction of calcium currents by Lambert-Eaton syndrome sera: motoneurons are preferentially affected, and L-type currents are spared. J Neurosci. 1996;16:4903–13.
63. Lang B, Newsom-Davis J, Peers C, Prior C, Wray DW. The effect of myasthenic syndrome antibody on presynaptic calcium channels in the mouse. J Physiol. 1987;390:257–70.
64. Yu Z, Lennon VA. Mechanism of intravenous immune globulin therapy in antibody-mediated autoimmune diseases. N Engl J Med. 1999;340:227–8.
65. Giuntoli RL, Lu J, Kobayashi H, Kennedy R, Celis E. Direct costimulation of tumor-reactive CTL by helper T cells potentiate their proliferation, survival and effector function. Clin Cancer Res. 2002;8:922–31.
66. Nakao YK, Motomura M, Fukudome T, Fukuda T, Shiraishi H, Yoshimura T, et al. Seronegative Lambert–Eaton myasthenic syndrome: study of 110 Japanese patients. Neurology. 2002;59:1773–5.
67. Oh SJ, Hatanaka Y, Claussen GC, Sher E. Electrophysiological differences in seropositive and seronegative Lambert–Eaton myasthenic syndrome. Muscle Nerve. 2007;35:178–83.
68. Hülsbrink R, Hashemolhosseini S. Lambert–Eaton myasthenic syndrome—diagnosis, pathogenesis and therapy. Clin Neurophysiol. 2014;125:2328–36.
69. Wirtz PW, Nijnuis MG, Sotodeh M, Willems LNA, Brahim JJ, Putter H, et al. The epidemiology of myasthenia gravis, Lambert-Eaton myasthenic syndrome and their associated tumours in the northern part of the province of South Holland. J Neurol. 2003;250:698–701.
70. Willcox N, Demaine AG, Newsom-Davis J, Welsh KI, Robb SA, Spiro SG. Increased frequency of IgG heavy chain marker Glm(2) and of HLA-B8 in Lambert-Eaton myasthenic syndrome with and without associated lung carcinoma. Hum Immunol. 1985;14:29–36.
71. Parsons KT, Kwok WW, Gaur LK, Nepom GT. Increased frequency of HLA class II alleles DRB1*0301 and DQB1*0201 in Lambert-Eaton myasthenic syndrome without associated cancer. Hum Immunol. 2000;61:828–33.
72. Elrington GM, Murray NME, Spiro SG, Newsom-Davis J. Neurological paraneoplastic syndromes in patients with small cell lung cancer: a prospective survey of 150 patients. J Neurol Neurosurg Psychiatry. 1991;54:764–7.
73. Payne M, Bradbury P, Lang B. Prospective study into the incidence of Lambert Eaton myasthenic syndrome in small cell lung cancer. J Thorac Oncol. 2010;5:34–8.
74. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics 2014. CA Cancer J Clin. 2014;64(1):9–29.
75. Lucchinetti CF, Kimmel DW, Lennon VA. Paraneoplastic and oncological profiles of patients seropositive for type 1 anti-neuronal nuclear autoantibodies. Neurology. 1998;50:652–7.
76. Yu Z, Kryzer TJ, Griesmann GE, Kim K-K, Benarroch E, Lennon VA. CRMP-5 neuronal autoantibody: marker of lung cancer and thymoma-related autoimmunity. Ann Neurol. 2001;49:146–54.
77. Iorio R, Lennon VA. Neural antigen-specific autoimmune disorders. Immunol Rev. 2012;248:104–21.
78. Gozzard P, Woodhall M, Chapman C, Nibber A, Waters P, Vincent A, et al. Paraneoplastic neurologic disorders in small cell lung carcinoma. A prospective study. Neurology. 2015;85:235–9.
79. Pittock SJ, Kryzer TJ, Lennon VA. Paraneoplastic antibodies coexist and predict cancer, not neurological syndrome. Ann Neurol. 2004;56:715–9.
80. Gutmann L, Phillips LH, Gutmann L. Trends in the association of Lambert-Eaton myasthenic syndrome with carcinoma. Neurology. 1992;42:848–50.
81. Argov Z, Shapira Y, Averbuch-Heller L, Wirguin I. Lambert-Eaton myasthenic syndrome (LES) in association with lymphoproliferative disorders. Muscle Nerve. 1995;18:715–9.
82. Burns TM, Juel VC, Sanders DB, Phillips LH. Neuroendocrine lung tumors and disorders of the neuromuscular junction. Neurology. 1999;52:1490–1.
83. Collins DR, Connolly S, Burns M, Offiah L, Grainger R, Walsh JB. Lambert-Eaton myasthenic syndrome in association with transitional cell carcinoma: a previously unrecognized association. Urology. 1999;54:162.
84. Dalmau J, Gultekin HS, Posner JB. Paraneoplastic neurologic syndromes: pathogenesis and physiopathology. Brain Pathol. 1999;9:275–84.
85. Oyaizu T, Okada Y, Sagawa M, Yamakawa K, Kuroda H, Fujihara K, et al. Lambert-Eaton myasthenic syndrome associated with an anterior mediastinal small cell carcinoma. J Thorac Cardiovasc Surg. 2001;121:1005–6.
86. Mckeon A, Apiwattanakul M, Lachance D, Lennon, Mandrekar JN, Boeve BF. Positron emission tomography-computed tomography in paraneoplastic neurologic disorders. Arch Neurol. 2010;67:322–9.
87. Kennelly KD, Dodick DW, Pascuzzi RM, Albain KS, Lennon VA. Neuronal autoantibodies and paraneoplastic neurological syndromes associated with extrapulmonary small cell carcinoma. Neurology. 1997;48(Suppl 2):A31.
88. Burns TM, Russell JA, Lachance DH, Jones HR. Oculobulbar involvement is typical with Lambert-Eaton myasthenic syndrome. Ann Neurol. 2003;53:270–3.

89. Young JD, Leavitt JA. Lambert-Eaton myasthenic syndrome: ocular signs and symptoms. J Neuroophthalmol. 2016;36:20–2.
90. Barr CW, Claussen G, Thomas D, Fesenmeier JT, Pearlman RL, Oh SJ. Primary respiratory failure as the presenting symptom in Lambert-Eaton myasthenic syndrome. Muscle Nerve. 1993;6:712–5.
91. Chan KH, Vernino S, Lennon VA. ANNA-3 antineuronal nuclear antibody: marker of lung cancer-related autoimmunity. Ann Neurol. 2001;50:301–11.
92. Sabater L, Titulaer M, Saiz A, Verschuuren J, Güre AO, Graus F. SOX1 antibodies are markers of paraneoplastic Lambert-Eaton myasthenic syndrome. Neurology. 2008;70:924–8.
93. Gadoth A, Kryzer TJ, Fryer J, McKeon A, Lennon VA, Pittock SJ. Microtubule associated protein 1B: novel paraneoplastic biomarker. Ann Neurol. 2017;81:266–77.
94. Lee H-R, Lennon VA, Camilleri M, Prather CM. Paraneoplastic gastrointestinal motor dysfunction: clinical and laboratory characteristics. Am J Gastroenterol. 2001;96:373–9.
95. Vernino S, Low PA, Fealey RD, Stewart JD, Farrugia G, Lennon VA. Autoantibodies to ganglionic acetylcholine receptors in autoimmune autonomic neuropathies. N Engl J Med. 2000;343:847–55.
96. Knowles CH, Lang B, Clover L, Scott SM, Gotti C, Vincent A, Martin JE. A role for autoantibodies in some cases of acquired non-paraneoplastic gut dysmotility. Scand J Gastroenterol. 2002;37:166–70.
97. Dhamija R, Tan KM, Pittock SJ, Fox-Orenstein A, Benarroch E, Lennon V. Serologic profiles aiding the diagnosis of autoimmune gastrointestinal dysmotility. Clin Gastroenterol Hepatol. 2008;6:988–92.
98. Titulaer MJ, Verschuuren JJ. Lambert-Eaton myasthenic syndrome: tumor versus nontumor forms. Ann N Y Acad Sci. 2008;1132:129–34.
99. Gozzard P, Chapman C, Vincent A, Lang B, Maddison P. Novel humoral prognostic markers in small-cell lung carcinoma: a prospective study. PLoS One. 2015;10(11):e0143558.
100. Galanis E, Frytak S, Rowland KM, Sloan A, Lennon VA. Neuronal autoantibody titers in the course of small-cell lung carcinoma and platinum-associated neuropathy. Cancer Immunol Immunother. 1999;48:85–90.
101. Harms L, Sieb JP, Williams AE, Graham R, Shlaen R, Claus V, et al. Long-term disease history, clinical symptoms, health status, and healthcare utilization in patients suffering from Lambert Eaton myasthenic syndrome: results of a patient interview survey in Germany. J Med Econ. 2012;15:521–30.
102. Lundh H, Nilsson O, Rosen I, Johansson S. Practical aspects of 3,4-diaminopyridine treatment of the Lambert-Eaton myasthenic syndrome. Acta Neurol Scand. 1993;88:136–40.
103. Sanders DB. 3,4-Diaminopyridine (DAP) in the treatment of Lambert-Eaton myasthenic syndrome (LES). Ann N Y Acad Sci. 1998;841:811–6.
104. Wirtz PW, Titulaer MJ, Gerven JM, Verschuuren JJ. 3,4-diaminopyridine for the treatment of Lambert-Eaton myasthenic syndrome. Expert Rev Clin Immunol. 2010;6:867–74.
105. Oh SJ, Shcherbakova N, Kostera-Pruszczyk A, Alsharabati M, Dimachkie M, Blanco JM, et al. Amifampridine phosphate [Firdapse] is effective and safe in a phase 3 clinical trial in LEMS. Muscle Nerve. 2016;53:717–25.
106. Maddison P, McConville J, Farrugia ME, Davies N, Rose M, Norwood F, et al. The use of rituximab in myasthenia gravis and Lambert–Eaton myasthenic syndrome. J Neurol Neurosurg Psychiatry. 2011;82:671–3.
107. Small S, Ali HH, Lennon VA, Brown RH, Carr DB, de Armendi A. Anesthesia for unsuspected Lambert-Eaton myasthenic syndrome with autoantibodies and occult small cell lung carcinoma. Anesthesiology. 1992;76:142–5.
108. Hatanaka Y, Oh SJ. Ten-second exercise is superior to 30-second exercise for post-exercise facilitation in diagnosing Lambert-Eaton myasthenic syndrome. Muscle Nerve. 2008;37(5):572.
109. Oh SJ, Ohira M. Single-fiber EMG and clinical correlation in Lambert-Eaton myasthenic syndrome. Muscle Nerve. 2013;47:664–7.
110. Sanders DB, Cao L, Massey JM, Juel VC, Hobson-Webb L, Guptill JT. Is the decremental pattern in Lambert-Eaton syndrome different from that in myasthenia gravis? Clin Neurophysiol. 2014;125:1274–7.
111. Oh SJ, Hatanaka Y, Ito E, Nagai T. Post-exercise exhaustion in Lambert–Eaton myasthenic syndrome. Clin Neurophysiol. 2014;125:411–4.
112. Chan KH, Lachance DH, Harper CM, Lennon VA. Frequency of seronegativity in adult-acquired generalized myasthenia gravis. Muscle Nerve. 2007;36:651–8.
113. Titulaer MJ, Maddison P, Sont JK, Wirtz PW, Hilton-Jones D, Klooster R, et al. Clinical Dutch-English Lambert-Eaton myasthenic syndrome (LEMS) tumor association prediction score accurately predicts small-cell lung cancer in the LEMS. J Clin Oncol. 2011;29:902–8.
114. Vernino S, Adamski J, Kryzer TJ, Lennon VA. Neuronal nicotinic AChR antibody in subacute autonomic neuropathy and cancer-related syndromes. Neurology. 1998;50:1806–13.
115. Lennon VA, Kryzer TJ. Correspondence: neuronal calcium channel autoantibodies coexisting with type 1 Purkinje cell cytoplasmic autoantibodies (PCA-1 or “anti-Yo”). Neurology. 1998;51:327–8.
116. Albers JW, Faulkner JA, Dorovini-Zis K. Abnormal neuromuscular transmission in an infantile myasthenic syndrome. Ann Neurol. 1984;16:28–34.
117. Bady B, Chauplannaz G, Carrier H. Congenital Lambert-Eaton myasthenic syndrome. J Neurol Neurosurg Psychiatry. 1987;50:476–8.

118. Katz JS, Wolfe GI, Bryan WW, Tintner R, Barohn RJ. Acetylcholine receptor antibodies in the Lambert-Eaton myasthenic syndrome. Neurology. 1998;50:470–5.
119. Kamenskaya MA, Elmqvist D, Thesleff S. Guanidine and neuromuscular transmission. IL effect on transmitter release in response to repetitive nerve stimulation. Arch Neurol. 1975;32:510–8.
120. Cherington M. Guanidine and germine in Eaton-Lambert syndrome. Neurology. 1976;26:944–6.
121. Blumhardt LD, Joekes AM, Marshall J, Philalithis PE. Guanidine treatment and impaired renal function in the Eaton-Lambert syndrome. Br Med J. 1977;1:946–7.
122. Silbert PL, Hankey GJ, Barr AL. Successful alternate day guanidine therapy following guanidine-induced neutropenia in the Lambert-Eaton myasthenic syndrome. Muscle Nerve. 1990;13:360–1.
123. Harper CM, McEvoy KM, Windebank AJ, Daube JR. Effect of 3,4-diaminopyridine on neuromuscular transmission in patients with Lambert-Eaton myasthenic syndrome. Electroencephalogr Clin Neurophysiol. 1990;75:557.
124. Sanders DB, Massey JM, Sanders LL, Edwards LJ. A randomized trial of 3,4-diaminopyridine in Lambert-Eaton myasthenic syndrome. Neurology. 2000;54:603–7.
125. Oh SJ, Claussen G, Hatanaka Y, Morgan MB. 3,4-Diaminopyridine is more effective than placebo in a randomized, double-blind, cross-over drug study in LEMS. Muscle Nerve. 2009;40:795–800.
126. Wirtz PW, Verschuuren JJ, van Dijk JG, de Kam ML, Schoemaker RC, van Hasselt JG, et al. Efficacy of 3,4-diaminopyridine and pyridostigmine in the treatment of Lambert Eaton myasthenic syndrome: a randomized, double-blind, placebo-controlled, crossover study. Clin Pharmacol Ther. 2009; 86:44–8.
127. Wu ZZ, Li DP, Chen SR, Pan HL. Aminopyridines potentiate synaptic and neuromuscular transmission by targeting the voltage-activated calcium channel beta subunit. J Biol Chem. 2009;284:36453–61.
128. Kim DS, Claussen GC, Oh SJ. Single-fiber electromyography improvement with 3,4-diaminopyridine in Lambert-Eaton myasthenic syndrome. Muscle Nerve. 1998;21(8):1107.
129. Flet L, Polard E, Guillard O, Leray E, Allain H, Loic J, et al. 3,4-Diaminopyridine safety in clinical practice: an observational, retrospective cohort study. J Neurol. 2010;257:937–46.
130. Boerma CE, Rommes JH, van Leeuwen RB, Bakker J. Cardiac arrest following an iatrogenic 3,4-diaminopyridine intoxication in a patient with Lambert-Eaton myasthenic syndrome. Clin Toxicol. 1995;33:249–51.
131. Tarr TB, Malick W, Liang M, Valdomir G, Frasso M, Lacomis D, et al. Evaluation of a novel calcium channel agonist for therapeutic potential in Lambert-Eaton myasthenic syndrome. J Neurosci. 2013;33:10559–67.
132. Tarr TB, Lacomis D, Reddel SW, Liang M, Valdomir G, Frasso M, et al. Complete reversal of Lambert-Eaton myasthenic syndrome synaptic impairment by the combined use of a K+ channel blocker and a Ca2+ channel agonist. J Physiol. 2014;592:3687–96.
133. Dau PC, Denys EH. Plasmapheresis and immunosuppressive drug therapy in the Eaton-Lambert syndrome. Ann Neurol. 1982;11:570–5.
134. Bird SJ. Clinical and electrophysiologic improvement in Lambert-Eaton syndrome with intravenous immunoglobulin therapy. Neurology. 1992;42:1422–3.
135. Motomura M, Tsujihata M, Takeo G, Matsuo H, Kinoshita I, Nakamura T, et al. The effect of high-dose gamma-globulin in Lambert-Eaton myasthenic syndrome. Neurol Ther. 1994;11:377–83.
136. Takano H, Tanaka M, Koike R, Nagai H, Arakawa M, Tsuji S. Effect of intravenous immunoglobulin in Lambert-Eaton myasthenic syndrome with small-cell lung cancer: correlation with the titer of anti-voltage-gated calcium channel antibody. Muscle Nerve. 1994;17:1074–5.
137. Pellkofer HL, Voltz R, Kuempfel T. Favorable response to rituximab in a patient with anti-VGCC-positive Lambert-Eaton myasthenic syndrome and cerebellar dysfunction. Muscle Nerve. 2009;40:305–8.
138. Bain PG, Motomura M, Newsom-Davis J, Misbah SA, Chapel HM, Lee ML, et al. Effects of intravenous immunoglobulin (IVIg) treatment on muscle weakness and calcium channel autoantibodies in the Lambert-Eaton myasthenic syndrome. Neurology. 1996;47:678–83.
139. Weiss N, Zamponi GW. Trafficking of neuronal calcium channels. Neuronal Signal. 2017;1. https://doi.org/10.1042/NS20160003.

Acquired Neuromyotonia

15

Hakan Cetin and Angela Vincent

Introduction

Neuromyotonia (NMT; Isaacs' syndrome) is a syndrome of nerve hyperexcitability that usually presents with clinical and electrodiagnostic features of continuous skeletal muscle hyperactivity. Although the syndrome can be caused by several different pathogenic mechanisms, this chapter will concentrate on the acquired autoimmune form. The clinical features, including autonomic and CNS symptoms, immune and tumor associations, and the role of antibodies to proteins that form part of a voltage-gated potassium channel (VGKC) complex will be reviewed.

This chapter is based on that written by Hart and Vincent in the first edition (2003). Dr. Ian Hart (Walton Centre for Neurology and Neurosurgery, Liverpool, UK) died unexpectedly in 2008, but his knowledge of neuromyotonia and contributions to its pathophysiology have been essential in revising this chapter for the third edition.

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Clinical and Electrophysiological Features of NMT and Related Syndromes

Clinical Manifestations

The typical presenting features are fasciculations, myokymia (i.e., irregularly undulating movements of the skin), and cramps [1–5], which can be associated with muscle stiffness, pseudomyotonia (i.e., delayed muscle relaxation after voluntary contraction), pseudotetany (e.g., Chvostek's and Trousseau's signs), and weakness. The limb and trunk muscles are most commonly affected, although facial, bulbar, and respiratory muscles can also be involved. Muscle contraction often provokes or exacerbates the symptoms. Reflexes are usually normal but can be reduced or absent when there is an accompanying neuropathy. Chronic cases may develop muscle hypertrophy.

Pain is often present and was reported to be the predominant symptom in an Indian NMT cohort [6]. Indeed, some patients with NMT have sensory symptoms such as paresthesia and numbness without evidence of peripheral neuropathy, suggesting that sensory as well as motor nerves may be hyperexcitable [7]. Moreover, autonomic symptoms such as constipation, tachycardia, and increased and sometimes patchy hyperhidrosis are often associated. Perhaps more interestingly, in view of the antibody-mediated pathology, cen-

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H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*, Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_15

tral nervous system (CNS) symptoms ranging from personality change and insomnia to a psychotic-like state with delusions and hallucinations can occur. This association is often called chorée fibrillaire or Morvan's syndrome [8, 9], which often includes autonomic dysfunction [10, 11]. Although the full-blown Morvan's syndrome is apparently rare, there is an emerging spectrum of related CNS disorders with mild or even absent peripheral features (see below).

Neuromyotonia can occur at any age, and since the severity of the clinical features ranges from the inconvenient to the incapacitating, it is not at all clear how common the disorder is. The course is variably progressive, although spontaneous remissions can occur and some forms may be monophasic. These have been related to established infections in a few cases [12, 13] or to a preceding anaphylactic shock [14].

A review of the clinical features of NMT showed that very similar features were found in patients with cramp-fasciculation syndrome (CFS, see [15]), a proportion of whom also had VGKC antibodies (Table 15.1 [15, 16]). This, together with other evidence for autoimmunity in some CFS patients [15], strongly indicates that CFS and NMT should be considered as parts of a spectrum of autoimmune peripheral nerve hyperexcitability (PNH), and some prefer to use this term exclusively. This may also apply to myokymia-cramp syndrome [17] and other conditions associated with muscle hyperexcitability and hyperhidrosis [18, 19]. Gutmann et al. [20] discussed the relationship between electrical neuromyotonic and myokymic discharges and the clinical disorder and came to a similar conclusion. Here we will mainly discuss NMT because most of the clinical and experimental studies have been performed in patients who fulfill this diagnosis.

Electrophysiological Features

The classical electrophysiological feature of NMT is the spontaneous firing of single motor units as doublet, triplet, or multiplet discharges (Fig. 15.1, taken from [21]). They occur at irregular intervals of 1–30 s and have an intraburst frequency of 40–400 Hz. They may be present in the absence of visible muscle twitching. By contrast, myokymic discharges are characterized by undulating contractions of muscle fibers associated with wormlike movements of the skin. They are composed of 2–10 motor unit action potentials (MUAPs) spontaneously firing in a regular or semirhythmic pattern at intervals of 0.1–10 s. The intraburst frequency is typically 40–60 Hz [22].

Neuromyotonic discharges consist of bursts of MUAPs firing at very high frequencies ranging from 100 to 300 Hz. The duration can last from hundreds of milliseconds to seconds. Typically, the burst amplitude decreases due to the inability of muscle fibers to maintain high firing rates above 100 Hz [22]. In addition, voluntary or electrical

Table 15.1 Similarities between neuromyotonia and the cramp-fasciculation syndrome[a]

Feature	Neuromyotonia (42 patients)%	Cramp-fasciculation syndrome (18 patients)%
Cramps	88	100
Muscle twitching	100	94
Pseudomyotonia	36	22
Sensory symptoms	33	39
CNS symptoms	29	22
Other autoimmune diseases	59	28
Thymoma	19	11
Lung tumor	10	1
Other autoantibodies	50	17
VGKC antibodies	38	28

[a]Data from [15]

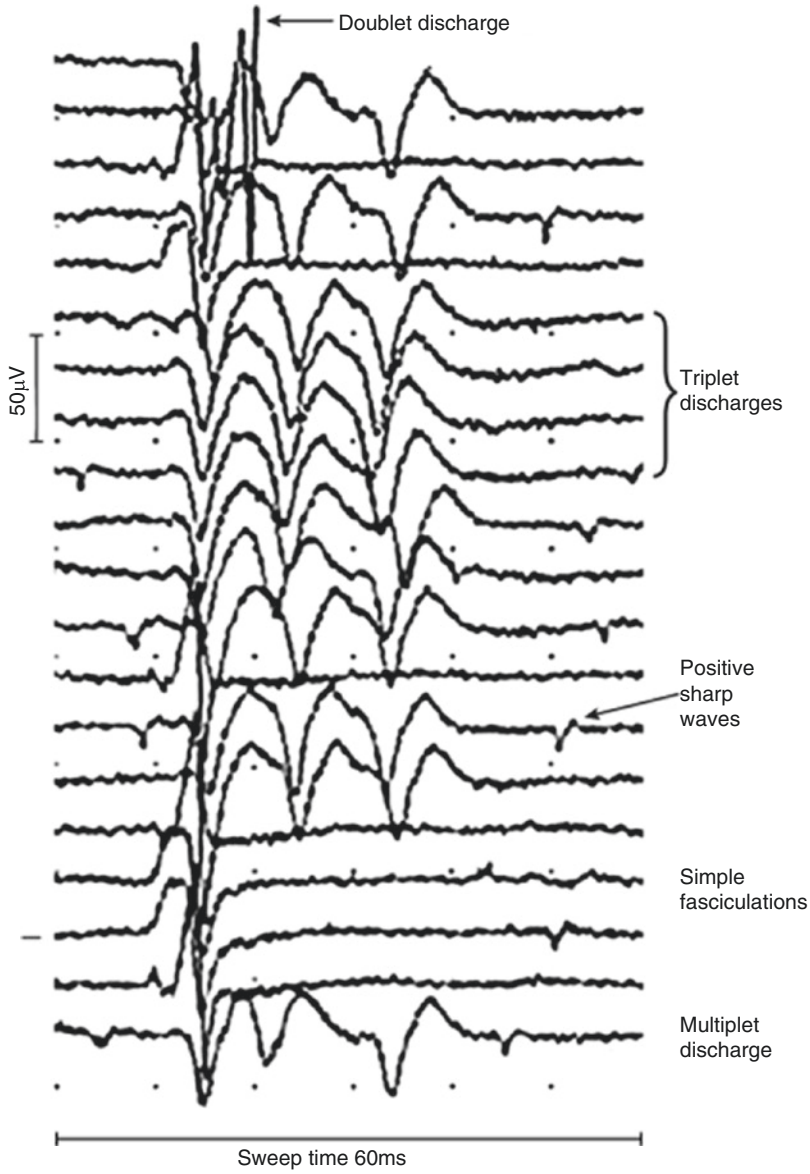

Fig. 15.1 A raster display of doublet and triplet discharges along with fasciculations in a representative patient with acquired neuromyotonia. Reproduced with permission of the authors from Vucic S, Cheah BC, Yiannikas C, Vincent A, and Kiernan MC. Corticomotoneuronal function and hyperexcitability in acquired neuromyotonia. Brain 2010; 133: 2727–2733

activation may trigger prolonged afterdischarges at similar frequencies. Fasciculation and fibrillation potentials can also occur. All electrophysiological phenomena in NMT are more prevalent in distal hand and foot muscles compared to proximal muscles, and there is a significant correlation of discharge frequency with the length of the nerve innervating a muscle being examined [22]. The presence of electrophysiological phenomena in NMT is summarized in Table 15.2 and [22, 23].

The spontaneous and repetitive muscle activity arises in the peripheral motor nerve, rather than in the muscle itself [4, 24]. Isaacs [24] showed that

Table 15.2 Frequency of different electrophysiological findings in NMT[a]

Electrophysiological finding	Frequency (%) in 11 patients
Spontaneous neuromyotonia	11 (100)
Stimulus-induced neuromyotonia	7 (65)
Fibrillation potentials	2 (18)
Fasciculations	11 (100)
Neuromyotonic discharges	
Doublets	11 (100)
Triplets	10 (90)
Multiplets	8 (73)
Maximum intraburst frequency (Hz)	50–220

[a]Data from [23]

the discharges were abolished when curare was used to block neuromuscular transmission but persisted after either proximal or distal nerve block. A macro EMG study also suggested that spontaneous discharges are generated distally [25]. These observations, and studies using botulinum toxin, suggested that the activity was generated in the terminal arborization of the motor axons [25, 26]. However, other studies indicated a more proximal initiation of the hyperactivity with reduction by proximal nerve block [27–29] or by epidural anesthesia [30]. The simplest explanation for these data is that the activity can originate at different sites including the lower motor neuron cell body in some cases.

Although it seems likely that NMT is usually a disorder of the motor nerve, rather than a secondary phenomenon caused by structural nerve or myelin damage, about 5–10% of patients have, in addition to NMT, electrical evidence of a subclinical idiopathic axonal or demyelinating polyneuropathy.

Serum and Cerebrospinal Fluid Tests

Although most patients with NMT reported so far do not have marked CNS features, it is common to find a raised total IgG and oligoclonal bands in the CSF [1]. Curiously, VGKC-complex (see below) antibodies are often not found in CSF, perhaps because the serum levels are relatively low and the corresponding CSF level is undetectable.

Physiological Basis of Excitability of Motor Axons in NMT Patients

It is possible to measure the response of subthreshold stimuli and the stimulus intensity (threshold) that is required to excite the motor nerve [31, 32]. One of these measures is the strength-duration time constant (SDTC), which is a property of the nodal membrane determined by the passive membrane time constant and the ion conductances acting at the nerve threshold. The SDTC was prolonged in the motor but not in the sensory axons in some NMT patients in one study [33]. However, a subsequent study, using more sensitive measures of both motor and sensory axonal excitability in the median nerve, found that all excitability indices were normal in all eight NMT patients examined [34]. Thus, a general alteration of axonal membrane permeability is probably not present in these patients, as predicted by the previous studies, which indicated a distal pathology in most patients (see above).

Clinical and Experimental Evidence for an Autoimmune Basis

Associated Autoimmune Syndromes

As in disorders of the other neuromuscular junction, one of the first indications that NMT had an autoimmune basis was the recognition of associated autoimmune diseases. These conditions include myasthenia gravis (MG) with or without thymoma, rheumatoid arthritis, systemic lupus erythematosus, and diabetes (see Table 15.1). Individual patients with NMT and systemic sclerosis or idiopathic thrombocytopenia or NMT occurring after bone marrow transplantation are also reported [35–43]. Some patients may have an inflammatory neuropathy, although in these cases one might argue that the neuropathy is the primary event [44].

The strongest tumor association is with thymoma. This occurs in patients with or without associated MG and particularly in the former can precede the neuromyotonia by several years. Small cell lung cancers are also described in a few patients (see Table 15.1 [29]), but in these cases the neuromyotonia usually precedes the cancer with latencies between onset of NMT and diagnosis of SCLC of up to 4 years [1]. There are isolated reports of neuromyotonia with Hodgkin's lymphoma [45] or with a plasmacytoma and paraproteinemia [46].

Response to Immunotherapies

The first clinical experiment that suggested an autoimmune etiology for NMT was in a patient in whom plasma exchange resulted in marked reduction in the frequency of spontaneous MUAPs [47]. Although there have been no formal trials of immunotherapies, there are many anecdotal reports of improvement following plasma exchange, intravenous immunoglobulins, or corticosteroids [1]. In most patients, clinical improvement begins within 2–8 days of plasma exchange and lasts for about 1 month.

Passive Transfer of Immunoglobulins to Mice

Passive transfer of NMT IgG to mice provided further evidence for the autoimmune basis of the disorder. After injection of purified IgG intraperitoneally for 10–12 days, there was an enhancement of neuromuscular transmission in mouse hemidiaphragm preparations, as demonstrated by a resistance to bath-applied curare [47]. Mice injected with NMT IgG released significantly more packets of acetylcholine from their motor nerve endings, consistent with the increased efficiency of neuromuscular transmission. However, there were no spontaneous MUAPs or double responses to single stimuli, as observed in the human disease [48]. Dorsal root ganglion cell cultures incubated with NMT IgG, however, showed repetitive action potentials [48]. Together these studies suggested that an IgG autoantibody is responsible for at least some of the features of neuromyotonia. Moreover, the striking similarity between the changes seen in the mouse preparations and those found after small doses of the VGKC antagonists, 3,4-diaminopyridine or 4-aminopyridine, made it likely that the target of the antibodies was a VGKC [48]. The main function of neuronal VGKC is to restore the membrane potential after each action potential, preventing the reopening of voltage-gated calcium channels. Thus, a reduction in VGKC numbers or function would lead to prolonged depolarization of the motor nerve with enhanced transmitter release. Indeed dendrotoxins from species of *Dendroaspis mambas* [49] that block the function of some of the VGKCs of the Kv1 family produce repetitive or spontaneous activity in the motor nerve terminal not dissimilar from that in NMT.

VGKCs function as delayed rectifiers or type A rapid inactivators and are involved in nerve repolarization [49]. A variety of studies suggest that they are mainly restricted to juxtaparanodal regions and nerve terminals of the peripheral motor nerves [50, 51]. In addition, they are also widely expressed in the central nervous system and peripheral sensory nerves. In the nodes, both Kv1.1 and 1.2 are expressed and co-localize with binding of NMT IgG [52]. The identity of the VGKCs at the motor nerve terminal is not so clear, but both Kv1.2 and 1.6 may be present as well as other types of potassium channels (K Kleopa, personal communication).

Antibodies to VGKC-Complex Proteins

The associated autoimmunity, response to plasma exchange, and partially successful passive transfer studies strongly suggested that acquired NMT had an autoantibody-mediated pathogenesis. In order to look for antibodies to VGKCs, the same approach was used as in MG (Chap. 8). VGKCs were extracted from human or mammalian brain and allowed to bind ^{125}I-α-dendrotoxin [48, 53]. The soluble labeled complex was then used in radioimmunoprecipitation assays; around 40% of NMT sera immunoprecipitated ^{125}I-α-dendrotoxin-labeled VGKCs (Fig. 15.2).

Patch clamp studies of cultured PC12 and NB-1 cell lines demonstrated that NMT IgG sup-

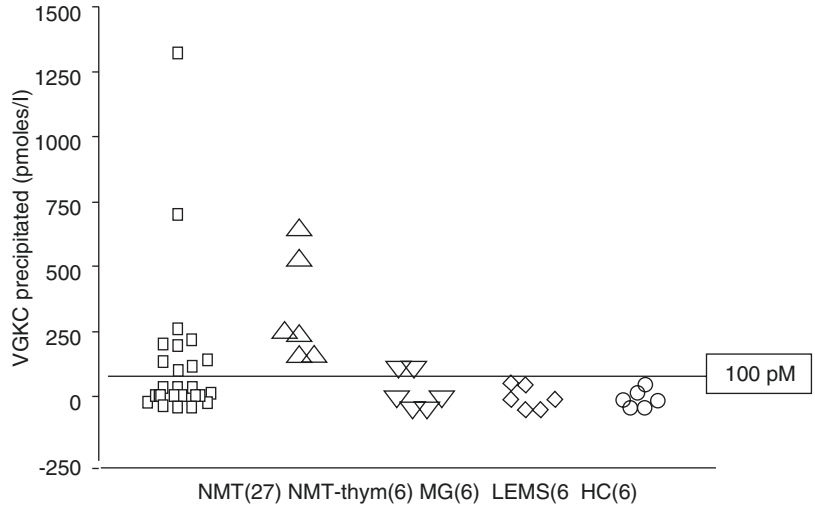

Fig. 15.2 VGKC-complex antibodies measured by radioimmunoprecipitation in neuromyotonia and cramp-fasciculation syndrome patients with and without a thymoma. Data taken from Hart et al. (2002). It is now clear that in many of the thymoma samples and around 40% of the non-thymoma samples, the VGKC-complex antibodies are actually binding to CASPR2, LGI1, or contactin-2 (see Fig. 15.3)

presses voltage-dependent potassium currents [54–56]. This effect did not occur within a few hours but was found after overnight incubation. Moreover, the effects were dependent on divalent antibodies or $F(ab')_2$ and not found with monovalent Fabs or Fc [56]. A direct block of function should be seen within 1 or 2 h, but when NMT IgG was applied to dorsal root ganglion cells, repetitive firing was observed after 24 h but not in the initial 2 h [48]. Similarly, in the patch clamp studies of PC12 cells, application of the NMT IgG for short periods did not have any effect, and positive results were only found after 3 or more days [54–56].

Functional VGKCs consist of four α subunits, each of which has six transmembrane regions (S1 to 6) [51]. Together, the S5 and S6 regions of the α subunits form the central ion pore. VGKC Kv1s are expressed as gene products that need to combine as homomultimeric or heteromultimeric tetramers and to associate with intracellular β subunits [57]. In the immunoprecipitation assay, only those complexes that include subtypes that bind dendrotoxin (Kv1.1, 1.2, and 1.6) are labeled, limiting detection of antibodies to those against these channels—or other proteins with which the channels are complexed. Indeed attempts to demonstrate that the antibodies were binding directly to the Kv1 channels themselves were mainly unsuccessful, and further studies demonstrated clearly that most of the VGKC-complex antibodies bound to other proteins [58].

The studies that were required to define more accurately the target(s) of the VGKC-complex antibodies were performed mainly in patients with high levels of antibodies that immunoprecipitated VGKC-complexes, and those tended to have CNS features (see further below). The main targets of the antibodies were identified as leucine-rich, glioma-inactivated 1 (LGI1) and contactin-associated protein-2 (CASPR2). Of the 11 patients with only NMT who were included in the study, seven had CASPR2 antibodies, and only one had LGI1 antibodies [58]. This contrasted with the much higher proportion of LGI1 antibodies in patients with purely CNS disease but is consistent with further studies of NMT (A Vincent, M Kiernan, and O Watanabe, in preparation). Whereas LGI1 is a CNS protein predominantly, CASPR2 is found at the juxtaparanodes of the nodes of Ranvier, both in the CNS and PNS, where it is closely associated with VGKCs and with another protein, contactin-2. Antibodies to contactin-2 can also be found in some patients with NMT or CNS disorder although they are uncommon [58, 59]. A diagram of the VGKC-

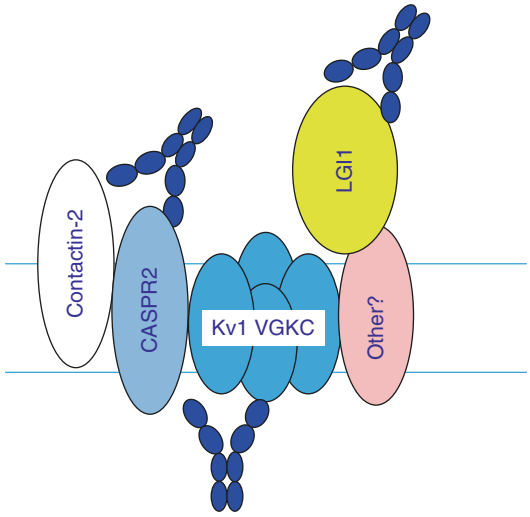

Fig. 15.3 The VGKC-complex. The CASPR2, LGI1, and contactin-2 antibodies are measured by cell-based assays as described in Cao and Vincent, Chap. 10. Because the VGKC-antibody assay is performed in solution, antibodies that bind to intracellular epitopes can be detected. It is therefore preferred to use live cell-based assays to measure antibodies to the extracellular epitopes on LGI1, CASPR2 or contactin-2

complex is shown in Fig. 15.3. Most VGKC-complex antibodies appear to be of both IgG1 and IgG4 subclasses [59]. IgG4 antibodies can, under certain circumstances, dissociate into two half molecules which can reassociate randomly with other IgG4 half molecules. This limits the immunopathological mechanisms as the resulting monovalent antibodies would not activate complement or be likely to lead to internalization of the VGKC-complex which requires divalent antibody binding. It is possible that the remaining IgG1 antibodies seen in CASPR2 antibodies, and much less so in LGI1 antibodies, are responsible for some loss of VGKC function in NMT and other disorders, but this has not yet been demonstrated.

VGKC-Complex Antibodies in Other Muscle Diseases

A study of 42 patients with NMT and 18 patients with cramp-fasciculation syndrome (CFS) suggests considerable overlap between the two syndromes [15], as summarized in Table 15.1. VGKC-complex antibodies were found in a patient with "neuromuscular hyperexcitability", very similar to a previous case of rippling muscle disease [60]. Both these patients had coexisting MG and are therefore assumed to have an autoimmune disease.

NMT can occur in patients with GBS [44], an acute inflammatory demyelinating polyneuropathy, and at least one patient had VGKC antibodies (G Ebers and A Vincent, unpublished observations). Defects in myelination by themselves can induce nerve hyperexcitability, presumably by secondary effects on the regional expression of ion channels along the length of the axon.

Central Nervous System Abnormalities in NMT

Morvan's Syndrome

The clinical association of neuromyotonia with hallucinations, delusions, insomnia, and personality change was first recognized by Auguste Morvan, who called it chorée fibrillaire [8, 9]. Although he described the case fully, the central symptoms were only recorded in one of the ten patients who otherwise had symptoms and signs of NMT. If they are sought specifically, the incidence of central nervous system manifestations in NMT is probably around 20% (see Table 15.1 [15]; and A Vincent, M Kiernan, and O Watanabe in preparation).

A detailed description of a patient with classical Morvan's syndrome was published in 2001 [11]. His onset was at 76 years of age, and no neoplasm was identified antemortem, although a small adenocarcinoma of the lung was found postmortem. He had developed severe insomnia, memory loss, confusion, and hallucinations over a few months, associated with extreme sweating, salivation, and severe constipation. An electroencephalogram showed complete lack of non-REM sleep and a tendency to oscillate between a state of sub-wakefulness and REM sleep. Polysomnographic studies showed florid neuro-

myotonic discharges and cardiac arrhythmias. He had very high levels of VGKC-complex antibodies, subsequently identified as directed against CASPR2 [11]. Strikingly, the peripheral, autonomic, and central features improved substantially after plasma exchange.

There have been a number of reports of Morvan's syndrome since with variable presentations including peripheral, autonomic, and central manifestations. VGKC-complex antibodies are present in the majority, and CASPR2 antibodies are the most frequent, although CASPR2 and LGI1 antibodies, or even in a few cases CASPR2, LGI1, and contactin-2 antibodies, can also be found [59]. Different antibodies can be found in rare patients with a similar sleep disorder of agrypnia [61]. Around 30% of the patients have a thymoma. In a few cases, the disease appears to associate with chemical treatment of scrotal hydrocele [62].

CNS Symptoms Without Overt Neuromyotonia

Two patients with VGKC-complex antibodies were reported who contrasted with Morvan's syndrome [63]. Both were diagnosed as having a limbic encephalitis on the basis of memory loss, confusion, and anxiety with abnormalities of the hippocampus on MRI before the antibody test was available. Neither was reported to have neuromyotonia or sleep disorders at the time or subsequently, but both had excessive secretions—sweating in one and salivation in the other. In a retrospective study, both patients had high levels of VGKC-complex antibodies at the time of their limbic manifestations, and both improved when VGKC-complex antibody levels fell—in one case after plasma exchange and in the other spontaneously. Interestingly, one of these patients was a woman with a long history of MG with thymoma. The limbic manifestations arose following a recurrence of the thymoma, 13 years after the tumor had been removed. At the time, the MG was poorly controlled, but there was a striking dissociation between acetylcholine receptor autoantibody levels that fluctuated quite widely and the single peak of VGKC-complex antibodies that coincided with the limbic abnormalities. Plasma exchange was effective in reducing the CNS signs [63].

Subsequently, VGKC-complex antibodies have been identified in a variety of patients with cognitive, psychiatric, or sleep disorders, similar to those previously associated with paraneoplastic limbic encephalitis but almost always non-paraneoplastic and with an excellent response to immunosuppressive treatment regimes [64, 65]. The VGKC-complex/LGI1-specific antibody levels in these patients are typically high (often >1000 pM) and fall dramatically following adequate immunosuppression. In many cases, the antibodies do not recur even when immunotherapies are stopped. This condition and the growing number of other "autoimmune encephalopathies" are reviewed in more detail elsewhere [66]. Here it is relevant that the presence of NMT in patients with CNS symptoms can be a clue to an immunotherapy-responsive condition.

Differential Diagnosis

The differential diagnosis of NMT comprises various disorders associated with peripheral nerve hyperexcitability and needs consideration especially in cases without detectable VGKC-complex antibodies.

Diseases Associated with Fasciculations

Fasciculations are commonly observed in motor neuron diseases including amyotrophic lateral sclerosis (ALS), spinal muscular atrophy, and Kennedy's syndrome. As progressive neurodegenerative disorders, they are usually associated with weakness and muscle atrophy, both of which do not predominate in NMT patients. In contrast to ALS, corticomotoneuronal pathways appear not to be involved in NMT, which might also be helpful in differential diagnostic evaluations [21]. Multifocal motor neuropathy (MMN) is

another immune-mediated disorder associated with fasciculations. However, muscle atrophy, the presence of antiganglioside (GM1) antibodies, and conduction block in electrodiagnostic studies are other typical MMN features guiding its differentiation from NMT.

Myopathies Associated with Spontaneous Activity in Electrodiagnostic Studies

Spontaneous activity in electrodiagnostic studies can be found in a variety of myopathies [22, 67], where it is of postsynaptic origin generated in the muscle itself. In NMT, however, spontaneous activity is of presynaptic origin associated with specific electrophysiological features that can help to distinguish it from postsynaptically generated spontaneous activity using needle electromyography. Presynaptically generated discharges are independent of needle insertions. The individual potentials derive from the activation of entire motor units and appear as normal motor unit action potentials [22]. Postsynaptically generated discharges, by contrast, are often associated with increased insertional activity. The potentials derive from individual muscle fibers rather than from the entire motor unit and are often shorter and smaller in amplitude.

Myopathies Associated with Muscle Contractions or Stiffness

Some patients with stiff person syndrome present with muscle hyperactivity and cramps. Electromyography recordings are characterized by the continuous firing of motor unit action potentials of normal morphology. Neuromyotonic and myokymic discharges are not seen. Recordings from antagonist muscle pairs often reveal the failure of reciprocal inhibition [68], which usually correlates with the clinical failure to relax affected muscles. Sleep or general anesthesia abolishes muscle activity in stiff person syndrome but not in NMT, and this is a clinically important distinction between the two conditions. Autoantibodies against glutamate decarboxylase (GAD) can often be found. Rippling muscle disease presents with muscle contractions provoked by percussion or stretching. They are electrically silent which can help to differentiate rippling muscle disease from NMT. The disease is caused by mutations in the gene encoding caveolin-3 although rare cases associated with myasthenia and AChR antibodies have been reported [69], that responded to immunotherapies. Brody disease is another rare myopathy associated with delayed muscle relaxation after phasic exercise and silent cramps (i.e., electrical silence during muscle stiffness on electromyography). The disease is caused by mutations in ATP2A1 encoding for SERCA1, a sarcoplasmic reticulum Ca^{2+} ATPase. A reduced SERCA1 activity results in a prolonged increase of intracellular calcium concentrations following muscle activation. Myotonias are a heterogeneous group of chloride or sodium channelopathies presenting with prolonged muscle contraction/delayed muscle relaxation after muscle activation and typically associated with percussion myotonia, which usually is not present in NMT. Electromyography often reveals extensive myotonic discharges.

Treatment of NMT

Antiepileptic drugs, such as carbamazepine and phenytoin, probably act by reducing sodium conductance and thus stabilize the nerve membrane making it less excitable. Many patients require a symptomatic treatment with these drugs only, either separately or together. In general, baclofen, benzodiazepines, carbonic anhydrase inhibitors, and quinine are unhelpful.

Plasma exchange often produces short-term relief [1], and a trial of plasma exchange can help establish the diagnosis in the difficult situation of a patient with suspected autoimmune NMT but with no VGKC antibodies. There is no evidence for a differential response to plasma exchange among patients with and without detectable VGKC antibodies. Nevertheless, a positive response to plasma exchange may be helpful in predicting those patients who are likely to benefit

from long-term immunosuppression. Intravenous immunoglobulin has also been used, although reports suggest that some patients do not do well [70] or do better with plasma exchange [71]. Combinations of prednisolone with azathioprine or methotrexate have helped some patients [1]. A recent review from Europe summarizes the main approaches to treatment of NMT [72], but randomized controlled trials are needed to evaluate these therapies more fully.

The potential association of NMT with tumors in paraneoplastic cases makes it necessary to search for and treat malignancies. This can have a beneficial effect on the NMT disease course but has been discussed controversially in literature [72, 73].

References

1. Newsom-Davis J, Mills KR. Immunological associations of acquired neuromyotonia (Isaacs' syndrome). Report of five cases and literature review. Brain. 1993;116:453–69.
2. Denny-Brown D, Foley DM. Myokymia and the benign fasciculation of muscular cramps. Trans Assoc Am Phys. 1948;61:88–96.
3. Gamstorp I, Wohlfart G. A syndrome characterized by myokymia, myotonia, muscular wasting and increased perspiration. Acta Psychiatr Scand. 1959;34:181–94.
4. Isaacs H. A syndrome of continuous muscle-fibre activity. J Neurol Neurosurg Psychiatry. 1961;24:319–25.
5. Mertens HG, Zschoke S. Neuromyotonie. Klin Wochenschr. 1965;43:917–25.
6. Panagariya A, Kumar H, Mathew V, Sharma B. Neuromyotonia: clinical profile of twenty cases from northwest India. Neurol India. 2006;54:382–6.
7. Lance JW, Burke D, Pollard J. Hyperexcitability of motor and sensory neurons in neuromyotonia. Ann Neurol. 1979;5:523–32.
8. Morvan A. De la chorée fibrillaire. Gaz Hebdon de Med Chirurg. 1890;27:173–200.
9. Serratrice G, Azulay JP. Que reste-t-il de la choreé fibrillaire de Morvan? Rev Neurol. 1994;150:257–65.
10. Halbach M, Homberg V, Freund HJ. Neuromuscular, autonomic and central cholinergic hyperactivity associated with thymoma and acetylcholine receptor-binding antibody. J Neurol (Berlin). 1987;234:433–6.
11. Liguori R, Vincent A, Clover L, Avoni P, Plazzi G, Cortelli P, et al. Morvan's syndrome: peripheral and central nervous system and cardiac involvement with antibodies to voltage-gated potassium channels. Brain. 2001;124(Pt 12):2417–26.
12. Maddison P, Lawn N, Mills KR, Vincent A, Donaghy M. Acquired neuromyotonia in a patient with spinal epidural abscess. Muscle Nerve. 1998;21:672–4.
13. Tahmouch AJ, Alonso RJ, Tahmouch GP, et al. Cramp-fasciculation syndrome: a treatable hyperexcitable peripheral nerve disorder. Neurology. 1991;41:1021–4.
14. Turner MR, Madkhana A, Ebers GC, Clover L, Vincent A, McGavin G, Sarrigiannis P, Kennett R, Warrell DA. Wasp sting induced autoimmune neuromyotonia. J Neurol Neurosurg Psychiatry. 2006;77:704–5.
15. Hart IK, Maddison P, Vincent A, Mills K, Newsom-Davis J. Phenotypic variants of autoimmune peripheral nerve hyperexcitability. Brain. 2002;125:1887–95.
16. Liewluck T, Klein CJ, Jones LK Jr. Cramp-fasciculation syndrome in patients with and without neural autoantibodies. Muscle Nerve. 2014;49:351–6.
17. Smith KKE, Claussen GY, Fesenmeier JT, Oh SJ. Myokymia-cramp syndrome: evidence of hyperexcitable peripheral nerve. Muscle Nerve. 1994;17:1065–7.
18. Wakayama Y, Ohbu S, Machida H. Myasthenia gravis, muscle twitch, hyperhidrosis, and limb pain associated with thymoma: proposal of a possible new myasthenic syndrome. Tohoku J Exp Med. 1991;164:285–91.
19. Ho WKH, Wilson JD. Hypothermia, hyperhidrosis, myokymia and increased urinary excretion of catecholamines associated with a thymoma. Med J Aust. 1993;158:787–8.
20. Gutmann L, Libell D, Gutmann L. When is myokymia neuromyotonia? Muscle Nerve. 2001;24:151–3.
21. Vucic S, Cheah BC, Yiannikas C, Vincent A, Kiernan MC. Corticomotoneuronal function and hyperexcitability in acquired neuromyotonia. Brain. 2010;133:2727–33.
22. Daube JR, Rubin DI. Needle electromyography. Muscle Nerve. 2009;39:244–70.
23. Maddison P, Mills KR, Newsom-Davis J. Clinical, electrophysiological characterization of the acquired neuromyotonia phenotype of autoimmune peripheral nerve hyperexcitability. Muscle Nerve. 2006;33:801–8.
24. Isaacs H. Continuous muscle fibre activity in an Indian male with additional evidence of terminal motor fibre abnormality. J Neurol Neurosurg Psychiatry. 1967;30:126–33.
25. Arimura K, Arimura Y, Ng A, et al. The origin of spontaneous discharges in acquired neuromyotonia. A macro EMG study. Muscle Nerve. 2005;116:1835–9.
26. Deymeer F, Oge AE, Serdaroglu P, et al. The use of botulinum toxin in localizing neuromyotonia to the terminal branches of the peripheral nerve. Muscle Nerve. 1998;21:643–6.
27. García-Merino A, Cabella A, Mora JS, Liaño H. Continuous muscle fiber activity, peripheral neuropathy, and thymoma. Ann Neurol. 1991;29:215–8.

28. Irani PF, Purohit AV, Wadia HH. The syndrome of continuous muscle fiber activity. Acta Neurol Scand. 1977;55:273–88.
29. Partanen VSJ, Soininen H, Saksa M, Riekkinen P. Electromyographic and nerve conduction findings in a patient with neuromyotonia, normocalcemic tetany and small-cell lung cancer. Acta Neurol Scand. 1980;61:216–26.
30. Hosokawa S, Shinoda H, Sakai T, Kato M, Kuroiwa Y. Electrophysiological study on limb myokymia in three women. J Neurol Neurosurg Psychiatry. 1987;50:877–81.
31. Bostock H, Cikurel K, Burke D. Threshold tracking techniques in the study of human peripheral nerve. Muscle Nerve. 1998;21:137–58.
32. Burke D. Excitability of motor axons in neuromyotonia. Muscle Nerve. 1999;22:797–9.
33. Maddison P, Newsom-Davis J, Mills KR. Strength-duration properties of peripheral nerve in acquired neuromyotonia. Muscle Nerve. 1999;22:823–30.
34. Kiernan MC, Hart IK, Bostock H. Excitability of motor axons in patients with spontaneous motor unit activity. J Neurol Neurosurg Psychiatry. 2001;70:56–64.
35. Harman JB, Richardson AT. Generalized myokymia in thyrotoxicosis: report of a case. Lancet. 1954;2:473–4.
36. Reeback J, Benton S, Swash M, Schwartz MS. Penicillamine-induced neuromyotonia. Br Med J. 1979;279:1464–5.
37. Vilchez JJ, Cabello A, Benedito J, Villarroya T. Hyperkalaemic paralysis, neuropathy and persistent motor unit discharges at rest in Addison's disease. J Neurol Neurosurg Psychiatry. 1980;43:818–22.
38. Benito-Leon J, Martin E, Vincent A, Fernandez-Lorente J, de Blas G. Neuromyotonia in association with essential thrombocythemia. J Neurol Sci. 2000;173:78–9.
39. Gutmann L, Gutmann L, Schochet SS. Neuromyotonia and type 1 myofiber predominance in amyloidosis. Muscle Nerve. 1996;19:1338–41.
40. Hadjivassiliou M, Chattopadhyay AK, Davies-Jones GA, et al. Neuromuscular disorder as a presenting feature of coeliac disease. J Neurol Neurosurg Psychiatry. 1997;63:770–5.
41. Le Gars L, Clerc D, Cariou D, Lavabre C, Metral S, Bisson M. Systemic juvenile rheumatoid arthritis and associated Isaacs' syndrome. J Rheumatol. 1997;24:178–80.
42. Benito-Leon J, Miguelez R, Vincent A, Masjuán J, de Blas G. Neuromyotonia in association with systemic sclerosis. J Neurol. 1999;246:976–7.
43. Liguori R, Vincent A, Avoni P, Valentino ML, D'Alessandro R, Zaccaria A, et al. Acquired neuromyotonia after bone marrow transplantation. Neurology. 2000;54:1390–931.
44. Vasilescu C, Alexianu M, Dan A. Muscle hypertrophy and a syndrome of continuous motor unit activity in prednisone-responsive Guillain-Barré polyneuropathy. J Neurol (Berlin). 1984;231:276–9.
45. Caress JB, Abend WK, Preston DC, Logigian EL. A case of Hodgkin's lymphoma producing neuromyotonia. Neurology. 1997;49:258–9.
46. Zifko U, Drlicek M, Machacek E, Jellinger K, Grisold W. Syndrome of continuous muscle fiber activity and plasmacytoma with IgM paraproteinemia. Neurology. 1994;44:560–1.
47. Sinha S, Newsom-Davis J, Mills K, Byrne N, Lang B, Vincent A. Autoimmune aetiology for acquired neuromyotonia (Isaacs' syndrome). Lancet. 1991;338:75–7.
48. Shillito P, Molenaar PC, Vincent A, Leys K, Zheng W, van den Berg RJ, et al. Acquired neuromyotonia: evidence for autoantibodies directed against K^+ channels of peripheral nerves. Ann Neurol. 1995;38:714–22.
49. Harvey AL, Rowan EG, Vatanpour H, Fatehi M, Castaneda O, Karlsson E. Potassium channel toxins and transmitter release. Ann N Y Acad Sci. 1994;710:1–10.
50. Faivre-Sarrailh C, Devaux JJ. Neuro-glial interactions at the nodes of Ranvier: implication in health and diseases. Front Cell Neurosci. 2013;7:196.
51. Ramaswami R, Gautam M, Kamb A, Rudy B, Tanouye MA, Mathew MK. Human potassium channel genes: molecular cloning and functional expression. Mol Cell Neurosci. 1990;1:214–23.
52. Kleopa KA, Elman LB, Lang B, Vincent A, Scherer SS. Neuromyotonia and limbic encephalitis sera target mature Shaker-type K+ channels: subunit specificity correlates with clinical manifestations. Brain. 2006;129:1570–84.
53. Hart IK, Waters C, Vincent A, Newland C, Beeson D, Pongs O, et al. Autoantibodies detected to expressed K^+ channels are implicated in neuromyotonia. Ann Neurol. 1997;41:238–46.
54. Sonoda Y, Arimura K, Kurono A, Suehara M, Kameyama M, Minato S, et al. Serum of Isaacs' syndrome suppresses potassium channels in PC-12 cell lines. Muscle Nerve. 1996;19:1439–46.
55. Nagado T, Arimura K, Sonoda Y, Kurono A, Horikiri Y, Kameyama A, et al. Potassium current suppression in patients with peripheral nerve hyperexcitability. Brain. 1999;122:2057–66.
56. Tomimitsu H, Arimura K, Nagado T, Watanabe O, Otsuka R, Kurono A, et al. Mechanism of action of voltage-gated K+ channel antibodies in acquired neuromyotonia. Ann Neurol. 2004;56:440–4.
57. Rettig J, Heinemann SH, Wunder F, Lorra C, Parcej DN, Dolly JO, et al. Inactivation properties of voltage-gated K+ channels altered by presence of beta-subunit. Nature. 1994;369:289–94.
58. Irani SR, Alexander S, Waters P, Kleopa KA, Pettingill P, Zuliani L, et al. Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactivated 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan's syndrome and acquired neuromyotonia. Brain. 2010;133:2734–48.
59. Irani SR, Pettingill P, Kleopa KA, Schiza N, Waters P, Mazia C, et al. Morvan syndrome: clinical and serological observations in 29 cases. Ann Neurol. 2012;72:241–55.

60. Vernino S, Auger RG, Emslie-Smith AM, Harper CM, Lennon VA. Myasthenia, thymoma, presynaptic antibodies, and a continuum of neuromuscular hyperexcitability. Neurology. 1999;53:1233–9.
61. Batocchi AP, Marca GD, Mirabella M, Caggiula M, Frisullo G, Mennuni GF, et al. Relapsing-remitting autoimmune agrypnia. Ann Neurol. 2001;50: 668–71.
62. Sharma S, Sharma P. Morvan syndrome: after scrotal sac drainage and chemical instillation in hydrocele. Neurol India. 2013;61:300–2.
63. Buckley C, Oger J, Clover L, Tüzün E, Carpenter K, Jackson M, et al. Potassium channel antibodies in two patients with reversible limbic encephalitis. Ann Neurol. 2001;50:74–9.
64. Vincent A, Buckley C, Schott JM, Baker I, Dewar BK, Detert N, et al. Potassium channel antibody-associated encephalopathy: a potentially immunotherapy-responsive form of limbic encephalitis. Brain. 2004;127:701–12.
65. Thieben MJ, Lennon VA, Boeve BF, Aksamit AJ, Keegan M, Vernino S. Potentially reversible autoimmune limbic encephalitis with neuronal potassium channel antibody. Neurology. 2004;62:1177–82.
66. Kleopa KA. Autoimmune channelopathies of the nervous system. Curr Neuropharmacol. 2011;9: 458–67.
67. Paganoni S, Amato A. Electrodiagnostic evaluation of myopathies. Phys Med Rehabil Clin N Am. 2013;24:193–207.
68. Rakocevic G, Floeter MK. Autoimmune stiff person syndrome and related myelopathies: understanding of electrophysiological and immunological processes. Muscle Nerve. 2012;45:623–34.
69. Schoser B, Jacob S, Hilton-Jones D, Müller-Felber W, Kubisch C, Claus D, et al. Immune-mediated rippling muscle disease with myasthenia gravis: a report of seven patients with long-term follow-up in two. Neuromuscul Disord. 2009;19:223–8.
70. Ishii A, Hayashi A, Ohkoshi N, Oguni E, Maeda M, Ueda Y, et al. Clinical evaluation of plasma exchange and high dose intravenous immunoglobulin in a patient with Isaacs' syndrome. J Neurol Neurosurg Psychiatry. 1994;57:840–2.
71. Van den Berg JS, van Engelen BG, Boerman RH, de Baets MH. Acquired neuromyotonia: superiority of plasma exchange over high-dose intravenous human immunoglobulin. J Neurol. 1999;246:623–5.
72. Skeie GO, Apostolski S, Evoli A, Gilhus NE, Illa I, Harms L, et al. Guidelines for treatment of autoimmune neuromuscular transmission disorders. Eur J Neurol. 2010;17:893–902.
73. Maddison P. Neuromyotonia. Clin Neurophysiol. 2006;117:2118–27.

Congenital Myasthenic Syndromes

16

David Beeson

Introduction

The congenital myasthenic syndromes (CMS) are rare inherited disorders of neuromuscular transmission characterized by fatiguable muscle weakness [1, 2]. Their overall prevalence is uncertain but is thought to be in the order of 1 in 100,000 of the population in the UK [3]. They are genetically determined (usually autosomal recessive, so a history of consanguinity is common), non-autoimmune disorders. Major clinical features include onset in infancy, fatiguable weakness, a decremental response to repetitive nerve stimulation, and absence of autoantibodies to the muscle acetylcholine receptor (AChR) or muscle-specific tyrosine kinase (MuSK) or low-density lipoprotein receptor-related protein 4 (LRP4), although a clear pathogenic role for LRP4 antibodies in myasthenia gravis patients has not yet been demonstrated. Remarkable differences in severity occur even within families harboring the same mutation. Although impairment of neuromuscular transmission may often give rise to similar clinical presentation, detailed analysis of intact biopsied muscle fibers from patients using electrophysiology, microscopy, and biochemical techniques demonstrates distinct molecular and cellular mechanisms. The syndromes may be classified on the basis of the site of the defect of neuromuscular transmission, but the ability to determine this may only be available at a few centers and is not always certain. Diagnosis depends upon electrophysiological tests, morphological studies of the endplate region in muscle biopsy specimens, and, increasingly, identification of the specific genetic defect (Table 16.1). Next-generation sequencing (NGS) is now helping to identify new CMS causative genes which are ubiquitously expressed, proving that protein defects not specifically confined to the neuromuscular junction (NMJ) can cause myasthenia [4–7]. Moreover, in many of the more recently identified CMS-associated genes, aberrant neuromuscular transmission is only one component of a more complex phenotype in which muscle, the central nervous system, and other organs may also be affected [8–12].

Molecular Genetic Classification

For ease of classification, the CMS can be broadly grouped according to the location of the defective neuromuscular junction protein and thus are often classed as presynaptic, synaptic, and postsynaptic disorders [13]. Within these headings

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© Springer International Publishing AG, part of Springer Nature 2018
H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*,
Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_16

Table 16.1 Classification of congenital myasthenic syndromes (CMS) and their genetic loci[a]

Presynaptic CMS	Gene
CMS with episodic apnea	*CHAT*
Unconventional myosin 9	*MYO9*
Munc13-1	*MUNC13*
Synaptosome-associated protein 25	*SNAP25B*
High-affinity choline transporter 1 (CHT)	*SLC5A7*
Vesicular ACh transporter (VAChT)	*SLC18A*
Synaptobrevin 1 deficiency	*VAMP1*
Prolyl-endopeptidase-like gene	*PREPL*
Synaptic CMS	
Congenital endplate acetylcholinesterase deficiency	*COLQ*
Agrin	*AGRN*
Collagen type XIII alpha 1 chain	*COL13A1*
Laminin α5 deficiency	*LAMA5*
Laminin ß2 deficiency	*LAMB2*
Postsynaptic CMS	
AChR deficiency syndromes	*CHRNA*, *CHRNB*, *CHRND*, *CHRNE*
Multiple pterygium syndromes due to AChR γ-subunit mutations	*CHRNG*
AChR deficiency syndromes due to mutations in rapsyn	*RAPSN*
Slow-channel CMS	*CHRNA*, *CHRNB*, *CHRND*, *CHRNE*
Fast-channel CMS	*CHRNA*, *CHRND*, *CHRNE*
Low conductance syndrome	*CHRNA*, *CHRNE*
CMS due to voltage-gated sodium channel mutations	*SCN4A*
CMS due to mutations in MuSK	*MUSK*
CMS due to mutations in DOK7	*DOK7*
CMS due to mutations in LRP4	*LRP4*
Plectin deficiency	*PLEC1*
Ubiquitously expressed proteins (glycosylation)	
Glutamine-fructose-6-phosphate transaminase-1	*GFPT1*
Dolichyl-phosphate-*N*-acetylglucosamine-phosphotransferase-1	*DPAGT1*
N-linked glycosylation protein 2	*ALG2*
N-linked glycosylation protein 13	*ALG13*
GDP-mannose pyrophosphorylase B	*GMPPB*

AChR muscle acetylcholine receptor, *MuSK* muscle-specific tyrosine kinase, *DOK7* downstream of kinase 7, *LRP4* low-density lipoprotein receptor-related protein 4

[a]The genetic origin of further CMS cases is yet to be defined or not yet reported

different genes may be defective. A diagrammatic representation of CMS-associated genes/proteins at the neuromuscular junction is shown in Fig. 16.1 and a list of proteins and their genetic loci in Table 16.1.

CMS with episodic apnea was the first neuromuscular junction presynaptic disorder in which the genetic origin was defined and is due to mutations in the enzyme choline acetyltransferase (CHAT) [14, 15]. Other presynaptic disorders have been identified that have a paucity of synaptic vesicles, reduced quantal release, or resemble the autoimmune Lambert-Eaton myasthenic syndrome, and now, with the advent of NGS, the underlying genetics for these disorders are being uncovered [9, 16]. Acetylcholinesterase (AChE) deficiency is the most common CMS directly affecting proteins in the synaptic space and results from mutations in *COLQ*, which encodes an AChE-associated collagen tail that both anchors and concentrates AChE in the synaptic cleft [17, 18]. However, other proteins located in the basal lamina that are involved in interactions between the nerve terminal and the muscle membrane are being uncovered. Agrin initiates a signaling pathway that underlies the maintenance of pre- and postsynaptic structures, and other proteins in the synaptic cleft such as laminins or COL13A1 although not directly participating in the agrin pathway are thought also to be important for maintaining synaptic structure. Impaired function of these proteins has been found to result in a CMS. A series of other genes have been found to cause postsynaptic disorders, and these form the most common causes for CMS. Mutations in the genes encoding the AChR subunits were the first to be identified. These may give rise to kinetic abnormalities of AChR function or AChR deficiency or a combination of altered AChR kinetics and AChR

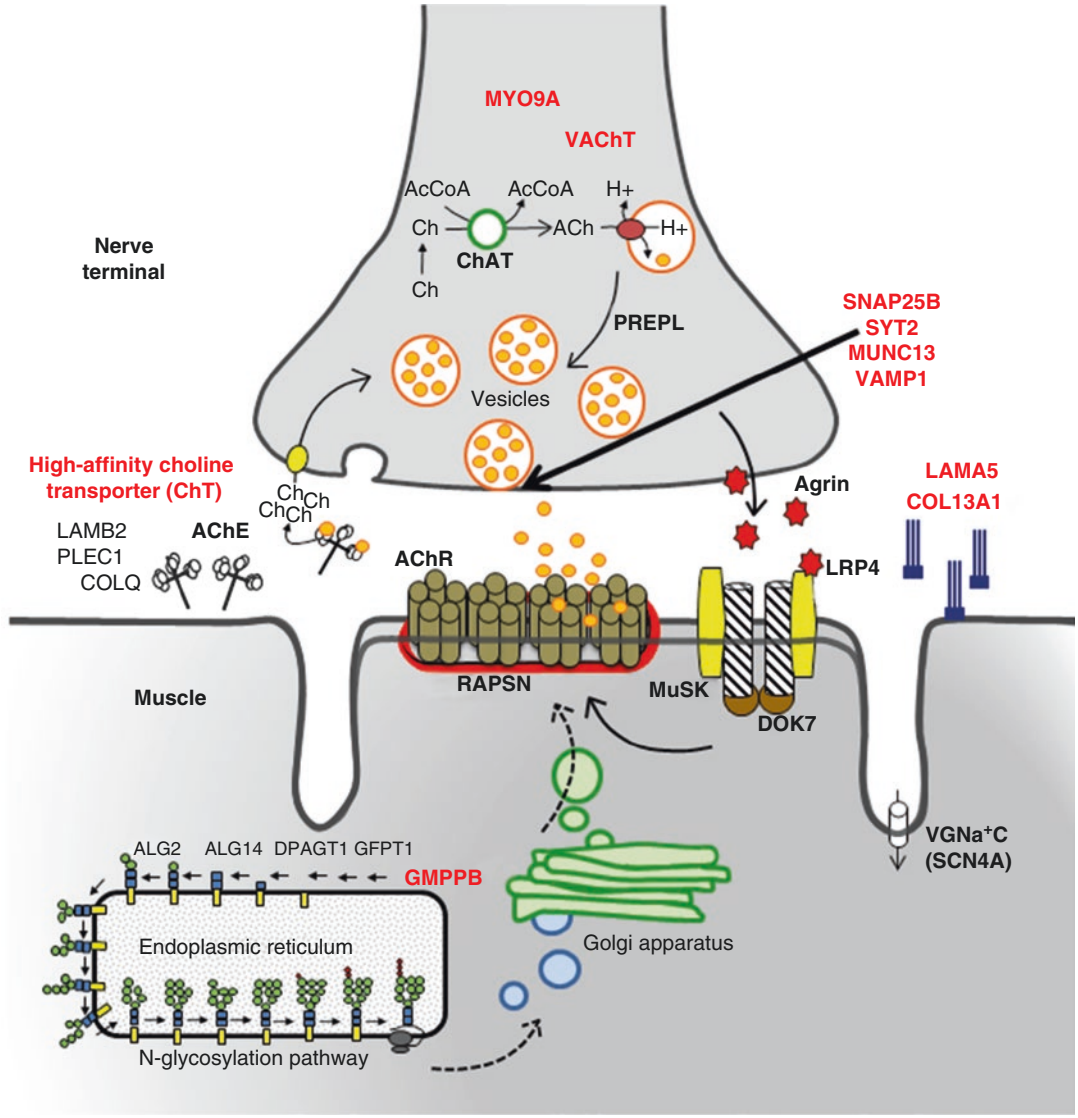


Fig. 16.1 Diagrammatic representation of the neuromuscular junction and the proteins in which mutations underlying congenital myasthenic syndromes have been found. Recently identified CMS genes are shown in red

deficiency [19–22]. However, mutations of many other proteins located on the postsynaptic side of the NMJ that are involved in synaptic function, in AChR clustering, or in the maturation of the synaptic structure can commonly underlie CMS (Fig. 16.2). Mutations in MUSK [23, 24], AGRN [25, 26], LRP4 [27], and SCN4A [28] have proved to be rare, but mutations of RAPSN [29] or DOK7 [30] are common causes of CMS.

More recently, a series of genes encoding enzymes involved in the asparagine-linked (N-linked) glycosylation pathway have also been found to harbor CMS-causing mutations [4–7] (Fig. 16.3). The phenotype for these subtypes often varies from the archetypal picture of myasthenia in that eye and facial muscles are often spared. As a result, many of these patients may go undiagnosed [2].

Diagnostic Methods

CMS should be considered in any person presenting with fatiguable muscle weakness during infancy or early childhood. Clinical features may help to pinpoint which of the diverse range of genetic loci is involved and provide clues about the underlying molecular pathogenesis. Electromyography (EMG), the clinical phenotype, and, in some cases, muscle biopsies may all provide important diagnostic pointers.

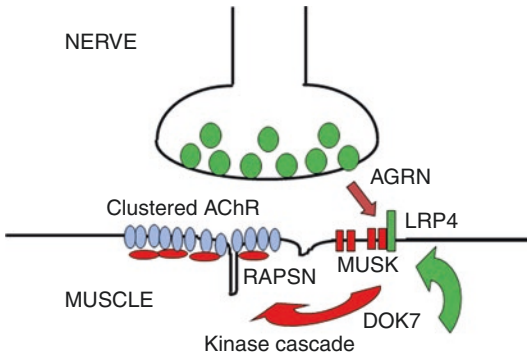


Fig. 16.2 Illustrative diagram highlighting the classical pathway believed to be responsible for formation and maintenance of the neuromuscular junction

Standard EMG can often detect impaired neuromuscular transmission, especially if the muscle under test is weak. Decrement in the compound muscle action potential (CMAP) elicited by repetitive nerve stimulation at low frequency (2–3 Hz) is suggestive of impaired neuromuscular transmission but is generally considered less sensitive than single-fiber electromyography (SFEMG). SFEMG revealing abnormal jitter and block can be operator-dependent and can occur in other disorders but is more sensitive than CMAP recording and will usually give an indication of defective neuromuscular transmission.

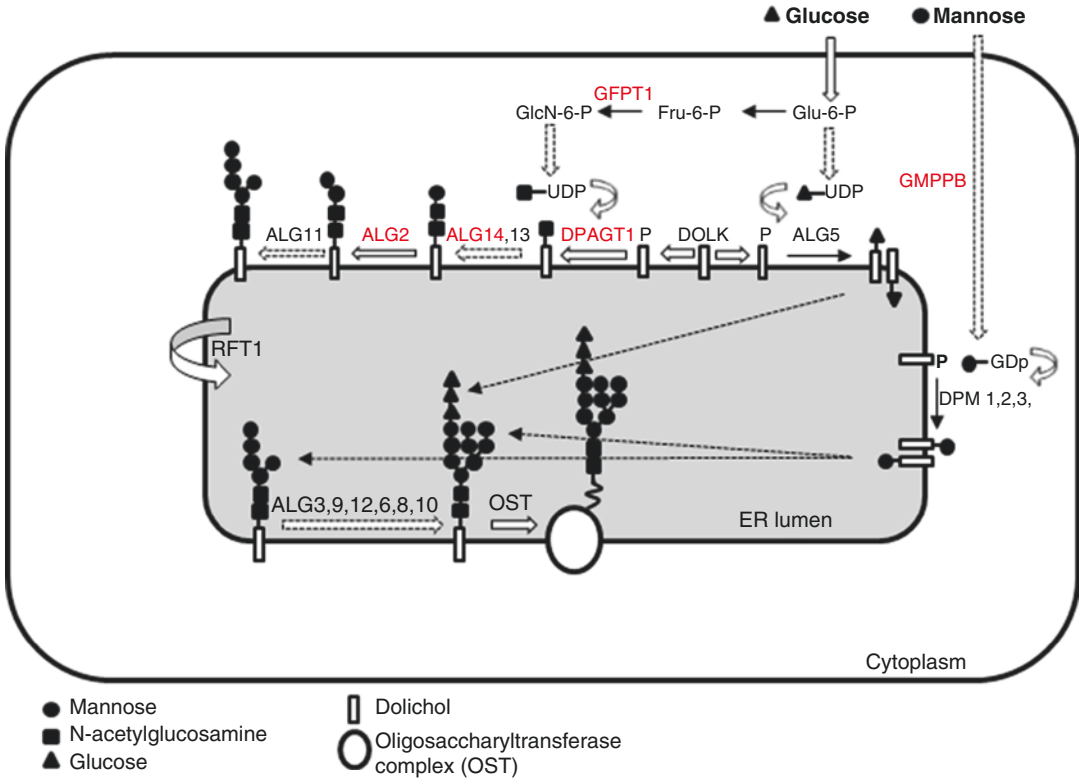


Fig. 16.3 Illustrative representation of the asparagine-linked (N-linked) glycosylation pathway in which glycan residues are stepwise added to dolichol phospholipid prior to transfer to a respective protein. CMS-associated genes are shown in red

Phenotypic clues may be gleaned for many of the CMS and greatly facilitate targeted genetic screens, although now in nonspecialized services whole exome or whole genome sequencing may provide the quickest route to a genetic diagnosis. More detailed descriptions will be given later in this chapter, but illustrative examples are that a repetitive CMAP in response to a single nerve stimulus is frequently seen in syndromes involving over-excitation such as the slow-channel congenital myasthenic syndromes or that AChE deficiency syndromes and mild arthrogryposis multiplex congenita are a strong pointer to AChR deficiency due to RAPSN mutations [31, 32], although it can also occur rarely with mutations in other genes, such as some of the presynaptic disorders.

In specialist centers, muscle biopsy is essential for characterizing disorders where the underlying genetic cause remains unknown. Electrophysiology of endplates can determine quantal content and the amplitude of miniature endplate potentials and currents (MEPPs and MEPCs) and endplate potentials and currents (EPPs and EPCs). Their size and decay times may suggest abnormalities in the number and kinetic properties of the endplate AChR. Electron microscopy can define the ultrastructure of the pre- and postsynaptic apparatus of the neuromuscular junction, providing clues to whether the defect is pre- or postsynaptic. Binding of iodinated or fluorescence-labeled α-neurotoxins, such as α-bungarotoxin (α-BuTX), can be used to determine the localization and number of the AChR. Similarly, histochemistry may be used to establish the presence or absence of AChE at the endplate [1].

Response to treatments may provide additional clues about the disorder, although the tensilon test, where the short-term response to intravenous edrophonium is measured, may give misleading results. In all CMS, autoimmune myasthenia gravis should be excluded through testing for antibodies to AChR or to MuSK. Parental consanguinity and a positive family history are both suggestive of hereditary rather than autoimmune myasthenia, and the onset of myasthenia gravis at less than 1 year is very rare. Although most CMS first present in infancy or early childhood and show recessive inheritance, an exception to this generalization is the slow-channel myasthenic syndrome, which may present in infancy or adult life and is usually inherited as an autosomal dominant trait, and late-onset CMS associated with mutations in *COLQ*, *RAPSN*, and *DOK7* have been reported [32–34]. Mutations affecting the N-linked glycosylation pathway tend to have a later onset in childhood or early adulthood.

Presynaptic Congenital Myasthenic Syndromes

These are the least well characterized of the myasthenic disorders. Electrophysiology and ultrastructure studies of the endplate regions in muscle biopsies identified apparent defects in the presynaptic apparatus in various CMS [1], and it is only with the advent of next-generation sequencing that identification of the genetic abnormalities has become apparent. These include disorders where the electrophysiology shows similarities to those seen for the Lambert-Eaton syndrome and others where there is a paucity of synaptic vesicles in the presynaptic bouton and reduced quantal release. Indeed a series of disorder have been identified that involve the process of neurotransmitter release into the synaptic cleft from the presynaptic vesicles. However, as might be expected, if there is a problem in the neurotransmitter release mechanism, then patients are likely to have problems at many sites other than at the neuromuscular junction; as a result, these patients will often suffer from a severe and often fatal multisystem disorder in which the myasthenic component only forms one small part. Thus, it is debatable as to whether they should truly be termed as a myasthenic syndrome.

Presynaptic CMS Associated with the Synthesis or Recycling of Acetylcholine

Clinical Features

The presynaptic CMS are autosomal recessive disorders that have previously been called familial

infantile myasthenia and CMS with episodic apnea [1]. However, this terminology is not ideal since similar severe episodic apneic attacks are now known to occur both in presynaptic and other postsynaptic forms of CMS. The presynaptic disorders share common clinical symptoms that typically manifest at birth and consist of hypotonia, bulbar and respiratory muscle weakness, ptosis, and varying degrees of extraocular muscle weakness. Respiratory insufficiency with recurrent apnea is a hallmark of these disorders. The episodic crises, which may be life-threatening in early life, are frequently induced by infections and fever, stress, or overexertion but become less frequent with age. Patients usually respond well to anticholinesterase medication, which may be taken prophylactically in anticipation of a crisis. The majority of these presynaptic disorders are due to mutations in choline acetyltransferase (CHAT), though recently mutations in the high-affinity choline transporter (*SLC5A7*) [9] responsible for choline uptake into the nerve terminal and the vesicular acetylcholine transporter (VAChT encoded by gene *SLC18A3*) [10] have been identified. Mutations in SLC5A7 and SLC18A3 tend to result in more severe phenotypes that are often associated with arthrogryposis and neonatal death.

Diagnosis of patients with CHAT mutations may be helped by the characteristic EMG profile. In rested muscle, the CMAP elicited by repetitive nerve stimulation at 3 Hz and SFEMG may be normal. However, after exercise or repeated nerve stimulation at 10 Hz for up to 5 min, a decremental response and abnormal jitter can be seen. In vitro studies similarly show normal EPP and MEPP amplitudes in rested muscle that decrease after continuous 10-Hz stimulation [14]. Endplate AChR shows normal number and distribution. Patients with mutations in SLC5A7 or SLC18A3 may show a similar EMG profile but may also show significant decrement at a standard repetitive 3-Hz stimulation. They both can result in fatal disorders with death in utero or as neonates.

Molecular Basis

CHAT catalyzes the reversible synthesis of ACh from acetyl coenzyme A and choline at the synapse. It has also long been hypothesized that mutations in both the choline uptake transporter (CHT) and the vesicular acetylcholine transporter (VAChT) might also result in myasthenic syndromes. Impairment in the ability to resynthesize ACh sufficiently fast, and thus reduction of ACh within the presynaptic vesicles, is responsible for the activity-dependent weakness, evident as decrement and reduced MEPP amplitude following continuous stimulation at 10 Hz. Similarly either the reduced ability of the motor terminal to take up choline (CHT) or to transport synthesized choline into synaptic vesicles (VACHT) will affect neurotransmitter release and lead to myasthenic weakness. Frameshift mutations are likely to be lethal, and so most of mutations identified are mostly missense and cause either a reduction in enzyme/transporter expression or in activity or a combination of the two [14, 35, 36].

Congenital Myasthenic Syndromes Associated with Impaired ACh Release

The mutations in the ACh recycling pathway clearly cause reduced ACh quantal release, but a second category of presynaptic disorders is formed by proteins involved in the actual docking of the synaptic vesicles and release of the neurotransmitter from the nerve terminals. This exocytosis is controlled by the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex. With the advent of next-generation sequencing, human mutations have been identified in synaptotagmin [37], SNAP25B [8], synaptobrevin [12], and Munc13-1 [11], which all feature in the docking and Ca^{2+}-triggered fusion of synaptic vesicles with the presynaptic membrane at both central and neuromuscular synapses.

Clinical Features

To date these can be classified as very rare disorders, and so it is difficult to generalize about the clinical features other than to state that they are often severe or fatal and are likely to involve developmental and central abnormality. Comorbidities

may involve cortical hyperexcitability, cerebellar ataxia, developmental delay, dysmorphic features, arthrogryposis, and intellectual disability. Unusual for CMS, the mutations identified in synaptotagmin 2 (*SYT2*) and SNAP25B are dominant. Mutations in *SYT2* result in both a motor neuropathy and a Lambert-Eaton myasthenia-like syndrome, with small CMAPs and post exercise facilitation. The patients respond to 3,4-DAP. For SNAP25B, neurophysiological studies show reduced MEPP frequencies and quantal content. Recessive mutations for VAMP1 (synaptobrevin) led to hypotonia, feeding difficulties, and ophthalmoparesis presenting at birth and again show a reduction of CMAP amplitude with facilitation at high-frequency stimulation, and patients were reported to respond to pyridostigmine medication.

Molecular Basis

The SNARE complex forms the principal component that controls the docking, priming, and then fusion of synaptic vesicles with the presynaptic plasma membrane. Synaptotagmin acts as a sensor of the synaptic vesicles for the calcium influx that results from the activation of the presynaptic voltage-gated calcium channels, and Munc13 interacts with syntaxin-1 in the SNARE complex. This complex for exocytosis is common to release mechanisms at many different synapses and in endocrine tissues, and thus it is not surprising that mutations in these proteins can cause multiple neurological problems and are often fatal, particularly if frameshift mutations are present. Further reports describe a presynaptic syndrome due to mutations in MYO9A, which encodes an atypical myosin, but it is not yet clear how this protein is involved in presynaptic neurotransmitter release.

Synaptic Congenital MyasthenicSyndrome

Endplate Acetylcholinesterase Deficiency

Clinical Features

Endplate acetylcholinesterase deficiency (EAD) is an autosomal recessive disorder with onset at birth or in early childhood. Weakness is often severe affecting facial, cervical axial, and limb muscles that may result in lordosis and kyphoscoliosis. Difficulty feeding, respiratory distress, and delayed motor milestones are common. In some patients delayed papillary light reflexes are evident and may be used to distinguish EAD from other CMS. Weakness is refractory to anticholinesterase medication, and patients do not respond well to AChR open-channel blockers, such as quinidine sulfate or fluoxetine, that have been used for slow-channel syndrome. Ephedrine and salbutamol have been reported to be beneficial [1, 38, 39].

EMG shows features associated with defective neuromuscular transmission, with CMAP decrement on repetitive stimulation, and jitter and block evident with SFEMG. Single nerve stimulation elicits a repetitive CMAP, which is another diagnostic hallmark and is only seen in EAD or the slow-channel syndrome. On muscle biopsy microelectrode studies show prolonged decay of the MEPPs and EPPs indicating prolonged activation of the AChR ion channels owing to the absence of AChE, and histochemical staining of the neuromuscular junctions shows this absence or severe reduction of AChE at the endplates. Further analysis of the endplate region often demonstrates a compensatory reduction in nerve terminal size and extension of Schwann cells into the synaptic cleft.

Molecular Basis

The *COLQ* gene encodes the collagen-like tail (ColQ) that attaches the asymmetric form of AChE to the basal lamina at the neuromuscular junction. Mutations in ColQ rather than AChE itself have been found to cause endplate EAD. The ColQ protein contains an N-terminal proline-rich domain (PRAD) that binds AChE tetramers, a collagen domain with 63 Gxy repeats, and a C-terminal domain responsible for anchoring in the basal lamina and for initiating the collagen triple-helix structure. Many mutations have been identified in COLQ [1, 40]. They occur in all the putative functional domains, although there is some evidence that

mutations in the C-terminal domain that effect the anchoring to the basal lamina are less severe [41]. The result of the mutations is the loss of the asymmetric form of AChE from the synaptic cleft and consequently the increase in the time that ACh is available to bind the AChR. Prolonged exposure to ACh will cause desensitization of AChR, and the persistent depolarization of the endplate will inactivate the voltage-gated sodium channels in the depths of the postsynaptic folds and thus block signal transmission. In addition, the prolonged stimulation of the endplates may lead to overload of calcium ions at the endplate and an endplate myopathy.

Rare Synaptic Forms of CMS

The formation and maintenance of the synapse involves cross talk between the pre- and postsynaptic sides. Basal lamina proteins help form scaffold structures that ensure the correct alignment of the functional components, but several may also play a role in anterograde or retrograde signals important for synaptic structure.

COL13A1 CMS Clinical Features

Patients identified to date with mutations in *COL13A1* [42] have all had onset of weakness at birth including respiratory and feeding difficulties, slight dysmorphic facial features, ptosis but normal eye movements, and marked weakness of neck flexion. All have shown a decrement of compound muscle action potentials (CMAP) on repetitive nerve stimulation. A common feature for COL13A1 patients that might help distinguish them from other forms of CMS is the presence of *pectus carinatum*, which may suggest that the mutant COL13A1 is affecting a separate developmental respiratory pathway and would be in keeping with high levels of COL13A1 expressed in the lung, and with age the fatigability of the ptosis becomes limited and it may appear as a fixed ptosis. There also appears to be a natural improvement of the overall myasthenic weakness over time through childhood. It is also notable that there is a lack of a beneficial response to anticholinesterase medication, but beneficial effect for 3,4-DAP and salbutamol has been reported.

Molecular Basis

COL13A1 has three extracellular collagenous domains but is anchored by a transmembrane domain in the N-terminal region. It has a proprotease recognition site just outside of the transmembrane domain and exists as an anchored form and a cleaved ectodomain form [43]. Next-generation sequencing of individuals with suspected CMS revealed three patients in two independent kinships with mutations in *COL13A1* [42]. A woman, without known consanguinity in the family, is homozygous for c.1171delG (p.Leu392Serfs*711), and two siblings from a consanguineous family were found to be homozygous for c.5231delG (p.Gly175Valfs*20). Much of our knowledge of the role of COL13A1 at the neuromuscular junction comes from animal models. Mice lacking col13a1 (*col13a1*$^{-/-}$) show abnormal endplates which remained small, immature, and fragmented when compared to wild-type animals [44]. It is thought that it is the membrane-bound form that plays the major role in neuromuscular junction development. The COL13A1 ectodomain is thought to interact with fibronectin, heparin, and basal membrane proteins nidogen-2 and perlican, and *col13a1*$^{-/-}$ mice also show abnormalities in the presynaptic motor terminal with aberrant localization of vesicles and ACh release, as well as showing differences in the positioning and form of the terminal Schwann cells. There is also the suggestion that COL13A1 might interact with COLQ and that it therefore may have a role in the precise localization of the asymmetric form of acetylcholinesterase (AChE) in the synaptic cleft [45].

LAMB2 and LAMA5 CMS

Single patients have been reported with myasthenic syndromes caused by mutations in laminin β2 (LAMB2) [46] and laminin α5 (LAMA5) [47]. Laminins are large heterotrimeric extracellular proteins that self-assemble into a cruciform structure but also interact with cell surface recep-

tors. The patient with *LAMB2* mutations suffered from congenital nephrosis, and the myasthenia became truly apparent following kidney transplants. The LAMA5 CMS patient had myopia and facial tics and showed neurophysiological features in keeping with a LEMS-like presynaptic disorder. Overall these studies emphasize the role of extracellular matrix proteins, distinct from those of the AGRN-LRP4-MUSK-DOK7 pathway, in the formation and maintenance of the neuromuscular synapse and further emphasize the importance of retrograde signaling across the synapse, although this process still remains poorly understood.

Postsynaptic Congenital Myasthenic Syndromes

The majority of CMS are caused by mutations in genes that encode postsynaptic proteins. Initial studies identified mutations in the genes encoding the AChR subunits that impair ion channel gating or reduce the number of endplate receptors or a combination of the two, giving rise to "slow-channel," "fast-channel," "reduced conductance," or AChR deficiency syndromes [19–22, 48–50]. However, CMS also arise from mutations in proteins involved in the formation and maintenance of the neuromuscular junction such as RAPSN [29, 31, 32], AGRN [25], LRP4 [27], MUSK [23], or DOK7 [30].

Genetic Disorders of the AChR

Muscle AChRs are glycosylated transmembrane molecules that mediate synaptic transmission. They are allosteric (existing in several different conformations), and the binding of two ACh to each receptor is thought to favor a conformational change that results in a brief activation of the channel and the influx of cations. Each AChR is made up of five subunits arranged in a pentameric structure around the central ion pore. In mammalian muscle there are two types of AChR: a form found in fetal muscle that consists of $\alpha_2\beta\gamma\delta$ and in adult muscle that consists of $\alpha_2\beta\delta\varepsilon$. The subunits are homologous, vary in size from 437 to 495 amino acids, and are encoded by separate genes of between 10 and 12 exons. In embryonic muscle, before innervation, fetal AChRs are distributed along the length of the muscle fibers. During innervation the AChRs are clustered on the postsynaptic membrane and are lost from extrasynaptic sites. At the same time, expression of the γ-subunit (fetal) mRNA is repressed, and it is replaced by ε-subunit (adult) mRNA transcribed from subsynaptic nuclei [50]. In humans the γ-subunit is readily detectable in neuromuscular junctions of fetal muscle up to around 31 weeks of gestation [51] and continues to be expressed at extremely low levels in adult muscle [52].

AChR Deficiency Due to Mutations in the AChR Subunits

Clinical Features

Hereditary AChR deficiency is the most common CMS [1, 2]. It is as an autosomal recessive disorder in which mutations in the AChR subunit genes cause a primary deficiency of AChR at the endplate. The phenotype may vary from mild to severe. Weakness is usually evident at birth or within the first year of life and is characterized by feeding difficulties, ptosis, impaired eye movements, and delayed motor milestones. Patients sometimes show improvement in adolescence, and in general the disease course is not progressive. In general, patients improve with anticholinesterase medication or 3,4-diaminopyridine. Electromyography typically shows decrement of CMAPs at 3-Hz stimulation, and single-fiber EMG shows increased jitter and block. Intracellular microelectrode recordings from the endplate region of biopsied muscle show that MEPPs and MEPCs may be decreased to around 8–26% and 20–42% of normal values. Consistent with MEPP and MEPC findings, staining with ^{125}I-α-BuTx or Alexa Fluor-conjugated α-BuTx shows reduced numbers of AChR that are often distributed abnormally along the muscle fiber [53]. In addition, electron microscopy shows a severe reduction of the postsynaptic folds.

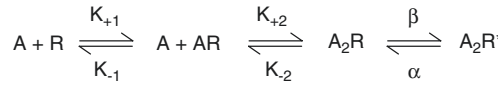
Molecular Basis

Mutations in each of the AChR subunits, CHRNA, CHRNB, CHRND, and CHRNE, may underlie AChR deficiency syndromes [1]. However, the overwhelming majority are in CHRNE. At least 100 different mutations, located along the length of the ε-subunit gene, have been identified and may cause premature termination of translation, affect the promoter [54–56] or the signal peptide, or affect the assembly of the AChR pentamer. Many are null mutations [57, 58]. It is thought that in these patients residual expression of the γ or fetal subunit is incorporated into the endplate receptors, which accumulate at low levels at the endplate, are able to mediate synaptic transmission, and partially compensate for the loss of adult AChR. Evidence to support this hypothesis includes recordings from endplates that demonstrate the receptors have functional properties of fetal AChR and that animal models in which the adult AChR is replaced by fetal AChR mimic the human condition [57]. The partial compensation through γ-subunit expression explains why the majority of AChR mutations are located in the ε-subunit gene. Most CHRNE mutations are limited to a few families although some evidence for founder effects is evident within European populations. In particular, the mutation ε1267delG [59, 60], which tends to manifest a relatively mild phenotype, is common in Southeastern Europe, particularly in the Romany gypsies [61]. Mutations CHRNA, CHRNB, and CHRND underlying AChR deficiency tend to cause a severe phenotype probably because they cannot be compensated by expression of an alternative subunit. In these cases the mutations usually affect assembly of the AChR pentamer [62].

Kinetic Abnormalities of the AChR

Mutations that underlie AChR deficiency may often also change the kinetics or functional properties of the AChR. In these cases the primary pathogenic defect is loss of endplate AChR, and the altered channel kinetics has a secondary effect. However, if the numbers of endplate AChR are not severely reduced, the phenotype is determined by the altered ion channel gating.

Slow-Channel Congenital Myasthenic Syndrome



Basic kinetic framework for analysis of the activation of the AChR. A, ACh; R, AChR; A_2R^* indicates the ion channel in the open state

Clinical Features

This syndrome was first described by Andrew Engel and colleagues [63]. It is an autosomal dominant disorder, with an age of onset of weakness that may occur neonatally or may not arise until adolescence and adulthood or during pregnancy. There is also a wide range of severity and in some patients variable penetrance [64]. Weakness and associated wasting of cervical and scapular muscles and of finger extensors are often early features. Frequently, there is only mild ptosis and extraocular muscle involvement. Unlike other CMS, this disorder is commonly slowly progressive, involving the respiratory, limb, and bulbar muscles.

On EMG decrement of CMAPs in response to 3-Hz stimulation may only be seen in affected muscles. However, as for endplate AChE deficiency, a characteristic repetitive CMAP response to a single nerve stimulus is often, but not always, present. In vitro microelectrode studies show prolongation of the EPPs and EPCs and of the MEPPs. Single-channel recordings directly from endplate regions show AChR with abnormally prolonged activations that account for the abnormally long decay of the EPPs and EPCs. Ultrastructural studies show an "endplate myopathy," in which there is a widening of the postsynaptic cleft, areas of degenerating junctional folds, degenerating and swollen

mitochondria, apoptotic subsynaptic nuclei, calcium deposits, and vacuole formation [65].

Molecular Basis

At least 32 different mutations have been identified that give rise to slow-channel congenital myasthenic syndromes [1]. They occur not only in each of the AChR subunits [66, 67] but also in different functional domains within each subunit [68], although mutations within the M2 channel pore region are most common [69]. They are single amino acid changes that result in a pathogenic gain of function for the AChR, explaining the dominant inheritance, although a single amino acid deletion may also cause a slow-channel syndrome [70].

The AChR is thought to adopt multiple conformations, and at least three interconvertible functional states have been recognized: a resting state in the absence of ACh in which the probability of opening is small, an active state in the presence of ACh in which the probability of opening is high, and a closed state that results from prolonged exposure to high ACh concentrations in which the AChR is "desensitized." A simplistic but illustrative mechanism for the activation of the AChR (derived from recordings of single-channel currents) is given in Fig. 16.4.

This scheme does not take into account channel openings of unliganded or monoliganded AChRs or various desensitized states and assumes the binding of the two ACh to a single receptor is equivalent. In neuromuscular transmission there is a transient saturating concentration of ACh, leading to rapid binding of two ACh (A_2R, where the receptor is bound but closed), and the rapid open rate β ensures fast opening of the channel (A_2R^*). The hydrolysis of ACh by AChE rapidly clears ACh from the synaptic cleft, lowering the ACh concentration so that there is no rebinding once ACh dissociates from the receptor. Because the opening rate β and the dissociation rate k_{-2} are roughly similar, the receptor will oscillate between an open and a closed state before the ACh finally dissociates from the receptor. The activation of wild channels depends largely upon β (the opening rate), α (the closing rate), and k_{-2} (the dissociation rate). Mutations of the AChR that affect channel kinetics are likely to alter one or more of these rates [67].

Single-channel recordings of AChR harboring slow-channel mutations show prolonged ion channel activations, both from mutant AChR expressed in HEK 293 cells or *Xenopus* oocytes and recordings direct from muscle biopsies. For mutation αG153S [19, 68] that is located close to the predicted ACh binding site, kinetic analysis shows that prolonged activations arise primarily through a reduction in the rate of dissociation of ACh from the AChR (k_{-2}), thereby increasing the number of channel openings during ACh occupancy. Thus, the primary effect of the αG153S mutation is to alter the affinity of AChR for ACh. However, the majority of slow-channel syndrome mutations are in the M2 transmembrane domains. Mutations in the M2 domain, such as εL264P [48], primarily slow the rate of channel closure (α), so that within activations the duration of individual openings is increased. Although severity of disease is variable, in general patients with mutations in the M2 region tend to be more severely affected. The prolonged ion channel activations explain the extended decay phase for the EPPs and MEPPs observed in slow-channel syndrome patients.

The evidence supports the theory that prolonged channel activations lead to excess entry of calcium or calcium overload which in turn activates a variety of enzymatic pathways leading to the degenerative changes on the postsynaptic side of the synapse. The endplate myopathy can lead to defective neuromuscular transmission through reducing the number of endplate AChR and reducing efficiency of transmission through widening of the synaptic cleft and decay of the postsynaptic folds. Neuromuscular transmission may also be compromised by an increased propensity of the mutant channels to desensitize and depolarization block of the voltage-gated sodium channels due to summation of the endplate potentials at physiological rates of stimulation [1].

Both quinidine and fluoxetine that, among other actions, block the AChR channel when it is open have been partially successful in treating

patients and have improved symptoms [70–73]. However, both of these drugs can have potentially serious adverse effects, and so patients should be carefully monitored.

Fast-Channel Congenital Myasthenic Syndrome

Clinical Features

The phenotypes of fast-channel congenital myasthenic syndromes, reduced conductance syndromes, and AChR deficiency syndromes share many features. Fast-channel syndromes show recessive inheritance, except in one reported case [74], with the fast-channel mutation usually found in combination with a low expressor or null allele. Weakness is usually evident at birth or in the first months and is characterized by feeding difficulties, ptosis, impaired eye movements, and delayed motor milestones [75]. Patients with fast-channel syndromes tend to be more severely affected than AChR deficiency syndromes due to ε-subunit mutations, and in one patient joint contractures at birth were reported [76]. EMG typically shows decrement at 3-Hz stimulation, and single-fiber EMG reveals an increase in jitter and block. Intracellular microelectrode recordings from the endplate of biopsied muscles show small MEPPs and MEPCs. However, biochemical and morphological analysis helps to differentiate the syndromes; endplates do not show a severe loss of receptors, postsynaptic folds, or other morphological changes associated with AChR deficiency. Fast-channel patients show a beneficial response to cholinesterase inhibitors to 3,4-diaminopyridine or to a combination of the two [75].

Molecular Basis

A series of mutations have been identified that alter the AChR channel properties causing abnormally brief channel activations, in direct contrast to the slow-channel mutations. The mutations have been identified in genes encoding each of the AChR subunits [20, 74, 76, 77]. They cause a loss of response to ACh and thus show recessive inheritance. When a fast-channel mutation segregates with a null mutation or a second fast-channel mutation, the fast-channel phenotypic footprint is uncovered. Mutations have been identified that reduce AChR affinity for ACh or alternatively effect the channel gating properties. For instance, εP121L has been identified in combination with εS143L, εG-8R, or Y15H. In each case the low-expressor second allele unmasks the phenotypic effects of εP121L that are generated by channel activations that are fewer and shorter than normal. εP121L slows the rate of channel opening (β) but has little effect on dissociation (k_{-2}). Since channel opening depends upon β/k_{-2}, εP121L will result in reduced channel reopening when ACh is bound and consequently shorter activations, resulting in a reduction in signal transmission. As perhaps might be expected, a third channel disease mechanism that causes a myasthenic syndrome is through reduced channel conductance. Like the fast-channel syndromes, these are recessive conditions in which the mutation causing reduced conductance is inherited alongside a null mutation, with the phenotype found to be similar to fast-channel syndrome [49].

Mutations Affecting AChR Clustering and Synaptic Structure

Efficient synaptic transmission depends upon the apposition of nerve terminal and postsynaptic apparatus and the correct localization of all the key functional components [78]. Just as studies of mutations underlying the fast- and slow-channel syndromes provide insights into AChR function, so studies of recently identified CMS are providing novel insights into synaptogenesis and the maintenance of the neuromuscular synapse. Rapsyn (*RAPSN*) is the crucial molecule that is thought to actually anchor AChR in the postsynaptic membrane, but the synaptic structure and AChR-rapsyn interaction are controlled by a pathway initiated from the nerve terminal by neural agrin and comprising of low-density lipoprotein receptor-related protein 4 (LRP4), MUSK, and DOK7 located at the postsynaptic membrane (see Fig. 16.2).

AChR Deficiency Due to *RAPSN* Mutations

Clinical Features

Mutations in the AChR-clustering protein rapsyn also cause endplate AChR deficiency [29]. Onset of manifestations is usually at birth, "early onset," although occasional "late-onset" patients presenting from early adulthood through to middle age have been reported [31]. Early-onset cases are frequently associated with hypotonia and marked bulbar dysfunction often necessitating nasogastric feeding and may require assisted ventilation. Joint contractures (arthrogryposis multiplex congenita) of hands and ankles are common. In childhood the course of disease is associated with severe exacerbations often presenting with life-threatening respiratory failure. Patients tend to improve over time, severe apneic episodes are rarer over the age of 6, and in many cases in adulthood disability is minimal. "Late-onset" patients may be mistaken for "seronegative" immune-mediated myasthenia gravis. Bulbar, speech, and respiratory problems were not observed. Weakness of ankle dorsiflexion, which is uncommon in myasthenia gravis, may provide a clue that RAPSN mutations underlie the condition [32]. Both early- and late-onset cases show abnormal decrement on EMG and jitter on single-fiber EMG, although it is not always easy to detect. Patients with RAPSN mutations respond well to anticholinesterase medication, although some may gain further benefit from the addition of 3,4-diaminopyridine.

On muscle biopsy endplates from patients with AChR deficiency due to ε-subunit mutations or AChR deficiency from rapsyn mutations appear similar; however, the two conditions differ in distinctive clinical features that may enable a targeted genetic screen [32]. The underlying cause of these differential features is unclear. However, whereas patients with ε-subunit null mutations most likely survive through maintained low-level expression of the fetal (γ) subunit, patients with rapsyn mutations express low levels of the ε-subunit. Thus, one clear difference between the rapsyn group and the ε-subunit group is the type of AChR that mediated synaptic transmission (Table 16.2).

Table 16.2 Distinguishing clinical characteristics of AChR deficiency due to mutations in CHRNE and RAPSN

Clinical feature	Early-onset rapsyn mutations	AChR deficiency ε-subunit mutations
Arthrogryposis	Common	Absent
Episodic crises	Common	Rare
Ophthalmoplegia	Absent	Common
Spontaneous improvement	Common	Rare

Molecular Basis

In about a third of AChR deficiency patients, mutations are not detected in the AChR subunits. Many of these cases are due to the recessive inheritance of mutations with the AChR-clustering protein rapsyn [79–81]. Various functional domains have been proposed for rapsyn, including an N-terminal myristoylation signal involved in membrane association, a string of tetratricopeptide repeats involved in rapsyn self-association, a zinc finger/coiled-coil domain implicated in the interaction of rapsyn with the AChR, and a RING-H2 domain thought to be involved in binding to scaffold proteins. Mutations are observed along the length of the rapsyn protein. To date more than 30 mutations have been identified, but there are no clear phenotypic associations with their positions within rapsyn. However, the overwhelming majority of patients harbor the missense mutation N88K on at least one allele, suggesting an original founder mutation [82]. The observations suggest rapsyn-N88K retains at least partially function, whereas many of the others are null mutations. This is supported by cell culture experiments, in which several mutations were found to drastically inhibit rapsyn function and AChR-rapsyn association, whereas rapsyn-N88K was able to mediate agrin-induced AChR clusters but these clusters were found to be less stable than clusters formed with wild-type rapsyn [83]. Thus, it may be that in patients with the N88K mutation AChR deficiency is

due to instability of the endplate rapsyn-N88K/AChR clusters.

Not all patients with AChR deficiency harbor N88K. Rarely, patients have other mutations in the coding region that result in partially functional rapsyn. In addition, a number of patients have been identified with mutations in the promoter region of the RAPSN gene. Some of these promoter mutations severely reduce rapsyn mRNA transcription, but one, −38A > G, has a less drastic effect on transcription and has been found homozygous in patients of Iranian-Jewish origin that commonly show facial malformation [84]. The facial malformation, high-arched palate, and joint contractures observed in patients with rapsyn mutations are all thought to result from akinesia in the womb presumably due to failed neuromuscular transmission at critical stages in fetal development. Thus, the rapsyn mutations must affect clustering of both the adult and the fetal AChR subtypes.

A mutation identified in the AChR δ-subunit gene, δE381K, does not affect AChR function but rather impairs rapsyn induced AChR clustering, presumably through impaired interaction with rapsyn [85]. The patient phenotype bears all the hallmarks of a "rapsyn deficiency" rather than an AChR ε-subunit deficiency. This mutation may shed light on the molecular basis for AChR-rapsyn interaction which has remained unresolved despite many years of study.

Congenital Myasthenic Syndromes Due to Mutations in the AGRN-LRP4-MUSK-DOK7 AChR-Clustering Pathway DOK7-CMS

Clinical Features

Muscle groups can be differentially affected by the CMS. A group of patients have been identified in which proximal muscles are more affected than distal muscle groups. These have been termed "limb-girdle" congenital myasthenia [86]. However, this term may lead to confusion with other non-myasthenic "limb-girdle" muscle disorders, and they may be better classified as CMS with proximal muscle weakness. Mutations in the AGRN-LRPR-MUSK-DOK7 AChR-clustering pathway give rise to hereditary "limb-girdle" myasthenic weakness, although mutations in DOK7 are by far the most common [30].

Clinical onset of disease is generally characterized by difficulty in walking after initial achievement of walking milestones. Patients occasionally have earlier signs of ptosis, floppy tone, and bulbar and respiratory problems, but even among these, walking difficulty is not appreciated. The walking and running impairment tends to worsen in childhood and is often accompanied by upper limb weakness and loss of ambulation in some patients. A waddling and lordotic gait is often seen associated with proximal lower limb and truncal weakness.

Ptosis is often present from an early age, though it may develop and progress in childhood. Eye movements are usually normal, while facial, jaw, and neck weakness is common and tongue wasting has been observed in around 50% of cases. Bulbar problems typically develop later in the clinical course than limb weakness. Features seen in patients with rapsyn mutations such as congenital joint deformity and weakness of ankle dorsiflexion, which are associated with reduced fetal movement in the womb, have not been observed. However, fluctuations in symptoms were common. In many cases studied diagnosis as a myasthenic disorder was delayed, and disorders such as muscular dystrophy and congenital myopathy were suggested [34, 87]. There is a remarkable improvement following treatment with the β2-adrenergic receptor agonists, ephedrine and salbutamol [88]. However, contrasting with the characteristic myasthenic response to pyridostigmine or 3,4-DAP which usually is felt within an hour, the response to β2-adrenergic receptor agonists occurs gradually over weeks and months, and it can take up to 2 years before the full benefit is achieved.

Molecular Basis

The classic view of the development of the neuromuscular synapse derives from a series of experiments in which components of the neuromuscular junction were "knocked out" [89–92]. They highlighted a pathway in which agrin, released from the motor nerve terminal, binds to

LRP4 which in turn interacts with and activates MuSK, a receptor tyrosine kinase, which in turn activates a kinase pathway in which the AChR β-subunit is phosphorylated, and rapsyn clusters and stabilizes the AChR on the postsynaptic membrane [93] (Fig. 16.3). The MuSK signaling is amplified by intracellular interaction with DOK7. DOK7 is found to bind specifically to MuSK and when expressed in cultured C2C12 cell-line myotubes is able to induce large AChR clusters in the absence of neural agrin. When the DOK7 gene was "knocked out" in mice, neuromuscular junctions failed to form, and offspring failed to survive past birth [94].

Splice site, missense, and frameshift mutations have been identified in *DOK7*, and recessive inheritance of these results in a myasthenic syndrome with the characteristic proximal or "limb-girdle"-type weakness described above. The majority of the mutations are located in the large 3′ exon of the gene that encodes the C-terminal region of DOK7. Mutation 1124_1127dupTGCC is common, occurring in at least one allele in 20/24 kinships reported in a recent study. It is thought that DOK7 activates MuSK through the DOK7 phosphotyrosine-binding domain interacting with the juxtamembrane phosphotyrosine-binding motif of MuSK. The binding can occur even when the C-terminal region is truncated [30]. Studies of the motor endplates from patients harboring *DOK7* mutations found that components of the neuromuscular junction were present at normal density and showed normal function but that the size of the pre- and postsynaptic structures was reduced [95]. These observations, in combination with functional studies of the action of mutant DOK7 on the AChR-clustering pathway, show that mutations of DOK7 result in impaired maturation and maintenance of neuromuscular junction structure [30].

AGRN, LRP4, and MUSK CMS

Clinical Features

Mutations in *AGRN*, *LRP4*, and *MUSK*, which are the other key components of the AChR-clustering pathway, are rare, but they are large genes, and NGS is beginning to uncover more cases. There is considerable variation of disease severity, but early respiratory failure and feeding difficulties accompanied by mild ptosis, very mild ophthalmoparesis, and moderate to severe proximal weakness appear to be common. Agrin has several isoforms, and it is only the neural isoform that is critical for control of the synaptic structure of the neuromuscular junction. CMS due to AGRN mutations often have an accompanying mild distal myopathy [26], and it may be that this is due to effects of the mutations on AGRN on non-neural isoforms that are known to be expressed by muscle. Post-exercise increment on repetitive nerve stimulation has been noted in some but not all AGRN-CMS. Similarly mutations in LRP4 are commonly the cause of Cenani-Lenz syndactyly syndrome [96] and only very rarely cause a myasthenic syndrome [27]. In keeping with the findings from DOK7, patients with CMS-causing mutations in these genes do not improve and often worsen with anticholinesterase medication, but show a good response to β2-adrenergic receptor agonists [88], though patients with *AGRN* mutations tend to respond less well than *LRP4*-, *MUSK*-, or *DOK7*-CMS patients.

Molecular Basis

AGRN mutations occasionally occur in the N-terminal region of the protein [26] but are mostly found nearer to the C-terminus in the regions that give rise to the splice variants for the neuronal AGRN form. Typical mutants are p.Gly1675Ser, p.Gly1709Arg, and p.Val1727Phe [25, 26, 97]. This suggests that the CMS mutations are likely affecting the protein in the region where AGRN interacts with LRP4, and thus there is impaired activation of MUSK and severe reduction of the signal for AChR clustering.

LRP4 encodes low-density lipoprotein receptor-related protein 4. As stated above, most LRP4 mutations give rise to Cenani-Lenz syndactyly syndrome and have no effect on neuromuscular transmission. However, a few mutations have been identified in the third β-propeller domain that cause CMS, such as p.Glu1233Lys and p.Arg1277His [27]. The implication from these

studies is that this domain is critical for the interaction between LRP4 and MUSK and that the mutations affect the interaction. MUSK is considered the major organizer of neuromuscular junction synaptic development, maintenance, and stability. MuSK mutations are extremely rare, and this may be because any severe loss-of-function mutations are not compatible with life. For the mutations that have been identified (p.Met605Ile, p.Ala727Val, p.Asp38Glu, and p.Pro344Arg), it has often been difficult to demonstrate a clear disease mechanism, although studies suggest that they affect processes that control the level of MUSK phosphorylation or may affect the interaction with DOK7 [98].

Prenatal Hereditary Myasthenia Due to Mutations in *CHRNG*

Neuromuscular transmission at nearly all normal adult muscle endplates is mediated by AChR consisting of $\alpha_2\beta\delta\varepsilon$ subunits. However, for crucial periods of fetal development, in utero transmission is mediated through the fetal form ($\alpha_2\beta\delta\gamma$) of the AChR [99]. Loss of fetal movement during these periods can lead to a series of developmental abnormalities.

Clinical Features

Multiple pterygia syndromes or Escobar's syndrome is an autosomal recessive condition that manifests with orthopedic and cranial abnormalities. Characteristically, there is short stature, arthrogryposis multiplex congenita, pterygia of the neck, and anomalies of the head including low-set ears, ptosis, a pointed and receding chin, and high-arched palate [100]. Intrauterine death and stillbirths are common.

Molecular Basis

Mutations of the AChR γ-subunit gene *CHRNG* have been found to underlie many cases of Escobar's syndrome [101, 102]. Mutations may be splice site, short duplication, missense, or nonsense mutations that result in either truncation or low expression levels of the γ-subunit. The loss of fetal AChR function associated with *CHRNG* mutations is thought to result in fetal akinesia, which in turn causes the associated multiple developmental abnormalities. Surprisingly, some patients that harbor γ-subunit null alleles can survive, suggesting early expression of the ε-subunit that partially compensates for loss of the γ-subunit. Similarly, the severity of the condition varies in patients with the same mutations. Following birth, neuromuscular transmission is mediated by the adult AChR, and patients show little or no progression of their condition. Since the disorder results from lack of neuromuscular transmission at crucial developmental phases, it might be expected that recessive inheritance of loss-of-function mutations in other essential components of the neuromuscular junction, such as RAPSN, MUSK, DOK7, or AChR subunits, would also result in fetal akinesia. This is indeed the case, but the resultant condition is not compatible with life.

CMS Due to Mutations in Glycosylation Pathways

The asparagine-linked (N-linked) glycosylation pathway is a ubiquitous process in eukaryotic cells involving to sequential addition of sugar moieties that are transferred to a protein at an asparagine residue at consensus sequence Asn-X-Ser or Asn-X-Thr [103]. Membrane-bound and exported proteins are modified in the endoplasmic reticulum in a process crucial for protein folding and multisubunit assembly. The glycans are often further processed during their intracellular transport through the Golgi to the plasma membrane. Mutations in components of this pathway produce a spectrum of severe multisystem disorders known as congenital disorders of glycosylation (CDGs) [104]. The neuromuscular junction is known to be highly glycosylated, and therefore it is perhaps not surprising that mutations affecting glycosylation can impair neuromuscular transmission [105]. What is surprising is that there are a considerable number of cases in which a defective neuromuscular transmission is the only presenting manifestation for mutations within the N-linked glycosylation pathway. Also

surprising is that it is mutations in genes encoding the enzymes/subunits for the initial steps of the pathway or providing substrates that feed into the early stages of N-linked glycosylation that manifest as a myasthenia. Mutations in proteins involved in later steps of the pathway causing myasthenia have so far not been identified.

GFPT1, DPAGT1, ALG2, and *ALG14* CMS Clinical Features

CMS due to "glycosylation mutations" share many similar phenotypic features [4–7]. Onset of symptoms tends to be in childhood rather than at birth. Proximal muscles are affected more than distal with a typical limb-girdle pattern of weakness. Whereas fatiguable ptosis may or may not be present, eye and facial muscles are usually unaffected. Indeed, a characteristic of these patients is the lack of cranial and bulbar muscle involvement, and ptosis may be completely absent. Biopsies in many but not all of the patients show the presence of tubular aggregates, and this is a useful pointer to the underlying genetics although they can be seen rarely in some other forms of CMS. In some cases, modestly elevated serum creatine kinase (CK) levels and additional myopathic changes on needle EMG suggest a concomitant myopathy. Cognitive manifestations can vary from none to mild learning disabilities or major intellectual disability [106]. Mutations in these genes, in particular in *DPAGT1*, can also cause a severe congenital disorder of glycosylation type Ij characterized by severe hypotonia, intractable seizures, mental retardation, and microcephaly [107].

GFPT1, DPAGT1, ALG2, and *ALG14* CMS Molecular Basis

GFPT1 encodes glutamine-fructose-6-phosphate transaminase-1, which catalyzes the first step in the biosynthesis of UDP-*N*-acetylglucosamine, an essential substrate for N- and O-glycosylation of protein [108]. Thus, it provides the rate-limiting step for feeding substrate into the first step of the N-glycosylation pathway. As with the majority of CMS forms, *GFPT1*-CMS is a recessive condition. Mutations are located through the length of the gene, are mostly missense, and are likely to affect either enzyme levels or catalytic activity. Knockdown of GFPT1 using siRNAs causes reduced surface expression of AChR suggesting that this may be at least one mechanism through which neurotransmission is affected [109]. *DPAGT1* encodes the enzyme dolichyl-phosphate-*N*-acetylglucosamine-phosphotransferase-1, a transmembrane ER protein essential for N-linked glycosylation that catalyzes the first step in the dolichol oligosaccharide pathway for glycoprotein biosynthesis [110]. *ALG14* encodes a membrane protein that together with DPAGT1 and ALG13 forms a functional multienzyme complex involved in the initial steps of N-glycosylation [111]. *ALG2* encodes an alpha-1,3-mannosyltransferase that catalyzes the second and third mannosylation steps for the elongation of the carbohydrate chain linked to dolichol [112].

Studies with tunicamycin (an inhibitor of DPAGT1) show the clear role of DPAGT1 in glycosylation of the AChR, in particular of the AChR δ-subunit [5]. Moreover, as with GFPT1, siRNA knockdown of DPAGT1, ALG14, or ALG2 all reduces cell surface expression of the AChR [5, 6]. Thus, it appears that impaired function of the early steps of the N-linked pathway affect the surface expression of the AChR. Muscle biopsies from a patient with DPAGT1 mutations show loss of postsynaptic junctional folds characteristic of AChR deficiency syndromes, and the patients respond to anticholinesterase medication, both of which argue for loss of endplate AChR as a key mechanism of disease [5, 6]. However, it might also be expected that other glycosylated proteins at the neuromuscular junction are affected, and there is evidence that synaptic structure and the presynaptic terminal are also affected [106].

GMPPB CMS Clinical Features

GMPPB CMS provides a useful illustration of the forms of CMS that are now being uncovered by NGS. Mutations in this ubiquitously expressed gene disturb both downstream O- and N-glycosylation that may manifest as a dystroglycanopathy [113] or a combination of myasthenia and a dystroglycanopathy [7]. The myasthenic

component responds to anticholinesterase medication with some additional improvement with the addition of β2-adrenergic receptor agonists, but this does not help the disease component due to the dystroglycanopathy, and therefore there is likely to be long-term progression of the disorder. Nevertheless, recognition is important since appropriate medication can significantly improve quality of life.

Patients with reduced α-dystroglycan O-mannosylation showed a wide range of disease severity varying from congenital muscular dystrophy with structural brain involvement, to a milder, later-onset limb-girdle muscular dystrophy [113]. Thus, mutations in *GMPPB* produce a wide phenotypic spectrum that includes CMS. CMS patients with *GMPPB* mutations usually present in adolescence or early adulthood; although some symptoms may be present at an earlier age, they have a predominantly limb-girdle pattern of weakness, more severe in lower than upper limbs, with associated waddling gait. As with the other "glycosylation CMS" subtypes, eye, facial, and bulbar muscles are frequently spared. Patients are defined as having CMS through the presence of fatiguable muscle weakness on examination and decrement of compound muscle action potentials. Surprisingly, many of the very severe congenital muscular dystrophy cases do not have decrement of CMAP, which appears largely restricted to the milder CMS presentations [114]. In addition, frequently no neurophysiological characteristics of CMS were identified when testing eye or facial muscles but were apparent when testing affected limb-girdle muscles. Thus, these cases reinforce the notion that for confirmation of a suspected myasthenia diagnosis, it is important to perform neurophysiological tests on muscles that show fatiguable weakness. In accordance with the dystroglycanopathy component, serum creatine kinase (CK) levels are markedly raised in GMPPB CMS (which is unusual in other forms of CMS) and therefore provide a pointer for genetic screening [114].

GMPPB CMS Molecular Basis

GMPPB encodes GDP-mannose pyrophosphorylase B that catalyzes the conversion of mannose-1-phosphate and GTP- to GDP-mannose. *GMPPB* contributes to both the N-glycosylation and the O-mannosylation pathways since mannose is a key glycan added in both. Mutations within the O-mannosylation pathway were originally identified in patients with a form of muscular dystrophy within the spectrum of dystroglycanopathies [113, 115]. These disorders are characterized by reduction in α-dystroglycan glycosylation, which is the most well characterized and functionally relevant O-mannosylated protein. Mutations are found throughout the gene, and there are no obvious genotype-phenotype correlations as yet. Indeed in some patients, the same mutations are present in severe dystroglycanopathies as are seen in mild CMS cases. Pathogenicity of genetic variants correlates well with a marked increase in the presence of protein aggregates following expression of mutant GMPPB in the TE671 muscle cell line [114].

Rare Congenital Myasthenic Syndromes

Patients have been reported of heteroallelic mutations in the postsynaptic voltage-gated sodium channel, SCN4A [28]. In a severely affected patient with mutations in SCN4A, endplate potentials of normal amplitude failed to activate the postsynaptic voltage-gated sodium channels. This is due to the rapid inactivation of the of $Na_v1.4$ channels resulting from the presence of mutation Val1442Glu. This mutation may show dominant inheritance although it was identified in a patient who also harbored a clinically silent mutation S246L that causes small but detectable biophysical changes [28]. More recently a further case homozygous for Arg1457His has been reported [116].

The prolyl-endopeptidase-like gene (*PREPL*) encodes a protein that belongs to the prolyl-oligopeptidase subfamily of serine peptidases: proteolytic enzymes that cleave peptides, in which serine serves as the nucleophilic acid at the active site. PREPL is ubiquitously expressed, with highest levels in the brain, kidney, and muscle [117]. One report to date has identified a patient with CMS due to an isolated PREPL deficiency and no

cystinuria [118]. A known function of PREPL at the NMJ is to act as an effector of the clathrin-associated adaptor protein 1 in the trafficking of the vesicular ACh transporter [119].

MYO9 encodes the protein myosin-IXA belonging to the superfamily of unconventional myosins. These are actin-based molecular motors implicated in diverse cellular processes [120]. The unconventional myosins are defined by myosin-like head (motor) domains attached to class-specific tail domains that differ greatly from myosin-II. In addition, they do not form bipolar thick filaments when binding to actin filaments [120]. There is increasing evidence that atypical myosins are expressed in peripheral neurons and might play an important role in axonal transport [121].

Three patients from two unrelated families with missense biallelic mutations in *MYO9* have been reported [122]. All patients had severe neonatal onset with ptosis, hypotonia, respiratory, and bulbar involvement. Additional features included developmental delay, nystagmus, and oculomotor apraxia. There was a positive response to pyridostigmine and 3,4-diaminopyridine.

Treatment

Response to pharmacological treatment depends on the CMS subtype. Classic treatments include acetylcholinesterase inhibitors to inhibit the acetylcholinesterase from breaking down acetylcholine [123]; 3,4-diaminopyridine that works by blocking presynaptic potassium channels and thus increases the action potential duration and acetylcholine release [124]; and fluoxetine and quinidine that work as open-channel blockers to restore synaptic currents in slow-channel syndrome [71, 72]. More recently, a number of studies have reported great benefit of therapy with β2-adrenergic agonists such as salbutamol and ephedrine in DOK7-CMS [88, 125, 126]. The use of these drugs is increasing in other CMS subtypes such as acetylcholinesterase deficiency [127, 128] and CMS due to abnormal glycosylation [106, 129]. The molecular mechanism for salbutamol and ephedrine at the NMJ is unknown, although for patients with mutations in the AGRN-MUSK-DOK7 pathway, there is a slow but progressive response starting within weeks and increasing in effect before stabilizing at between 6 and 24 months. Additionally, patients with severe AChR deficiency syndrome on long-term anticholinesterase medication may also show remarkable improvement when β2-agonists are added [130]. Indeed β2-agonists are likely to be helpful to anyone on long-term anticholinesterase medication. Chronic anticholinesterase medication is known to be detrimental to synaptic structure, as is impaired signaling through the AGRN-LRP4-MUSK-DOK7 pathway, and therefore an attractive hypothesis is that β2-agonists act to rebuild and stabilize the neuromuscular junction synaptic structure [2].

References

1. Engel AG, Shen XM, Selcen D, Sine SM. Congenital myasthenic syndromes: pathogenesis, diagnosis, and treatment. Lancet Neurol. 2015;14:420–34.
2. Beeson D. Congenital myasthenic syndromes: recent advances. Curr Opin Neurol. 2016;29:565–71.
3. Parr JR, Andrew MJ, Finnis M, Beeson D, Vincent A, Jayawant S. How common is childhood myasthenia? The UK incidence and prevalence of autoimmune and congenital myasthenia. Arch Dis Child. 2014;99:539–42.
4. Senderek J, Müller JS, Dusl M, Strom TM, Guergueltcheva V, Diepolder I, et al. Hexosamine biosynthetic pathway mutations cause neuromuscular transmission defect. Am J Hum Genet. 2011;88:162–72.
5. Belaya K, Finlayson S, Slater C, Cossins J, Liu WW, Maxwell S, et al. Mutations in DPAGT1 cause a limb-girdle congenital myasthenic syndrome with tubular aggregates. Am J Hum Genet. 2012;91:193–201.
6. Cossins J, Belaya K, Hicks D, Salih MA, Finlayson S, Carboni N, et al. Congenital myasthenic syndromes due to mutations in ALG2 and ALG14. Brain. 2013;136:944–56.
7. Belaya K, Rodríguez Cruz PM, Liu WW, Maxwell S, McGowan S, Farrugia ME, et al. Mutations in GMPPB cause congenital myasthenic syndrome and bridge myasthenic disorders with dystroglycanopathies. Brain. 2015;138:2493–504.
8. Shen XM, Selcen D, Brengman J, Engel AG. Mutant SNAP25B causes myasthenia, cortical hyperexcitability, ataxia, and intellectual disability. Neurology. 2014;83:2247–55.

9. Bauché S, O'Regan S, Azuma Y, Laffargue F, McMacken G, Sternberg D, et al. Impaired presynaptic high-affinity choline transporter causes a congenital myasthenic syndrome with episodic apnea. Am J Hum Genet. 2016;99:753–61.
10. O'Grady GL, Verschuuren J, Yuen M, Webster R, Menezes M, Fock JM, et al. Variants in SLC18A3, vesicular acetylcholine transporter, cause congenital myasthenic syndrome. Neurology. 2016;87:1442–8.
11. Engel AG, Selcen D, Shen XM, Milone M, Harper CM. Loss of MUNC13-1 function causes microcephaly, cortical hyperexcitability, and fatal myasthenia. Neurol Genet. 2016;2:e105.
12. Shen XM, Scola RH, Lorenzoni PJ, Kay CS, Werneck LC, Brengman J, et al. Novel synaptobrevin-1 mutation causes fatal congenital myasthenic syndrome. Ann Clin Transl Neurol. 2017;4:130–8.
13. Engel AG, Ohno K, Sine SM. Sleuthing molecular targets for neurological diseases at the neuromuscular junction. Nat Rev Neurosci. 2003;4:339–52.
14. Ohno K, Tsujino A, Brengman JM, Harper CM, Bajzer Z, Udd B, et al. Choline acetyltransferase mutations cause myasthenic syndrome associated with episodic apnea in humans. Proc Natl Acad Sci U S A. 2001;98:2017–22.
15. Maselli RA, Chen D, Mo D, Bowe C, Fenton G, Wollmann RL. Choline acetyltransferase mutations in myasthenic syndrome due to deficient acetylcholine resynthesis. Muscle Nerve. 2003;27:180–7.
16. Milone M, Fukuda T, Shen XM, Tsujino A, Brengman J, Engel AG. Novel congenital myasthenic syndromes associated with defects in quantal release. Neurology. 2006;66:1223–9.
17. Donger C, Krejci E, Serradell AP, Eymard B, Bon S, Nicole S, et al. Mutation in the human acetylcholinesterase-associated collagen gene, COLQ, is responsible for congenital myasthenic syndrome with endplate acetylcholinesterase deficiency (type 1c). Am J Hum Genet. 1998;63:967–75.
18. Ohno K, Brengman J, Tsujino A, Engel AG. Human endplate acetylcholinesterase deficiency caused by mutations in the collagen-like tail subunit (ColQ) of the asymmetric enzyme. Proc Natl Acad Sci U S A. 1998;95:9654–9.
19. Sine SM, Ohno K, Bouzat C, Auerbach A, Milone M, Pruitt JN, et al. Mutation of the acetylcholine receptor alpha subunit causes a slow-channel myasthenic syndrome by enhancing agonist binding affinity. Neuron. 1995;15:229–39.
20. Ohno K, Wang HL, Milone M, Bren N, Brengman JM, Nakano S, et al. Congenital myasthenic syndrome caused by decreased agonist binding affinity due to a mutation in the acetylcholine receptor epsilon subunit. Neuron. 1996;17:157–70.
21. Engel AG, Ohno K, Bouzat C, Sine SM, Griggs RC. End-plate acetylcholine receptor deficiency due to nonsense mutations in the epsilon subunit. Ann Neurol. 1996;40:810–7.
22. Ohno K, Quiram PA, Milone M, Wang HL, Harper MC, Pruitt JN 2nd, et al. Congenital myasthenic syndromes due to heteroallelic nonsense/missense mutations in the acetylcholine receptor epsilon subunit gene: identification and functional characterization of six new mutations. Hum Mol Genet. 1997;6:753–66.
23. Chevessier F, Faraut B, Ravel-Chapuis A, Richard P, Gaudon K, Bauché S, et al. MuSK, a new target for mutations causing congenital myasthenic syndrome. Hum Mol Genet. 2004;13:3229–40.
24. Maselli RA, Arredondo J, Cagney O, Ng JJ, Anderson JA, Williams C, et al. Mutations in MUSK causing congenital myasthenic syndrome impair MuSK-Dok-7 interaction. Hum Molec Genet. 2010;19:2370–9.
25. Huze C, Bauche S, Richard P, Chevessier F, Goillot E, Gaudon K, et al. Identification of an agrin mutation that causes congenital myasthenia and affects synapse function. Am J Hum Genet. 2009;85:155–67.
26. Nicole S, Chaouch A, Torbergsen T, Bauché S, de Bruyckere E, Fontenille MJ, et al. Agrin mutations lead to a congenital myasthenic syndrome with distal muscle weakness and atrophy. Brain. 2014;137:2429–43.
27. Selcen D, Ohkawara B, Shen XM, McEvoy K, Ohno K, Engel AG. Impaired synaptic development, maintenance, and neuromuscular transmission in LRP4-related myasthenia. JAMA Neurol. 2015;72:889–96.
28. Tsujino A, Maertens C, Ohno K, Shen XM, Fukuda T, Harper CM, et al. Myasthenic syndrome caused by mutation of the SCN4A sodium channel. Proc Natl Acad Sci U S A. 2003;100:7377–82.
29. Ohno K, Engel AG, Shen XM, Selcen D, Brengman J, Harper CM, et al. Rapsyn mutations in humans cause endplate acetylcholine-receptor deficiency and myasthenic syndrome. Am J Hum Genet. 2002;70:875–85.
30. Beeson D, Higuchi O, Palace J, Cossins J, Spearman H, Maxwell S, et al. Dok-7 mutations underlie a neuromuscular junction synaptopathy. Science. 2006;313:1975–8.
31. Burke G, Cossins J, Maxwell S, Owens G, Vincent A, Robb S, et al. Rapsyn mutations in hereditary myasthenia; distinct early- and late-onset phenotypes. Neurology. 2003;61:826–8.
32. Burke G, Cossins J, Maxwell S, Robb S, Nicolle M, Vincent A, et al. Distinct phenotypes of congenital acetylcholine receptor deficiency. Neuromuscul Disord. 2004;14:356–64.
33. Wargon I, Richard P, Kuntzer T, Sternberg D, Nafissi S, Gaudon K, et al. Long-term follow-up of patients with congenital myasthenic syndrome caused by COLQ mutations. Neuromuscul Disord. 2012;22:318–24.
34. Palace J, Lashley D, Newsom-Davis J, Cossins J, Maxwell S, Kennett R, et al. Clinical features of the DOK7 neuromuscular junction synaptopathy. Brain. 2007;130:1507–15.
35. Barisic N, Muller JS, Paucic-Kirincic E, Gazdik M, Lah-Tomulic K, Pertl A, et al. Clinical variability of CMS-EA (congenital myasthenic syndrome with epi-

sodic apnea) due to identical CHAT mutations in two infants. Eur J Paediatr Neurol. 2005;9:7–12.
36. Shen XM, TO C, Brengman J, Acsadi G, Iannaconne S, Karaca E, et al. Functional consequences and structural interpretation of mutations of human choline acetyltransferase. Hum Mutat. 2011;32:1259–67.
37. Herrmann DN, Horvath R, Sowden JE, Gonzalez M, Sanchez-Mejias A, Guan Z, et al. Synaptotagmin 2 mutations cause an autosomal-dominant form of lambert-eaton myasthenic syndrome and nonprogressive motor neuropathy. Am J Hum Genet. 2014;95:332–9.
38. Bestue-Cardiel M, Saenz de Cabezon-Alvarez A, López-Pisón J, Peña-Segura JL, Martin-Martinez J, et al. Congenital endplate acetylcholinesterase deficiency responsive to ephedrine. Neurology. 2005;65:144–6.
39. Padmanabha H, Saini AG, Sankhyan N, Singhi P. COLQ-related congenital myasthenic syndrome and response to salbutamol therapy. J Clin Neuromuscul Dis. 2017;18:162–3.
40. Ohno K, Engel AG, Brengman JM, Shen XM, Heidenreich F, Vincent A, et al. The spectrum of mutations causing end-plate acetylcholinesterase deficiency. Ann Neurol. 2000;47:162–70.
41. Kimbell LM, Ohno K, Engel AG, Rotundo RL. C-terminal and heparin-binding domains of collagenic tail subunit are both essential for anchoring acetylcholinesterase at the synapse. J Biol Chem. 2004;279:10997–1005.
42. Logan CV, Cossins J, Rodríguez Cruz P, Parry DA, Maxwell S, Martínez-Martínez P, et al. Congenital myasthenic syndrome type 19 is caused by mutations in COL13A1, encoding the atypical non-fibrillar collagen type XIII α1 chain. Am J Hum Genet. 2015;97:878–85.
43. Hägg P, Rehn M, Huhtala P, Väisänen T, Tamminen M, Pihlajaniemi T. Type XIII collagen is identified as a plasma membrane protein. J Biol Chem. 1998;273:15590–7.
44. Latvanlehto A, Fox M, Sormunen R, Tu H, Oikarainen T, Koski A, et al. Muscle-derived collagen XIII regulates maturation of the skeletal neuromuscular junction. J Neurosci. 2010;30:12230–41.
45. Härönen H, Zainul Z, Tu H, Naumenko N, Sormunen R, Miinalainen I, et al. Collagen XIII secures pre- and postsynaptic integrity of the neuromuscular synapse. Hum Mol Genet. 2017;26:2076–90.
46. Maselli RA, Ng JJ, Anderson JA, Cagney O, Arredondo J, Williams C, et al. Mutations in LAMB2 causing a severe form of synaptic congenital myasthenic syndrome. J Med Genet. 2009;46:203–8.
47. Maselli RA, Arredondo J, Vázquez J, Chong JX, University of Washington Center for Mendelian Genomics, Bamshad MJ, et al. Presynaptic congenital myasthenic syndrome with a homozygous sequence variant in LAMA5 combines myopia, facial tics, and failure of neuromuscular transmission. Am J Med Genet A. 2017;173:2240–5.
48. Ohno K, Hutchinson DO, Milone M, Brengman JM, Bouzat C, Sine SM, et al. Congenital myasthenic syndrome caused by prolonged acetylcholine receptor channel openings due to a mutation in the M2 domain of the epsilon subunit. Proc Natl Acad Sci U S A. 1995;92:758–62.
49. Webster R, Maxwell S, Spearman H, Tai K, Beckstein O, Sansom M, et al. A novel congenital myasthenic syndrome due to decreased acetylcholine receptor ion-channel conductance. Brain. 2012;135:1070–80.
50. Croxen R, Young C, Slater C, Haslam S, Brydson M, Vincent A, et al. End-plate gamma- and epsilon-subunit mRNA levels in AChR deficiency syndrome due to epsilon-subunit null mutations. Brain. 2001;124:1362–72.
51. Hesselmans LF, Jennekens FG, Van den Oord CJ, Veldman H, Vincent A. Development of innervation of skeletal muscle fibers in man: relation to acetylcholine receptors. Anat Rec. 1993;236:553–62.
52. MacLennan C, Beeson D, Buijs AM, Vincent A, Newsom-Davis J. Acetylcholine receptor expression in human extraocular muscles and their susceptibility to myasthenia gravis. Ann Neurol. 1997;41:423–31.
53. Vincent A, Cull-Candy SG, Newsom-Davis J, Trautmann A, Molenaar PC, Polak RL. Congenital myasthenia: end-plate acetylcholine receptors and electrophysiology in five cases. Muscle Nerve. 1981;4:306–18.
54. Nichols P, Croxen R, Vincent A, Rutter R, Hutchinson M, Newsom-Davis J, et al. Mutation of the acetylcholine receptor ε-subunit promoter in congenital myasthenic syndrome. Ann Neurol. 1999;45:439–43.
55. Ohno K, Anlar B, Engel AG. Congenital myasthenic syndrome caused by a mutation in the Ets-binding site of the promoter region of the acetylcholine receptor epsilon subunit gene. Neuromuscul Disord. 1999;9:131–5.
56. Abicht A, Stucka R, Schmidt C, Briguet A, Höpfner S, Song IH, et al. A newly identified chromosomal microdeletion and an N-box mutation of the AChR epsilon gene cause a congenital myasthenic syndrome. Brain. 2002;125:1005–13.
57. Cossins J, Webster R, Maxwell S, Burke G, Vincent A, Beeson D. A mouse model of AChR deficiency syndrome with a phenotype reflecting the human condition. Hum Mol Genet. 2004;13:2947–57.
58. Ealing J, Webster R, Brownlow S, Abdelgany A, Oosterhuis H, Muntoni F, et al. Mutations in congenital myasthenic syndromes reveal an ε-subunit C-terminal cysteine, C470, crucial for maturation and surface expression of adult AChR. Hum Mol Genet. 2002;11:3087–96.
59. Croxen R, Newland C, Betty M, Vincent A, Newsom-Davis J, Beeson D. Novel functional epsilon-subunit polypeptide generated by a single nucleotide deletion in acetylcholine receptor deficiency congenital myasthenic syndrome. Ann Neurol. 1999;46:639–47.
60. Abicht A, Stucka R, Karcagi V, Herczegfalvi A, Horváth R, Mortier W, et al. A common mutation

(epsilon1267delG) in congenital myasthenic patients of gypsy ethnic origin. Neurology. 1999;53:1564–9.
61. Morar B, Gresham D, Angelicheva D, Tournev I, Gooding R, Guergueltcheva V, et al. Mutation history of the Roma gypsies. Am J Hum Genet. 2004;75:596–609.
62. Quiram PA, Ohno K, Milone M, Patterson MC, Pruitt NJ, Brengman JM, et al. Mutation causing congenital myasthenia reveals acetylcholine receptor beta/delta subunit interaction essential for assembly. J Clin Invest. 1999;104:1403–10.
63. Engel AG, Lambert EH, Mulder DM, Torres CF, Sahashi K, Bertorini TE, et al. A newly recognized congenital myasthenic syndrome attributed to a prolonged open time of the acetylcholine-induced ion channel. Ann Neurol. 1982;11:553–69.
64. Croxen R, Hatton C, Shelley C, Brydson M, Chauplannaz G, Oosterhuis H, et al. Recessive inheritance and variable penetrance of slow-channel congenital myasthenic syndromes. Neurology. 2002;59:162–8.
65. Gomez CM, Maselli R, Gammack J, Lasalde J, Tamamizu S, Cornblath DR, et al. A beta-subunit mutation in the acetylcholine receptor channel gate causes severe slow-channel syndrome. Ann Neurol. 1996;39:712–23.
66. Gomez CM, Maselli RA, Vohra BP, Navedo M, Stiles JR, Charnet P, et al. Novel delta subunit mutation in slow-channel syndrome causes severe weakness by novel mechanisms. Ann Neurol. 2002;51:102–12.
67. Hatton CJ, Shelley C, Brydson M, Beeson D, Colquhoun D. Properties of the human muscle nicotinic receptor, and of the slow-channel myasthenic syndrome mutant epsilonL221F, inferred from maximum likelihood fits. J Physiol. 2003;547:729–60.
68. Croxen R, Newland C, Beeson D, Oosterhuis H, Chauplannaz G, Vincent A, et al. Mutations in different functional domains of the human muscle acetylcholine receptor alpha subunit in patients with the slow-channel congenital myasthenic syndrome. Hum Mol Genet. 1997;6:767–74.
69. Engel AG, Ohno K, Milone M, Wang HL, Nakano S, Bouzat C, et al. New mutations in acetylcholine receptor subunit genes reveal heterogeneity in the slow channel congenital myasthenic syndrome. Hum Mol Genet. 1996;5:1217–27.
70. Finlayson S, Spillane J, Kullmann DM, Howard R, Webster R, Palace J, et al. Slow channel congenital myasthenic syndrome responsive to a combination of fluoxetine and salbutamol. Muscle Nerve. 2013;47:279–82.
71. Harper CM, Engel AG. Quinidine sulfate therapy for the slow-channel congenital myasthenic syndrome. Ann Neurol. 1998;43:480–4.
72. Harper CM, Fukodome T, Engel AG. Treatment of slow-channel congenital myasthenic syndrome with fluoxetine. Neurology. 2003;60:1710–3.
73. Colomer J, Muller JS, Vernet A, Nascimento A, Pons M, Gonzalez V, et al. Long-term improvement of slow-channel congenital myasthenic syndrome with fluoxetine. Neuromuscul Disord. 2006;16:329–33.
74. Webster R, Brydson M, Croxen R, Newsom-Davis J, Vincent A, Beeson D. Mutation in the AChR ion channel gate underlies a fast channel congenital myasthenic syndrome. Neurology. 2004;62:1090–6.
75. Palace J, Lashley D, Bailey S, Jayawant S, Carr A, McConville J, et al. Clinical features in a series of fast channel congenital myasthenia syndrome. Neuromuscul Disord. 2012;22:112–7.
76. Brownlow S, Webster R, Croxen R, Brydson M, Neville B, Lin JP, et al. Acetylcholine receptor delta subunit mutations underlie a fast-channel myasthenic syndrome and arthrogryposis multiplex congenita. J Clin Invest. 2001;108:125–30.
77. Wang HL, Milone M, Ohno K, Shen XM, Tsujino A, Batocchi AP, et al. Acetylcholine receptor M3 domain: stereochemical and volume contributions to channel gating. Nat Neurosci. 1999;2:226–33.
78. Sanes JR, Lichtman JW. Induction, assembly, maturation and maintenance of a postsynaptic apparatus. Nat Rev Neurosci. 2001;2:791–805.
79. Muller JS, Mildner G, Muller-Felber W, Schara U, Krampfl K, Petersen B, et al. Rapsyn N88K is a frequent cause of congenital myasthenic syndromes in European patients. Neurology. 2003;60:1805–10.
80. Dunne V, Maselli RA. Identification of pathogenic mutations in the human rapsyn gene. J Hum Genet. 2003;48:204–7.
81. Maselli RA, Dunne V, Pascual-Pascual SI. Rapsyn mutations in myasthenic syndrome due to impaired receptor clustering. Muscle Nerve. 2003;28:293–301.
82. Muller JS, Abicht A, Burke G, Cossins J, Richard P, Baumeister SK, et al. The congenital myasthenic syndrome mutation RAPSN N88K derives from an ancient indo-European founder. J Med Genet. 2004;41:e104.
83. Cossins J, Burke G, Maxwell S, Spearman H, Man S, Kuks J, et al. Diverse molecular mechanisms involved in AChR deficiency due to rapsyn mutations. Brain. 2006;129:2773–83.
84. Ohno K, Sadeh M, Blatt I, Brengman JM, Engel AG. E-box mutations in the RAPSN promoter region in eight cases with congenital myasthenic syndrome. Hum Mol Genet. 2003;12:739–48.
85. Muller JS, Baumeister SK, Schara U, Cossins J, Krause S, von der Hagen M, et al. CHRND mutation causes a congenital myasthenic syndrome by impairing co-clustering of the acetylcholine receptor with rapsyn. Brain. 2006;129:2784–93.
86. McQuillen MP. Familial limb-girdle myasthenia. Brain. 1966;89:121–32.
87. Muller JS, Herczegfalvi A, Vilchez JJ, Colomer J, Bachinski LL, Mihaylova V, et al. Phenotypical spectrum of DOK7 mutations in congenital myasthenic syndromes. Brain. 2007;130:1497–506.
88. Lashley D, Palace J, Jayawant S, Robb S, Beeson D. Ephedrine treatment in congenital myasthenic syndrome due to mutations in DOK7. Neurology. 2010;74:1517–23.

89. Missias AC, Mudd J, Cunningham JM, Steinbach JH, Merlie JP, Sanes JR. Deficient development and maintenance of postsynaptic specializations in mutant mice lacking an 'adult' acetylcholine receptor subunit. Development. 1997;124:5075–86.
90. Gautam M, Noakes PG, Mudd J, Nichol M, Chu GC, Sanes JR, et al. Failure of postsynaptic specialization to develop at neuromuscular junctions of rapsyn-deficient mice. Nature. 1995;377:232–6.
91. Gautam M, Noakes PG, Moscoso L, Rupp F, Scheller RH, Merlie JP, et al. Defective neuromuscular synaptogenesis in agrin-deficient mutant mice. Cell. 1996;85:525–35.
92. DeChiara TM, Bowen DC, Valenzuela DM, Simmons MV, Poueymirou WT, Thomas S, et al. The receptor tyrosine kinase MuSK is required for neuromuscular junction formation in vivo. Cell. 1996;85:501–12.
93. Willmann R, Fuhrer C. Neuromuscular synaptogenesis: clustering of acetylcholine receptors revisited. Cell Mol Life Sci. 2002;59:1296–316.
94. Okada K, Inoue A, Okada A, Murata Y, Kakuta S, Jigami T, et al. The muscle protein Dok-7 is essential for neuromuscular synaptogenesis. Science. 2006;312:1802–5.
95. Slater CR, Fawcett PR, Walls TJ, Lyons PR, Bailey SJ, Beeson D, et al. Pre- and post-synaptic abnormalities associated with impaired neuromuscular transmission in a group of patients with 'limb-girdle' myasthenia. Brain. 2006;129:2061–76.
96. Kariminejad A, Stollfuß B, Li Y, Bögershausen N, Boss K, Hennekam RC, et al. Severe Cenani-Lenz syndrome caused by loss of LRP4 function. Am J Med Genet A. 2013;161A:1475–9.
97. Maselli RA, Fernandez JM, Arredondo J, Navarro C, Ngo M, Beeson D, et al. LG2 agrin mutation causing severe congenital myasthenic syndrome mimics functional characteristics of non-neural (z-) agrin. Hum Genet. 2012;131:1123–35.
98. Ben Ammar A, Soltanzadeh P, Bauché S, Richard P, Goillot E, Herbst R, et al. A mutation causes MuSK reduced sensitivity to agrin and congenital myasthenia. PLoS One. 2013;8(1):e53826.
99. Mishina M, Takai T, Imoto K, Noda M, Takahashi T, Numa S, et al. Molecular distinction between fetal and adult forms of muscle acetylcholine receptor. Nature. 1986;321:406–11.
100. Escobar V, Bixler D, Gleiser S, Weaver DD, Gibbs T. Multiple pterygium syndrome. Am J Dis Child. 1978;132:609–11.
101. Morgan NV, Brueton LA, Cox P, Greally MT, Tolmie J, Pasha S, et al. Mutations in the embryonal subunit of the acetylcholine receptor (CHRNG) cause lethal and Escobar variants of multiple pterygium syndrome. Am J Hum Genet. 2006;79:390–5.
102. Hoffmann K, Muller JS, Stricker S, Megarbane A, Rajab A, Lindner TH, et al. Escobar syndrome is a prenatal myasthenia caused by disruption of the acetylcholine receptor fetal gamma subunit. Am J Hum Genet. 2006;79:303–12.
103. Moremen K, Tiemeyer M, Nairn A. Vertebrate protein glycosylation: diversity, synthesis and function. Nat Rev Mol Cell Biol. 2012;13:448–62.
104. Jaeken J, Matthijs G. Congenital disorders of glycosylation: a rapidly expanding disease family. Annu Rev Genomics Hum Genet. 2007;8:261–78.
105. Gehle VM, Walcott EC, Nishizaki T, Sumikawa K. N-glycosylation at the conserved sites ensures the expression of properly folded functional ACh receptors. Brain Res Mol Brain Res. 1997;45:219–29.
106. Selcen D, Shen XM, Brengman J, Li Y, Stans AA, Wieben E, et al. DPAGT1 myasthenia and myopathy: genetic, phenotypic, and expression studies. Neurology. 2014;82:1822–30.
107. Würde AE, Reunert J, Rust S, Hertzberg C, Haverkämper S, Nürnberg G, et al. Congenital disorder of glycosylation type Ij (CDG-Ij, DPAGT1-CDG): extending the clinical and molecular spectrum of a rare disease. Mol Genet Metab. 2012;105:634–41.
108. Haltiwanger RS, Lowe JB. Role of glycosylation in development. Annu Rev Biochem. 2004;73:491–537.
109. Zoltowska K, Webster R, Finlayson S, Maxwell S, Cossins J, Müller J, et al. Mutations in GFPT1 that underlie limb-girdle congenital myasthenic syndrome result in reduced cell-surface expression of muscle AChR. Hum Mol Genet. 2013;22:2905–13.
110. Bretthauer RK. Structure, expression, and regulation of UDP-GlcNAc: dolichol phosphate GlcNAc-1-phosphate transferase (DPAGT1). Curr Drug Targets. 2009;10:477–82.
111. Lu J, Takahashi T, Ohoka A, Nakajima K, Hashimoto R, Miura N, et al. Alg14 organizes the formation of a multiglycosyltransferase complex involved in initiation of lipid-linked oligosaccharide biosynthesis. Glycobiology. 2012;22:504–16.
112. Jackson BJ, Kukuruzinska MA, Robbins P. Biosynthesis of asparagine-linked oligosaccharides in Saccharomyces cerevisiae: the alg2 mutation. Glycobiology. 1993;3:357–64.
113. Carss KJ, Stevens E, Foley AR, Cirak S, Riemersma M, Torelli S, et al. Mutations in GDP-mannose pyrophosphorylase B cause congenital and limb-girdle muscular dystrophies associated with hypoglycosylation of α-dystroglycan. Am J Hum Genet. 2013;93:29–41.
114. Rodríguez Cruz PM, Belaya K, Basiri K, Sedghi M, Farrugia ME, Holton JL, et al. Clinical features of the myasthenic syndrome arising from mutations in GMPPB. J Neurol Neurosurg Psychiatry. 2016;87:802–9.

115. Muntoni F, Torelli S, Wells DJ, Brown SC. Muscular dystrophies due to glycosylation defects: diagnosis and therapeutic strategies. Curr Opin Neurol. 2011;24:437–42.
116. Habbout K, Poulin H, Rivier F, Giuliano S. A recessive Nav1.4 mutation underlies congenital myasthenic syndrome with periodic paralysis. Neurology. 2016;86:161–9.
117. Martens K, Derua R, Meulemans S, Waelkens E, Jaeken J, Matthijs G, et al. PREPL: a putative novel oligopeptidase propelled into the limelight. Biol Chem. 2006;387:879–83.
118. Régal L, Shen XM, Selcen D, Verhille C, Meulemans S, Creemers JW, et al. PREPL deficiency with or without cystinuria causes a novel myasthenic syndrome. Neurology. 2014;82:1254–60.
119. Kim MH, Hersh LB. The vesicular acetylcholine transporter interacts with clathrin-associated adaptor complexes AP-1 and AP-2. J Biol Chem. 2004;279:12580–7.
120. Hartman MA, Finan D, Sivaramakrishnan S, Spudich JA. Principles of unconventional myosin function and targeting. Annu Rev Cell Dev Biol. 2011;27:133–55.
121. Bridgman PC. Myosin motor proteins in the cell biology of the axon and other neuronal compartments. In: Koenig E, editor. Cell biology of the axon. Berlin: Springer; 2009. p. 91–105.
122. O'Connor E, Töpf A, Müller JS, Cox D, Evangelista T, Colomer J, et al. Identification of mutations in the MYO9A gene in patients with congenital myasthenic syndrome. Brain. 2016;139:2143–53.
123. Schwab RS, Timberlake WH. Pyridostigmin (mestinon) in the treatment of myasthenia gravis. N Engl J Med. 1954;251:271–2.
124. Palace J, Wiles CM, Newsom-Davis J. 3,4-Diaminopyridine in the treatment of congenital (hereditary) myasthenia. J Neurol Neurosurg Psychiatry. 1991;54:1069–72.
125. Burke G, Hiscock A, Klein A, Niks EH, Main M, Manzur AY, et al. Salbutamol benefits children with congenital myasthenic syndrome due to DOK7 mutations. Neuromuscul Disord. 2013;23:170–5.
126. Lorenzoni PJ, Scola RH, Kay CS, Filla L, Miranda AP, Pinheiro JM, et al. Salbutamol therapy in congenital myasthenic syndrome due to DOK7 mutation. J Neurol Sci. 2013;331:155–7.
127. Mihaylova V, Müller JS, Vilchez JJ, Salih MA, Kabiraj MM, D'Amico A, et al. Clinical and molecular genetic findings in COLQ-mutant congenital myasthenic syndromes. Brain. 2008;131:747–59.
128. Liewluck T, Selcen D, Engel AG. Beneficial effects of albuterol in congenital endplate acetylcholinesterase deficiency and Dok-7 myasthenia. Muscle Nerve. 2011;44:789–94.
129. Finlayson S, Palace J, Belaya K, Walls TJ, Norwood F, Burke G, et al. Clinical features of congenital myasthenic syndrome due to mutations in DPAGT1. J Neurol Neurosurg Psychiatry. 2013;84:1119–25.
130. Rodríguez Cruz PM, Palace J, Ramjattan H, Jayawant S, Robb SA, Beeson D. Salbutamol and ephedrine in the treatment of severe AChR deficiency syndromes. Neurology. 2015;85:1043–7.

Toxic Neuromuscular Transmission Disorders

17

James F. Howard Jr.

Introduction

The neuromuscular junction (NMJ) is uniquely sensitive to the effects of neurotoxins. There are no barriers to protect the NMJ from the deleterious effects of these agents unlike the blood-brain barrier, which protects the brain and spinal cord, and the blood-nerve barrier, which protects peripheral nerve. Several types of neurotoxins are directed against the NMJ. Many occur as natural substances of animals or plants, others result from the actions of widely prescribed pharmaceutical compounds, and still others are environmental hazards. In nearly all instances of NMJ neurotoxicity, there is a reduction in the safety factor of neuromuscular transmission by one of several mechanisms. These neurotoxins may affect either the presynaptic or the postsynaptic elements of the NMJ and rarely both. The clinical features of these neurotoxins are quite varied, as many have associated toxicity to other parts of the central, peripheral, or autonomic nervous systems. Many will have other systemic effects as well. While feared as the purveyor of morbidity and mortality, many of these neurotoxins have led to significant advances in our understanding of the molecular mechanisms of pharmacology and physiology and their associated diseases. For example, were it not for the recognition that α-bungarotoxin binds to the acetylcholine receptor (AChR), and omega-conotoxin binds to the voltage-gated calcium channel (VGCC), advances in the diagnosis and treatment of myasthenia gravis (MG) and the Lambert-Eaton syndrome (LES) would have been delayed [1, 2]. Worldwide, the most common neurotoxicity of the neuromuscular junction results from envenomation. Of more concern to the clinician are those situations that result from the direct effects of various pharmaceuticals routinely used in the practice of medicine that produce significant aberrations of neuromuscular transmission in susceptible individuals. The potential for environmental intoxication has been limited by the stringent regulation of federal and international regulatory agencies in most countries.

The neurotoxins of interest may be broadly classified into three major categories: pharmacological, biological, and environmental. This chapter focuses on the effects of neurotoxins affecting neuromuscular transmission in man and, of recent concern, those drugs that dysregulate the immune system and induce an autoimmune attack against synaptic elements of the NMJ. It is not meant to be a treatise on the broad topic of neuromuscular neurotoxicology. It is not possible to elaborate on all of the pharmacological and physiological effects of particular toxins

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H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*, Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_17

beyond the scope of the NMJ, nor will it discuss in detail the neuromuscular blocking effects of these neurotoxins in animals or experimental preparations.

All forms of NMJ neurotoxicity are characterized by progressive, typically symmetrical, muscle weakness. Muscles of eye movement or the eyelid are most often involved as well as the muscles of neck flexion and the pectoral and pelvic girdles. There may be involvement of bulbar and respiratory musculature depending upon the toxin involved, the dose acquired, and the duration of toxin exposure. Cognition and sensation are spared, unless other elements of the central nervous system are simultaneously involved. Muscle stretch reflexes are often preserved or only minimally diminished, particularly during the early phases of the illness but may be lost if the weakness is severe.

Pharmacological Neurotoxicity

The adverse reaction of drugs on synaptic transmission may be classified broadly either as acting *presynaptically*, with a reduction in acetylcholine (ACh) release due to local anesthetic-like activity on the nerve terminal, alteration or impairment of calcium flux into the nerve terminal, or a hemicholinium effect; *postsynaptically*, with antibody blockade of ACh receptors, curare-like effects, or potentiation of depolarizing or nondepolarizing neuromuscular blocking agents; or, in varying degrees, *both*. Each of these pharmacological interactions may result in any of the clinical situations described above. Since the publication of the summaries of Barrons, Howard, and Kaeser, describing disorders of neuromuscular transmission occurring as the result of adverse drug reactions, many more reports have surfaced adding to the list of potentially dangerous drugs [3, 4]. An up-to-date list of these potential drug-disorder interactions is maintained on the web site of the Myasthenia Gravis Foundation of America (http://www.myasthenia.org/LinkClick.aspx?fileticket=JuFvZPPq2vg%3D). Unfortunately, much of the literature is anecdotal, and there are only a few comprehensive in vitro studies of drug effects on neuromuscular transmission in animal or human nerve-muscle preparations. The potential adverse effects of these medications must be taken into consideration when deciding which drugs to use in treating patients who have disorders of synaptic transmission.

With the exception of telithromycin (no longer available in the United States) and the possible exceptions of D-penicillamine, α-interferon, and botulinum toxin, there are no drugs that are absolutely contraindicated in patients with MG and LES. Numerous drugs, however, will interfere with neuromuscular transmission and will make the weakness of these patients worse or prolong the duration of neuromuscular block in patients receiving muscle relaxants. Drug-induced disturbances of synaptic transmission resemble MG with varying degrees of ptosis and ocular, facial, bulbar, respiratory, and generalized muscle weakness. Treatment includes discontinuation of the offending drug and, when necessary, reversing the neuromuscular block with intravenous infusions of calcium, potassium, or cholinesterase inhibitors. In rare instances, these drugs may induce an autoimmune form of MG (D-penicillamine, interferon alpha, and checkpoint inhibitors). The latter is only a recently recognized phenomenon. In these situations, the treating physician must utilize therapies that are typically used for other forms of autoimmune MG.

While it is most desirable to avoid drugs that may adversely affect neuromuscular transmission, in certain instances they must be used for the management of another illness. In such situations, a thorough knowledge of the deleterious side effects can minimize their potential danger. If at all possible, it is wise to use the drug within a class of drugs that has been shown to have the least effect on neuromuscular transmission. Unfortunately, studies that allow such comparisons are quite few.

The most frequently encountered problems are the effects of antibiotics (macrolides, fluoroquinolones, and aminoglycosides) and β-adrenergic blocking agents acutely worsening the strength of patients with MG. Less commonly encountered is prolonged muscle weakness and

respiratory embarrassment postoperatively in patients with disorders of neuromuscular transmission.

Antibiotics

Two classes of antibiotics carry the FDA black box warning designation. The ketolide, telithromycin, and the fluoroquinolone class of antibiotics received this designation due to repeated reports of myasthenic exacerbation and death following their use [5–7]. The aminoglycoside antibiotics may produce neuromuscular weakness irrespective of their route of administration [8]. The weakness is related to serum levels of the drug and is reversible in part by cholinesterase inhibitors, calcium infusion, and the aminopyridines [9]. These drugs have pre- and postsynaptic actions; many have elements of both. Neuromuscular toxicity data exist for several of the antibiotics including amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, and tobramycin [10]. Of the group neomycin is the most toxic; tobramycin the least. Clinically, gentamicin, kanamycin, neomycin, tobramycin, and streptomycin have been implicated in producing muscle weakness in non-myasthenic patients [11]. Neuromuscular blockade is not limited to the aminoglycoside antibiotics. Myasthenic patients given the macrolides, erythromycin, or azithromycin will often report a mild to moderate exacerbation of their weakness as well as myasthenic crisis; the latter has been reversed with calcium gluconate. The ketolide, telithromycin, has produced abrupt and severe worsening in MG or unmasked previously undiagnosed MG (Howard, JF, personal observation) [6, 12]. The polypeptide and monobasic amino acid antibiotics, penicillins, sulfonamides, tetracyclines, and fluoroquinolones cause transient worsening of myasthenic weakness, potentiate the weakness of neuromuscular blocking agents, or have theoretical reasons for blocking synaptic transmission [13]. Lincomycin and clindamycin can cause neuromuscular blocking which is not readily reversible with cholinesterase inhibitors [14, 15]. Polymyxin B, colistimethate, and colistin are also reported to produce neuromuscular weakness, particularly in patients with renal disease or when used in combination with other antibiotics or neuromuscular blocking agents [8, 16]. These drugs, ampicillin, the tetracycline analogs, oxytetracycline, and rolitetracycline, may exacerbate MG, although the mechanism for each medication is not fully understood [17, 18].

The fluoroquinolones, particularly levofloxacin and ciprofloxacin, are reported to acutely exacerbate myasthenic weakness [7]. By virtue of their quinolone ring, they are structurally similar to quinine, quinidine, and chloroquine which have significant neuromuscular blocking capabilities [19, 20]. The severity of these exacerbations is typically severe, and for that reason this drug class carries a black box warning regarding their use in MG.

Cardiovascular Drugs

Many cardiovascular drugs are implicated in adversely affecting the strength of patients with MG and LES, and they (along with the antibiotics) account for the majority of adverse drug reactions in patients with neuromuscular disorders. Beta-adrenergic blockers may cause exacerbation of MG, or their use may coincide with the onset of myasthenic manifestations [21]. Even those drugs instilled topically on the cornea are capable of producing such weakness [22, 23]. Atenolol, labetalol, metoprolol, nadolol, propranolol, and timolol cause a dose-dependent reduction in the efficacy of neuromuscular transmission in normal rat skeletal muscle and human myasthenic intercostal muscle biopsies [24]. Different ß-blockers have reproducibly different pre- and postsynaptic effects on neuromuscular transmission. Of the group, propranolol is most effective in blocking neuromuscular transmission, and atenolol the least.

The effects of calcium channel blockers on skeletal muscle are not understood fully, and studies have provided conflicting information. Some demonstrated neuromuscular blockade with postsynaptic curare-like effects, presynaptic inhibition of ACh release, and both pre- and

postsynaptic effects [25–28]. The oral administration of calcium channel blockers to cardiac patients without neuromuscular disease does not produce altered neuromuscular transmission by single-fiber electromyography (SFEMG) measures [25]. Acute respiratory failure was temporally associated with administration of oral dose of verapamil in a patient with LES and small cell carcinoma of the lung [29]. One patient with moderately severe, generalized MG developed acute respiratory failure following verapamil initiation (author's observations). Low doses of verapamil and its timed-release preparation have been used successfully for the treatment of hypertension in patients with MG receiving cyclosporine (author's observations).

Procainamide may produce acute worsening of strength in patients with MG [30]. The rapid onset of neuromuscular block and the rapid resolution of symptoms following discontinuation of the drug suggest the drug has a direct toxic effect on synaptic transmission, rather than the induction of an autoimmune response against the neuromuscular junction. The postulated mechanism of action is primarily at the presynaptic membrane with impaired formation of ACh or its release, although it is known to have postsynaptic blocking effects as well. Two case reports suggest that the anti-arrhythmic P-glycoprotein inhibitor, propafenone, may cause acute exacerbations of myasthenic weakness [31, 32]. Like the effects of procainamide, the rapid onset of worsening and resolution following the discontinuation of the drug implicates a direct toxic effect on neuromuscular transmission.

The earliest report of quinidine (the stereoisomer of quinine) administration aggravating MG was by Weisman [33]. There are several reports of the unmasking of previously unrecognized MG following treatment with quinidine [34, 35]. The neuromuscular block is both presynaptic; impairing either the formation or release of ACh or, in larger doses, postsynaptic with a curare-like action [36]. It has been claimed the ingestion of small amounts of quinine, for example, in a gin and tonic, may acutely worsen weakness in a myasthenic patient, although this cannot be substantiated with objective reports. Despite the potential for these drugs to acutely exacerbate weakness in acquired autoimmune MG, their use in congenital myasthenic syndromes as a therapeutic agent is well recognized [37].

Cholesterol-Lowering Agents

Several works suggest that the statin cholesterol-lowering agents may be causal in the exacerbation of myasthenic weakness although this remains controversial [38–45]. The mechanism(s) for this worsening is not clear but several postulates have been proposed. It is well recognized that HMG-CoA reductase therapy may produce a myopathy [46, 47]. Is it possible the myasthenic worsening could be due to a coexisting disorder of the muscle membrane? Statins have immunomodulatory properties, with the ability to induce production of the Th2 cytokines interleukin (IL)-4, IL-5, and IL-10 [48]. Animal and human studies suggest that these Th2 cytokines may influence the development of MG [49] and, therefore, that upregulation of Th2 cytokine production could lead to worsening MG. Statins have been postulated to cause mitochondrial dysfunction by depleting endogenous coenzyme Q10 [50]. Statin-induced mitochondrial failure in the nerve terminal has been proposed as a mechanism to impair neuromuscular transmission given the high content of mitochondria in the nerve terminal [39]. There is no evidence to suggest that HMG-CoA reductase is known to directly interfere with neuromuscular transmission [51].

Magnesium

Hypermagnesemia is an uncommon clinical complication from the use of magnesium-containing drugs [52]. Renal failure predisposes to hypermagnesemia and is a reason to avoid magnesium-containing antacids and laxatives [53, 54]. Excessive use of enemas containing Mg^{2+} may produce hypermagnesemia, but this is usually in patients with underlying gastrointestinal (GI) tract disease [55, 56]. Hypermagnesemia commonly results from administration of high

doses of parenteral $MgSO_4$ for treatment of low serum magnesium level or eclampsia, at times resulting in myasthenic crisis or serious side effects to the mother or the newborn [57–60]. The clinical manifestations of hypermagnesemia correlate with serum Mg^{2+} levels [56, 61]. Muscle stretch reflexes become reduced when the serum Mg^{2+} level exceeds 5 mEq/L; levels of 9–10 mEq/L are associated with absent reflexes and muscle weakness. During treatment of preeclampsia, muscle stretch reflexes are monitored, and Mg^{2+} administration is discontinued if the reflexes disappear [59]. Fatal respiratory failure can occur at levels greater than 10 mEq/L [57, 59]. Serum levels greater than 14 mEq/L can induce acute cardiac arrhythmia, including heart block and arrest. Symptoms of autonomic dysfunction in hypermagnesemia include dry mouth, dilated pupils, urinary retention, hypotension, and flushing and are thought to result from presynaptic blockade at autonomic ganglia [62]. The muscles of ocular motility tend to be spared, and the clinical findings of hypermagnesemia resemble those of LES rather than MG [63]. Magnesium inhibits release of ACh by competitively blocking calcium entry at the motor nerve terminal [64]. There may also be a milder postsynaptic affect. Magnesium also potentiates the action of neuromuscular blocking agents, and this must be considered in women undergoing cesarean section after receiving Mg^{2+} for preeclampsia [65, 66]. Patients with underlying junctional disorders are more sensitive to Mg^{2+}-induced weakness. Patients with MG and LES may become weaker after receiving Mg^{2+} even when serum Mg^{2+} levels are normal or only slightly elevated [67–70]. Reports exist of the uncovering of previously unrecognized MG following treatment of preeclampsia with magnesium salts (Howard JF, unpublished observation) [71]. Increased MG symptoms most often occur when Mg^{2+} is administered parenterally, but on occasion is seen with oral use [70]. Therefore, parenteral Mg^{2+} administration should be avoided, and oral Mg^{2+} preparations should be used with caution in patients with known disorders of synaptic transmission, such as MG, LES, and botulism. Patients with MG or LES respond poorly to calcium and may respond better to cholinesterase inhibitors [67].

Recreational Drugs

Several patients with MG exacerbation following recreational use of cocaine have been reported [72–75]. These attacks frequently include respiratory insufficiency requiring ventilatory support and improve with therapeutic apheresis or high-dose intravenous immunoglobulin [74, 75]. Cocaine reduces the skeletal muscle response to repetitive nerve stimulation in mice by decreasing the muscle and nerve excitability, but without apparent effect on NMT. Cocaine inhibits nicotinic AChR in cultured muscle cells [76].

Rheumatologic Drugs

D-Penicillamine (D-P) is used in the treatment of rheumatoid arthritis (RA), Wilson's disease, and cystinuria. A number of autoimmune diseases occur in patients receiving D-P of which MG is most frequent [77–79]. The MG induced by D-P is usually mild and may be restricted to the ocular muscles. In many patients the manifestations are not recognized, and it may be difficult to demonstrate mild weakness of the limbs in the presence of severe arthritis. Both AChR+ and MuSK (muscle-specific kinase antibodies) have been reported in a single patient with D-P-induced MG [80]. It is unlikely that D-P has a direct effect on neuromuscular transmission as MG begins after prolonged D-P therapy in most patients and has a relatively low incidence in patients receiving D-P for Wilson's disease compared with those receiving it for RA [81]. It is more likely that D-P induces MG by stimulating or enhancing an immunological reaction against the neuromuscular junction. When MG begins while the patient is receiving D-P, it remits in 70% of patients within 1 year after the discontinuation of the drug [82]. In a few patients, the MG persists after D-P is discontinued, implying that a subclinical myasthenic state existed prior to the initiation of the D-P (author's observations).

Chloroquine is used as an antimalarial drug, but in higher doses it is also provided for treatment of several collagen vascular disorders including RA, discoid lupus erythematosus, and porphyria cutanea. It may produce a number of neurological adverse effects among which are disorders of neuromuscular transmission [83]. Reported mechanisms of action for this have been both pre- and postsynaptic, but chloroquine may also alter immune regulation producing a clinical syndrome of MG similar to that reported with D-P. One patient with RA and another with systemic lupus erythematosus developed the typical clinical, physiological, and pharmacological picture of MG following prolonged treatment with chloroquine. Antibodies to the AChR were identified and subsequently slowly disappeared, as did the clinical and electrophysiological abnormalities, with discontinuation of the drug [84, 85]. A patient is described with a transient postsynaptic disorder of neuromuscular transmission following 1 week of chloroquine therapy that was thought to be due to a direct toxic effect on the neuromuscular junction rather than a derangement of immune function [86].

Other

Checkpoint Inhibitors

Checkpoint inhibitors are an emerging class of immunotherapeutic drugs across a broad spectrum of malignancies that are designed to block selective proteins (checkpoint proteins) on the surface of tumor cells and on T-cells. Checkpoint proteins modulate the immune system and defeat normal host defense systems and allow the proliferation of tumor cells by evading the normal T-cell attack against the tumor [87]. There are several types of checkpoint proteins found on cancer cells or T-cells including PD-1/PD-L1 and CTLA-4/B7-1/B7-2. Checkpoint inhibitors block these perturbed normal proteins on the surface of tumor cells thus allowing T-cells to recognize the cell as cancerous and allow the immune surveillance system to act [88, 89]. Despite the major advances made with these drugs, the side effect profile is substantial, ranging from 30% to 60% [90, 91]. Uniquely, these drugs have the ability to "unleash" the immune systems with the development of multiple autoimmune disorders, including myasthenia gravis [92–102]. It is not clear at this time what are predisposing characteristics for the development of autoimmune disease.

Interferon Alpha

Generalized MG may be exacerbated by or occur after starting interferon alpha therapy for leukemia, during interferon alpha-2b treatment for malignancy, and during treatment for chronic active hepatitis C [103–108]. Myasthenic crisis may even develop with interferon alpha therapy [109]. The mechanism of interferon-induced MG is not known but may relate in part to background genetic and environmental factors [110]. Expression of interferon gamma at motor endplates of transgenic mice results in generalized weakness and abnormal NMJ function, which improves with cholinesterase inhibitors. Immunoprecipitation studies identified an 87-kD target antigen recognized by sera from these transgenic mice and from human MG patients. Such studies suggest that the expression of interferon gamma at the motor endplates provoked an autoimmune humoral response, similar to that which occurs in human MG [111].

Botulinum Neurotoxin

Botulinum neurotoxin, when used therapeutically for focal dystonia, has unmasked subclinical LES and MG [112–114]. Worsening of weakness or crisis has also been reported in MG following injections of botulinum toxin [115–117]. A known defect of NMT is considered to be a relative contraindication to the use of botulinum toxin although others have reported its successful use in patients with MG [118, 119].

Biological Neurotoxins

Botulism

Botulism is caused by a clostridial neurotoxin that blocks the release of ACh from the motor

Table 17.1 Clinical classification of botulism

Classic form
Infantile form
Wound botulism
Traumatic or surgical
Drug abuse
Intranasal
Intravenous
Hidden form

nerve terminal[1] [120]. The result is a long-lasting, severe muscle paralysis. Botulism may be classified clinically according to presentation as noted in Table 17.1. Of eight different types of botulinum toxins, types A and B cause most cases of botulism in the United States. Type E is transmitted in seafood and Type A is thought to produce the most severe manifestations. The classic form of the disease usually follows ingestion of foods that were contaminated by inadequate sterilization. Not all persons ingesting contaminated food become symptomatic. Nausea and vomiting are the first symptoms and the neuromuscular features begin 12–36 h after exposure. The clinical findings are stereotypical. Ptosis, blurred vision, dysphagia, and dysarthria are the presenting features. The pupils may be dilated and poorly reactive to light. Descending weakness progresses for 4–5 days and then plateaus. Respiratory paralysis may occur rapidly. Most patients have autonomic dysfunction, such as dry mouth, constipation, urinary retention, and cardiovascular instability.

Infantile botulism, the most common form of botulism found in the United States, is caused by the chronic absorption of toxin from *Clostridium botulinum* in the infant gastrointestinal tract after ingesting spores [121, 122]. Honey is a common source of contamination. The onset of symptoms, lethargy, and poor suck usually occur between 1 week and 12 months of age, most commonly between 2 and 8 months of age. Constipation, a common finding in children with botulism, is quite difficult to determine in the infant. Fever is typically not a feature of this syndrome. The majority of reported cases occur with Type A or B toxin. A descending pattern of weakness occurs and may produce widespread cranial nerve and limb muscle involvement. The pupils are poorly reactive and the tendon reflexes are hypoactive. Most babies will require ventilatory support.

Wound botulism occurs due to the contamination of a wound with *Clostridium botulinum*. Its rarity may be related to the difficulty of spore germination in a wound environment. The clinical presentation of wound botulism is similar to the classical form of the disease as noted above. It may occur as a complication of intranasal or parenteral use of cocaine [123–126].

Hidden botulism refers to those cases in which no source of botulism can be identified and in whom no apparent source or exposure is known [127, 128]. Some argue that these cases represent adult forms of infantile botulism [129]. This is suggested by the high prevalence of colonic diverting procedures, achlorhydria, Crohn's disease, or recent antibiotic treatment among these patients.

Response to antitoxin treatment is generally poor probably because once toxin binds to the nerve terminal, it is no longer accessible to the antitoxin. Antibiotic therapy is not effective unless the botulism is the infantile or hidden form of the disease. Otherwise, treatment is supportive with respiratory assistance when necessary. Cholinesterase inhibitors are not usually beneficial; guanidine or 3,4-diaminopyridine (3,4-DAP) may improve strength but not respiratory function. Recovery takes many months but is usually complete.

EMG abnormalities evolve as the disease progresses and may not be present at onset of symptoms. CMAP amplitude is decreased in affected muscles, but motor and sensory nerve conduction is normal. Some patients demonstrate a decremental pattern, and most have post-tetanic facilitation in some muscles of 30–100% at some time to low-frequency stimulation. These findings are

[1]While tetanus toxin also binds to the neuromuscular junction, its mechanism of action is distinctly different. This toxin is translocated into the nerve terminal and then moves in a retro-axonal fashion to the synaptic space between the alpha motoneuron and inhibitory neurons. There it inhibits exocytosis resulting in paresis. Because it does not directly involve the motor nerve terminal, it will not be discussed further.

similar to LES but have a more restricted distribution. SF-EMG shows markedly increased jitter and blocking. The organism can be recovered from the stool of infected infants or in the hidden form of the disease.

Envenomation

Most biological toxins of animal origin affect the cholinergic system and either facilitate the release of neurotransmitter from the presynaptic nerve terminal or block the AChR. In general, bites from snakes, scorpions, and ticks are more common during summer months when they are inadvertently encountered. In contrast, exposure to marine toxins may occur at any time as they are acquired through ingestion and less rarely by injection or penetration. Specific geographic loci can be demonstrated for each of these vectors. For example, tick envenomation predominates in states west of the Rocky Mountains, the western provinces of Canada, and Australia. The geography of snake envenomation is species specific. The cobras are found in Asia and Africa, the kraits in Southeast Asia, the mambas in Africa, the coral snake in North America, and the sea snakes in the waters of the Pacific near Australia and New Guinea.

Arthropods

The venoms of the phylum Arthropod are used to incapacitate prey for feeding or as a defense against predators [130]. An observation made in antiquity. Few of the arthropods, however, produce toxicity at the NMJ, but when produced, it is by three mechanisms (Table 17.2). In the first there is an initial augmentation of ACh release followed by presynaptic depletion of neurotransmitter. The second leads to a facilitation of ACh release without a subsequent presynaptic depletion of neurotransmitter. The third mechanism causes a depletion of ACh release without a subsequent presynaptic depletion of neurotransmitter.

Table 17.2 Mechanisms of arthropod blockade of synaptic transmission

Facilitation of ACh release with subsequent exhaustion of neurotransmitter
Facilitation of ACh release without subsequent exhaustion of neurotransmitter
Depletion of ACh release with subsequent exhaustion of neurotransmitter

Spider Bites

Only a few spider venoms affect the neuromuscular junction. The funnel web spider and the redback spider of Australia are the most dangerous spiders in this group. In North America, only the bite of the black widow spider is of concern. The usual victim of a black widow spider bite is a small boy, perhaps the result of their inquisitiveness of nooks and crannies.

Latrotoxins found in the venoms from the spider genus *Latrodectus* (black widow spider) cause systemic latrodectism. These toxins produce a marked facilitation in neurotransmitter release by depolarization of the presynaptic nerve terminal and increasing Ca^{++} influx into the nerve terminal at all neurosecretory synapses including the neuromuscular junction [131–133]. There is a subsequent depletion of neurotransmitter from the nerve terminal resulting in a blockade of synaptic transmission. This toxin exerts its effects on the presynaptic nerve terminal by several mechanisms. The toxin binds to neurexin and thereby activates the presynaptic protein complex of neurexin, syntaxin, synaptotagmin, and the N-type calcium channel to massively facilitate ACh release [134]. Neurotransmitter release in nerve-muscle preparations, as measured by MEPP frequency, increases several hundredfold within a few minutes [135]. There is a subsequent depletion of synaptic vesicles, disruption of the highly organized active zone region of the presynaptic nerve terminal, thus inhibiting the docking of synaptic vesicles to the terminal membrane and effective recycling of vesicular membrane [136–140].

Signs of a black widow spider bite begin within minutes of the bite and reflect the massive release of neurotransmitter from peripheral, autonomic, and central synapses [141]. Severe muscle rigidity and cramps precede generalized muscle weakness due to the depolarizing

neuromuscular blockade. The black widow spider bite is rarely fatal, but cardiovascular collapse may occur in the elderly or in young children. Treatment is primarily supportive. The administration of calcium gluconate may be helpful in alleviating muscle cramps and rigidity [142]. Magnesium salts may be beneficial by reducing neurotransmitter release [141]. The administration of horse serum antivenom is effective and rapidly reverses the neurotoxic effects [143].

Tick Paralysis

Tick paralysis, a worldwide disorder, was first described at the turn of the twentieth century in North America and Australia although there is vague reference to an earlier case in the early 1800s [144–147]. It is one of several kinds of neuromuscular disorders that result from tick venom exposure [148]. Tick paralysis results from the introduction of a neurotoxin from one of more than 60 tick species [149, 150]. In North America, the *Dermacentor andersoni, D. variabilis, D. occidentalis, Amblyomma americanum, and A. maculatum* species are toxic. The vectors in Europe and the Pacific are *Ixodes ricinus* and *I. cornuatus*, and in Australia it is *I. holocyclus*. Geographically, tick paralysis is more common in states west of the Rocky Mountains and in British Columbia and Alberta [151].

The symptoms are stereotypical. Within 5–6 days of attachment, there is a prodrome of paresthesia, headache, malaise, nausea, and vomiting. The prodromal period parallels the feeding pattern of the tick. Over the next 24–48 h, an ascending paralysis occurs. It begins symmetrically in the lower extremities and progresses to involve the trunk and arms. In most instances when a tick is found, it is fully engorged. In contrast to the vectors found most commonly in North America (*Dermacentor* and *Amblyomma species*), the weakness of the Australian tick is more severe and much slower to resolve. In these patients there is often a worsening of clinical signs 24–48 h following the removal of the tick [152]. Sensation is preserved, but muscle stretch reflexes are often diminished or not present suggesting the Landry-Guillain-Barré syndrome (Table 17.3), a common misdiagnosis [153]. There is no demonstrable response to cholinesterase inhibitors [154, 155]. There is some indication of an association between the proximity of the site of attachment to the brain and the severity of the disease. Antitoxin may be of benefit in some situations, but the high frequency of acute allergic reactions makes its widespread use less useful [156]. Resolution of manifestations is dependent in part on how quickly the tick is removed, suggesting that the amount of muscle weakness is a dose-dependent process. Often, improvement begins within hours of removing the tick. Paralyses due to the *Dermacentor* species may continue over several days. However, prolonged weakness is reported [157]. Death may occur due to respiratory failure from severe bulbar and respiratory muscle weakness, and the clinical picture may be clouded by the presences of central nervous system manifestations [151, 158].

Table 17.3 Comparative features of ascending paralysis

Clinical and laboratory features	Tick paralysis	Landry-Guillain-Barré syndrome
Rate of progression	Hours to days	Days to 1–2 weeks
Sensory loss	Absent	Mild
Muscle stretch reflexes	Diminished or absent	Diminished or absent
Time to recovery	<24 h after tick removal	Weeks to months
CSF WBC count	<10 per mm^2	<10 per mm^2
CSF protein	Normal	Elevated

CSF cerebrospinal fluid, *mm^2* per square millimeter, < less than

Where once these envenomations carried a 12–25% mortality, the improvement in critical care over the latter half of the twentieth century makes these intoxications rarely fatal [144, 159]. Children are more prone to the disorder than adults. This may be due in part to their play habits and their lower body mass relative to the amount of toxin acquired. The head and neck is the most common site for tick attachment, although any part of the body may be bitten. Some studies suggest that girls are more often affected because their on average longer hair than

boys allows the tick to remain hidden for longer periods and therefore allows prolonged feeding [153, 160]. The identification of a tick bite is often delayed resulting in misdiagnosis. Tick paralysis may be confused with Landry-Guillain-Barré syndrome, myasthenia gravis, spinal cord disease, periodic paralysis, diphtheria, heavy-metal intoxication, insecticide poisoning, porphyria, and hysteria [153, 161]. In many instances the tick is located by the nurse, the house officer, the mortician, or autopsy personnel [153, 159, 162]. Careful, systematic inspection of the scalp, neck, and perineum, often with a fine-toothed comb, is necessary to locate the tick.

The mechanisms of paralysis following tick envenomation remain controversial. The most potent toxin is from the Australian tick, *Ixodes holocyclus*. Holocyclotoxin, isolated from the salivary glands of female ticks, causes a temperature-dependent blockade of neuronally evoked release of ACh [163]. Others have suggested a postsynaptic block of neuromuscular transmission [164]. The tick paralysis of the *Dermacentor* species is understood less clearly, and no direct abnormality of synaptic transmission may occur. Rather, the abnormality may be due to impaired depolarization of the nerve terminal with the consequence of decreased ACh release [165, 166]. Prolonged distal motor latencies, slowed nerve conduction velocities, and reduced compound muscle action potential (CMAP) amplitudes are described [149, 154, 167–169]. Others have postulated a direct effect on muscle membrane [170].

Scorpion Bites

The peptides contained in scorpion neurotoxins may cause a variety of neurological effects, the most significant of which are those that modulate Na^+ and K^+ channel function. Many cause a slowing of the inactivation of Nav channels [171]. Some, however, affect the neuromuscular junction and produce an enhanced presynaptic depolarization resulting in neurotransmitter release [172]. Increased excretion of catecholamines is demonstrated after scorpion sting and may relate to the primary effect of the venom or to a secondary sympathetic adrenergic surge. Treatment is nonspecific and focuses on maintaining respiratory, cardiac, and coagulation function. Antivenom appears not to be efficacious [173, 174].

Snakebites

Nearly 3,000,000 people a year develop serious illness following a snakebite. More than 95,000 individuals die from snakebites yearly with an additional 300,000 who survive the snakebite but are left with permanent disability or disfigurement [175]. Envenomation by snakebite occurs from four major groups: Viperidae (true vipers), Crotalidae (rattlesnakes and pit vipers), Elapidae (American coral snake, cobras, kraits, mambas), and Hydrophiidae (sea snakes). The incidence of snakebites is influenced by the density of snakes, which is related to altitude and climate, the specific preferences of the snake for an environment suitable for their development, and the density of the human population [176]. Neuromuscular blockade occurs primarily from the Elapidae and Hydrophiidae species [177–179]. One Crotalidae species, *Crotalus durissus terrificus*, a South American rattlesnake, has a very potent neuromuscular blocking venom. Other rattlesnakes and pit vipers act through hematological and cardiovascular mechanisms. Venom is produced and stored in salivary glands, and inoculation occurs through fangs or modified premaxillary teeth [177].

Snake toxins may act by presynaptic or postsynaptic mechanisms. Presynaptic toxins, β-neurotoxins (β-bungarotoxin, notexin, and taipoxin), act to inhibit the normal release of acetylcholine from the presynaptic cell of the neuromuscular junction. Often, there is an initial augmentation of acetylcholine release followed by presynaptic depletion of neurotransmitter. They tend to be more potent than postsynaptic toxins. Postsynaptic neurotoxins, α-neurotoxins, produce a curare-mimetic, nondepolarizing neuromuscular block and vary in the degree of the reversibility of the block in experimental preparations.

Most venoms are a mixture of the two types of neurotoxin, although one type may predominate in a given venom. For example, the venom of the

Thai cobra is composed primarily of a single postsynaptic neurotoxin [180]. In contrast, the venom of *Bungarus multicinctus* contains β-bungarotoxin, four other presynaptic toxins, α-bungarotoxin, and two other postsynaptic toxins [181]. The venoms of Hydrophiidae species are more toxic than land snakes although the amount of toxin injected by sea snakes is smaller than that of land-based snakes [182, 183]. The α-neurotoxins (postsynaptic), like curare, bind to the muscle nicotinic AChR. They have a slower onset of action and a longer duration of effect and are 15–40 times more potent than d-tubocurarine [184]. There are numerous subforms of β-neurotoxins (presynaptic). Most have a phospholipase component that is essential for the presynaptic effects of the toxin. All suppress the release of ACh from the nerve terminal although there is some variability in the precise mechanism by which this occurs. In experimental preparations, toxins from different species potentiate each other suggesting different binding sites at the neuromuscular junction [185]. Taipoxin from the Australian and Papua New Guinean taipan snake is unique. In addition to its potent presynaptic blockade of synaptic transmission, it also has a direct myotoxic component. This produces rapid muscle necrosis and degeneration. There is species variation in the susceptibility to toxin exposure. The venom of the Australian mulga snake is fatal in man, produces ptosis in monkeys, and does not produce a neuromuscular block in the rabbit [186, 187].

The clinical course of snake envenomation follows a specific pattern. After the bite of a pit viper or cobra, there is local pain, which is often absent following the bite of other Elapidae (mambas, kraits, coral snakes) and Hydrophiidae. Swelling typically follows within an hour of the bites from Viperidae, Crotalidae, or the cobra but is not seen following bites from other Elapidae and Hydrophiidae. A preparalytic stage develops with headache, vomiting, loss of consciousness, paresthesias, hematuria, or hemoptysis [188]. These manifestations are not common after envenomation by cobras or mambas. The time between snakebite and paralysis may vary from 0.5–19 h [189]. The first signs of neuromuscular toxicity are usually ptosis and ophthalmoparesis though these are absent following the bite of the South American rattlesnake. Facial and bulbar weakness develops over hours following the ocular signs [190]. For 2–3 days, limb, diaphragmatic, and intercostal weakness follows and may continue to evolve [177, 191], and without appropriate treatment, cardiovascular collapse, seizures, and coma ensue. There is no sensory abnormality other than that from the bite itself. Other systemic effects of neurologic importance relate to coagulation deficits. Cerebral and subarachnoid hemorrhage may occur after bites from many species and is the leading cause of death following viper bites in several regions of the world [192, 193].

Treatment consists of antivenom which are most effective in bites that do not contain significant amounts of phospholipase, a component of presynaptic neurotoxins. If the type of snake is known, a high-titer monovalent type is administered, but more often snake variety is not known necessitating the use of polyvalent antivenom [175, 194]. The goal of antivenom is to shorten the duration of weakness, and frequently the addition of respiratory, cardiovascular, and hematological support is required. Supportive measures are the mainstay of care for most victims of coral snakebite. Intensive care treatment and airway maintenance is similar to patients with myasthenia gravis. Some authors recommend treatment with cholinesterase inhibitors in cases that are predominantly caused by a postsynaptic abnormality and suggest that electrodiagnostic testing may be useful in determining their effectiveness [195, 196].

Marine Toxins

The rapid rise in marine pollution has spurred a renewed interest in marine toxins. Previously they were only of interest to the physiologist and pharmacologist who use them in the investigation of biological systems. Examples of marine neurotoxicology are scattered throughout the literature dating to biblical times (Exodus 7:20–21). The reader is referred to Southcott's paper for an excellent review of the subject [197]. Marine neurotoxins affecting the neuromuscular junction

are rare and occur primarily from poisonous fish, a few mollusks, and perhaps dinoflagellates. Unlike the poisoning from arthropods and snakes, the majority of marine intoxications occur as the result of ingestion. Unique to some marine toxins is the increase in concentration of toxin through successive predatory transvection up the food chain.

Dinoflagellates are single-celled, biflagellated, algae-like organisms. Diatoms, similar to dinoflagellates, are not flagellated and are encased by a silica shell. The toxins produced by these organisms cause a variety of systemic and neurological effects, but neuromuscular junction effects are rare and indirect. Paralytic shellfish poisoning results from neurotoxins produced by less than 1% of the 2000–3000 species of known dinoflagellates and diatoms [198]. These toxins are rapidly absorbed through the gastrointestinal tract and symptoms begin within 30 min of ingestion. Characteristically, there is an initial burning or paresthesia of the face and mouth, spreading quickly to involve the neck and limbs. Slowly, the sensations abate and are replaced with numbness, some ataxia and, in severe cases, progressive generalized weakness, and respiratory failure. Overall, the mortality approaches 10%. Most neurotoxins from dinoflagellates and diatoms are sodium channel blockers (e.g., saxitoxin and tetrodotoxin). Brevetoxin, a milder neurotoxin that causes the nonlethal neurotoxic shellfish poisoning, depolarizes cholinergic systems, by opening sodium channels and resulting in neuromuscular transmission alterations indirectly [199, 200]. The cyclic imine group of toxins, gymnodimine and spirolides, are potent antagonists of both cholinergic and muscarinic nicotinic acetylcholine receptors [201]. Isolated from New Zealand shellfish, these toxins are rapid in onset and lethal. Ciguatoxin, from *Gambierdiscus toxicus*, is commonly found in the Caribbean, Australia, and South Pacific island countries and territories. The incidence is rapidly increasing and is now considered a major health hazard [202]. This heat-stable lipid enhances the release of ACh from the neuromuscular junction by prolonged sodium channel opening. Onset of symptoms is within hours of ingestion and respiratory muscle paralysis may occur quickly. Recovery usually occurs within a few weeks.

Conotoxins are a diverse group of toxins from predatory cone snails that inject their venom through a small harpoon-like dart [203]. It is only the fish predatory species (*Conus geographus*, *C. textile*, *C. marmoreus*, and *C. omaria*) of this mollusk that appear dangerous to humans [204–206]. The effects of these toxins are variable among species and within a single species, and several have direct effects on the neuromuscular junction. α-Conotoxins block the binding of ACh to the ligand-binding site [207–209]. These venoms function similarly to the snake α-neurotoxins described earlier. The ω-conotoxins block the voltage-gated calcium channel of the presynaptic nerve terminal [210]. The latter toxin has played an important role in understanding of the LES and serves as the basis for the currently used antibody assay [2, 211]. Recent studies show a large repertoire of neurotoxins that have the potential for therapeutic benefit [212]. Following the injection of toxin, there is intense local pain quickly followed by malaise, headache, and within 30 min progressive generalized weakness. Respiratory failure often occurs within 1–2 h. Most cone shell bites are preventable. These shells should be handled carefully with forceps and thick gloves. The proboscis protrudes from the small end of the shell, but it is flexible and long enough to sting the holder at the other end. The live shells should never be placed in a pocket as the dart may penetrate cloth [197]. Treatment is directed toward respiratory and cardiovascular support. There is no available antivenom. There is no literature discussing the potential efficacy of cholinesterase inhibitors. More than 60% of stings are fatal [206, 213].

The most venomous fish is the stonefish, *Synanceia horrida*, *S. trachynis*, and *S. verrucosa*, found in the Indo-Pacific oceans and Red Sea as well as the genus *Inimicus* found off the coast of Japan [214]. The toxin, stonustoxin, is inflicted by injection through the 13 dorsal spines when the victim steps on the small fish that is buried in the sand. Neuromuscular blockade results from induced neurotransmitter release with depletion of ACh stores, similar to that of

other presynaptic toxins [215, 216]. Envenomation results in immediate, excruciating pain that may last for 1–2 days. Severe edema occurs due to the actions of hyaluronidase that promotes the rapid spread of venom through the tissue, and tissue necrosis may occur [197]. In addition to gastrointestinal, autonomic, and cognitive effects, the victim may experience generalized muscle weakness due to the mechanism noted above. Death occurs from cardiotoxicity. Treatment is supportive and in some patients a specific antitoxin may be administered.

Plant Toxins

Rarely, plant neurotoxins affect the human NMJ, but more toxic effects are observed in animals. Neurotoxicity is dependent upon the potency, concentration, and interaction with other toxins or substrates in the victim. Many are alkaloids. Coniine, the neurotoxin from the herb, *Conium maculatum* (poison hemlock), produces a rapidly ascending paralysis often resulting in death. Sensory abnormalities are common and prominent [217]. The death of Socrates is attributed to hemlock [218]. The mechanisms of action of this piperidine alkaloid neurotoxin are not completely understood but are thought to act agonistically at nicotinic-type acetylcholine (cholinergic) receptors (nAChRs) [219, 220]. Many of these alkaloid neurotoxins are teratogenic to the fetus and by desensitizing nicotinic acetylcholine receptors produce arthrogryposis, scoliosis, kyphosis, and lordosis [221].

Occupational Neurotoxins

Heavy Metals

Numerous polyvalent cations affect neuromuscular transmission and are often used to study basic mechanisms of synaptic transmission. These include barium, erbium, cadmium, cobalt, gadolinium, lanthanum, manganese, nickel, praseodymium, triethyltin, and zinc [222–234]. Nearly all of these intoxicants have multiple effects on synaptic transmission, but they predominantly block the release of ACh as well as facilitating spontaneous quantal release of neurotransmitter. They exert their effects by the block of the flux of Ca++ through voltage-gated calcium channels and disrupt intracellular stores of Ca++ [235].

Heavy-metal intoxication is a rare cause of clinical NMJ toxicity. Interest in this topic arose from the 1971 contamination of grain in Iraq with a methylmercury fungicide. Despite appropriate warnings, the grain was fed to animals, ground for flour, and used for making bread [236]. Symptoms began within 1 month of consumption ultimately affecting more than 6500 people and killing nearly 8% [237]. Patients experienced ataxia, fatigue, generalized muscle weakness, and occasionally optic atrophy. While one of the expected abnormalities following mercurial poisoning is a peripheral neuropathy (based on the Minamata experience), extensive electrodiagnostic examinations of the affected population did not demonstrate this [238, 239]. Repetitive nerve stimulation studies demonstrated a decremental response that was partially reversible with cholinesterase inhibitors [240]. Similar abnormalities were demonstrated in experimental animals [241]. Drinking water contaminated with heavy metals is becoming the major health concern for public and healthcare professionals in the twenty-first century [242].

Organophosphate and Carbamate Poisoning

The earliest use of a cholinesterase inhibitor as a neurotoxin is attributed to tribesman in Africa who used the Calabar bean as a rite of passage (*Physostigma venenosum Balfour*) or an "ordeal poison" [243]. Organophosphates (OP) are a class of more than 20,000 compounds that irreversibly inhibit cholinesterases including acetylcholinesterase (AChE) [244]. They are widely used in the agricultural, manufacturing, and pharmaceutical industries as well as a weapon of mass destruction [245, 246]. Exposure to OP compounds occurs in the workplace, in food, in drinking water, and in the environment. OP intoxication is infrequent in the United States because OP-containing insecticides are not

readily available. However, these are used commonly in many other countries, where intoxication results from attempted suicide by ingestion of insecticides, indiscriminate handling, and storage by poorly informed workers or from contaminated food sources [247–250].

The physiochemical properties of these compounds vary. They may be solid, liquid, or gaseous and soluble in various media. Some are highly corrosive, others are not; some highly volatile, others not. Dermal contact, respiratory inhalation, and gastrointestinal absorption may lead to OP absorption. These various physiochemical properties lend themselves to the wide range of applications noted above as well as to the inherent danger of their use [251].

Four neuromuscular toxicological syndromes occur from OP poisoning: an acute cholinergic crisis (types 1 and 2), an intermediate syndrome, a myopathy, and a delayed toxin-induce neuropathy (Table 17.4) [252]. Only the type 2 cholinergic crisis and the intermediate syndrome are the result of NMJ toxicity. OP compounds exert their NMJ toxicity by the irreversible inhibition of AChE. This results in the excessive accumulation of ACh at the NMJ as well as other cholinergic synapses of the central, peripheral, and autonomic nervous systems [253]. The excessive accumulation of ACh produces a depolarizing neuromuscular block at the NMJ that is followed by desensitization of the AChR [254, 255]. Electrodiagnostic studies demonstrate normal nerve conduction studies, reduced CMAP amplitudes, a decremental response to repetitive nerve stimulation, and CMAP afterdischarges to a single nerve stimulus [256, 257].

Carbamate salts and esters, which are primarily used as pesticides, are synthetic analogs of the alkaloid physostigmine (eserine). They may directly or indirectly affect the NMJ. Like the OP compounds, carbamates also inhibit the action of AChE at cholinergic synapses [258]. They are easily absorbed into the central nervous system because of their lipid solubility characteristics. Unlike OP compounds, the effects of carbamate agents are reversible. However, the manifestations of carbamate poisoning are indistinguishable from OP poisoning. Neurotoxicity occurs rapidly following significant exposure to both classes of compounds. Mortality rates are high with death usually occurring from respiratory paralysis, which develops in 40% of poison victims [259].

Table 17.4 Neuromuscular syndromes of organophosphate poisoning

Acute cholinergic crisis
Intermediate syndrome
Myopathy
Delayed toxin-induced neuropathy

Pesticides

OP chemistry had it origin around 1820 when Lassainge synthesized triethyl phosphate, but not until the turn of the century did they become commonly used for their insecticidal properties. The OP insecticides are all derivatives of phosphoric acid. There are many subclasses within this group of compounds, and their various moieties (e.g., sulfur, amides) confer variation in overall toxicity. Despite recognition of their toxicity, their use continues to rise, particularly in developing countries where demand was predicted to more than double in the 1990s [260]. Most fatal intoxications result from suicidal ingestion [249, 256, 261–266]. Reports of carbamate NMJ toxicity are few [267]. The largest episode of carbamate poisoning occurred in 1985 when aldicarb was illegally used as an insecticide on watermelons [268]. Seventy-seven percent of 1376 exposed individuals were poisoned, each exhibiting a dose-related spectrum of nicotinic and muscarinic cholinergic receptor toxicity. Fatalities are rare and only occur at high exposure levels [269–272]. Symptoms appear rapidly, often within an hour, peaking in 2–3 h with full recovery within 72 h [273].

Agents of War and Terrorism

The highly dangerous toxicity of OP compounds was recognized in 1932 leading to the development of a series of G-agents (G = German). These compounds, GA (ethyl *N*,*N*-dimethylphosphoramidocyanidate; tabun) in 1936, GB (*O*-isopropyl methylphosphonofluoridate; sarin) in

1938, GD (*O*-pinacolyl methylphosphonofluoridate; soman) in 1944, and GF (cyclohexyl methylphosphonofluoridate; cyclosarin), were developed specifically as agents of war [274]. The V-series (V = venomous) followed and include four compounds, VE (*S*-(diethylamino) ethyl *O*-ethyl ethylphosphonothioate), VG (*O*,*O*-diethyl-*S*-[2-(diethylamino)ethyl] phosphorothioate; also called Amiton or Tetram), VM (phosphonothioic acid, methyl-, *S*-(2-(diethylamino)ethyl) *O*-ethyl ester), and VX (*O*-ethyl-*S*-[2(diisopropylamino)ethyl] methylphosphonothioate; venom X) in 1952. Little is known about a Russian agent, coded VR-55 [275]. Great Britain ceased nerve gas weapons research in 1959, and the United States transiently discontinued their efforts between 1969 and 1981 [276]. Other countries continue to develop their weapons programs. It is speculated that GA was used in the 1980s during the Iraq-Iran conflict, causing innumerable deaths [275]. G-agents share a number of similar characteristic properties. They are highly volatile in room air. As a result their toxicity may be from either inhalation or by contact. These compounds are soluble in fat and water. This allows ready absorption through the skin eyes and mucous membranes. Vapor agents are absorbed initially through the eyes producing local irritation and then through the respiratory tree. Liquid agents penetrate through the skin at the point of contact and are able to produce more severe and generalized symptoms.

The V-series toxins are the most highly toxic chemical warfare agents. These agents of war are termed persistent agents as they remain active on skin, clothes, and other surfaces for prolonged periods of time. VX was synthesized by the British in 1957. Other agents in the series (Ve, Vg, Vm, and V-gas) are less well known as information about them is limited.

Local effects (sweating, mucosal irritation) occur within seconds, and paralysis and apnea may occur as quickly as 1–2 min of exposure. Comparative inhalation toxicities are summarized in Table 17.5. Absorption may be through inhalation, ingestion, or cutaneous contact. VX is the most potent and GA the least. Terrorist attacks in Japan resulted in the exposure of civilians to GB and VX [277–281].

Table 17.5 Comparative human LCt_{50} and LD_{50} to nerve agents

Nerve agent	Aerosolized (LCt_{50})	Percutaneous (LD_{50})
VX vapor	10 mg·min/m^3	6–10 mg
Soman vapor	50 mg·min/m^3	350 mg
Sarin vapor	100 mg·min/m^3	1700 mg
Tabun vapor	400 mg·min/m^3	1000 mg

LCt_{50}, the dose of vapor necessary to cause death in 50% of the exposed population where C is concentration and t is time; LD_{50}, the dose of cutaneous exposure necessary to cause death in 50% of the exposed population; mg milligram, min/m^3 minutes per meter squared

Pathophysiology

The G- and V-series nerve agents inhibit the hydrolysis of acetylcholine by acetylcholinesterase by binding to and phosphorylating the active site of AChE. The resulting depolarizing neuromuscular block leads to rapid and profound muscle weakness. Death occurs by respiratory failure. The inhibition of AChE by these agents becomes irreversible, a phenomenon known as "aging." The aging halftime is variable, as short as 2 min for GD and as long as 48 h for VX [282]. Aging is an irreversible phenomenon. Prior to this reaction, the enzyme can be reactivated with the use of oximes which remove the neurotoxin from the AChE molecule. Oximes dissociate the toxic phosphate moiety from the esteratic site on AChE, thus reactivating esterase and restoring normal NMT [283]. Following attachment of the nerve agent to AChE, a portion of the nerve agent, called the leaving group, is cleaved from the bound molecule. This is followed by a second reaction during which an alkyl group leaves the nerve agent. This results in an aged complex for which oximes have no effect [284].

Treatment

Treatment is directed toward the prevention of chemical exposure by appropriate clothing and the decontamination of exposed victims [285, 286]. Aggressive cardiopulmonary support is necessary. Atropine is an effective antidote to

block excessive cholinergic activity at muscarinic receptors in both organophosphate and carbamate intoxications. AChE reactivators (oximes, e.g., 2-PAM) and anticholinergics are often helpful for acute OP intoxications but appear to have little effect to reverse weakness caused by the intermediate syndrome. Oximes dissociate the toxic phosphate moiety from the esteratic site on AChE, thus reactivating esterase and restoring normal neuromuscular transmission [283]. Soman is the least responsive of the nerve agents to oxime therapy because the agent-enzyme complex rapidly undergoes "aging." This refers to the compound undergoing a time-dependent conformational change that is no longer responsive to reactivators [287]. In contrast to OP intoxications, oxime reactivators are contraindicated in carbamate poisoning as these compounds enhance the effects of the carbamate and promote further junctional toxicity [288]. In cases where the offending compound is not known, it is possible to assay the reactivation of AChE activity in vivo and possibly differentiate between OP and carbamate poisoning [289]. Neuromuscular blockade with curariform drugs blocks repetitive discharges although it is not clear whether there is any clinical benefit to their use [290]. The carbamate, pyridostigmine, was used as a "pretreatment" for organophosphorus poisoning during the Gulf War, but their use is controversial. While studies showed that a 40% inhibition in AChE activity by physostigmine protected experimental animals from acute cholinergic toxicity following exposure to soman, such findings have not been conclusively demonstrated for pyridostigmine [291]. The expanding footprint of terrorism and the adaptability of biological, pharmacological, and environmental compounds to agents of war necessitate that physicians become familiar with the management of toxic neurotransmission disorders [292].

References

1. Fambrough DM, Drachman DB, Satymurti S. Neuromuscular function in myasthenia gravis: decreased acetylcholine receptors. Science. 1973;182:293–5.
2. De Aizpurua HJ, Lambert EH, Griesmann GE, Olivera M, Lennon VA. Antagonism of voltage-gated calcium channels in small cell carcinomas of patients with and without Lambert-Eaton myasthenic syndrome by autoantibodies omega-conotoxin and adenosine. Cancer Res. 1988;48:4719–24.
3. Howard JF. Adverse drug interactions in disorders of neuromuscular transmission. J Neurol Orthop Med Surg. 1991;12:26–34.
4. Barrons RW. Drug-induced neuromuscular blockade and myasthenia gravis. Pharmacotherapy. 1997;17:1220–32.
5. Nieman RB, Sharma K, Edelberg H, Caffe SE. Telithromycin and myasthenia gravis. Clin Infect Dis. 2003;37:1579.
6. Telithromycin: review of adverse effects. Prescrire Int. 2014;23:264–6.
7. Jones SC, Sorbello A, Boucher RM. Fluoroquinolone-associated myasthenia gravis exacerbation: evaluation of postmarketing reports from the US FDA adverse event reporting system and a literature review. Drug Saf. 2011;34:839–47.
8. Pittinger C, Adamson R. Antibiotic blockade of neuromuscular function. Annu Rev Pharmacol. 1972;12:109–84.
9. Singh YN, Marshall IG, Harvey AL. Reversal of antibiotic-induced muscle paralysis by 3, 4-diaminopyridine. J Pharm Pharmacol. 1978;30:249–50.
10. Caputy AJ, Kim YI, Sanders DB. The neuromuscular blocking effects of therapeutic concentrations of various antibiotics on normal rat skeletal muscle: a quantitative comparison. J Pharmacol Exp Ther. 1981;217:369–78.
11. Kaeser HE. Drug-induced myasthenic syndromes. Acta Neurol Scand Suppl. 1984;100:39–47.
12. Telithromycin: visual disorders, myasthenia. Case fatalities: this macrolide has a negative risk-benefit balance; its use not justified. Prescrire Int. 2008;17:66.
13. Roquer J, Cano A, Seoane JL, Pou SA. Myasthenia gravis and ciprofloxacin [letter]. Acta Neurol Scand. 1996;94:419–20.
14. Samuelson RJ, Giesecke AHJ, Kallus FT, Stanley VF. Lincomycin-curare interaction. Anesth Analg. 1975;54:103–5.
15. Fogdall RP, Miller RD. Prolongation of a pancuronium-induced neuromuscular blockade by clindamycin. Anesthesiology. 1974;41:407–8.
16. McQuillen MP, Engbaek L. Mechanism of colistin-induced neuromuscular depression. Arch Neurol. 1975;32:235–8.
17. Decker DA, Fincham RW. Respiratory arrest in myasthenia gravis with colistimethate therapy. Arch Neurol. 1971;25:141–4.
18. Argov Z, Brenner T, Abramsky O. Ampicillin may aggravate clinical and experimental myasthenia gravis. Arch Neurol. 1986;43:255–6.
19. Sieb JP. Fluoroquinolone antibiotics block neuromuscular transmission. Neurology. 1998;50:807.

20. Sieb JP, Milone M, Engel AG. Effects of the quinoline derivative quinine, quinidine and chloroquine in neuromuscular transmission. Brain Res. 1996;712:179–89.
21. Howard JF Jr. Adverse drug effects on neuromuscular transmission. [review] [202 refs]. Semin Neurol. 1990;10:89–102.
22. Coppeto JR. Timolol-associated myasthenia gravis. Am J Ophthalmol. 1984;98:244–5.
23. Verkijk A. Worsening of myasthenia gravis with timolol maleate eyedrops. Ann Neurol. 1985;17:211–2.
24. Howard JF, Johnson BR, Quint SR. The effects of beta-adrenergic antagonists on neuromuscular transmission in rat skeletal muscle. Soc Neurosci Abstr. 1987;13:147.
25. Adams RJ, Rivner MH, Salazar J, Swift TR. Effects of oral calcium antagonists on neuromuscular transmission. Neurology. 1984;34:132–3.
26. Bikhazi GB, Leung I, Foldes FF. Interaction of neuromuscular blocking agents with calcium channel blockers. Anesthesiology. 1982;57:A268.
27. Van der Kloot W, Kita H. The effects of verapamil on muscle action potentials in the frog and crayfish and on neuromuscular transmission in the crayfish. Comp Biochem Physiol C. 1975;50C:121–5.
28. Ribera AB, Nastuk WL. The actions of verapamil at the neuromuscular junction. Comp Biochem Physiol C. 1989;93C:137–41.
29. Krendel DA, Hopkins LC. Adverse effect of verapamil in a patient with the Lambert-Eaton syndrome. Muscle Nerve. 1986;9:519–22.
30. Kornfeld P, Horowitz SH, Genkins G, Papatestas AE. Myasthenia gravis unmasked by antiarrhythmic agents. Mt Sinai J Med. 1976;43:10–4.
31. Lecky BR, Weir D, Chong E. Exacerbation of myasthenia by propafenone [letter]. J Neurol Neurosurg Psychiatry. 1991;54:377.
32. Fierro B, Castiglione MG, Salemi G, Savettieri G. Myasthenia-like syndrome induced by cardiovascular agents. Report of a case. Ital J Neurol Sci. 1987;8:167–9.
33. Weisman SJ. Masked myasthenia gravis. JAMA. 1949;141:917–8.
34. Shy ME, Lange DJ, Howard JF, Gold AP, Lovelace RE, Penn AS. Quinidine exacerbating myasthenia gravis: a case report and intracellular recordings. Ann Neurol. 1985;18:120.
35. Stoffer SS, Chandler JH. Quinidine-induced exacerbation of myasthenia gravis in patient with Graves' disease. Arch Intern Med. 1980;140:283–4.
36. Miller RD, Way WL, Katzung BG. The neuromuscular effects of quinidine. Proc Soc Exp Biol Med. 1968;129:215–8.
37. de la Vaissiere S, Toutain A, Chene MA, Lagrue E, Cantagrel S, Provost S, et al. Congenital myasthenic syndromes in childhood: drug therapeutic strategies. Arch Pediatr. 2015;22:724–8.
38. O'Riordan J, Javed M, Doherty C, Hutchinson M. Worsening of myasthenia gravis on treatment with imipenem/cilastatin. J Neurol Neurosurg Psychiatry. 1994;57:383.
39. Cartwright MS, Jeffery DR, Nuss GR, Donofrio PD. Statin-associated exacerbation of myasthenia gravis. Neurology. 2004;63:2188.
40. Purvin V, Kawasaki A, Smith KH, Kesler A. Statin-associated myasthenia gravis: report of 4 cases and review of the literature. Medicine. 2006;85:82–5.
41. Oh SJ, Dhall R, Young A, Morgan MB, Lu L, Claussen GC. Statins may aggravate myasthenia gravis. Muscle Nerve. 2008;38:1101–7.
42. Pasutharnchat N, Phanthumchinda K. Statin-associated myasthenic weakness. J Med Assoc Thail. 2011;94:256–8.
43. Gale J, Danesh-Meyer HV. Statins can induce myasthenia gravis. J Clin Neurosci. 2014;21:195–7.
44. Khalid R, Ibad A, Thompson PD. Statins and myasthenia gravis. Muscle Nerve. 2016;54:509.
45. de Sousa E, Howard J. More evidence for the association between statins and myasthenia gravis. Muscle Nerve. 2008;38:1085–6.
46. du Souich P, Roederer G, Dufour R. Myotoxicity of statins: mechanism of action. Pharmacol Ther. 2017;175:1–16.
47. Ramachandran R, Wierzbicki AS. Statins, muscle disease and mitochondria. J Clin Med. 2017;6:75.
48. Youssef S, Stuve O, Patarroyo JC, Ruiz PJ, Radosevich JL, Hur EM, et al. The HMG-CoA reductase inhibitor, atorvastatin, promotes a Th2 bias and reverses paralysis in central nervous system autoimmune disease. Nature. 2002;420:78–84.
49. Milani M, Ostlie N, Wang W, Conti-Fine BM. T cells and cytokines in the pathogenesis of acquired myasthenia gravis. Ann N Y Acad Sci. 2003;998:284–307.
50. Hargreaves IP, Heales S. Statins and myopathy. Lancet. 2002;359:711–2.
51. Wierzbicki AS, Poston R, Ferro A. The lipid and non-lipid effects of statins. Pharmacol Ther. 2003;99:95–112.
52. Krendel DA. Hypermagnesemia and neuromuscular transmission. Semin Neurol. 1990;10:42–5.
53. Castlebaum AR, Donofrio PD, Walker FO, Troost BT. Laxative abuse causing hypermagnesemia quadriparesis and neuromuscular junction defect. Neurology. 1989;39:746–7.
54. Randall RE, Cohen MD, Spray CC, Rossmeise EC. Hypermagnesemia in renal failure. Ann Intern Med. 1964;61:73–88.
55. Collins EN, Russell P. Fatal magnesium poisoning following magnesium sulfate, glycerine and water enema in primary megacolon. Cleve Clin Q. 1949;16:162–6.
56. Mordes JP, Wacker WEC. Excess magnesium. Pharmacol Rev. 1978;29:273–300.
57. Flowers CJ. Magnesium in obstetrics. Am J Obstet Gynecol. 1965;91:763–76.
58. Lipsitz PJ. The clinical and biochemical effects of excess magnesium in the newborn. Pediatrics. 1971;47:501–9.

59. Pritchard JA. The use of magnesium sulfate in preeclampsia. J Reprod Med. 1979;23:107–14.
60. Singh P, Idowu O, Malik I, Nates JL. Acute respiratory failure induced by magnesium replacement in a 62-year-old woman with myasthenia gravis. Tex Heart Inst J. 2015;42:495–7.
61. Fishman RA. Neurological aspects of magnesium metabolism. Arch Neurol. 1965;12:562–96.
62. Hutter OF, Kostial K. Effect of magnesium ions upon the release of acetylcholine. J Physiol. 1953; 120:53P.
63. Swift TR. Weakness from magnesium containing cathartics. Muscle Nerve. 1979;2:295–8.
64. Del Castillo J, Engback L. The nature of the neuromuscular block produced by magnesium. J Physiol. 1954;124:370–84.
65. De Silva AJC. Magnesium intoxication: an uncommon cause of prolonged curarization. Br J Anaesth. 1973;45:1228–9.
66. Ghoneim MM, Long JP. The interaction between magnesium and other neuromuscular blocking agents. Anesthesiology. 1970;32:23–7.
67. Cohen BA, London RS, Goldstein PJ. Myasthenia gravis and preeclampsia. Obstet Gynecol. 1976;48:35S–7S.
68. George WK, Han CL. Calcium and magnesium administration in myasthenia gravis. Lancet. 1962;ii:561.
69. Gutmann L, Takamori M. Effect of Mg++ on neuromuscular transmission in the Eaton-Lambert syndrome. Neurology. 1973;23:977–80.
70. Strieb EW. Adverse effects of magnesium salt cathartics in a patient with the myasthenic syndrome. Ann Neurol. 1973;2:175–6.
71. Bashuk RG, Krendel DA. Myasthenia gravis presenting as weakness after magnesium administration. Muscle Nerve. 1990;13:708–12.
72. Valmaggia C, Gottlob IM. Cocaine abuse, generalized myasthenia, complete external ophthalmoplegia, and pseudotonic pupil. Strabismus. 2001;9:9–12.
73. Berciano J, Oterino A, Rebollo M, Pascual J. Myasthenia gravis unmasked by cocaine abuse [letter]. N Engl J Med. 1991;325:892.
74. Daras M, Samkoff LM, Koppel BS. Exacerbation of myasthenia gravis associated with cocaine use. Neurology. 1996;46:271–2.
75. Venkatesh S, Rao A, Gupta R. Exacerbation of myasthenia gravis with cocaine use [letter]. Muscle Nerve. 1996;19:1364.
76. Krivoshein AV, Hess GP. Mechanism-based approach to the successful prevention of cocaine inhibition of the neuronal (alpha 3 beta 4) nicotinic acetylcholine receptor. Biochemistry. 2004;43:481–9.
77. Balint G, Szobor A, Temesvari P, Zahumenszky Z, Bozsoky S. Myasthenia gravis developed under d-penicillamine treatment. Scand J Rheumatol. 1975;4(Suppl 8):12–21.
78. Bucknall RC, Balint G, Dawkins RL. Myasthenia associated with D-penicillamine therapy in rheumatoid arthritis. Scand J Rheumatol Suppl. 1979;8(28):91–3.
79. Czlonskowska A. Myasthenia syndrome during penicillamine treatment. Br Med J. 1975;2:726–7.
80. Poulas K, Koutsouraki E, Kordas G, Kokla A, Tzartos SJ. Anti-MuSK- and anti-AChR-positive myasthenia gravis induced by d-penicillamine. J Neuroimmunol. 2012;250:94–8.
81. Masters CL, Dawkins RL, Zilko PJ, Simpson JA, Leedman RJ. Penicillamine-associated myasthenia gravis, antiacetylcholine receptor and antistriational antibodies. Am J Med. 1977;63:689–94.
82. Albers JW, Beals CA, Levine SP. Neuromuscular transmission in rheumatoid arthritis, with and without penicillamine treatment. Neurology. 1981;31:1562–4.
83. Varan O, Kucuk H, Tufan A. Myasthenia gravis due to hydroxychloroquine. Reumatismo. 2015;67:849.
84. O'Riordan J, Javed M, Doherty C, Hutchinson M. Worsening of myasthenia gravis on treatment with imipenem/cilastatin [letter]. J Neurol Neurosurg Psychiatry. 1994;57:383.
85. Parmar B, Francis PJ, Ragge NK. Statins, fibrates, and ocular myasthenia. Lancet. 2002;360:717.
86. Robberecht W, Bednarik J, Bourgeois P, Van Hees J, Carton H. Myasthenic syndrome caused by direct effect of chloroquine on neuromuscular junction. Arch Neurol. 1989;46:464–8.
87. Fife BT, Bluestone JA. Control of peripheral T-cell tolerance and autoimmunity via the CTLA-4 and PD-1 pathways. Immunol Rev. 2008;224:166–82.
88. Li B, Chan HL, Chen P. Immune checkpoint inhibitors: basics and challenges. Curr Med Chem. 2017;24:1–15.
89. de Miguel-Luken MJ, Mansinho A, Boni V, Calvo E. Immunotherapy-based combinations: current status and perspectives. Curr Opin Oncol. 2017;29:382–94.
90. Roberts K, Culleton V, Lwin Z, O'Byrne K, Hughes BG. Immune checkpoint inhibitors: navigating a new paradigm of treatment toxicities. Asia Pac J Clin Oncol. 2017;13:277–88.
91. Cousin S, Italiano A. Toxicity profiles of immunotherapy. Pharmacol Ther. 2018;181:91–100.
92. Makarious D, Horwood K, Coward JIG. Myasthenia gravis: an emerging toxicity of immune checkpoint inhibitors. Eur J Cancer. 2017;82:128–36.
93. Chang E, Sabichi AL, Sada YH. Myasthenia gravis after nivolumab therapy for squamous cell carcinoma of the bladder. J Immunother. 2017;40:114–6.
94. Cooper DS, Meriggioli MN, Bonomi PD, Malik R. Severe exacerbation of myasthenia gravis associated with checkpoint inhibitor immunotherapy. J Neuromuscul Dis. 2017;4:169–73.
95. Gonzalez NL, Puwanant A, Lu A, Marks SM, Živković SA. Myasthenia triggered by immune checkpoint inhibitors: new case and literature review. Neuromuscul Disord. 2017;27:266–8.

96. Kimura T, Fukushima S, Miyashita A, Aoi J, Jinnin M, Kosaka T, et al. Myasthenic crisis and polymyositis induced by one dose of nivolumab. Cancer Sci. 2016;107:1055–8.
97. Konoeda F, Suzuki S, Nishimoto Y, Hoshino H, Takagi M. A case of myasthenia gravis and myositis induced by nivolumab. Rinsho Shinkeigaku. 2017;57:373–7.
98. Lau KH, Kumar A, Yang IH, Nowak RJ. Exacerbation of myasthenia gravis in a patient with melanoma treated with pembrolizumab. Muscle Nerve. 2016;54:157–61.
99. Nguyen BH, Kuo J, Budiman A, Christie H, Ali S. Two cases of clinical myasthenia gravis associated with pembrolizumab use in responding melanoma patients. Melanoma Res. 2017;27:152–4.
100. Phadke SD, Ghabour R, Swick BL, Swenson A, Milhem M, Zakharia Y. Pembrolizumab therapy triggering an exacerbation of preexisting autoimmune disease: a report of 2 patient cases. J Investig Med High Impact Case Rep. 2016;4:2324709616674316.
101. Shirai T, Sano T, Kamijo F, Saito N, Miyake T, Kodaira M, et al. Acetylcholine receptor binding antibody-associated myasthenia gravis and rhabdomyolysis induced by nivolumab in a patient with melanoma. Jpn J Clin Oncol. 2016;46:86–8.
102. Suzuki S, Ishikawa N, Konoeda F, Seki N, Fukushima S, Takahashi K, et al. Nivolumab-related myasthenia gravis with myositis and myocarditis in Japan. Neurology. 2017;89(11):1127–34. https://doi.org/10.1212/WNL.0000000000004359.
103. Saleem A. Unmasking of myasthenia gravis during pegylated Alfa 2 a interferon and ribavirin therapy for chronic hepatitis C. J Pak Med Assoc. 2016;66:618–20.
104. Baik SJ, Kim TH, Kim HI, Rhie JY. Myasthenia crisis induced by pegylated-interferon in patient with chronic hepatitis C: a case report. Medicine. 2016;95:e3782.
105. Congeni JP, Kirkpatrick RB. Pegylated interferon induced myasthenia crisis—a case report. J Clin Neuromuscul Dis. 2013;14:123–5.
106. Kang HM, Park MJ, Hwang JM, Kim JW, Jeong SH. Development of ocular myasthenia during pegylated interferon and ribavirin treatment for chronic hepatitis C. Korean J Hepatol. 2009;15:209–15.
107. Wolfe CM, Tafuri N, Hatfield K. Exacerbation of myasthenia gravis during imiquimod treatment. J Drugs Dermatol. 2007;6:745–6.
108. Borgia G, Reynaud L, Gentile I, Cerini R, Ciampi R, Dello Russo M, et al. Myasthenia gravis during low-dose IFN-alpha therapy for chronic hepatitis C. J Interf Cytokine Res. 2001;21:469–70.
109. Konishi T. [A case of myasthenia gravis which developed myasthenic crisis after alpha-interferon therapy for chronic hepatitis C]. [Review] [14 refs] [Japanese]. Rinsho Shinkeigaku Clin Neurol. 1996;36:980–5.
110. Selmi C, Lleo A, Zuin M, Podda M, Rossaro L, Gershwin ME. Interferon alpha and its contribution to autoimmunity. Curr Opin Investig Drugs. 2006;7:451–6.
111. Gu D, Wogensen L, Calcutt N, Xia C, Zhu S, Merlie JP, et al. Myasthenia gravis-like syndrome induced by expression of interferon in the neuromuscular junction. J Exp Med. 1995;18:547–57.
112. Glick ZR, Vaphiades MS, Northington ME. Onabotulinumtoxin a unmasking myasthenia gravis. Dermatol Surg. 2013;39:472–3.
113. Erbguth F, Claus D, Engelhardt A, Dressler D. Systemic effect of local botulinum toxin injections unmasks subclinical Lambert-Eaton myasthenic syndrome. J Neurol Neurosurg Psychiatry. 1993;56:1235–6.
114. Iwase T, Iwase C. Systemic effect of local and small-dose botulinum toxin injection to unmask subclinical myasthenia gravis. Graefes Arch Clin Exp Ophthalmol. 2006;244:415–6.
115. Watts J, Brew B, Tisch S. Myasthenia gravis exacerbation with low dose ocular botulinum toxin for epiphoria. J Clin Neurosci. 2015;22:1979–81.
116. Tarsy D, Bhattacharyya N, Borodic G. Myasthenia gravis after botulinum toxin a for Meige syndrome. Mov Disord. 2000;15:736–8.
117. Borodic G. Myasthenic crisis after botulinum toxin. Lancet. 1998;352:1832.
118. Goncalves MR, Barbosa ER, Zambon AA, Marchiori PE. Treatment of cervical dystonia with botulinum toxin in a patient with myasthenia gravis. Arq Neuropsiquiatr. 1999;57:683–5.
119. Fasano A, Bentivoglio AR, Ialongo T, Soleti F, Evoli A. Treatment with botulinum toxin in a patient with myasthenia gravis and cervical dystonia. Neurology. 2005;64:2155–6.
120. Cherington M. Clinical spectrum of botulism. Muscle Nerve. 1998;21:701–10.
121. Jackson L, Madan-Khetarpal S, Naik M, Michaels MG, Riley M. Infant botulism in the very young neonate: a case series. AJP Rep. 2017;7:e163–6.
122. Pickett J, Berg B, Chaplin E, Brunstetter-Shafer M. Syndrome of botulism in infancy: clinical and electrophysiologic study. N Engl J Med. 1976;295:770–92.
123. MacDonald KL, Rutherford SM, Friedman SM, Dietz JR, Kaye BR, McKinley GF, et al. Botulism and botulism-like illness in chronic drug users. Ann Intern Med. 1985;102:616–8.
124. Abavare L, Abavare C. Wound botulism resulting from heroin abuse: can you recognize it? J Emerg Nurs. 2012;38:301–3.
125. Martin SJ, Penrice G, Amar C, Grant K, Gorrie GH. Wound botulism, its neurological manifestations, treatment and outcomes: a case series from the Glasgow outbreak, 2015. Scott Med J. 2017;62(4):136–41. https://doi.org/10.1177/0036933017707165.

126. Qureshi IA, Qureshi MA, Rauf Afzal M, Maud A, Rodriguez GJ, Cruz-Flores S, et al. Black tar heroin skin popping as a cause of wound botulism. Neurocrit Care. 2017;27(3):415–9.
127. Griffin PM, Hatheway CL, Rosenbaum RB, Sokolow R. Endogenous antibody production to botulinum toxin in an adult with intestinal colonization botulism and underlying Crohn's disease. J Infect Dis. 1997;175:633–7.
128. Chia JK, Clark JB, Ryan CA, Pollack M. Botulism in an adult associated with food-borne intestinal infection with clostridium botulinum. N Engl J Med. 1986;315:239–41.
129. McCroskey LM, Hatheway CL, Woodruff BA, Greenberg JA, Jurgenson P, Type F. Botulism due to neurotoxigenic *Clostridium baratii* from an unknown source in an adult. J Clin Microbiol. 1991;29:2618–20.
130. Maretic Z. Epidemiology of envenomation. In: Bettini S, editor. Arthropod venoms. Berlin: Springer; 1978. p. 185–212.
131. Rosenthal L. Alpha-latrotoxin and related toxins. Pharmacol Ther. 1989;42:115–34.
132. Hurlbut WP, Iezzi N, Fesce R, Ceccarelli B. Correlation between quantal secretion and vesicle loss at the frog neuromuscular junction. J Physiol. 1990;424:501–26.
133. Henkel AW, Sankaranarayanan S. Mechanisms of a-latrotoxin action. Cell Tissue Res. 1999;296:229–33.
134. Ushkaryov YA, Petrenko AG, Geppert M, Sudhof TC. Neurexins: synaptic cell surface proteins related to the a-lathrotoxin receptor and laminin. Science. 1992;257:50–6.
135. Longenecker HE, Hurlbut WP, Mauro A, Clark AW. Effects of black widow spider venom on the frog neuromuscular junction. Effects on end-plate potential, miniature end-plate potential and nerve terminal spike. Nature. 1970;225:701–3.
136. Ceccarelli B, Grohovaz F, Hurlbut WP. Freeze-fracture studies of frog neuromuscular junctions during intense release of neurotransmitter. I. Effects of black widow spider venom and Ca2+-free solutions on the structure of the active zone. J Cell Biol. 1979;81:163–77.
137. Clark AW, Hurlbut WP, Mauro A. Changes in the fine structure of the neuromuscular junction of the frog caused by black widow spider venom. J Cell Biol. 1972;52:1–14.
138. Clark AW, Mauro A, He L, Hurlbut WP. Effects of black widow spider venom on the frog neuromuscular junction. Effects on the fine structure of the frog neuromuscular junction. Nature. 1970;225:703–5.
139. Gorio A, Rubin LL, Mauro A. Double mode of action of black widow spider venom on frog neuromuscular junction. J Neurocytol. 1978;7:193–202.
140. Howard BD. Effects and mechanisms of polypeptide neurotoxins that act presynaptically. Annu Rev Pharmacol Toxicol. 1980;20:307–36.
141. Gilbert WW, Stewart CM. Effective treatment of arachiodism by calcium salts. Am J Med Sci. 1935;189:532–6.
142. Miller TA. Bite of the black widow spider. Am Fam Physician. 1992;45:181–7.
143. D'Amour EF, Becker FE, Van Riper W. The black widow spider. Q Rev Med. 1936;11:123–60.
144. Temple IU. Acute ascending paralysis, or tick paralysis. Med Sentinel. 1912;20:507–14.
145. Todd JL. Tick bite in British Columbia. CMAJ. 1912;2:1118–9.
146. Cleland JB. Injuries and diseases of man in Australia attributable to animals (except insects). Australas Med Gaz. 1912;32:295–9.
147. Gregson JD. Tick paralysis—an appraisal of natural and experimental data 1973. Canada: Department of Agriculture; 1973.
148. Edlow JA. Tick paralysis. Curr Treat Options Neurol. 2010;12:167–77.
149. Gothe R, Kunze K, Hoogstraal H. The mechanisms of pathogenicity in the tick paralyses. J Med Entomol. 1979;16:357–69.
150. Anonymous. Tick paralysis—Washington, 1995. From the centers for disease control and prevention. JAMA. 1996;275:1470.
151. Weingart JL. Tick paralysis. Minn Med. 1967;50:383–6.
152. Brown AF, Hamilton DL. Tick bite anaphylaxis in Australia. J Accid Emerg Med. 1998;15:111–3.
153. Felz MW, Smith CD, Swift TR. A six-year-old girl with tick paralysis [see comments]. N Engl J Med. 2000;342:90–4.
154. Cherington M, Snyder RD. Tick paralysis: neurophysiological studies. N Engl J Med. 1968;278:95–7.
155. Swift TR, Ignacio OJ. Tick paralysis: electrophysiologic studies. Neurology. 1975;25:1130–3.
156. Grattan-Smith PJ, Morris JG, Johnston HM, Yiannikas C, Malik R, Russell R, et al. Clinical and neurophysiological features of tick paralysis. Brain. 1997;120:1975–87.
157. Donat JR, Donat JF. Tick paralysis with persistent weakness and electromyographic abnormalities. Arch Neurol. 1981;38:59–61.
158. Lagos JC, Thies RE. Tick paralysis without muscle weakness. Arch Neurol. 1969;21:471–4.
159. Rose IA. Review of tick paralysis. Can Med Assoc J. 1954;70:175–6.
160. Dworkin MS, Shoemaker PC, Anderson DE. Tick paralysis: 33 human cases in Washington state, 1946–1996. Clin Infect Dis. 1999;29:1435–9.
161. Jones HR Jr. Guillain-Barre syndrome: perspectives with infants and children. Semin Pediatr Neurol. 2000;7:91–102.
162. Stanbury JB, Huyck JH. Tick paralysis: critical review. Medicine. 1945;24:219–42.
163. Stone BF, Aylward JH. Holocyclotoxin—the paralysing toxin of the Australian paralysis tick Ixodes holocyclus; chemical and immunological characterization. Toxicon. 1992;30:552–3.

164. Rose I, Gregson JD. Evidence of neuromuscular block in tick paralysis. Nature. 1959;178:95–6.
165. Gothe R, Neitz AWH. Tick paralyses: pathogenesis and etiology. Adv Dis Vector Res. 1991;8:177–204.
166. Stone BF. Tick paralysis, particularly involving Ixodes holocyclus and other *Ixodes* species. Adv Dis Vector Res. 1988;5:61–85.
167. Esplin DW, Phillip CB, Hughes LE. Impairment of muscle stretch reflexes in tick paralysis. Science. 1960;132:958–9.
168. DeBusk FL, O'Connor S. Tick toxicosis. Pediatrics. 1972;50:328–9.
169. Haller JS, Fabara JA. Tick paralysis. Case report with emphasis on neurological toxicity. Am J Dis Child. 1972;124:915–7.
170. Lin J, Verma S. Electrodiagnostic abnormalities in tick paralysis: a case report and review of literature. J Clin Neuromuscul Dis. 2016;17:215–9.
171. Zhang S, Gao B, Zhu S. Target-driven evolution of scorpion toxins. Sci Rep. 2015;5:14973.
172. Warnick JE, Albuquerque EX, Diniz CR. Electrophysiological observations on the action of the purified scorpion venom, Tityus-toxin, on nerve and skeletal muscle of the rat. J Pharmacol Exp Ther. 1976;198:155–67.
173. Sofer S, Shahak E, Gueron M. Scorpion envenomation and antivenom therapy. Pediatrics. 1994;124:973–8.
174. Belghith M, Boussarsar M, Haguiga H, Besbes L, Elatrous S, Touzi N, et al. Efficacy of serotherapy in scorpion sting: a matched-pair study. J Toxicol Clin Toxicol. 1999;37:51–7.
175. The Lancet. Snake-bite envenoming: a priority neglected tropical disease. Lancet. 2017;390:2.
176. Chippaux JP. Incidence and mortality due to snakebite in the Americas. PLoS Negl Trop Dis. 2017;11:e0005662.
177. Campbell CH. The effects of snake venoms and their neurotoxins on the nervous system of man and animals. Contemp Neurol Ser. 1975;12:259–93.
178. Vital-Brazil O. Venoms: their inhibitory action on neuromuscular transmission. In: Cheymol J, editor. Neuromuscular blocking and stimulating agents. New York: Pergamon Press; 1972. p. 145–67.
179. Lee CY. Elapid neurotoxins and their mode of action. Clin Toxicol. 1970;3:457–72.
180. Karlsson E, Arnberg H, Eaker D. Isolation of the principal neurotoxin of tow *Naja naja* subspecies. Eur J Biochem. 1971;21:1–16.
181. Lee CY, Chang SL, Kau ST, Luh SH. Chromatographic separation of the venon of *Bungarus multicinctus* and characteristics of its components. J Chromatogr. 1972;72:71–82.
182. Barme M. Venomous sea snakes of Vietnam and their venoms. In: Keegan HL, MacFarlane W, editors. Venomous and poisonous animals and noxious plants of the Pacific region. Oxford: Pergamon Press; 1963. p. 373–8.
183. Tu AT, Tuh T. Sea snakes from southeast Asia and far east and their venoms. In: Halstead BW, editor. Poisonous and venomous marine animals of the world. Washington, DC: US Government Printing Office; 1970. p. 885–903.
184. Karlsson E. Chemistry of protein toxins in snake venoms. In: Lee CY, editor. Snake venoms. New York: Springer; 1979. p. 159–212.
185. Chang CC, Su MJ. Mutual potentiation a nerve terminals, between toxins from snake venoms that contain phospholipase a activity: b-bungarotoxin, crotoxin, taipoxin. Toxicon. 1980;18:641–8.
186. Kellaway CH. The peripheral action of the Australian snake venoms. 2. The curari-like action in mammals. Aust J Exp Biol Med Sci. 1932;10:181–94.
187. Rowlands JB, Mastaglia FL, Kakulas BA, Hainsworth D. Clinical and pathological aspects of a fatal case of mulga (Pseudechis Australis) snakebite. Med J Aust. 1969;1:226–30.
188. Bouquier JJ, Guibert J, Dupont C, Umdenstock R. Les piqures de vipere chez l'enfant. Arch Fr Pediatr. 1974;31:285–96.
189. Mitrakul C, Dhamkrong-At A, Futrakul P, Thisyakorn C, Vongsrisart K, Varavithya C, et al. Clinical features of neurotoxic snake bite and response to antivenom in 47 children. Am J Trop Med Hyg. 1984;33:1258–66.
190. Reid HA. Cobra-bites. Br Med J. 1964;2:540–5.
191. Warrell DA, Barnes HJ, Piburn MF. Neurotoxic effects of bites by the Egyptian cobra (Naja Haje) in Nigeria. Trans R Soc Trop Med Hyg. 1976;70:78–9.
192. Kerrigan KR. Venomous snake bites in eastern Ecuador. Am J Trop Med Hyg. 1991;44:93–9.
193. Ouyang C, Teng C-M, Huang T-F. Characterization of snake venom components acting on blood coagulation and platelet function. Toxicon. 1992;30:945–66.
194. Johnston CI, Ryan NM, Page CB, Buckley NA, Brown SG, O'Leary MA, et al. The Australian snakebite project, 2005–2015 (ASP-20). Med J Aust. 2017;207:119–25.
195. Kumar S, Usgaonkar RS. Myasthenia gravis like picture resulting from snake bite. J Indian Med Assoc. 1968;50:428–9.
196. Pettigrew LC, Glass JP. Neurologic complications of coral snake bite. Neurology. 1985;35:589–92.
197. Southcott RV. The neurologic effects of noxious marine creatures. In: Hornabrook RW, editor. Topics on tropical neurology. Philadelphia: F.A. Davis Company; 1975. p. 165–258.
198. Steidinger KA, Steinfield HJ. Toxic marine dinoflagellates. In: Spector DL, editor. Dinoflagellates. New York: Academic Press; 1984. p. 201–6.
199. Gallagher JP, Shinnick-Gallagher P. Effects of crude brevetoxin on membrane potential and spontaneous or evoked end-plate potentials in rat hemidiaphragm. Toxicon. 1985;23:489–96.
200. Tsai MC, Chou HN, Chen ML. Effect of brevetoxin-B on the neuromuscular transmission of the mouse diaphragm. J Formos Med Assoc. 1991;90:431–6.

201. Molgo J, Marchot P, Araoz R, Benoit E, Iorga BI, Zakarian A, et al. Cyclic imine toxins from dinoflagellates: a growing family of potent antagonists of the nicotinic acetylcholine receptors. J Neurochem. 2017;142(Suppl 2):41–51.
202. Robertson A, Garcia AC, Quintana HA, Smith TB, Castillo BF 2nd, Reale-Munroe K, et al. Invasive lionfish (Pterois Volitans): a potential human health threat for ciguatera fish poisoning in tropical waters. Mar Drugs. 2013;12:88–97.
203. Olivera BM, Gray WR, Zeikus R, et al. Peptide neurotoxins from fish-hunting cone snails. Science. 1985;230:1338–43.
204. Kohn AJ. Recent cases of human injury due to venomous marine snails of the genus *Conus*. Hawaii Med J. 1958;17:528–32.
205. Kohn AJ. Venomous marine snails of the genus Conus. In: Keegan HC, McFarlane WV, editors. Venomous and poisonous animals and noxious plants of the Pacific region. Oxford: Permagon Press; 1963. p. 1–456.
206. Cruz LJ, White J. Clinical toxicology of *Conus* snail stings. In: Meier J, White J, editors. CRC handbook on clinical toxicology of animal venoms and poisons. Boca Raton: CRC Press; 1995.
207. Gray WR, Luque A, Olivera BM, Barrett J, Cruz LJ. Peptide toxins from *Conus geographus* venom. J Biol Chem. 1981;256:4734–40.
208. Hopkins C, Grilley M, Miller C, Shon KJ, Cruz LJ, Gray WR, et al. A new family of Conus peptides targeted tothe nicotinic acetylcholine receptor. J Biol Chem. 1995;270:22361–7.
209. McIntosh M, Cruz LJ, Hunkapiller MW, Gray WR, Olivera BM. Isolation and structure of a peptide toxin from the marine snail *Conus magnus*. Arch Biochem Biophys. 1982;218:329–34.
210. McCleskey EW, Fox AP, Feldman D, Cruz LJ, Olivera BM, Tsien RW, et al. Calcium channel blockade by a peptide from *Conus*: specificity and mechanism. Proc Natl Acad Sci U S A. 1987;84:4327–31.
211. Adams DJ, Alewood PF, Craik DJ, Drinkwater RD, Lewis RJ. Conotoxins and their potential pharmaceutical applications. Drug Dev Res. 1999;46:219–34.
212. Robinson SD, Li Q, Lu A, Bandyopadhyay PK, Yandell M, Olivera BM, et al. The venom repertoire of Conus Gloriamaris (Chemnitz, 1777), the glory of the sea. Mar Drugs. 2017;15:145.
213. Yoshiba S. [An estimation of the most dangerous species of cone shell, Conus (Gastridium) geographus Linne, 1758, venom's lethal dose in humans]. J Hyg. 1984;39:565–72.
214. Halstead BW. Poisonous and venomous marine animals of the world. Washington, DC: US Government Printing Office; 1970.
215. Gwee MC, Gopalakrishnakone P, Yuen R, Khoo HE, Low KS. A review of stonefish venoms and toxins. Pharmacol Ther. 1994;64:509–28.
216. Kreger AS, Molgo J, Comella JX, Hansson B, Thesleff S. Effects of stonefish (*Synanceia trachynis*) venom on murine and frog neuromuscular junctions. Toxicon. 1993;31:307–17.
217. Hardin JW, Arena JW. Human poisoning from native and cultivated plants. 1st ed. Durham, NC: Duke University Press; 1974.
218. Davies M, Davies TA. Hemlock: murder before the Lord. Med Sci Law. 1994;34:331–3.
219. Panter KE, Keeler RF. Piperidine alkaloids of poison hemlock *(Conium maculatum)*. In: Cheeke P, editor. Toxicants of plant origin: alkaloids, vol. 1. Boca Raton: CRC Press; 1989.
220. Schep LJ, Slaughter RJ, Beasley DM. Nicotinic plant poisoning. Clin Toxicol. 2009;47:771–81.
221. Green BT, Lee ST, Welch KD, Panter KE. Plant alkaloids that cause developmental defects through the disruption of cholinergic neurotransmission. Birth Defects Res C Embryo Today. 2013;99:235–46.
222. Silinsky EM. On the role of barium in supporting the asynchronous release of acetylcholine quanta by motor nerve impulses. J Physiol. 1978;274:157–71.
223. Silinsky EM. Can barium support the release of acetylcholine by nerve impulses? Br J Pharmacol. 1977;59:215–7.
224. Metral S, Bonneton C, Hort-Legrand C, Reynes J. Dual action of erbium on transmitter release at the from neuromuscular synapse. Nature. 1978;271:773–5.
225. Cooper GP, Manalis RS. Cadmium: effects on transmitter release a the frog neuromuscular junction. Eur J Pharmacol. 1984;99:251–6.
226. Forshaw PJ. The inhibitory effect of cadmium on neuromuscular transmission in the rat. Eur J Pharmacol. 1977;42:371–7.
227. Weakly JN. The action of cobalt ions on neruomusculal transmission in the frog. J Physiol. 1973;234:597–612.
228. Molgo J, del Pozo E, Banos JE, Angaut-Petit D. Changes in quantal transmitter release caused by gadolinium ions at the frog neuromuscular junction. Br J Pharmacol. 1991;104:133–8.
229. Kajimoto N, Kirpekar SM. Effects of manganese and lanthanum on spontaneous release of acetylcholine at frog motor nerve terminals. Nature. 1972;235:29–30.
230. Balnave RJ, Gage PW. The inhibitory effect of manganese on transmitter release at the neuromuscular junction of the toad. Br J Pharmacol. 1973;47:339–52.
231. Kita H, Van der Kloot W. Action of Co and Ni at the frog neuromuscular junction. Nature. 1973;245:52–3.
232. Alnaes E, Rahaminoff R. Dual action of praseodymium (Pr3+) on transmitter release at the frog neuromuscular synapse. Nature. 1975;247:478–9.
233. Allen JE, Gage PW, Leaver DD, Leow ACT. Triethyltin decreases evoked transmitter release at the mouse neuromuscular junction. Chem Biol Interact. 1980;31:227–31.

234. Benoit PR, Mambrini J. Modification of transmitter release by ions which prolong the presynaptic action potential. J Physiol. 1970;210:681–95.
235. Cooper GP, Manalis RS. Influence of heavy metals on synaptic transmission. Neurotoxicology. 2001;4:69–84.
236. Rustam H, Hamdi T. Methylmercury poisoning n Iraq; a neurological study. Brain. 1974;97:499–510.
237. Bakir F, Damluji SF, Amin-Saki L, et al. Methylmercury poisoning in Iraq. Science. 1973;181:230–41.
238. Igata A. Neurological aspects of methylmercury poisoning in Minamata. In: Tsubaki T, Takahashi H, editors. Recent advances in Minamata disease studies. Tokyo: Kodansha; 1986. p. 41–57.
239. LeQuense P, Damluji SF, Berlin M. Electrophysiological studies of peripheral nerves in patients with organic mercury poisoning. J Neurol Neurosurg Psychiatry. 1974;37:333–9.
240. Rustam H, von Burg R, Amin-Saki L, Elhassani S. Evidence of a neuromuscular disorder in methylmercury poisoning. Arch Environ Health. 1975;30:190–5.
241. Atchinson WD, Narahashi T. Methylmercury induced depression of neuromuscular transmission in the rat. Neurotoxicology. 1982;3:37–50.
242. Rehman K, Fatima F, Waheed I, Akash MSH. Prevalence of exposure of heavy metals and their impact on health consequences. J Cell Biochem. 2017;119(1):157–84.
243. Proudfoot A. The early toxicology of physostigmine: a tale of beans, great men and egos. Toxicol Rev. 2006;25:99–138.
244. Taylor P. Anticholinesterase agents. In: Gilman AG, Goodman LS, Rall TW, Murad F, editors. The pharmacological basis of therapeutics. 7th ed. New York: MacMillan; 1985. p. 110–29.
245. Edmundson RS. Dictionary of organophosphorus compounds *[electronic resource]*. London: Chapman and Hall; 1988.
246. Gunderson CH, Lehmann CR, Sidell FR, Jabari B. Nerve effects: a review. Neurology. 1992;42:946–50.
247. Pedersen B, Ssemugabo C, Nabankema V, Jors E. Characteristics of pesticide poisoning in rural and urban settings in Uganda. Environ Health Insights. 2017;11:1178630217713015.
248. Alinejad S, Zamani N, Abdollahi M, Mehrpour OA. Narrative review of acute adult poisoning in Iran. Iran J Med Sci. 2017;42:327–46.
249. Fernando R. Pesticides in Sri Lanka. Friedrich-Ebert-Stiftung: Colombo; 1989.
250. Adinew GM, Asrie AB, Birru EM. Pattern of acute organophosphorus poisoning at University of Gondar Teaching Hospital, Northwest Ethiopia. BMC Res Notes. 2017;10:149.
251. Aldridge WN, Reiner E. Enzyme inhibitors as substrates. Amsterdam: North-Holland Publishing Co.; 1972.
252. Marrs TC. Organophosphate poisoning. Pharmacol Ther. 1993;58:51–66.
253. Namba T, Nolte CT, Jackrel J, Grob D. Poisoning due to organophosphorous insecticides. Am J Med. 2001;50:475–92.
254. De Wilde V, Vogblaers D, Colarddyn F, et al. Postsynaptic neuromuscular dysfunction in orgaophosphate induced intermediate syndrome. Klin Wochenschr. 1991;69:177–83.
255. Good JL, Khurana RK, Mayer RF, Cintra WM, Albuquerque EX. Pathophysiological studies of neuromuscular function in subacute organophosphate poisoning induced by phosmet. J Neurol Neurosurg Psychiatry. 1993;56:290–4.
256. Gutmann L, Besser R. Organophosphate intoxication: pharmacologic, neurophysiologic, clinical, and therapeutic considerations. Semin Neurol. 1990;10:46–51.
257. Maselli RA, Soliven BC. Analysis of the organophosphate-induced electromyographic response to repetitive nerve stimulation: paradoxical response to edrophonium and D-tubocurarine. Muscle Nerve. 1991;14:1182–8.
258. Vale A, Lotti M. Organophosphorus and carbamate insecticide poisoning. Handb Clin Neurol. 2015;131:149–68.
259. Tsao TC, Juang Y, Lan R, Shieh W, Lee C. Respiratory failure of acute organophosphate and carbamate poisoning. Chest. 1990;98:631–6.
260. WHO/UNEP. Public health impact of pesticides used in agriculture. Geneva: World Heath Organization; 1990. p. 1–128.
261. Besser R, Gutmann L, Dilimann U, Weilemann LS, Hopf HC. End plate dysfunction in acute organophosphate intoxication. Neurology. 1989;39:561–7.
262. Jeyaratnam J. Acute pesticide poisoning: a major health problem. World Health Stat Q. 1990;43:139–45.
263. Haddad LM. Organophosphate poisoning—intermediate syndrome. J Toxicol Clin Toxicol. 1992;30:331–2.
264. De Bleecker J, Willems J, Van Den Neucker K, De Reuck J, Vogelaers D. Prolonged toxicity with intermediate syndrome after combined parathion and methyl parathion poisoning. Clin Toxicol. 1992;30:333–45.
265. Güler K, Tascioglu C, Özbey N. Organophosphate poisoning. Isr J Med Sci. 1996;32:791–2.
266. Chaudhry R, Lall SB, Mishra B, Dhawan B. Lesson of the week—a foodborne outbreak of organophosphate poisoning. Br Med J. 1998;317:268–9.
267. Cranmer MF. Carbaryl. A toxicological review and risk analysis. Neurotoxicology. 1986;7:247–328.
268. Goldman LR, Smith DF, Neutra RR, Saunders LD, Pond EM, Stratton J, et al. Pesticide food poisoning from contaminated watermelons in California. Arch Environ Health. 1990;45:229–36.
269. Freslew KE, Hagardorn AN, McCormick WF. Poisoning from oral ingestion of carbofuran

(Furadan 4F), a cholinesterase-inhibiting carbamate insecticide, and its effects on cholinesterase activity in various biological fluids. J Forensic Sci. 1992;37:337–44.
270. Jenis EH, Payne RJ, Goldbaum LR. Acute meprobamate poisoning: a fatal case following a lucid interval. JAMA. 1969;207:361–2.
271. Klys M, Kosún J, Pach J, Kamenczak A. Carbofuran poisoning of pregnant women and fetus per ingestion. J Forensic Sci. 1989;34:1413–6.
272. Maddock RK, Bloomer HA. Meprobamate overdosage. Evaluation of its severity and methods of treatment. JAMA. 1967;201:123–7.
273. Ecobichon DJ. Carbamates. In: Spencer PS, Schaumburg HH, editors. Experimental and clinical neurotoxicology. 2nd ed. New York: Oxford University Press; 2000. p. 289–98.
274. Maynard RL. Toxicology of chemical warfare agents. In: Ballantyne B, Marrs T, Turner T, editors. General and applied toxicology. New York: Stockton Press; 1993. p. 1253.
275. Spencer PS, Wilson BW, Albuquerque EX. Sarin, other "nerve agents" and their antidotes. In: Spencer PS, Schaumburg HH, editors. Experimental and clinical neurotoxicology. 2nd ed. New York: Oxford University Press; 2000. p. 1073–93.
276. Meselson M, Perry Robinson J. Chemical warfare and disarmament. Sci Am. 1980;242:38–47.
277. Nozaki H, Aikawa N, Fujishima S, Suzuki M, Shinozawa Y, Hori S, et al. A case of VX poisoning and the difference from Sarin. Lancet. 1995;346:698–9.
278. Nozaki H, Aikawa N, Shinozawa Y, Hori S, Fujishima S, Takuma K, et al. Sarin poisoning in Tokyo subway. Lancet. 1995;345:980–1.
279. Morita H, Yanagisawa N, Nakajima T, Shimizu M, Hirabayashi H, Okudera H, et al. Sarin poisoning in Matsumoto, Japan. Lancet. 1995;346:290–3.
280. Nakajima T, Saro S, Morita H, Yanagisawa N. Sarin poisoning of a rescue team in the Matsumoto sarin incident in Japan. Occup Environ Med. 1997;54:697–701.
281. Woodall J. Tokyo subway gas attack. Lancet. 1997;350:296.
282. Dunn MA, Hackley BE, Sidell FR. Pretreatment for nerve agent exposure. In: Sidell FR, Talafuji ET, Franz DR, editors. Textbook of military medicine: medical aspects of chemical and biological warfare. Washington, DC: Borden Institute, Walter Reed Army Medical Center; 1997. p. 181–96.
283. Dawson RM. Review of oximes available for treatment of nerve agent poisoning. J Appl Toxicol. 1994;14:317–31.
284. Worek F, Thiermann H, Wille T. Oximes in organophosphate poisoning: 60 years of hope and despair. Chem Biol Interact. 2016;259:93–8.
285. Holstege CP, Kirk M, Sidell FR. Chemical warfare. Nerve agent poisoning. Crit Care Clin. 1997;13:923–42.
286. King AM, Aaron CK. Organophosphate and carbamate poisoning. Emerg Med Clin North Am. 2015;33:133–51.
287. Becker G, Kawan A, Szinicz L. Direct reaction of oximes with sarin, soman or tabun in vitro. Arch Toxicol. 1997;71:714–8.
288. Ecobichon DJ. Carbamic acid ester insecticides. In: Ecobichon DJ, Joy RM, editors. Pesticides and neurological disease. 2nd ed. Boca Raton: CRC Press; 1994. p. 251–89.
289. Rotenberg M, Shefi M, Dany S, Dore I, Tirosh M, Almog S. Differentiation between organophosphate and carbamate poisoning. Clin Chim Acta. 1995;234:11–21.
290. Besser R, Vogt T, Gutmann L. High pancuronium sensitivity of axonal nicotinic-acetylcholine receptors in humans during organophosphate intoxication. Muscle Nerve. 1991;14:1197–201.
291. Miller SA, Blick DW, Kerenyi SZ, Murphy MR. Efficacy of physostigmine as a pretreatment for organophosphate poisoning. Pharmacol Biochem Behav. 1993;44:343–7.
292. De Cauwer H, Somville FJ, Joillet M. Neurological aspects of chemical and biological terrorism: guidelines for neurologists. Acta Neurol Belg. 2017;117:603–11.

Predictors of Psychological Health in Myasthenia Gravis

18

Robert H. Paul and Paola García-Egan

Introduction

In the absence of a cure, individuals diagnosed with myasthenia gravis (MG) are confronted with a chronic and potentially disruptive medical disease. The mental health burden of MG has not been a primary focus of research, which is an unfortunate reality likely associated with the low prevalence of the disease compared to other neurological conditions centered in the modern cultural vernacular (e.g., Alzheimer's disease [AD]). Nevertheless, it is well known from work conducted in other neuroimmune conditions that patients and their loved ones are at heightened risk for psychological turmoil and reduced quality of life compared to the general population. In this chapter, we provide an updated review focused on mental health and disease progression in MG, as well as individual patient factors and disease factors that serve to challenge or enhance optimal mental health outcomes. The goal is to provide clinicians with the most effective, evidence-based resources to facilitate treatment and optimize patient care.

We begin with an updated review of cognitive dysfunction. We elected to start with mental performance because cognitive schemas define the lens through which individuals view the world, including chronic symptoms of disease and illness. The next section is focused on potential interactions between mental health and disease expression, followed by a review of four essential support systems to foster mental health in the context of chronic disease. Finally, examples of specific psychosocial resources are provided.

Neuropsychological Health in MG

Nicotinic acetylcholine receptors exist in both the central and peripheral nervous systems. The distribution of nicotinic receptors in the brain has prompted an intriguing question as to whether the antibodies that damage peripheral receptors at the neuromuscular junction also damage receptors in the brain parenchyma (for reviews see Paul et al. [1] and Mao et al. [2]). Since nicotinic receptors are abundantly distributed throughout the frontal and temporal regions of the brain and are known from both animal and human studies to mediate cognitive processes, MG-related cognitive impairment carries both clinical and research interest. This is particularly true in terms of mental health given

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© Springer International Publishing AG, part of Springer Nature 2018
H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*,
Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_18

the interdependence between mental health and self-reported cognitive difficulties.

Concern was first raised that MG patients exhibit cognitive impairment following a survey in which 60% of respondents reported memory loss [3]. This frequency was significantly higher than what was observed among demographically similar peers without MG. However, self-reported cognitive impairment is susceptible to reporting error. For example, among individuals infected with human immunodeficiency virus (HIV), self-reported ratings of cognitive impairment correlate with symptoms of depression but not with performance on cognitive testing [4].

Neuropsychological Function in MG

In a comprehensive review of the literature, we reported that most studies supported the position that cognition is disrupted in MG [1]. Our group conducted a methodologically rigorous study to begin to unravel the etiology of impairment in MG through cognitive phenotyping [5]. We administered a standardized battery of neuropsychological tests to measure attention, language fluency, information processing speed, and verbal/visual learning and retention. When compared to demographically similar healthy controls, MG patients exhibited worse performance on most tests with the exception of measures tapping attention and measures of memory retention. Contrasting this cognitive phenotype with the pattern observed in neurodegenerative diseases affecting the central cholinergic system (e.g., AD) revealed little overlap. The reduced information processing speed and learning inefficiencies without loss of learned information exhibited by the MG group are most consistent with a subcortical pattern rather than the cortical pattern of AD. Examples of other neurological disorders that exhibit a subcortical cognitive phenotype include multiple sclerosis [6], Parkinson's disease [7], and Huntington's disease [8]. Similarly, depression and fatigue mirror the subcortical neuropsychological phenotype.

Etiology of Neuropsychological Symptoms in MG

Results from our initial study suggest that the cognitive complaints reported by MG patients are not likely due to antibody-mediated damage to central nicotinic receptors. This is consistent with the limited work conducted to date on MG antibody binding in the brain [9] and the low level of antibodies in the CNS [10]. Alternative etiologies to cognitive symptoms that have been proposed in the literature include cytokine activity in the brain, sleep apnea, depression, and fatigue. Work by our group and others indicates that of these candidate mechanisms, fatigue is the most robust predictor of cognitive dysfunction in MG.

Fatigue and MG

Fatigue is the cardinal symptom of MG. The frequency of fatigue is more common in MG than other neurological conditions, including multiple sclerosis, Parkinson's disease, stroke, and traumatic brain injury [11]. Physiologically, MG-related fatigue manifests as impaired ability to sustain muscle contraction. Chronically affected individuals describe a more broad experience of feeling mentally and physically "tired" [12–14]. While previous groups delineate general "fatigue" (tiredness, lack of energy, and difficulty concentrating) from "physical fatigue" (difficulty initiating and sustaining muscle contractions) [11, 14], the two dimensions covary with effort. Specifically, healthy control subjects, individuals with neurological conditions other than MG, and MG patients experience increased levels of mental and physical fatigue with either cognitive or physical effort [15–17]. Interestingly, subjective ratings of fatigue in MG are more tightly linked to autonomic dysfunction than muscle fatigue recorded by neurophysiology [18].

Results from two studies reveal that cognitive dysfunction is related to increased fatigue in MG [17, 19]. In a study conducted by our group, MG patients reported higher mental and physical fatigue than controls before and after completing

a battery of demanding tests. Further, change in mental fatigue correlated with cognitive performance on the tests in which the patients differed from healthy controls. Similar results were reported by Jordan et al. [19]. In this study, cognitive performances over time intervals served as objective markers of cognitive fatigue. MG patients performed more poorly on the cognitive tests and demonstrated more fatigue than controls over the testing session. MG patients did not report a significant increase in subjective ratings of fatigue which is probably due to the relatively short test duration compared to what was utilized in our previous study.

Few studies have examined the impact of fatigue and cognitive symptoms on the ability of MG patients to complete activities of daily living (ADLs). A study of 20 MG patients with generalized disease reported more severe fatigue and more difficulties with ADLs than patients with ocular disease. Further, MG patients reported significant interference in ability to complete physical activities and to participate in social functions [20]. Controlled studies are needed to delineate the relationship between cognitive difficulties associated with fatigue and performance of instrumental ADLs (IADLs) (e.g., shopping, financial management, laundry, telephone use) in MG patients. This represents an important area of research given the known association between executive difficulties and reduced IADLs that have been described in other patient populations [21–23].

Effects of Psychological Health on Disease

Concern that psychological health directly affects disease expression is predicated on the notion that psychological distress causes immune dysregulation through activation of the hypothalamic pituitary adrenal axis [24] and direct binding of cortisol to immune cells [25–27]. A few clinical reports describe a relationship between mental health and the precipitation of MG [28–31]; however, these studies were uncontrolled and included a small number of MG patients. In a more recent study, MG was one of several autoimmune disorders identified as more common among individuals with a history of early life adversity [32]. However, of the 15,000 hospital records reviewed, MG accounted for only ten cases. Childhood adversity and immune dysregulation have been described in non-MG populations [33, 34], but the link to MG remains conjecture at this time. Previous work also identified personality subtype as a predictor of MG onset [35], but participants in this study were self-selected from psychological treatment studies and this referral bias confounds the study results. Indeed, other studies reveal no association between personality type and MG onset or progression [36], arguing against the notion of a premorbid or reactive "myasthenic personality."

A few descriptive reports [28, 36–38] suggest that psychological health affects the clinical course of MG. While intriguing from a pathophysiological perspective, the sequencing of events is challenging to define in clinical studies, and no study has empirically established that mental health alters disease progression in MG. Patients may experience greater symptom severity during times of emotional distress, despite no change in disease activity. Previous researchers have referred to the former as the "disease" and the latter as the "illness" [39]. These two dimensions of health covary, but they do not perfectly overlap. A full range of illness severity is possible at a given level of disease, and psychological health is an important factor that determines the point on the continuum that illness severity is expressed. This effect emanates from a patient's cognitive framework and psychological valence assigned to the various experiences. During times of stress or depressed mood, individuals perceive more severe symptoms. Patients may be prone to identify these episodes as exacerbations of the "disease," though they reflect exacerbations of the "illness." This relationship highlights the importance of focusing on variables that affect illness behavior in chronic disease.

Impact of MG on Psychological Health

Intuitively, MG would be expected to cause some degree of psychological discomfort in public settings [12], social anxiety, and avoidance [40]. However, remarkably, limited research has focused on mental health in this population [41–45]. In prior studies, the patient samples were relatively small, comparisons to norms were not consistently reported, and cultural differences (e.g., [46]) did not allow for broad generalization. In one frequently cited study, Paradis et al. [41] reported a high frequency of anxiety disorders in MG patients, but the sample was combined with patients diagnosed with polymyositis/dermatomyositis (PMD), and the frequency of anxiety was not reported for each subgroup.

Magni et al. [42] identified psychiatric disorders in half of MG patients. Adjustment disorders represented the most common diagnosis (22%) followed by personality (18%) and affective disorders (14%). Anxiety and somatoform disorders were uncommon (<6%). Psychopathology corresponded with disease severity, but not with medications or thymectomy. Ybarra et al. [46] also observed anxiety disorders in nearly half of MG patients and mood disorder in about one quarter of the sample. Older age, longer disease duration, and female sex correlated with mental health diagnoses. No correlation was evident between mental health symptoms and treatment regimen.

Our research group quantified psychiatric symptom severity in MG [43, 44] using a self-report measure that separately assessed mood (e.g., feeling sad) and vegetative symptoms (e.g., feeling tired) of depression [47]. MG patients did not differ from healthy controls on the mood subscale, but a significant difference was observed on the vegetative subscale, reflecting the prominent expression of physical symptoms in MG. Interestingly, the median daily dose of prednisone correlated only with scores on the vegetative subscale. The latter observation is noteworthy since use of prednisone is known to disrupt mood [48].

MG patients also report lower scores than healthy controls on scales of self-reported quality of life [44, 49–51]. When compared to individuals with arthritis, hypertension, or congestive heart failure, MG patients reported greater disruption in physical activities related to quality of life [52, 53]. Lower ratings were predicted by disease severity but not by anti-acetylcholine receptor antibodies, thymectomy status, or medication regimen. The findings suggest that mental health in MG is independent of disease severity.

Factors That Support Psychological Health in MG

Perceived Control and Mental Health

Perceived control significantly influences how individuals respond to threat. We are engineered to control our environment, and the absence of such control leads to distress manifested biologically and psychologically [54]. Diseases, especially those with chronic symptoms, challenge one's sense of control. For many MG patients, lack of control begins soon after symptom onset and continues even after treatment has been initiated following diagnosis [29]. As most medical conditions, a treatment program cannot be managed by the patient alone, and therefore control over one's own health must be conceded to other individuals. This is personally challenging, even when care is in the hands of a known expert.

Uncertainty in Illness and Mental Health

Uncertainty is the second predictor of mental health in chronic illness. Illness uncertainty is a cognitive experience in which the meaning of illness-related events is unclear and outcomes are unpredictable. Mishel [55] conceptualized illness uncertainty as four subcomponents, including ambiguity regarding the cues and the state of the illness, unpredictability of illness course and outcomes, complexity regarding the treatment and the health care system, and lack (or inconsistency) of information regarding the illness or treatments. A robust association exists between perceptions of uncertainty and psychological dis-

tress at the time of diagnosis, during treatment, and throughout stabilization periods [56–61]. Clearly, perceptions of uncertainty increase the risk of poor adaptation to illness and/or clinically significant psychological distress. Uncertainty also represents a potential target for intervention. Psychosocial interventions that target uncertainty in cancer patients demonstrate improved adaptation and mental health outcomes [62–66]. No studies have targeted uncertainty as a therapeutic focus in MG, but this represents an important area of future research.

Illness Intrusiveness and Mental Health

Devins and colleagues refer to illness intrusiveness as both the objective and perceived intrusiveness of an illness resulting from "barriers" that restrict engagement in activities [67, 68]. Disease factors reduce quality of life through perceived illness intrusiveness, which in turn challenge psychological adjustment. From a conceptual framework, illness intrusiveness and perceived control are related, but distinct constructs [67]. Both disease (e.g., disease severity, fatigue) and treatment variables (e.g., type of treatment and time required for treatment) are strongly associated with illness intrusiveness [69, 70]. Further, studies reveal a high level of correspondence between illness intrusiveness and depression, lower self-esteem, and poorer marital satisfaction [71]. Not surprisingly, age and perceived stigma moderate the impact of illness intrusiveness [56, 72] on the severity of mental health outcomes.

Social Support and Mental Health

The fourth major factor that influences psychological health in chronic disease is social support. Social networks consisting of family, friends, and colleagues provide an important buffer to the psychological challenges associated with any stressful experience [58, 73–79]. Individuals with greater levels of social support experience better coping and adjustment in the face of stressors. Adequate social support contributes to "adaptive" coping strategies that involve enacting a plan to reduce distress. By contrast, the absence of social support is associated with reliance on avoidant coping strategies, which represents a less effective coping response [77].

Social support influences patients' sense of personal control and overall well-being. Rheumatoid arthritis patients that report inadequate support also report poor coping mechanisms, more severe depression, and lower ratings of life satisfaction [78]. Blixen and Kippes [79] revealed that social support is strongly associated with better quality of life in patients with osteoarthritis even in the context of high levels of pain and discomfort. Relatedly, MG patients who live with a partner report more social support than patients who live alone [80].

Sources of social support for MG patients are available through national organizations (http://www.myastheniagravis.org/; http://www.myasthenia.org/; https://www.dailystrength.org/group/myasthenia-gravis; https://www.myaware.org; http://www.mdjunction.com/myasthenia-gravis). Regional chapters provide a forum for patients to discuss concerns and share insights, including effective coping strategies. Newsletters sponsored by these organizations keep patients abreast of research, clinical care issues, and regional social functions. Another key resource is available online. MG "chat rooms" (http://www.mdjunction.com/myasthenia-gravis; http://neurotalk.psychcentral.com/forum77.html and http://www.dailystrength.org/c/ Myasthenia-Gravis/support-group) link patients from across the world with 24/7 access to patient-centered information and discussion forums. These online resources are especially valuable to patients with restricted access to local chapters of the national organizations due to distance, transportation issues, work schedules, disease severity, etc.

Summary

Slightly more than one half of MG patients complain of memory loss, yet there is no empirical evidence that MG affects the CNS. A subset of

patients exhibit difficulties on tests of information processing and learning of information, which is likely caused by fatigue. Current data do not support the notion that psychological distress affects the onset or the course of MG. Most patients adjust well to the disease, with generally good levels of mental health and preserved quality of life. Anxiety is the most common mental health concern identified in previous studies, and this may be present at any stage of the disease. As such, clinicians are urged to evaluate mental health status as part of patient care regardless of disease severity.

Perceived control over personal health is an important buffer to mental health symptoms. Differentiation between factors that can be controlled using adaptive coping mechanisms and factors that cannot be controlled also fosters healthy adjustment to chronic illness. Coping with uncertainty is essential for individuals managing a disease with potential for fluctuations in symptom expression and severity. Finally, high levels of social support promote psychological well-being and quality of life. New and robust relationships can be established in person through local support groups and online through internet chat forums.

Clinicians are in a unique position to positively influence the psychological health of their patients. A physician-patient partnership that includes discussion of diagnostic methods, treatment options, and new scientific discoveries allows patients to be engaged in the management of their disease and care. Clinicians are also in an ideal position to develop the social support networks for their patients. Posting fliers in the waiting room announcing local support group meetings and events, dissemination of online resources, and monitoring of mental health symptoms and quality of life during routine clinic visits all represent key elements of holistic and most effective healthcare.

References

1. Paul RH, Cohen RA, Zawacki T, Gilchrist JM, Aloia MS. What have we learned about cognition in myasthenia gravis? A review of methods and results. Neurosci Biobehav Rev. 2001;25:75–81.
2. Mao Z, Yin J, Lu Z, Hu X. Association between myasthenia gravis and cognitive function: a systematic review and meta-analysis. Ann Indian Acad Neurol. 2015;18(2):131–7.
3. Ochs C, Bradley J, Katholi CR, Byl NN, Brown VM, Jones LL, et al. Symptoms of patients with myasthenia gravis receiving treatment. J Med. 1988;29:1–12.
4. Carter SL, Rourke SB, Murji S, Shore D, Rourke BP. Cognitive complaints, depression, medical symptoms, and their association with neuropsychological functioning in HIV infection: a structural equation model analysis. Neuropsychology. 2003;17(3):410–9.
5. Paul RH, Cohen RA, Gilchrist J, Aloia MS, Goldstein JM. Cognitive dysfunction in individuals with myasthenia gravis. J Neurol Sci. 2000;179:59–64.
6. Benedict RHB, Weinstock-Guttman B, Fishman I, Sharma J, Tjoa CW, Bakshi R. Prediction of neuropsychological impairment in multiple sclerosis: comparison of conventional magnetic resonance imaging measures of atrophy and lesion burden. Arch Neurol. 2004;61(2):226–30.
7. Green J, McDonald WM, Vitek JL, Evatt M, Freeman A, Haber M, et al. Cognitive impairments in advanced PD without dementia. Neurology. 2002;59(9):1320–4.
8. Bäckman L, Robins-Wahlin TB, Lundin A, Ginovart N, Farde L. Cognitive deficits in Huntington's disease are predicted by dopaminergic PET markers and brain volumes. Brain. 1997;120(12):2207–17.
9. Whiting J, Cooper J, Lindstrom JM. Antibodies in sera from patients with myasthenia gravis do not bind to nicotinic acetylcholine receptors from human brain. J Neuroimmunol. 1987;16:205–13.
10. Keesey JC. Does myasthenia affect the brain? J Neurol Sci. 1999;170:77–89.
11. Kluger BM, Krupp LB, Enoka RM. Fatigue and fatigability in neurologic illnesses proposal for a unified taxonomy. Neurology. 2013;80(4):409–16.
12. Sneddon J. Myasthenia gravis: a study of social, medical, and emotional problems in 26 patients. Lancet. 1980;1(8167):526–8.
13. Grohar-Murray ME, Becker A, Reilly S, Ricci M. Self-care actions to manage fatigue among myasthenia gravis patients. J Neurosci Nurs. 1998;30:191–9.
14. Elsais A, Wyller VB, Loge JH, Kerty E. Fatigue in myasthenia gravis: is it more than muscular weakness? BMC Neurol. 2013;13(1):132.
15. Steptoe A, Bolton J. The short-term influence of high and low intensity physical exercise on mood. Psychol Health. 1988;2:91–106.
16. Fiske AD, Schneider W. Controlled and automatic processing during tasks requiring sustained attention: a new approach to vigilance. Hum Factors. 1981;23:737–50.
17. Paul RH, Cohen RA, Gilchrist J. Ratings of subjective mental fatigue relates to cognitive performance in patients with myasthenia gravis. J Clin Neurosci. 2002;9:243–6.
18. Symonette CJ, Watson BV, Koopman WJ, Nicolle MW, Doherty TJ. Muscle strength and fatigue in patients with generalized myasthenia gravis. Muscle Nerve. 2010;41(3):362–9.

19. Jordan B, Schweden TL, Mehl T, Menge U, Zierz S. Cognitive fatigue in patients with myasthenia gravis. Muscle Nerve. 2017;56(3):449–57.
20. Paul RH, Cohen RA, Gilchrist J, Goldstein J. Fatigue and its impact on patients with myasthenia gravis. Muscle Nerve. 2000;23:1402–6.
21. Jefferson AL, Paul RH, Ozonoff AL, Cohen RA. Evaluating elements of executive functioning as predictors of instrumental activities of daily living (IADLs). Arch Clin Neuropsychol. 2006;21(4):311–20.
22. Cahn-Weiner DA, Farias ST, Julian L, Harvey DJ, Kramer JH, Reed BR, et al. Cognitive and neuroimaging predictors of instrumental activities of daily living. J Int Neuropsychol Soc. 2007;13(05):747–57.
23. Boyle PA, Paul RH, Moser DJ, Cohen RA. Executive impairments predict functional declines in vascular dementia. Clin Neuropsychol. 2004;18(1):75–82.
24. Phillips LH. The epidemiology of myasthenia gravis. Ann N Y Acad Sci. 2003;998:407–12.
25. Sherwood L. Human physiology: from cells to systems. 2nd ed. New York: West Publishing Company; 1993.
26. Blalock JE, Smith EM. A complete regulatory loop between the immune and neuroendocrine systems. Fed Proc. 1985;44:108–11.
27. Maes M, Bosmans E, Meltzer HY. Immunoendocrine aspects of major depression. Relationships between plasma interleukin-6 and soluble interleukin-2 receptor, prolactin and cortisol. Eur Arch Psychiatry Clin Neurosci. 1995;245:172–8.
28. Meyer E. Psychological disturbances in myasthenia gravis: a predictive study. Ann N Y Acad Sci. 1966;135:417–23.
29. Martin RD, Flegenheimer WV. Psychiatric aspects of the management of the myasthenic patient. Mt Sinai J Med. 1971;38:594–601.
30. Shinkai K, Ohmori O, Ueda N, Nakamura J, Amano T, Tsuji S. A case of myasthenia gravis preceded by major depression. J Neuropsychiatry Clin Neurosci. 2001;13:116–7.
31. Chafetz ME. Psychological disturbances in myasthenia gravis. Ann N Y Acad Sci. 1966;135:424–7.
32. Dube SR, Fairweather D, Pearson WS, Felitti VJ, Anda RF, Croft JB. Cumulative childhood stress and autoimmune diseases in adults. Psychosom Med. 2009;71(2):243–50.
33. Danese A, Pariante CM, Caspi A, Taylor A, Poulton R. Childhood maltreatment predicts adult inflammation in a life-course study. Proc Natl Acad SciU S A. 2007;104:1319–24.
34. Danese A, Moffitt TE, Harrington H, Milne BJ, Polanczyk G, Pariante CM, et al. Adverse childhood experiences and adult risk factors for age-related disease: depression, inflammation, and clustering of metabolic risk markers. Arch Pediatr Adolesc Med. 2009;163(12):1135–43.
35. Schwartz ML, Cahill R. Psychopathology associated with myasthenia gravis and its treatment by psychotherapeutically oriented group counseling. J Chronic Dis. 1971;24:543–52.
36. MacKenzie KR, Martin MH, Howard FM. Myasthenia gravis: psychiatric concomitants. Can Med Assoc J. 1969;100:988–91.
37. Santy PA. Underdiagnosed myasthenia gravis in emergency psychiatric referrals. Ann Emerg Med. 1983;12:397–8.
38. Beydoun SR, Wang J, Levine RL, Farvid A. Emotional stress as a trigger of myasthenic crisis and concomitant takotsubo cardiomyopathy: a case report. J Med Case Rep. 2010;4(1):393.
39. Kleinman A. The illness narratives. New York: Basic Books; 1988.
40. Richards HS, Jenkinson E, Rumsey N, Harrad RA. The psychosocial impact of ptosis as a symptom of myasthenia gravis: a qualitative study. Orbit. 2014;33(4):263–9.
41. Paradis CM, Friedman S, Lazar RM, Kula RW. Anxiety disorders in a neuromuscular clinic. Am J Psychiatry. 1993;150:1102–4.
42. Magni G, Micaglio GF, Lalli R, Bejato L, Candeago MR, Merskey H, Angelini C. Psychiatric disturbances associated with myasthenia gravis. Acta Psychiatr Scand. 1988;77:443–5.
43. Paul RH, Cohen RA, Goldstein J, Gilchrist J. Severity of mood, self-evaluative and vegetative symptoms in myasthenia gravis. J Neuropsychiatry Clin Neurosci. 2000;12:499–501.
44. Paul RH, Nash JM, Cohen RA, Gilchrist JM, Goldstein JM. Quality of life and well-being of patients with myasthenia gravis. Muscle Nerve. 2001;24:512–6.
45. Sabre L, Westerberg E, Liik M, Punga AR. Diversity in mental fatigue and social profile of patients with myasthenia gravis in two different Northern European countries. Brain Behav. 2017;7(4):e00653.
46. Ybarra MI, Kummer A, Frota ERC, Oliveira JTD, Gomez RS, Teixeira AL. Psychiatric disorders in myasthenia gravis. Arq Neuropsiquiatr. 2011;69(2A):176–9.
47. Nyenhuis DL, Rao SM, Zajecka JM, Luchetta T, Bernardin L, Garron DC. Mood disturbance versus other symptoms of depression in multiple sclerosis. JINS. 1995;1:291–6.
48. Brown ES, Suppes T. Mood symptoms during corticosteroid therapy: a review. Harv Rev Psychiatry. 1998;5:239–46.
49. Hoffmann S, Ramm J, Grittner U, Kohler S, Siedler J, Meisel A. Fatigue in myasthenia gravis: risk factors and impact on quality of life. Brain Behav. 2016;6(10):e00538.
50. Cioncoloni D, Casali S, Ginanneschi F, Carone M, Veronica B, Rossi A, et al. Major motor-functional determinants associated with poor self-reported health-related quality of life in myasthenia gravis patients. Neurol Sci. 2016;37(5):717–23.
51. Raggi A, Schiavolin S, Leonardi M, Antozzi C, Baggi F, Maggi L, et al. Development of the MG-DIS: an ICF-based disability assessment instrument for myasthenia gravis. Disabil Rehabil. 2014;36(7):546–55.

52. Padua L, Evoli A, Aprile I, Caliandro P, Mazza S, Padua R, et al. Health-related quality of life in patients with myasthenia gravis and the relationship between patient-oriented assessment and conventional measurements. Neurol Sci. 2001;22:363–9.
53. Rostedt A, Padua L, Stalberg EV. Correlation between regional myasthenic weakness and mental aspects of quality of life. Eur J Neurol. 2006;13:191–3.
54. House JS, Landis KR, Umberson D. Social relationships and health. Science. 1988;241:540–4.
55. Mishel MH. Reconceptualization of the uncertainty in illness theory. J Nurs Scholarsh. 1990;22:256–62.
56. Mishel MH. Perceived uncertainty and stress in illness. Res Nurs Health. 1984;7:163–71.
57. Mishel MH, Braden CJ. Uncertainty: a mediator between support and adjustment. West J Nurs Res. 1987;9:43–57.
58. Bennett SJ. Relationships among selected antecedent variables and coping effectiveness in postmyocardial infarction patients. Res Nurs Health. 1993;16:131–9.
59. Mullins LL, Cote MP, Fuemmeler BF, Jean VM, Beatty WW, Paul RH. Illness intrusiveness, uncertainty, and distress in individuals with multiple sclerosis. Rehabil Psychol. 2001;46:139–53.
60. Mishel MH, Sorenson DS. Uncertainty in gynecological cancer: a test of the mediating functions of mastery and coping. Nurs Res. 1991;40:167–71.
61. Mast ME. Adult uncertainty in illness: a critical review of research. Sch Inq Nurs Pract. 1995;9:3–23.
62. Braden CJ, Mishel MH, Longman AJ, Burns LR. Self-help intervention project: women receiving treatment for breast cancer. Cancer Pract. 1998;6:87–98.
63. Badger TA, Braden C, Mishel MH. Depression burden, self-help interventions, and side effect experience in women receiving treatment for breast cancer. Oncol Nurs Forum. 2001;28:567–74.
64. Mishel MH, Belyea M, Germino BB, Stewart JL, Bailey DE Jr, Robertson C, et al. Helping patients with localized prostate cancer manage uncertainty and treatment side effects: nurse delivered psychoeducational intervention via telephone. Cancer. 2002;94(6):1854–66.
65. Mishel MH, Germino BB, Belyea M, Stewart JL, Bailey DE Jr, Mohler J, et al. Moderators of outcomes from an uncertainty management intervention for men with localized prostate cancer. Nurs Res. 2003;52(2):89–97.
66. Mishel MH, Germino BB, Gil KM, Belyea M, Laney IC, Stewart J, et al. Benefits from an uncertainty management intervention for African-American and Caucasian older long-term breast cancer survivors. Psychooncology. 2005;14:962–78.
67. Devins GM, Binik YM, Hutchinson TA, Hollomby DJ, Barre PE, Guttmann RD. The emotional impact of end-stage renal disease: importance of patients' perceptions of intrusiveness and control. Int J Psychiatry Med. 1983–1984;13:327–43.
68. Devins GM. Illness intrusiveness and the psychosocial impact of lifestyle disruptions in chronic life-threatening disease. Adv Ren Replace Ther. 1994;1:251–63.
69. Devins GM, Seland TP, Klein GM, Edworthy SM, Saary MJ. Stability and determinants of psychosocial well-being in multiple sclerosis. Rehabil Psychol. 1993;38:11–26.
70. Devins GM. Illness intrusiveness and the psychosocial impact of end-stage renal disease. In: Hadry MA, Kiernan J, Kutscher AH, Cahill L, Bevenitsky AI, editors. Psychosocial aspects of end-stage renal disease: issues of our times. New York: Haworth Press; 1991. p. 83–102.
71. Devins GM, Shnek ZM. Multiple sclerosis. In: Frank RG, Elliott TR, editors. Handbook of rehabilitation psychology. Washington, DC: American Psychological Association; 2000. p. 163–84.
72. Devins GM, Stam HJ, Koopmans JP. Psychosocial impact of laryngectomy mediated by perceived stigma and illness intrusiveness. Can J Psychiatry. 1994;39:608–16.
73. Hough ES, Brumitt GA, Templin TN. Social support, demands of illness, and depression in chronically ill urban women. Health Care Women Int. 2000;20:349–62.
74. Cott CA, Gignac MAM, Badley EM. Determinants of self-rated health for Canadians with chronic disease and disability. J Epidemiol Community Health. 1999;53:731–6.
75. Brown GW, Andrews B, Harris T, Adler Z, Bridge L. Social support, self-esteem and depression. Psychol Med. 1986;6:238–47.
76. Holahan CK, Holahan CJ. Self-efficacy, social support, and depression in aging: a longitudinal analysis. J Gerontol. 1987;42(1):65–8.
77. Schreurs KMG, de Ridder DT. Integration of coping and social support perspectives: implications for the study of adaptation to chronic diseases. Clin Psychol Rev. 1997;17:89–112.
78. Smith CA, Dobbins CJ, Wallston KA. The mediational role of perceived competence in psychological adjustment to rheumatoid arthritis. J Appl Soc Psychol. 1991;21:1218–47.
79. Blixen CE, Kippes C. Depression, social support, and quality of life in older adults with osteoarthritis. Image J Nurs Sch. 1999;31:221–6.
80. Raggi A, Leonardi M, Mantegazza R, Casale S, Fioravanti G. Social support and self-efficacy in patients with Myasthenia Gravis: a common pathway towards positive health outcomes. Neurol Sci. 2010;31(2):231–5.

Myasthenia Gravis: Classification and Outcome Measurements

19

Nicholas J. Silvestri and Gil I. Wolfe

Clinical Classification of MG

Clinicians struggled to devise an adequate classification system for MG [1] over the second half of the twentieth century. In 1958, Osserman and colleagues [2, 3] proposed placing patients in five groups: I, localized (ocular); II, generalized (mild or moderate); III, acute fulminating; IV, late severe; and V, muscle atrophy. A separate category for neonatal and juvenile forms was created. Osserman and Genkins [4] later divided Group II into A (mild) and B (moderate) subclassifications and dropped the muscle atrophy group. Various modified Osserman criteria were suggested over the years, and some new schemes were developed [5–10]. The most widely used modification of Osserman's original classification is found in Table 19.1. However, the Osserman approach has been criticized for several shortcomings. These include the vague descriptive terminology, the potential for subjects to satisfy descriptions for more than one class at the same point in time, and the absence of a category for asymptomatic patients.

Table 19.1 Modified Osserman classification for MG

Group 1: Ocular
Group 2: Mild generalized
Group 3: Moderate to severe generalized
Group 4: Acute, severe, developing over weeks to months
Group 5: Late, severe with marked bulbar involvement

It became clear early on to the MGFA task force that they would have to tackle the difficult topic of MG classification, even if it did not bear directly on research outcomes [11, 12]. The task force defined five broad disease subdivisions, and the descriptors "mild, moderate, and severe" were borrowed from prior classifications with full realization of the subjective nature of such terminology (Table 19.2) [13]. For instance, two experienced MG clinicians might not always agree on whether a patient has mild or moderate generalized disease (Class II or III). A brief summary of the MGFA clinical classification follows. A pure ocular MG patient is denoted as Class I; there can be orbicularis oculi weakness, but any additional axial, limb, or oropharyngeal weakness places the patient in Classes II to V. The task force appreciated that some patients with MG have selective bulbar muscular weakness; therefore, separate distinctions for Classes II through IV are designated "a" if weakness is

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© Springer International Publishing AG, part of Springer Nature 2018
H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*, Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_19

Table 19.2 MGFA clinical classification [13]

Class I	Any ocular muscle weakness; may have weakness of eye closure. All other muscle strength is normal
Class II	Mild weakness affecting muscles other than ocular muscles; may also have ocular muscle weakness of any severity
	IIa. Predominantly affecting limb, axial muscles, or both. May also have lesser involvement of oropharyngeal muscles
	IIb. Predominantly affecting oropharyngeal, respiratory muscles, or both. May also have lesser or equal involvement of limb, axial muscles, or both
Class III	Moderate weakness affecting muscles other than ocular muscles; may also have ocular muscle weakness of any severity
	IIIa. Predominantly affecting limb, axial muscles, or both. May also have lesser involvement of oropharyngeal muscles
	IIIb. Predominantly affecting oropharyngeal, respiratory muscles, or both. May also have lesser or equal involvement of limb, axial muscles, or both
Class IV	Severe weakness affecting muscles other than ocular muscles; may also have ocular muscle weakness of any severity
	IVa. Predominantly affecting limb, axial muscles, or both. May also have lesser involvement of oropharyngeal muscles
	IVb. Predominantly affecting oropharyngeal, respiratory muscles, or both. May also have lesser or equal involvement of limb, axial muscles, or both
Class V	Defined as intubation, with or without mechanical ventilation, except when employed during routine postoperative management. The use of a feeding tube without intubation places the patient in class IVb

predominantly limb/axial or "b" if weakness is predominantly oropharyngeal/respiratory. A patient requiring intubation is Class V. Use of a feeding tube without intubation places the patient in Class IVb. The most severely affected muscle groups should guide class assignment, and a "maximum severity designation" denotes the most severe pretreatment classification, which can be used as a historical point of reference. The clinical classification is not intended for use as an outcome measure. Its primary purpose is to identify subgroups of patients who share similar clinical features. The classification continues to be used for patient entry criteria in many clinical trials including the NIH-funded study of thymectomy in non-thymomatous MG [14] and a randomized trial of methotrexate in generalized disease [15].

Quantitative MG Score (QMG)

The QMG is likely the best studied objective outcome measure in MG. The current QMG is an expansion and modification of the scale first developed by Bessinger and colleagues in the early 1980s [7, 16]. The original QMG consisted of eight items, each graded 0–3, with a score of 3 being the most severe. Tindall et al. expanded the scale to 13 items and used this version of the QMG as the primary efficacy measurement in 2 trials that determined that cyclosporine was an effective therapy for MG [17, 18]. Barohn et al. [19] replaced three items in Tindall's scale that were subjective in nature—facial muscles, chewing, and swallowing—so that each item on the QMG was scored objectively (Table 19.3). Interrater reliability testing was performed on this scale in advance of a randomized, placebo-controlled study of intravenous gamma globulin in MG that utilized the modified QMG as a primary outcome measure [20]. At a 95% confidence level, QMG scores did not differ by more than 2.63 units, translating to a required sample size of 17 patients per treatment arm in a placebo-controlled trial to detect a significant difference at a power of 0.80 [19].

Tindall's version of the QMG was found to have concurrent validity, meaning that in a single visit, the QMG, manual muscle testing, functional scores, and patient self-evaluation show good agreement [21]. The latest version of the QMG was tested for responsiveness, the QMG's sensitivity to clinical change versus "noise" that can be expected even in clinically unchanged subjects, as well as longitudinal construct valid-

Table 19.3 Quantitative MG score (QMG)

Test items weakness (score)	None (0)	Mild (1)	Moderate (2)	Severe (3)	Item score (0, 1, 2 or 3)
1. Double vision on lateral gaze right or left (*circle one*), seconds	61	11–60	1–10	Spontaneous	
2. Ptosis (upward gaze), seconds	61	11–60	1–10	Spontaneous	
3. Facial muscles	Normal lid closure	Complete, weak, some resistance	Complete, without resistance	Incomplete	
4. Swallowing 4 oz./120 mL water	Normal	Minimal coughing or throat clearing	Severe coughing/ choking or nasal regurgitation	Cannot swallow (test not attempted)	
5. Speech following counting aloud from 1 to 50 (onset of dysarthria)	None at #50	Dysarthria at #30–49	Dysarthria at #10–29	Dysarthria at #9	
6. Right arm outstretched (90° sitting), seconds	240	90–239	10–89	0–9	
7. Left arm outstretched (90° sitting), seconds	240	90–239	10–89	0–9	
8. Vital capacity (% predicted) mouthpiece or facemask (*circle one; best of 3*)	≥80%	65–79%	50–64%	<50%	
9. Right hand grip: (*best of 2*) male (KgW) female	≥45 ≥30	15–44 10–29	5–14 5–9	0–4 0–4	
10. Left hand grip: (*best of 2*) male (KgW) female	≥35 ≥25	15–34 10–24	5–14 5–9	0–4 0–4	
11. Head, lifted (45° supine), seconds	120	30–119	1–29	0	
12. Right leg outstretched (45° supine), seconds	100	31–99	1–30	0	
13. Left leg outstretched (45° supine), seconds	100	31–99	1–30	0	
				Total QMG Score (range 0–39)	

ity, meaning how changes in the score correspond to clinically relevant change [22]. The calculated index of responsiveness of 1.45 is considered excellent for clinical outcome measures. A drop of 2.3 points in the score was correlated with clinical improvement as assessed by neurologist experts. The direction of QMG change validly reflected gestalt impressions of patient status (improved, unchanged, or worse) and correlated tightly with changes in a manual muscle testing score ($p < 0.0001$).

The 2000 MGFA task force report recommended the QMG be used in all prospective studies of therapy for MG [13]. In addition, it encouraged proposals to improve the QMG, including "weighting" of subscores derived from the total scale to reflect regional impairment seen in many MG patients, a point emphasized by other authors [22]. In the 2012 task force report, the MG Composite, a simpler instrument to be discussed in a later section, received the panel recommendation [23].

The QMG has been used in studies of mycophenolate mofetil, tacrolimus, etanercept, thymectomy, and eculizumab [14, 24–28]. An instructional training video and manual for the QMG are available from the MGFA [29]. The QMG can be completed in 20–30 min. A spirometer and handheld dynamometer are the only required equipment.

Myasthenia Gravis Manual Muscle Test (MG-MMT)

Investigators at the Duke University Medical Center developed a disease-specific manual muscle test that can be performed at the bedside without specialized equipment (Table 19.4) [30]. Thirty muscle groups usually affected by MG (6 cranial nerve/24 axial-limb) are measured on a 0–4 scale (0 = normal; 1 = 25% weak/mild impairment; 2 = 50% weak/moderate impairment; 3 = 75% weak/severe impairment; 4 = paralyzed/unable to do). The MG-MMT demonstrates good interrater reliability with a mean difference between scores of 1.3 ± 1.8 points. It correlates well with the QMG [22, 30]. However, there is wide scatter of MG-MMT values within general disease classifications, an issue also observed with the QMG. Advantages of the MG-MMT over the QMG are that it can be performed by the physician as part of a routine clinic visit, takes less time, and requires no specialized equipment. Unlike the QMG and MG Composite, the MG-MMT does not directly assess swallowing, speech, or respiratory function. The MG-MMT has been used in studies of mycophenolate mofetil, etanercept, and methotrexate in MG [15, 24, 27, 31]. Of note, the MG-MMT was found to be more sensitive to change than the QMG in the mycophenolate mofetil trial at the end of the blinded phase (week 12) and open-label extension (week 36) [32].

Table 19.4 Myasthenia gravis manual muscle test

	Right	Left	Sum
Lid ptosis	___	___	___
Diplopia	___	___	___
Eye closure			___
Cheek puff			___
Tongue protrusion			___
Jaw closure			___
Neck flexion			___
Neck extension			___
Shoulder abduction (deltoid)	___	___	___
Elbow flexion (biceps)	___	___	___
Elbow extension (triceps)	___	___	___
Wrist extension	___	___	___
Grip	___	___	___
Hip flexion (iliopsoas)	___	___	___
Knee extension (quadriceps)	___	___	___
Knee flexion (hamstrings)	___	___	___
Ankle dorsiflexion	___	___	___
Ankle plantar flexion	___	___	___
Total score			___

Score each function as follows: 0, normal; 1, 25% weak/mild impairment; 2, 50% weak/moderate impairment; 3, 75% weak/severe impairment; 4, paralyzed/unable to do. In addition, record any condition other than MG causing weakness in any of these muscles

Myasthenic Muscle Score (MMS)

This scoring system has been used by French investigators and was developed by Gajdos and colleagues in 1983 [33]. The MMS summates nine independent functions that encompass cranial, neck, truncal, and limb strength (Table 19.5). The total score ranges between 0 and 100. Unlike the scales described earlier, a

Table 19.5 Myasthenic muscle score

Task	Score
Maintain upper limbs horizontally outstretched: 1 point per 10 s	0–15
Maintain lower limbs above bed plane while lying on back: 1 point per 5 s	0–15
Raise head above bed plane while lying on back	
Against resistance	10
Without resistance	5
Impossible	0
Sit up from lying position	
Without help of hands	10
Impossible	0
Extrinsic ocular musculature	
Normal	10
Ptosis	5
Double vision	0
Eyelid occlusion	
Complete	10
Mild weakness	7
Incomplete with corneal covering	5
Incomplete without corneal covering	0
Chewing	
Normal	10
Weak	5
Impossible	0
Swallowing	
Normal	10
Impaired without aspiration	5
Impaired with aspiration	0
Speech	
Normal	10
Nasal	5
Slurred	0
Total score	

higher score on the MMS connotes better strength and function. Pulmonary function is not assessed in the MMS. The MMS has high interrater reliability and showed slightly higher agreement between observers than the QMG in one study [34]. This study also determined that the high interobserver agreement was not dependent to a significant degree on any single item, suggesting a high reliability for individual constituents of both the MMS and QMG. Both the MMS and QMG correlated well with a five-grade functional disease classification. Less robust was the correlation with a patient self-evaluation tool [21, 34].

MG Activities of Daily Living (MG-ADL) Profile

The MG-ADL is a simple eight-point questionnaire which focuses on common symptoms reported by MG patients (Table 19.6) [35]. It was developed at the University of Texas Southwestern Medical Center to complement the QMG. Each item of the profile is graded 0 (normal) to 3 (most severe). A provider asks the patient the eight questions and records the responses. It can also be self-administered after minimal training. It is worthy to note that the MG-ADL is not a quality of life (QOL) scale. The MG-ADL has correlated well with the QMG [35], MG Composite, and MG-Quality of Life 15 (MG-QOL15) [36]. The MG-ADL was found to be more sensitive to change than the QMG in the mycophenolate mofetil trial at the end of the blinded phase (week 12) and open-label extension (week 36) [32].

A two-point reduction in the score predicted clinical improvement [36]. No specialized training is required, and it can be administered in 5 min or less. The MG-ADL has been applied widely in retrospective and prospective clinical studies [20, 37–39]. It recently served as the primary outcome measure in a Phase 3 study of eculizumab in myasthenia gravis.

Table 19.6 MG activities of daily living (MG-ADL) profile

Grade	0	1	2	3	Score (0, 1, 2 or 3)
1. Talking	Normal	Intermittent slurring or nasal speech	Constant slurring or nasal, but can be understood	Difficult to understand speech	
2. Chewing	Normal	Fatigue with solid food	Fatigue with soft food	Gastric tube	
3. Swallowing	Normal	Rare episode of choking	Frequent choking necessitating changes in diet	Gastric tube	
4. Breathing	Normal	Shortness of breath with exertion	Shortness of breath at rest	Ventilator dependence	
5. Impairment of ability to brush teeth or comb hair	None	Extra effort, but no rest periods needed	Rest periods needed	Cannot do one of these functions	
6. Impairment of ability to arise from a chair	None	Mild, sometimes uses arms	Moderate, always uses arms	Severe, requires assistance	
7. Double vision	None	Occurs, but not daily	Daily, but not constant	Constant	
8. Eyelid droop	None	Occurs, but not daily	Daily, but not constant	Constant	
				MG-ADL score total (items 1–8)	**=**

MG Composite (MGC)

One of the newer outcome measures for MG, the MGC, was constructed from items in the QMG, MG-ADL, and MMT based on item performance observed in two multicenter clinical trials of mycophenolate mofetil in generalized disease [40]. Ten items were initially chosen, and response options were weighted based on input from an international panel of MG experts. The weighting was based on health risk, quality of life, and prognosis among other factors. For instance, breathing item response categories were weighed more heavily (maximal score of 9) than those for ptosis (maximal score of 3). Both weighted and unweighted versions of the MGC correlated well with investigator global impressions from the Muscle Study Group mycophenolate mofetil trial [40]. The ten-item MGC contains two ocular, five bulbar/facial/neck, one respiratory, and two limb items (Table 19.7). Four of the items were borrowed from the MG-MMT and four from the MG-ADL.

Concurrent and construct validation of the MGC was assessed in over 150 patients over two consecutive office visits, comparing the MGC to the MG-MMT, MG-ADL, and MG-QOL15 [41]. The MGC demonstrated excellent concurrent validity for both visits with the other MG scales. Longitudinal construct validity and responsiveness analyses across the two visits showed that a three-point change in the MGC score signified a change in clinical status. A smaller cohort was studied to demonstrate excellent test-retest reliability, with a coefficient of 98% [41].

Psychometric evaluation of the MGC using a Rasch model has been performed [42]. This type of analysis ranks the ability of different measure items to differentiate between levels of disability (item difficulty) and how individual patients relate to each other (person ability). As a weighted scale, the MGC provided a unique opportunity for psychometric analysis to assess the appropriateness or fit of the weighting of each response category. The Rasch analysis showed that there was no significant order distortion between response categories, that the expected category response values fit well with the item weighting, and that it was appropriate to summate the items for a total score. The only items that performed

Table 19.7 MG composite

Ptosis, upward gaze (physician examination)	>45 s = *0*	11–45 s = *1*	1–10 s = *2*	Immediate = *3*
Double vision on lateral gaze, left or right (physician examination)	>45 s = *0*	11–45 s = *1*	1–10 s = *3*	Immediate = *4*
Eye closure (physician examination)	Normal = *0*	Mild weakness (can be forced open with effort) = *0*	Moderate weakness (can be forced open easily) = *1*	Severe weakness (unable to keep eyes closed) = *2*
Talking (patient history)	Normal = *0*	Intermittent slurring or nasal speech = *2*	Constant slurring or nasal but can be understood = *4*	Difficult to understand speech = *6*
Chewing (patient history)	Normal = *0*	Fatigue with solid food = *2*	Fatigue with soft food = *4*	Gastric tube = *6*
Swallowing (patient history)	Normal = *0*	Rare episode of choking or trouble swallowing = *2*	Frequent trouble swallowing e.g. necessitating changes in diet = *5*	Gastric tube = *6*
Breathing (thought to be caused by MG)	Normal = *0*	Shortness of breath with exertion = *2*	Shortness of breath at rest = *4*	Ventilator dependence = *9*
Neck flexion or extension (weakest) (physician examination)	Normal = *0*	Mild weakness = *1*	Moderate weakness (i.e. ~50% weak, ±15%) = *3*	Severe weakness = *4*
Shoulder abduction (physician examination)	Normal = *0*	Mild weakness = *2*	Moderate weakness (i.e. ~50% weak, ±15%) = *4*	Severe weakness = *5*
Hip flexion (physician examination)	Normal = *0*	Mild weakness = *2*	Moderate weakness (i.e. ~50% weak, ±15%) = *4*	Severe weakness = *5*
			Total score	

Please note that "moderate weakness" for neck and limb items should be construed as weakness that equals roughly 50 ± 15% of expected normal strength. Any weakness milder than that would be "mild" and any weakness more severe than that would be classified as "severe"

less than optimally were diplopia and ptosis, but the impact of the misfitting or order distortion was found to be minor, and no changes in the MGC were proposed [42]. Of note, in another psychometric analysis using the Rasch model in over 200 patients, an argument was made in favor of some modification of both the MGC and the QMG [43]. Score thresholds demonstrated suboptimal order, suggesting poor discrimination between response options such as fatigue with soft vs. solid food. Such thresholds had not been reported in the earlier Rasch analysis [42]. Disordered thresholds were observed in 6 of 10 MGC items and 4 of 13 QMG items [43]. Test-retest reliability was good for both measures.

The MGC requires no equipment and can be completed in 5 min. It was used as a secondary outcome in the methotrexate trial [15].

MG Disability Assessment (MG-DIS)

This novel patient-reported disability scale addresses impairments and activity limitations in MG [44]. From 31 items identified by patients to address disease-related limitations, a final scale of 20 items was generated [45]. Items can be divided into four categories: generalized impairment, bulbar-related problems, mental health and fatigue, and vision-related problems. In a valida-

tion study, the MG-DIS was superior to general health/disability surveys including the World Health Organization Disability Assessment Schedule 2.0 (WHODAS 2.0) and the Short Form-36 in discriminating between different MG clinical classes [45]. It showed good test-retest reliability and correlated favorably with the MGC ($r = 0.642$, $p < 0.001$).

MG-Quality of Life 15 (MG-QOL15) Score

In general, health-related quality of life (QOL) measures attempt to survey patients' subjective assessment of their disability caused by a disease state and their degree of satisfaction or dissatisfaction with function. The use of patient-reported outcome measures has gained wide acceptance both in the clinical setting and in clinical trials, particularly with regard to assessment of QOL [46]. The MG-QOL15 is a 15-item MG-specific, self-administered scale, which includes test items that focus on psychological well-being and social functioning [47]. It is designed to inform the treating physician of the patient's own perception of disability due to MG which can then be factored into treatment decisions. The MG-QOL15 can also be followed over time to determine the efficacy of interventions. From a clinical trial standpoint, the MG-QOL15 has been used as a secondary outcome measure in clinical trials of methotrexate [15] and eculizumab [28].

The MG-QOL15 was revised using Rasch analysis (MG-QOL 15R), a process that improved slightly its clinimetric properties and both face and content validity (Table 19.8) [48]. While both scales are validated, the MG-QOL15R is preferred given its superior content validity and simpler interpretation in the clinical setting. The MG-QOL15R takes less than 5 min to administer and can be completed independently by the patient after a brief, straightforward instruction.

Table 19.8 MG-QOL15R

Please indicate how true each statement has been (over the past few weeks)	Not at all 0	Somewhat 1	Very much 2
1. I am frustrated by my MG			
2. I have trouble with my eyes because of my MG (e.g. double vision)			
3. I have trouble eating because of MG			
4. I have limited my social activity because of my MG			
5. My MG limits my ability to enjoy hobbies and fun activities			
6. I have trouble meeting the needs of my family because of my MG			
7. I have to make plans around my MG			
8. I am bothered by limitations in performing my work (include work at home) because of my MG.			
9. I have difficulty speaking due to MG			
10. I have lost some personal independence because of my MG (e.g. driving, shopping, running errands)			
11. I am depressed about my MG			
12. I have trouble walking due to MG			
13. I have trouble getting around public places because of my MG			
14. I feel overwhelmed by my MG			
15. I have trouble performing my personal grooming needs due to MG			
		Total MGQOL15R score	

MG Impairment Index (MGII)

The recently developed MGII is unique in that it followed Food and Drug Administration and Consensus-Based Standards for the Selection of Health Measurement Instruments Group guidelines in incorporating patient input in its development [49]. The MGII measures 22 items judged to be most relevant by patients with MG, particularly those triggered by activity or that fluctuate and might not otherwise be easily assessed during a routine clinic visit. Of the 22 patient-reported items gleaned from more than 90 surveys of patients and MG experts, 6 relate to ocular, 3 to eating, 7 to speaking/breathing, and 6 to general/trunk/limb function. Similar to the MGC and to add an element of objectivity, six examination items were incorporated: diplopia, ptosis, lower facial strength, arm endurance, leg endurance, and neck endurance. The MGII has excellent reliability and is easy to implement in the clinical setting. MGII scores correlate well with other validated scales including the QMG, MG-ADL, MGC, and MG-QOL15R. It will likely be used in future clinical trials.

MGFA Therapy Status

The MGFA task force developed a system to describe treatment regimens for MG patients enrolled in clinical studies (Table 19.9) [13]. The various abbreviations may be used individually or in combination to summarize management. In addition, the duration of treatment, current and prior doses of pertinent medications, and schedule of plasma exchange or intravenous gamma globulin infusions should be recorded.

Table 19.9 MGFA MG therapy status

NT	No therapy
SPT	Status-post thymectomy (record type of resection)
CH	Cholinesterase inhibitors
PR	Prednisone
IM	Immunosuppressive therapy other than prednisone (define)
PE(a)	Plasma exchange therapy, acute (for exacerbations or preoperatively)
PE(c)	Plasma exchange therapy, chronic (used on a regular basis)
IG(a)	IVIg therapy, acute (for exacerbations or preoperatively)
IG(c)	IVIg therapy, chronic (used on a regular basis)
OT	Other forms of therapy (define)

MGFA Postintervention Status (PIS)

Assessment of postintervention status (PIS) designates the clinical state of MG patients at any time after initiation of treatment [13]. It can be used for routine follow-up or in formal clinical trials (Table 19.10). PIS should be determined by a clinician skilled in the evaluation of MG patients as it is based on careful clinical evaluation with both symptoms and signs taken into account. Of note, the MGFA task force concluded that isolated weakness of eyelid closure does not necessarily represent a sign of active disease, and, therefore, this finding would not exclude patients from qualifying for complete stable remission or pharmacologic remission. Patients

Table 19.10 MGFA postintervention status

Complete stable remission (CSR)	The patient has had no symptoms or signs of MG for at least 1 year and has received no therapy for MG during that time. There is no weakness of any muscle on careful examination by someone skilled in the evaluation of neuromuscular disease. Isolated weakness of eye closure is accepted
Pharmacologic remission (PR)	The same criteria as for CSR except that the patient continues to take some form of therapy for MG. Patients taking cholinesterase inhibitors are excluded from this category because their use suggests the presence of weakness
Minimal manifestation (MM)	The patient has no symptoms or functional limitations from MG but has some weakness on examination of some muscles. This class recognizes that some patients who otherwise meet the definition of CSR or PR do have weakness that is only detectable by careful examination

(continued)

Table 19.10 (continued)

	MM-0: The patient has received no MG treatment for at least 1 year MM-1: The patient continues to receive some form of immunosuppression but no cholinesterase inhibitors or other symptomatic therapy MM-2: The patient has received only low dose cholinesterase inhibitors (<120 mg pyridostigmine per day) for at least 1 year MM-3: The patient has received cholinesterase inhibitors or other symptomatic therapy and some form of immunosuppression during the past year
Change in status	
Improved (I)	A substantial decrease in pretreatment clinical manifestations or a sustained substantial reduction in MG medications as defined in the protocol. In prospective studies, this should be defined as a specific decrease in QMG score
Unchanged (U)	No substantial change in pretreatment clinical manifestations or reduction in MG medications as defined in the protocol. In prospective studies, this should be defined in terms of a maximum change in QMG score
Worse (W)	A substantial increase in pretreatment clinical manifestations or a substantial increase in MG medications as defined in the protocol. In prospective studies, this should be defined as a specific increase in QMG score
Exacerbation (E)	Patients who have fulfilled criteria for CSR, PR, or MM but subsequently developed clinical findings greater than permitted by these criteria
Died of MG (D of MG)	Patients who died of MG, of complications of MG therapy, or within 30 days after thymectomy. List the cause (see morbidity and mortality table)

receiving cholinesterase inhibitors were excluded from the pharmacologic remission category, however, since these agents may mask myasthenic symptoms and signs.

For prospective studies, it was recommended that PIS change of status categories (improved, unchanged, or worse) be linked to predetermined changes in the QMG score. In certain studies, the PIS may be the most important element in outcome determination. PIS classification was used to guide corticosteroid dosing adjustments in prospective trials of mycophenolate mofetil [50], methotrexate [15], and transsternal thymectomy [14, 20] and was included as outcome assessments in open-label studies of tacrolimus [26, 51] and a blinded, crossover trial of eculizumab [28]. A PIS category of minimal manifestations or better has been used to define the goals of treatment in the MGFA-sponsored international consensus guidance statement for MG management [52].

References

1. Vincent A, Palace J, Hilton-Jones D. Myasthenia gravis. Lancet. 2001;357:2122–8.
2. Osserman KE. Clinical aspects. In: Osserman KE, editor. Myasthenia gravis. New York: Grune & Stratton; 1958. p. 79–80.
3. Osserman KE, Kornfeld P, Cohen E, Genkins G. Studies in myasthenia gravis: review of two hundred eighty-two cases at the Mount Sinai Hospital, New York City. AMA Arch Intern Med. 1958;102:72–81.
4. Osserman KE, Genkins G. Studies in myasthenia gravis: review of a twenty-year experience in over 1200 patients. Mt Sinai J Med. 1971;38:497–537.
5. Jaretzki A III, Penn AS, Younger DS, Wolff M, Olarte MR, Lovelace RE, et al. "Maximal" thymectomy for myasthenia gravis. Results. J Thorac Cardiovasc Surg. 1988;95:747–57.
6. Barohn RJ, Jackson CE. New classification system for myasthenia gravis (abstract). J Child Neurol. 1994;9:205.
7. Besinger UA, Toyka KV, Heininger K, et al. Long-term correlation of clinical course and acetylcholine receptor antibody in patients with myasthenia gravis. Ann N Y Acad Sci. 1981;377:812–5.
8. Olanow CW, Wechsler AS, Sirotkin-Roses M, Stajich J, Roses AD. Thymectomy as primary therapy in myasthenia gravis. Ann N Y Acad Sci. 1987;505:595–606.
9. Wolfe GI, Barohn RJ. Neuromuscular junction disorders of childhood. In: Swaiman KF, Ashwal S, Ferriero DM, editors. Pediatric neurology: principles and practice. 4th ed. Philadelphia: Mosby Elsevier; 2006. p. 1941–68.

10. Oosterhuis HJ. Observations of the natural history of myasthenia gravis and the effect of thymectomy. Ann N Y Acad Sci. 1981;377:678–90.
11. Barohn RJ. Standards of measurement in myasthenia gravis. Ann N Y Acad Sci. 2003;998:432–9.
12. Jaretzki AI. Thymectomy for myasthenia gravis: analysis of the controversies regarding technique and result. Neurology. 1997;48(Suppl 5):S52–63.
13. Jaretzki A III, Barohn RJ, Ernstoff RM, Kaminski HJ, Keesey JC, Penn AS, et al. Myasthenia gravis: recommendations for clinical research standards. Neurology. 2000;55:16–23.
14. Wolfe GI, Kaminski HJ, Aban IB, Minisman G, Kuo H-C, Marx A, et al. Randomized trial of thymectomy in myasthenia gravis. N Engl J Med. 2016;375:511–2.
15. Pasnoor M, He J, Herbelin L, Burns TM, Nations S, Bril V, et al. A randomized controlled trial of methotrexate for patients with generalized myasthenia gravis. Neurology. 2016;87:57–64.
16. Besinger UA, Toyka KV, Homberg M, Heininger K, Hohlfeld R, Fateh-Moghadam A. Myasthenia gravis: long-term correlation of binding and bungarotoxin blocking antibodies against acetylcholine receptors with changes in disease severity. Neurology. 1983;33:1316–21.
17. Tindall RSA, Phillips JT, Rollins JA, Wells L, Hall K. A clinical therapeutic trial of cyclosporine in myasthenia gravis. Ann N Y Acad Sci. 1993;681:539–51.
18. Tindall RSA, Rollins JA, Phillips JT, Greenlee RG, Wells L, Belendiuk G. Preliminary results of a double-blind, randomized, placebo-controlled trial of cyclosporine in myasthenia gravis. N Engl J Med. 1987;316:719–24.
19. Barohn RJ, McIntire D, Herbelin L, Wolfe GI, Nations S, Bryan WW. Reliability testing of the quantitative myasthenia gravis score. Ann N Y Acad Sci. 1998;841:769–72.
20. Wolfe GI, Barohn RJ, Foster BM, Jackson CE, Kissel JT, Day JW, et al. Randomized, controlled trial of intravenous immunoglobulin in myasthenia gravis. Muscle Nerve. 2002;26:549–52.
21. Sharshar T, Chevret S, Mazighi M, Chillet P, Huberfeld G, Berreotta C, et al. Validity and reliability of two muscle strength scores commonly used as endpoints in assessing treatment of myasthenia gravis. J Neurol. 2000;246:286–9.
22. Bedlack RS, Simel DL, Bosworth H, Samsa G, Tucker-Lipscomb B, Sanders DB. Quantitative myasthenia gravis score: assessment of responsiveness and longitudinal validity. Neurology. 2005;64:1968–70.
23. Benatar M, Sanders DB, Burns TM, Cutter GR, Guptill JT, Baggi F, et al. Recommendations for myasthenia gravis clinical trials. Muscle Nerve. 2012;45:909–17.
24. Meriggioli MN, Ciafaloni E, Al-Hayk KA, Rowin J, Tucker-Lipscomb B, Massey JM, et al. Mycophenolate mofetil for myasthenia gravis: an analysis of efficacy, safety, and tolerability. Neurology. 2003;61:1438–40.
25. Nagane Y, Utsugisawa K, Obara D, Kondoh R, Terayama Y. Efficacy of low-dose FK506 in the treatment of myasthenia gravis—a randomized pilot study. Eur Neurol. 2005;53:146–50.
26. Ponseti JM, Azem J, Fort JM, Codina A, Montoro JB, Armengol M. Benefits of FK506 (tacrolimus) for residual, cyclosporin- and prednisone-resistant myasthenia gravis: one-year follow-up of an open-label study. Clin Neurol Neurosurg. 2005;107:187–90.
27. Rowin J, Meriggioli MN, Tüzün E, Leurgans S, Christadoss P. Etanercept treatment in cortocosteroid-dependent myasthenia gravis. Neurology. 2004;63:2390–2.
28. Howard JF, Barohn RJ, Cutter GR, Freimer M, Juel VC, Mozaffar T, et al. A randomized, double-blind, placebo-controlled phase II study of eculizumab in patients with refractory generalized myasthenia gravis. Muscle Nerve. 2013;48:76–84.
29. Barohn RJ. Video: how to administer the quantitative myasthenia test. In. St. Paul, MN 55104: Myasthenia Gravis Foundation of America, Inc. 1821 University Ave.W. Suite S256; 1996.
30. Sanders DB, Tucker-Lipscomb B, Massey JM. A simple manual muscle test for myasthenia gravis. Ann N Y Acad Sci. 2003;998:440–4.
31. Ciafaloni E, Massey JM, Tucker-Lipscomb B, Sanders DB. Mycophenolate mofetil for myasthenia gravis: an open-label pilot study. Neurology. 2001;56:97–9.
32. Wolfe GI, Barohn RJ, Sanders DB, McDermott MP, Muscle Study Group. Comparison of outcome measures from a trial of mycophenolate mofetil in myasthenia gravis. Muscle Nerve. 2008;38:1429–33.
33. Gajdos P, Simon N, de Rohan Chabot P, Goulon M. Effets a long terme des echanges plasmatiques au cours de la myasthenie. Resultats d'une etude randomisee. Presse Med. 1983;12:939–42.
34. Gajdos P, Sharshar T, Chevret S. Standards of measurement in myasthenia gravis. Ann N Y Acad Sci. 2003;998:445–52.
35. Wolfe GI, Herbelin L, Nations SP, Foster B, Bryan WW, Barohn RJ. Myasthenia gravis activities of daily living profile. Neurology. 1999;52:1487–9.
36. Muppidi S, Wolfe GI, Conaway M, Burns TM. MG-ADL: still a relevant outcome measure. Muscle Nerve. 2011;44:727–31.
37. Wolfe GI, Kaminski HJ, Jaretzki A III, Swan A, Newsom-Davis J. Development of a thymectomy trial in nonthymomatous myasthenia gravis patients receiving immunosuppressive therapy. Ann N Y Acad Sci. 2003;998:473–80.
38. Takamori M, Motomura M, Kawaguchi N, Nemoto Y, Hattori T, Yoshikawa Y, et al. Anti-ryanodine receptor antibodies and FK506 in myasthenia gravis. Neurology. 2004;62:1894–6.
39. Kawaguchi N, Yoshiyama Y, Nemoto Y, Munakata S, Fukutake T, Hattori T. Low-dose tacrolimus treatment in thymectomised and steroid-dependent myasthenia gravis. Curr Med Res Opin. 2004;20:1269–73.

40. Burns TM, Conaway MR, Cutter GR, Sanders DB, Muscle Study Group. Construction of an efficient evaluative instrument for myasthenia gravis: the MG composite. Muscle Nerve. 2008;38:1553–62.
41. Burns TM, Conaway M, Sanders DB. The MG composite: a valid and reliable outcome measure for myasthenia gravis. Neurology. 2010;74:1434–40.
42. Sadjadi R, Conaway M, Cutter G, Sanders DB, Burns TM. Psychometric evaluation of the myasthenia gravis composite using Rasch analysis. Muscle Nerve. 2012;45:820–5.
43. Barnett C, Merkies ISJ, Katzberg H, Bril V. Psychometric properties of the quantitative myasthenia gravis score and the myasthenia gravis composite scale. J Neuromuscul Dis. 2015;2:301–11.
44. Raggi A, Schiavolin S, Leonardi M, Antozzi C, Baggi F, Maggi L, et al. Development of the MG-DIS: an ICF-based disability assessment instrument for myasthenia gravis. Disabil Rehabil. 2014;36:546–55.
45. Raggi A, Leonardi M, Schiavolin S, Antozzi C, Brenna G, Maggi L, et al. Validation of the MG-DIS: a disability assessment for myasthenia gravis. J Neurol. 2016;263:871–82.
46. Burns TM. History of outcome measures for myasthenia gravis. Muscle Nerve. 2010;42:5–13.
47. Burns TM, Grouse C, Wolfe GI, Conaway MR, Sanders DB, et al. The MG-QOL15 for following the health-related quality of life of patients with myasthenia gravis. Muscle Nerve. 2011;43:14–8.
48. Burns TM, Sadjadi R, Utsugisawa K, Gwathmey KG, Joshi A, Jones S, et al. International clinimetric evaluation of the MG-QOL15, resulting in slight revision and subsequent validation of the MG-QOL15R. Muscle Nerve. 2016;54:1015–22.
49. Barnett C, Bril V, Kapral M, Kulkarni A, Davis AM. Development and validation of the Myasthenia Gravis Impairment Index. Neurology. 2016;87:879–86.
50. Sanders DB, Hart IK, Mantegazza R, Shukla SS, Siddiqi ZA, De Baets MHV, et al. An international, phase III, randomized trial of mycophenolate mofetil in myasthenia gravis. Neurology. 2008;71:400–6.
51. Ponseti JM, Azem J, Fort JM, López-Cano M, Vilallonga R, Buera M, et al. Long-term results of tacrolimus in cyclosporine- and prednisone-dependent myasthenia gravis. Neurology. 2005;64:1641–3.
52. Sanders DM, Wolfe GI, Benatar M, Evoli A, Gilhus NE, Illa I, et al. International consensus guidance for the management of myasthenia gravis: executive summary. Neurology. 2016;87:419–25.

Emerging Therapeutics for Myasthenia Gravis

20

Anna Rostedt Punga, Henry J. Kaminski, and Jeffrey T. Guptill

Established Therapies

Treatment of myasthenia gravis (MG) can be divided into two classes: immunosuppressive and symptomatic. Since MG is a chronic disorder, long-term immunosuppressive medications are often applied and include corticosteroids, general corticosteroid-sparing immunosuppressive agents such as azathioprine and mycophenolate (which inhibit T cells), and more specific targeting treatment with rituximab (which inhibits B cell response). MG patients may present with heterogeneous clinical manifestations and antibodies; therefore, they may respond differently to similar treatments, and thus, each regimen needs to be tailored for each patient. The European Federation of Neurological Societies has developed evidence-based consensus recommendations for MG [1], and consensus guidelines for the management of MG have been provided by a group with international representation [2]. For the acute treatment of exacerbations of weakness, it may be necessary to employ plasmapheresis or intravenous immunoglobulins, which result in a prompt reduction of the circulating autoimmune response. Patients with a thymoma usually undergo a thymectomy, removing the thymus. Further, the majority of acetylcholine receptor antibody-seropositive (AChR+) patients have a hyperplastic thymus, which is also an indication for undergoing thymectomy, particularly for young patients. The most commonly used chronic symptomatic treatment consists of nonselective acetylcholinesterase inhibitors. These inhibitors render more acetylcholine available at the neuromuscular junction (NMJ), thus temporarily decreasing fatigue. In general, AChR+ patients respond better to both immunosuppressive and symptomatic medications than muscle-specific kinase-seropositive (MuSK+) patients.

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Symptomatic Medication

Symptomatic medication in MG includes acetylcholinesterase inhibitors (AChEIs) as well as ß2 adrenoceptor agonists.

AChEIs are the most common symptomatic treatment, inducing its effect by inhibiting the enzymatic breakdown of acetylcholine, rendering this neurotransmitter available for a longer

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H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*, Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_20

time period at the motor endplate and the nicotinic AChRs. The most common form of nonselective AChEI is pyridostigmine bromide (Mestinon®), and its beneficial effect of improved muscle fatigue is most prominent at disease onset and in AChR+ MG. AChEIs may be used as an only therapy in ocular or mild generalized MG, if the therapeutic response is regarded sufficient. Common dose ranges from 60 to 120 mg given three to five times daily, although especially in elderly patients one has to be observant of cholinergic adverse effects [3]. Since AChEIs influence both the nicotinic AChRs and the muscarinic receptors in exocrine glands, adverse effects include increased gut motility, increased gastric acid secretion, hyperhidrosis, increased salivation, and muscle fatigue and fasciculations as possible signs of AChEI overdose. Nicotinergic side effects have been observed more often in MuSK+ patients, who also have been reported to clinically benefit less from pyridostigmine bromide [1, 4–6]. Cholinergic overdose can easily be identified with a motor nerve conduction study [6], as extra discharges after the compound motor action potential (CMAP) on motor neurography appear [3].

Other available symptomatic agents, **ß2 adrenoceptor agonists**, include mainly terbutaline and salbutamol. These agents are beneficial in both MG and congenital MG, and, based on experience in some neurology clinics in both Europe and the US, particularly in patients with limb-girdle phenotype and bulbar weakness [7, 8]. Studies in cattle with clenbuterol indicate skeletal muscle hypertrophy [9] resulting from this medication, and studies on MuSK+ mouse models have shown reduced muscle weakness upon treatment with albuterol [10].

Immunosuppressive Agents

Corticosteroids are generally considered to be an effective immunosuppressant for MG and therefore commonly applied as first-line immunosuppression. Corticosteroids reduce the inflammatory response through inhibition of transcription of inflammatory cytokines (interleukins) and adhesion molecules, causing a reduction in trafficking of inflammatory cells such as T cells. High doses also induce apoptosis of inflammatory cells [11]. Despite wide acceptance as an appropriate immunosuppressive therapy, only a few randomized controlled trials have studied the efficacy of corticosteroid treatment in MG [12, 13]. Lindberg et al. reported significant improvement in muscle function in the group of MG patients who received 2 g intravenous methylprednisolone on 2 consecutive days compared to the placebo group; duration of improvement ranged from 4 to 14 weeks, although the concentration of AChR antibodies remained unchanged. When high doses of corticosteroids are employed, there is also the risk of worsening during the first days of initiation, usually lasting less than 1 week. Gradually increasing the steroid dose over 1–2 months may significantly reduce this risk. Pascuzzi et al. reported improvement in as many as 95% of MG patients receiving long-term treatment with prednisone and remission (defined as no more than minimal eye closure weakness) in 28% of patients [14]. Additionally, corticosteroids appear to have a direct effect on the neuromuscular junction, which may explain the early alterations and short-term fluctuations in myasthenic weakness seen in patients being treated with corticosteroids. The effect is believed to be a facilitation of the spontaneous release of acetylcholine and reduction of miniature endplate potential (MEPP) amplitude by about 50% [15].

T Cell Inhibiting Medications

Azathioprine (Aza) is considered an important steroid-sparing agent in the treatment of MG, although most studies have described its usefulness in conjunction with corticosteroids. The mechanism of action is inhibition of purine synthesis especially of the most rapidly dividing cells, including lymphocytes involved in the autoimmune response [16]. Prolonged administration of Aza prevents the induction of EAMG, for at least 4 months, in rabbits immunized with AChR [17]. Aza has a delayed onset of effect, on average 4–6 months, and maximal effect is

obtained after a time period of approximately 14 months. Nevertheless, the majority of patients report reduced myasthenic fatigue after a few months [18]. Aza is administered orally with a preferred maintenance dose of 2–3 mg/kg/day. The initial dose is 50 mg/day and increases by 50 mg/day every week while monitoring for adverse effects.

Cyclosporine A (CyA) is another steroid-sparing medication, which inhibits calcineurin and thereby prohibits transcription of cytokine genes, including those of IL-2 and IL-4, in activated T cells [19]. CyA specifically inhibits T helper cell activation [20] and is widely used to prevent transplant rejection. CyA has a positive effect in MG by resulting in improved muscle strength and reduced AChR antibody titer [21]. Most patients improve maximally after 2–4 months of therapy. The commonly applied dose is 3–5 mg/kg/day, given orally at 12-h intervals.

Tacrolimus (Prograf®) is another calcineurin inhibitor indicated for the prevention of transplant rejection. Unlike CyA, tacrolimus is more potent and causes less nephrotoxicity when used at low doses [22]. Its intracellular binding to the protein FKBP-12 interferes with the activity of calcineurin, which in turn prevents the nuclear factor of activated T cells from translocation and initiation of gene transcription for lymphokines such as interleukin-2 (IL-2). There is limited, yet promising, data to suggest a beneficial role for tacrolimus in reducing muscle fatigue and corticosteroid burden in patients with refractory or newly diagnosed MG. This beneficial treatment response may appear as early as 4 months after initiation, with subjective improvement noted after only 1–2 months [23, 24].

Mycophenolate mofetil (MyM, Cellcept®) inhibits the proliferation of B and T lymphocytes through noncompetitive, reversible inhibition of inosine monophosphate dehydrogenase, a key enzyme in the synthetic pathway of guanine nucleotides. The proliferation of lymphocytes is reduced through selective inhibition of purine synthesis [25]. In a large retrospective study, MyM was associated with clinical improvement in approximately 70% of patients after a period of approximately 11 weeks [26]. MyM is also well tolerated in most patients and only discontinued due to the adverse effects in a very small fraction of patients, the most common reason being gastrointestinal intolerance, such as diarrhea. The long-term safety of MyM is not known, but rates of malignancy do not appear to be higher in transplant recipients who receive MyM chronically [27].

Cyclophosphamide is a nitrogen mustard with potent immunosuppressant effects and is used to treat MG patients who are resistant to other therapies. It affects the proliferation of B cells and thereby reduces antibody production. The use of cyclophosphamide in MG is limited, but undoubtedly beneficial in severe cases [28]. The usual dose ranges from 1 to 3 mg/kg/day, orally. Nevertheless, adverse effects are prominent and include alopecia, hemorrhagic cystitis, leukopenia, and nausea. Long-term treatment increases the risk of infertility and malignancy.

Limitations of Present Therapeutics

As should be appreciated from the discussion thus far, therapeutics for MG do not cure the disease, immune-therapies are non-specific, and all have the potential for significant adverse effects. Although great advances have been made in understanding of disease pathogenesis and in therapy, more than a third of patients experience MG exacerbations, which require hospitalization, and disease- and treatment-related morbidity remains high. Non-immunosuppressive treatments often do not completely relieve symptoms, and immunosuppressive treatments (high-dose corticosteroids, cyclosporine, azathioprine, cytoxan, mycophenolate mofetil, intravenous immunoglobulin, plasmapheresis) have poor side effect profiles with variable benefit. There is also a concern for increased frequency of neoplasia for all the immunosuppressives. Eculizumab, which was recently approved by the US Food and Drug Administration for the treatment of generalized AChR+ MG, is a disease moderating monoclonal antibody. There is no evidence presently that it impacts the cellular autoimmune

pathology. Although mortality of MG patients has improved over the decades, MG remains a disease with high morbidity and, at times, mortality. Improvements in acute and chronic disease management are needed. There is a significant need to improve MG therapeutics.

Pipeline for Development: Preclinical and Clinical Trials

The past decade has seen a dramatic expansion in therapeutic development for MG. A search of clinicaltrials.gov 2007–2016 for interventional therapeutic clinical trials for MG in adults resulted in 31 studies. A search of the previous 10 years yielded only seven trials (clinicaltrials.gov search 4/3/2017). Though reporting requirements for clinicaltrials.gov have changed over the years, these results support the increased interest in therapeutic development for MG that has been experienced by clinicians and patients. This increased attention is the result of many factors which include a progressively better understanding of the immunopathology of autoimmunity in general, and MG specifically; advances in therapeutic design and manufacturing, particularly monoclonal antibodies; and the success of clinical development programs in other autoimmune diseases, such as rheumatoid arthritis and multiple sclerosis. With several options now on the market for more common autoimmune diseases, the pharmaceutical industry has shifted some focus to developing therapies for rarer diseases (e.g., MG) to provide therapeutics for these populations with unmet need. In a turn of events that many clinicians would not have predicted, the number of planned or active clinical trials for MG has actually resulted in competition between studies for eligible patients.

Therapeutics in preclinical or clinical development for MG target many mechanisms aimed primarily at altering immune system function rather than providing symptomatic therapy. The immune system targets may be broadly categorized as direct lymphocyte modulators, cytokine and cytokine receptor targeting, complement inhibitors, and immunoglobulin/antibody regulators. After a brief description of some of the patient factors that make clinical testing of new therapies in MG challenging, the remainder of the chapter discusses the current state of therapeutics directed at these targets. Despite the challenges described below, the increased interest in MG will hopefully lead to regulatory approval of therapeutics with improved efficacy and reduced side effects through targeted immune system modulation.

Challenges in Therapeutic Development Based on Disease Heterogeneity

Recognition of several distinct disease subtypes makes therapeutic development for MG challenging. MG can be classified in several different ways: by age (early onset vs. late onset), antibody status (AChR, MuSK, seronegative), distribution of disease (ocular vs. generalized), thymoma and thymectomy status, and response to existing therapy (refractory vs. nonrefractory or treatment naïve). These challenges are further compounded by inherent fluctuation of the disease, changes in response that may occur in relation to the duration of disease (e.g., fixed weakness that may occur with prolonged inadequately treated MG), potential use of multiple concomitant immunosuppressive drugs, and even anatomic variability in the distribution of disease among patients with generalized disease (e.g., bulbar vs. limb predominant). In an effort to manage many of these factors and to study therapeutics in populations felt most appropriate for a therapy under development, many clinical trial protocols are written to exclude certain patient groups. Some of these exclusions make biologic sense when considering the mechanism of action of new therapies. For example, it would be generally recommended to exclude MuSK+ MG patients from clinical trials of complement inhibitors where complement is not thought to play a prominent role in disease pathophysiology. The overall effect , in many cases is to significantly reduce the available number of eligible patients, resulting in slow enroll-

ment. Appropriate measures to manage these limitations will be key to continued interest in therapeutic development in the field.

Several advances may help overcome some of these difficulties. Common data elements were developed to standardize data collection and facilitate the use of appropriate outcome measures [29]. Patient registries have been developed in the United States and Europe to collect clinical data from patients with MG to inform clinical trial design and to enhance communication with patients about ongoing clinical trials [30, 31]. Finally, international consensus treatment guidelines were developed by a panel of experts that define goals of therapy, disease status, and appropriate treatment of patients with MG [2].

Lymphocyte Modulators in Preclinical and Clinical Testing for MG

Plasma Cell Therapeutics

Plasma cells are an attractive target for treating MG and other B cell-mediated disorders [32]. Autoreactive plasma cells are responsible for the production of pathogenic antibodies targeting the postsynaptic neuromuscular junction [33]. In AChR+ MG, IgG1 and IgG3 autoantibodies destroy the postsynaptic endplate of the neuromuscular junction predominantly through complement-mediated pathways [34, 35]. In contrast, autoantibodies in MuSK MG are mainly IgG4 and do no fix complement [36]. No therapies currently in routine use for MG specifically target plasma cells. Among therapeutics commonly used for MG, therapeutic plasma exchange directly removes the autoantibodies from the blood and is highly effective for treating AChR+ and MuSK+ MG (>90% of patients improve). However, these procedures are time consuming, often require central lines that result in complications, and necessitate highly trained staff that are not available at all centers [37].

A couple therapeutics that specifically target plasma are currently available to clinicians, though experience with these agents in patients with MG is limited. The first therapy to become available is bortezomib [38]. Bortezomib is a proteasome inhibitor approved for the treatment of mantle cell lymphoma and multiple myeloma, which causes accumulation of nondegraded, misfolded proteins within plasma cells leading to apoptosis. It has been shown to reduce autoantibody titers and improve clinical outcomes in animal models of autoimmunity [39, 40]. Studies of bortezomib in EAMG and in vitro studies of MG patient thymus cultures have suggested that plasma cell depletion is a promising target [41]. In EAMG reduced AChR antibody production, reduced postsynaptic muscle membrane damage, and clinical improvement have been observed [42]. Clinical trials of bortezomib to treat MG are currently underway [43]. More recently the monoclonal antibody daratumumab was approved for treatment of refractory multiple myeloma [44]. Daratumumab is directed against the plasmablast and plasma cell marker CD38. Specifically targeting these B cell subsets would appear to have distinct advantages for treating blood cancers as well as antibody-mediated autoimmunity [45].

Therapies targeting plasma cells that are in development or currently available have important limitations. Bortezomib has a common toxicity of painful neuropathy [46]. This adverse effect is seen in approximately 35% of patients, potentially making it unsuitable for patients with MG that may be otherwise relatively healthy [47]. In addition, an effect of daratumumab observed in multiple myeloma patients is depletion of immunosuppressive CD38-expressing regulatory T cells and expansion of helper and cytotoxic T cell populations [48]. These effects may be undesirable for MG, where dysfunctional regulatory T cells and abnormal activation of T cell populations have already been documented, raising concern that daratumumab may exacerbate the disease [49, 50]. Next-generation proteasome inhibitors are currently under investigation and presumably have the advantage of fewer associated adverse effects [51]. Given the prime role of plasma cells in autoantibody production, a therapeutic that selectively targets plasma cells and overcomes the current limitations of existing

therapies will be likely be of prime interest in antibody-mediated diseases such as MG.

Rituximab (see below) depletes CD20-positive B cells, but does not affect autoantibody-producing plasmablasts or plasma cells that do not express CD20. Thus, these cell subsets are not directly affected, and the dramatic effects observed in some MG patients administered rituximab are likely accounted for through another mechanism, either indirect modulation of plasma cell populations (i.e., cell signaling) or depletion of plasma cell precursors, such as activated B cells.

Targeting T Cells

Despite longstanding knowledge of the critical role of T cells in MG immunopathogenesis, few novel T cell-directed therapies are being studied in MG. Detailed understanding of T cell reactivity and interactions with other cells in the MG autoimmune pathway will likely lead to a stronger rationale for T cell-directed therapies in MG and a better understanding of potential off-target effects of these therapies in patients with MG. Strategies aimed at reducing T cell activation, improving regulatory T cell (Treg) function, and inhibiting stimulatory interactions between activated T cells and B cells hold promise for MG therapy. This discussion will focus primarily on therapeutics currently available to treat other diseases that could be applied to MG and selected studies completed in EAMG or pilot studies in patients.

Studies have demonstrated strong Th1 and Th17 cell involvement in both AChR+ and MuSK+ MG. Th17 cells represent a subset of CD4+ T cells characterized by the production of inflammatory cytokines interleukin-17 (IL-17) and IL-21 [52–54]. Several lines of evidence in both animals and humans suggest Th17 cells play a prominent role in MG immunopathology [55]. An EAMG study demonstrated increased serum IL-17 and a shift toward Th17 cell subsets after immunization with AChR peptides and reduced disease severity in IL-17 knockout mice [56]. IL-17 stimulation also induced T cell proliferation and B cell production of AChR autoantibodies [57]. Studies in human MG showed upregulation of Th17 genes and increased peripheral IL-17A levels [58–60]. More recently, studies using peripheral blood mononuclear cells have shown increased frequencies of Th17 cells in MuSK+ MG patients compared with controls following polyclonal T cell stimulation and AChR+ MG patients demonstrate a memory response with high IL-17 production [50, 61]. These findings strongly suggest that MG patients are primed for proinflammatory IL-17 responses.

Multiple subcutaneously administered monoclonal antibodies are in development that target the inflammatory cytokine IL-17 or its receptor and may have applications to MG. The IL-17 receptor A is targeted by brodalumab and approved for the treatment of adults with moderate to severe plaque psoriasis. Two approved monoclonal antibodies target the IL-17A cytokine itself. Ixekizumab is also indicated in moderate to severe plaque psoriasis, while secukinumab has additional indications for psoriatic arthritis and ankylosing spondylitis.

Another approved therapy with potential application to MG is the fusion protein abatacept. Abatacept is approved for the treatment of juvenile idiopathic arthritis and adult rheumatoid arthritis. The mechanism of action is inhibition of T cell activation, resulting in reduced levels of proinflammatory cytokines that are implicated in MG, such as TNF-α, interferon-γ, and IL-2. More specifically, abatacept consists of the extracellular domain of human cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and a modified Fc region of human immunoglobulin G1. It blocks the required co-stimulatory signal to activate T cells that is mediated by CD80/CD86 (also known as B7-1 and B7-2) on antigen-presenting cells. Abatacept failed in clinical trials in multiple sclerosis and ulcerative colitis, and it has been hypothesized that abatacept may not be as effective in diseases with strong IL-17-mediated responses. A pilot clinical trial is ongoing in MG (NCT03059888).

Antigen-presenting cells, including B cells, macrophages, and dendritic cells, constitutively express CD40 on their surface. Activated CD4+ T cells interact with CD40 through CD40L (CD154) leading to dendritic cell activation, production of proinflammatory cytokines, and enhanced humoral immune responses [62]. Modulation of this pathway has been a goal for treating organ transplant-mediated rejection, though early clinical studies raised concern for thromboembolic events [63]. Studies in EAMG demonstrated clinical improvement in established disease following delivery of anti-CD40L monoclonal antibodies and a reduction in Th1-related inflammatory cytokines [64]. Next-generation monoclonal antibodies targeting the CD40-CD40L interaction with an improved safety profile reducing concerns for thromboembolic events are currently in clinical testing in MG (NCT02565576).

Multiple studies have shown regulatory T cell (Treg) dysfunction in both the thymus and periphery [49, 59, 65]. Methods for selectively improving Treg function are currently under development, but are still several years away. In the meantime, EAMG studies and a single case report in MG suggest that granulocyte-macrophage colony-stimulating factor (GM-CSF) can increase Treg frequencies and function and ameliorate disease [66–69].

B Cell Targets

Rituximab (MabThera®) is a chimeric IgG1 monoclonal antibody that targets CD20, a transmembrane phosphoprotein available on most B cells. Rituximab depletes B cells by binding to the CD20 molecule and initiating complement-dependent cytolysis or antibody-dependent cell-mediated cytotoxicity [70]. A phase 2 randomized controlled study of rituximab in patients with AChR+ MG has recently completed enrollment with results expected soon (NCT02110706). A recent study of 16 patients indicated complete stable remission, pharmacologic remission, or minimal manifestation status according to the MGFA criteria in all AChR+ MG patients with a corresponding reduction in AChR antibodies [71]. In MuSK+ MG patients, rituximab also reduces prednisone dose and induces withdrawal of concomitant immunosuppressants along with clinical improvement and significant decrease in the MuSK antibody titers [72]. Further, several case reports have demonstrated the effect of rituximab in MuSK+ MG patients on the clinical course of bulbar and respiratory symptoms [73, 74]. Thus, although based on the retrospective studies available, rituximab comes across as an effective option in patients both with refractory AChR+ and MuSK+ MG.

B cell activating factor (BAFF) There is accumulating evidence implying the involvement of B cell activating factor (BAFF) in the pathogenesis of MG [75]. Increased serum levels and expression of BAFF and its receptors have been demonstrated in the thymus of MG patients [76]. BAFF contributes to autoimmunity by promoting the survival, growth, and maturation of B cells, including autoreactive B cells and rescue B cells from apoptosis [77]. The genetic associations found between early-onset MG (EOMG) and BAFF are novel and interesting since BAFF plays important roles in the proliferation and differentiation of B cells [78]. The potential therapeutic effects of a conjugate of BAFF receptor-specific monoclonal antibody and short interference RNA in EAMG mice have been examined [79]. Whereas high-dose siRNA conjugate resulted in significant accumulation of Fas expressing CD19+/B220+ cells and concurrent expression of type 1 interferon in lymph nodes, low-dose conjugate did not induce FAS expression but caused marked BAFF receptor deficiency in lymph nodes that was further associated with improved MG manifestations. Unexpectedly, despite inhibiting BAFF receptor significantly in PBMCs, conjugate treatment did not reduce the AChR antibody titer. These data indicate a dose-dependent, immunomodulatory distant effect resulting from BAFF receptor-specific mAb-siRNA conjugate treatment in EAMG [79]. A clinical trial of belimumab, an antibody directed toward BAFF, has been completed, but results are not published at the time of this writing (NCT01480596).

Cytokine-Targeted Therapies

Tumor necrosis factor alfa (TNF-α) has been implicated in the development of both MG [80] and EAMG [81]. Patients with MG have been shown to have increased numbers of TNF-α mRNA-expressing cells among blood mononuclear cells compared to controls, and T cells from MG patients have significantly more TNF-α receptors [80].

Etanercept, a soluble, recombinant human TNF-receptor that competitively blocks the action of TNF-α, has been shown to have steroid-sparing effects in studies in small MG cohorts [82, 83]. In approximately half of patients, with low plasma IL-6 and IFN-γ levels, the clinical scores improved, and patients with increased cytokine levels (IL-6, IFN-γ, and TNF-α) had worse clinical outcomes [83]. TNF-α is involved in the generation of AChR-specific T and B cell responses during the development of EAMG, and preclinical studies on AChR-immunized mice have shown that etanercept can suppress established EAMG without inducing significant immunosuppression [84]. Nevertheless, case reports have also alerted on the possible association of TNF-α agents and onset of MG, and exploration of this therapeutic class in MG has been stalled [85].

Stem Cell Transplant

Allogenic hematopoietic stem cell transplantation has been reported in one young MG patient [86] whose muscle weakness completely resolved after transplantation. Recently, a case series of seven patients as well as another case study of one patient treated with autologous hematopoietic stem cell transplantation (AHSCT) for MG [87, 88] reported all patients achieved durable MGFA complete stable remission with no residual MG manifestations and freedom from ongoing MG therapy. Compared to allogenic HSCT, AHSCT does not require a compatible donor and does not result in graft-versus-host disease. Still, treatment-related morbidity and mortality of AHSCT must be taken into account when considering this treatment, as well as costs. Nevertheless, based on these few reports, autologous hematopoietic stem cell transplantation can be an effective therapeutic option for carefully selected patients of severe, treatment refractory MG [88].

Complement Inhibitors

Complement plays a vital role in MG pathology by reducing the viable NMJs (review Conti-Fine [89]). AChR antibodies binding to the NMJs activate the classical complement pathway and result in membrane attack complex deposition. Studies have shown that inhibition of complement ablates weakness and improves the synaptic transmission [90–92]. Various complement proteins have been the targets for therapeutic development potential for MG. In 1989, the first potential therapeutic approach was used with antibody directed against C6 and successfully inhibited the development of passive transfer MG (PTMG) [93]. C3 deposition at the NMJ was still evident, indicating the requirement for MAC formation to produce AChR loss and weakness. Piddlesden and colleagues in 1994 administered soluble complement receptor 1 simultaneously using the PTMG model and reduced the severity of weakness and loss of AchR [94]. Neither of these early approaches moved on to clinical therapeutic development [95].

The most extensively assessed target for complement inhibition is C5. Eculizumab is a monoclonal antibody against C5 and produced by Alexion to treat paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS). Preclinical evaluation in a rat model of passive transfer MG (PTMG) demonstrated resistance to disease induction with pretreatment and moderation of disease severity when given after weakness had developed [96]. A phase 3 trial of eculizumab has demonstrated efficacy [97], and the drug has been approved for use in the United States by the Food and Drug Administration in 2017.

Other approaches for inhibiting the action of C5 are under development. RA101495 is a small molecule inhibitor of C5 that has moved to phase

2 testing in MG (NCT03315130). The C5 inhibitor, rEV576, specifically targets the C5 activation step by directly binding to C5 [98]. Treatment with rEV576 in an EAMG rat model drastically improved strength and weight [99]. rEV576, now designated Coversin® and produced by Akari Pharmaceutics, has been used in humans for PNH. Finally, small interfering RNAs (siRNAs) to suppress the synthesis of liver-derived C5 have also been used as a therapeutic approach and have reduced the severity of PTMG and EAMG (unpublished data HK and Alnylam Pharmaceuticals).

A monoclonal antibody directed against a conserved epitope of factor B inhibits complement activation by blocking the alternative pathway C3 proconvertase, and factor B inhibition moderated weakness and limited loss of AChR in a PTMG model [100]. Although complement-mediated destruction of the postsynaptic surface of the junction is mediated by the classical pathway, its high efficiency depends on alternative pathway amplification in which C3b generated by the C3 convertase forms more C3 convertases and thus exponentially amplifies activation.

Complement inhibitors targeting early points in complement activation have been evaluated by the Christadoss lab. Antibody directed against C1q proved deleterious or beneficial depending on dose. Administration of low doses of anti-C1q decreased weakness, IL-6 production in lymph nodes, and moderated pathogenic T cell expression, but a dose tenfold higher increased AChR antibody and IgG deposition and led to immune complex deposition in the kidney. Such dose-related adverse effects reduce enthusiasm for C1q as a reasonable therapeutic target. Using a siRNA approach, suppression of C2 expression reduced EAMG-induced weakness and neuromuscular junction injury [101]. As of yet, this target has not moved toward human testing.

With the success of eculizumab moving into the clinic, there is now a need to move toward second-generation complement inhibitors that will be easier to use compared to intravenous infusions and those that may reduce systemic complement inhibition [91, 102].

Cholinesterase Inhibition

EN101 is a selective AChEI, an antisense oligodeoxynucleotide that acts at the mRNA level and selectively reduces the enzymatic isoform of AChE-R. This compound selectively lowers the levels of AChE-R in both blood and muscle, yet leaves the synaptic variant (AChE-S) unchanged. Based on a successful EN101 treatment trial in rats with experimental autoimmune MG (EAMG) demonstrating improved survival, muscle strength, and disease severity [103], a phase 1b open-label trial with oral EN101 (Monarsen) was conducted in 16 MG patients [104]. This study reported an overall clinical improvement in approximately 47% of patients, as well as an improvement in the swallowing time component. Further, the effects of EN101 lasted for greater than 24 h, indicating the possibility of a reduction in multiple dosing through the use of antisense therapy. Thus far, there has been no movement to bring this compound to phase 3 evaluation.

Antibody Inhibitors/Regulators

Induction of Tolerance

Antigen-specific therapy that specifically suppresses the autoimmune response and does not compromise immune function is the first step to cure MG [105]. The first attempt to induce tolerance dates back to the time when the AChR was identified as the primary autoantigen. Administration of denatured *Torpedo* AChR moderated EAMG severity in rabbits [106]. Nasal and oral administration of large doses of *Torpedo* AChR will also moderate EAMG [107–109]. The challenge with translation of these early attempts to human use is the immunogenicity of the *Torpedo* AChR and large amounts required. Lindstrom and colleagues have used bacterially expressed human AChR cytoplasmic domains and found that when administered either after acute EAMG or chronic EAMG its established weakness can be reversed and animals were resistant to further induction of EAMG [110, 111]. The work builds on the observation

that EAMG weakness is moderated despite continued production of AChR antibody; however, these antibodies are primarily directed toward cytoplasmic domains. Hence, these AChR autoantibodies are not pathogenic. Presently, this approach is slowly moving to clinical trials.

CV-MG01 is a combination of two synthetic peptides that are designed to complement the structure of the main immunogenic region of the AChR. The expectation is that administration of the exogenous peptide will induce anti-idiotype and anti-clonotypic responses against binding sites of antigen receptors on autoreactive lymphocytes. This will then reduce AChR-specific T cell help and anergize autoreactive B cells [112–114]. An evaluation in canine MG suggested that the peptide vaccination reduced weakness and antibodies more rapidly to historical controls [115]. However, the study has been criticized because of the high spontaneous remission rates of canine MG [116]. A phase 1b study in patient with MG is ongoing of CV-MG-101 at the time of this writing.

FcRn Lowering Strategies

Multiple groups are pursuing modulation of antibody lifecycle to treat autoimmunity. As IgG molecules move through the circulation, they are naturally endocytosed by endothelial cells. Within the endocytosed vesicle, IgG interacts under acidic pH with the neonatal Fc receptor (FcRn). IgGs bound to FcRn are returned to the circulation, thereby extending their half-life to approximately 21 days [117]. Novel therapeutics are currently in clinical development to prevent the FcRn-IgG interaction which results in IgG catabolism and lowering of serum IgG levels. This approach would be expected to have a clinical effect similar to therapeutic plasma exchange, but with higher specificity. IgG antibodies would be rapidly removed from the circulation without interfering with other plasma proteins [118], such as clotting factors and other immunoglobulin molecules, that are removed during plasma exchange. This therapeutic approach could be used for MG patients experiencing exacerbations as an alternative to therapeutic plasma exchange or IVIg, and chronic administration targeting moderate IgG reductions could be envisioned as a substitute for current oral drugs that broadly suppress the immune system. Both intravenous and subcutaneous formulations of anti-FcRn monoclonal antibodies are currently in clinical trials in MG and other autoimmune diseases [119, 120]. Recombinant multimerized IgG2a Fc applied to EAMG rats also demonstrated suppression of weakness but has not advanced to the clinic [121].

Antigen-Specific Immunoadsorption

Attempts have been made to improve the specificity of plasmapheresis to preferentially remove autoantibodies, rather than all plasma components. Attempts are focused on preferential absorption of autoantibodies through the use of protein A, which binds immunoglobulin, or antibodies specifically directed toward human IgG [122–124]. Although the procedures have demonstrated more specific antibody removal, there has been no evidence of improved efficacy or safety [125]. Attempts at increasing the specificity of immunoadsorption to the specific autoantigen have been made by constructing resins with bound AChR or peptides containing the extracellular domains of the AChR subunits [126–128]. At present, these approaches have not moved to clinical assessment.

Conclusion

The pipeline for MG therapies is a rich one ranging from several lines of preclinical work to agents entering early-phase evaluations and, recently, the first approval of an immunomodulatory therapeutic for MG. The targets of these agents range from antibody removal and complement inhibition to moderation of autoimmune activity. There never has been a richer time for bringing new and better treatments to MG patients.

References

1. Skeie GO, Apostolski S, Evoli A, Gilhus NE, Illa I, Harms L, et al. Guidelines for treatment of autoimmune neuromuscular transmission disorders. Eur J Neurol. 2010;17:893–902.
2. Sanders DB, Wolfe GI, Benatar M, Evoli A, Gilhus NE, Illa I, et al. International consensus guidance for management of myasthenia gravis: executive summary. Neurology. 2016;87:419–25.
3. Punga AR, Sawada M, Stalberg EV. Electrophysiological signs and the prevalence of adverse effects of acetylcholinesterase inhibitors in patients with myasthenia gravis. Muscle Nerve. 2008;37:300–7.
4. Evoli A, Bianchi MR, Riso R, Minicuci GM, Batocchi AP, Servidei S, et al. Response to therapy in myasthenia gravis with anti-MuSK antibodies. Ann N Y Acad Sci. 2008;1132:76–83.
5. Evoli A, Tonali PA, Padua L, Monaco ML, Scuderi F, Batocchi AP, et al. Clinical correlates with anti-MuSK antibodies in generalized seronegative myasthenia gravis. Brain. 2003;126:2304–11.
6. Punga AR, Flink R, Askmark H, Stalberg EV. Cholinergic neuromuscular hyperactivity in patients with myasthenia gravis seropositive for MuSK antibody. Muscle Nerve. 2006;34:111–5.
7. Liewluck T, Selcen D, Engel AG. Beneficial effects of albuterol in congenital endplate acetylcholinesterase deficiency and Dok-7 myasthenia. Muscle Nerve. 2011;44:789–94.
8. Gallenmuller C, Muller-Felber W, Dusl M, Stucka R, Guergueltcheva V, Blaschek A, et al. Salbutamol-responsive limb-girdle congenital myasthenic syndrome due to a novel missense mutation and heteroallelic deletion in MUSK. Neuromuscul Disord. 2014;24:31–5.
9. Hinkle RT, Hodge KM, Cody DB, Sheldon RJ, Kobilka BK, Isfort RJ. Skeletal muscle hypertrophy and anti-atrophy effects of clenbuterol are mediated by the beta2-adrenergic receptor. Muscle Nerve. 2002;25:729–34.
10. Ghazanfari N, Morsch M, Tse N, Reddel SW, Phillips WD. Effects of the ss2-adrenoceptor agonist, albuterol, in a mouse model of anti-MuSK myasthenia gravis. PLoS One. 2014;9:e87840.
11. Barnes PJ. Molecular mechanisms of corticosteroids in allergic diseases. Allergy. 2001;56:928–36.
12. Howard FM Jr, Duane DD, Lambert EH, Daube JR. Alternate-day prednisone: preliminary report of a double-blind controlled study. Ann N Y Acad Sci. 1976;274:596–607.
13. Lindberg C, Andersen O, Lefvert AK. Treatment of myasthenia gravis with methylprednisolone pulse: a double blind study. Acta Neurol Scand. 1998;97:370–3.
14. Pascuzzi RM, Coslett HB, Johns TR. Long-term corticosteroid treatment of myasthenia gravis: report of 116 patients. Ann Neurol. 1984;15:291–8.
15. Wilson RW, Ward MD, Johns TR. Corticosteroids: a direct effect at the neuromuscular junction. Neurology. 1974;24:1091–5.
16. Elion GB. Significance of azathioprine metabolites. Proc R Soc Med. 1972;65:257–60.
17. Abramsky O, Tarrab-Hazdai R, Aharonov A, Fuchs S. Immunosuppression of experimental autoimmune myasthenia gravis by hydrocortisone and azathioprine. J Immunol. 1976;117:225–8.
18. Lewis RA, Selwa JF, Lisak RP. Myasthenia gravis: immunological mechanisms and immunotherapy. Ann Neurol. 1995;37(Suppl 1):S51–62.
19. Kronke M, Leonard WJ, Depper JM, Arya SK, Wong-Staal F, Gallo RC, et al. Cyclosporin A inhibits T-cell growth factor gene expression at the level of mRNA transcription. Proc Natl Acad Sci U S A. 1984;81:5214–8.
20. Matsuda S, Moriguchi T, Koyasu S, Nishida E. T lymphocyte activation signals for interleukin-2 production involve activation of MKK6-p38 and MKK7-SAPK/JNK signaling pathways sensitive to cyclosporin A. J Biol Chem. 1998;273:12378–82.
21. Tindall RS, Phillips JT, Rollins JA, Wells L, Hall K. A clinical therapeutic trial of cyclosporine in myasthenia gravis. Ann N Y Acad Sci. 1993;681:539–51.
22. Sanders DB, Evoli A. Immunosuppressive therapies in myasthenia gravis. Autoimmunity. 2010;43:428–35.
23. Nagaishi A, Yukitake M, Kuroda Y. Long-term treatment of steroid-dependent myasthenia gravis patients with low-dose tacrolimus. Intern Med. 2008;47:731–6.
24. Shimojima Y, Matsuda M, Gono T, Ishii W, Tokuda T, Ikeda S. Tacrolimus in refractory patients with myasthenia gravis: coadministration and tapering of oral prednisolone. J Clin Neurosci. 2006;13:39–44.
25. Allison AC, Kowalski WJ, Muller CD, Eugui EM. Mechanisms of action of mycophenolic acid. Ann N Y Acad Sci. 1993;696:63–87.
26. Meriggioli MN, Rowin J. Single fiber EMG as an outcome measure in myasthenia gravis: results from a double-blind, placebo-controlled trial. J Clin Neurophysiol. 2003;20:382–5.
27. Haberal M, Karakayali H, Emiroglu R, Basaran O, Moray G, Bilgin N. Malignant tumors after renal transplantation. Artif Organs. 2002;26:778–81.
28. Perez MC, Buot WL, Mercado-Danguilan C, Bagabaldo ZG, Renales LD. Stable remissions in myasthenia gravis. Neurology. 1981;31:32–7.
29. NINDS. Common data elements: Myasthenia Gravis [online]. https://www.commondataelements.ninds.nih.gov/MG.aspx#tab=Data_Standards. Accessed 10 Apr 2017.
30. Fulvio B, Mantegazza R. European database for myasthenia gravis: a model for an international disease registry. Neurology. 2014;83:189–91.
31. Myasthenia Gravis Foundation of America. MG Patient Registry [online]. http://mgregistry.org/. Accessed 10 Apr 2017.

32. Hiepe F, Radbruch A. Plasma cells as an innovative target in autoimmune disease with renal manifestations. Nat Rev Nephrol. 2016;12:232–40.
33. Willcox HN, Newsom-Davis J, Calder LR. Cell types required for anti-acetylcholine receptor antibody synthesis by cultured thymocytes and blood lymphocytes in myasthenia gravis. Clin Exp Immunol. 1984;58:97–106.
34. Vincent A. Unravelling the pathogenesis of myasthenia gravis. Nat Rev Immunol. 2002;2:797–804.
35. Liu Y, Wang W, Li J. Evaluation of serum IgG subclass concentrations in myasthenia gravis patients. Int J Neurosci. 2011;121:570–4.
36. Niks EH, van Leeuwen Y, Leite MI, Dekker FW, Wintzen AR, Wirtz PW, et al. Clinical fluctuations in MuSK myasthenia gravis are related to antigen-specific IgG4 instead of IgG1. J Neuroimmunol. 2008;195:151–6.
37. Guptill JT, Oakley D, Kuchibhatla M, Guidon AC, Hobson-Webb LD, Massey JM, et al. A retrospective study of complications of therapeutic plasma exchange in myasthenia. Muscle Nerve. 2013;47:170–6.
38. Kane RC, Farrell AT, Sridhara R, Pazdur R. United States Food and Drug Administration approval summary: bortezomib for the treatment of progressive multiple myeloma after one prior therapy. Clin Cancer Res. 2006;12:2955–60.
39. Bontscho J, Schreiber A, Manz RA, Schneider W, Luft FC, Kettritz R. Myeloperoxidase-specific plasma cell depletion by bortezomib protects from anti-neutrophil cytoplasmic autoantibodies-induced glomerulonephritis. JASN. 2011;22:336–48.
40. Neubert K, Meister S, Moser K, Weisel F, Maseda D, Amann K, et al. The proteasome inhibitor bortezomib depletes plasma cells and protects mice with lupus-like disease from nephritis. Nat Med. 2008;14:748–55.
41. Gomez AM, Willcox N, Vrolix K, Hummel J, Nogales-Gadea G, Saxena A, et al. Proteasome inhibition with bortezomib depletes plasma cells and specific autoantibody production in primary thymic cell cultures from early-onset myasthenia gravis patients. J Immunol. 2014;193:1055–63.
42. Gomez AM, Vrolix K, Martinez-Martinez P, Molenaar PC, Phernambucq M, van der Esch E, et al. Proteasome inhibition with bortezomib depletes plasma cells and autoantibodies in experimental autoimmune myasthenia gravis. J Immunol. 2011;186:2503–13.
43. Hiepe F. Therapy of antibody-mediated autoimmune diseases by bortezomib (TAVAB) [online]. https://clinicaltrials.gov/ct2/show/NCT02102594. Accessed 18 Apr 2017.
44. Palumbo A, Chanan-Khan A, Weisel K, Nooka AK, Masszi T, Beksac M, et al. Daratumumab, bortezomib, and dexamethasone for multiple myeloma. N Engl J Med. 2016;375:754–66.
45. Moreau P, van de Donk NW, San Miguel J, Lokhorst H, Nahi H, Ben-Yehuda D, et al. Practical considerations for the use of daratumumab, a novel CD38 monoclonal antibody, in myeloma. Drugs. 2016;76:853–67.
46. Arastu-Kapur S, Anderl JL, Kraus M, Parlati F, Shenk KD, Lee SJ, et al. Nonproteasomal targets of the proteasome inhibitors bortezomib and carfilzomib: a link to clinical adverse events. Clin Cancer Res. 2011;17:2734–43.
47. Moreau P, Pylypenko H, Grosicki S, Karamanesht I, Leleu X, Grishunina M, et al. Subcutaneous versus intravenous administration of bortezomib in patients with relapsed multiple myeloma: a randomised, phase 3, non-inferiority study. Lancet Oncol. 2011;12:431–40.
48. Krejcik J, Casneuf T, Nijhof IS, Verbist B, Bald J, Plesner T, et al. Daratumumab depletes CD38+ immune regulatory cells, promotes T-cell expansion, and skews T-cell repertoire in multiple myeloma. Blood. 2016;128:384–94.
49. Thiruppathi M, Rowin J, Ganesh B, Sheng JR, Prabhakar BS, Meriggioli MN. Impaired regulatory function in circulating CD4(+)CD25(high) CD127(low/-) T cells in patients with myasthenia gravis. Clin Immunol. 2012;145:209–23.
50. Cao Y, Amezquita RA, Kleinstein SH, Stathopoulos P, Nowak RJ, O'Connor KC. Autoreactive T cells from patients with myasthenia gravis are characterized by elevated IL-17, IFN-gamma, and GM-CSF and diminished IL-10 production. J Immunol. 2016;196:2075–84.
51. Gomez AM, Willcox N, Molenaar PC, Buurman W, Martinez-Martinez P, De Baets MH, et al. Targeting plasma cells with proteasome inhibitors: possible roles in treating myasthenia gravis? Ann N Y Acad Sci. 2012;1274:48–59.
52. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol. 2005;6:1123–32.
53. Waite JC, Skokos D. Th17 response and inflammatory autoimmune diseases. Int J Inflamm. 2012;2012:819467.
54. Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol. 2005;6:1133–41.
55. Aguilo-Seara G, Xie Y, Sheehan J, Kusner LL, Kaminski HJ. Ablation of IL-17 expression moderates experimental autoimmune myasthenia gravis disease severity. Cytokine. 2017;96:279–85.
56. Schaffert H, Pelz A, Saxena A, Losen M, Meisel A, Thiel A, et al. IL-17-producing CD4(+) T cells contribute to the loss of B-cell tolerance in experimental autoimmune myasthenia gravis. Eur J Immunol. 2015;45:1339–47.

57. Mu LL, Sun B, Kong QF, Wang J, Wang G, Zhang S, et al. Disequilibrium of T helper type 1, 2 and 17 cells and regulatory T cells during the development of experimental autoimmune myasthenia gravis. Immunology. 2009;128:e826–36.
58. Roche JC, Capablo JL, Larrad L, Gervas-Arruga J, Ara JR, Sánchez A, et al. Increased serum interleukin-17 levels in patients with myasthenia gravis. Muscle Nerve. 2011;44:278–80.
59. Gradolatto A, Nazzal D, Truffault F, Bismuth J, Fadel E, Foti M, et al. Both Treg cells and Tconv cells are defective in the myasthenia gravis thymus: roles of IL-17 and TNF-alpha. J Autoimmun. 2014;52:53–63. https://doi.org/10.1016/j.jaut.2013.12.015. Epub 2014 Jan 7.
60. Xie Y, Li HF, Jiang B, Li Y, Kaminski HJ, Kusner LL. Elevated plasma interleukin-17A in a subgroup of myasthenia gravis patients. Cytokine. 2016;78:44–6.
61. Yi JS, Guidon A, Sparks S, Osborne R, Juel VC, Massey JM, et al. Characterization of CD4 and CD8 T cell responses in MuSK myasthenia gravis. J Autoimmun. 2014;52:130–8.
62. Pinelli DF, Ford ML. Novel insights into anti-CD40/CD154 immunotherapy in transplant tolerance. Immunotherapy. 2015;7:399–410.
63. Chen J, Yin H, Xu J, Wang Q, Edelblum KL, Sciammas R, et al. Reversing endogenous alloreactive B cell GC responses with anti-CD154 or CTLA-4Ig. Am J Transplant. 2013;13:2280–92.
64. Im SH, Barchan D, Maiti PK, Fuchs S, Souroujon MC. Blockade of CD40 ligand suppresses chronic experimental myasthenia gravis by down-regulation of Th1 differentiation and up-regulation of CTLA-4. J Immunol. 2001;166:6893–8.
65. Balandina A, Lecart S, Dartevelle P, Saoudi A, Berrih-Aknin S. Functional defect of regulatory CD4(+)CD25+ T cells in the thymus of patients with autoimmune myasthenia gravis. Blood. 2005;105:735–41.
66. Sheng JR, Li L, Ganesh BB, Vasu C, Prabhakar BS, Meriggioli MN. Suppression of experimental autoimmune myasthenia gravis by granulocyte-macrophage colony-stimulating factor is associated with an expansion of FoxP3+ regulatory T cells. J Immunol. 2006;177:5296–306.
67. Sheng JR, Li LC, Ganesh BB, Prabhakar BS, Meriggioli MN. Regulatory T cells induced by GM-CSF suppress ongoing experimental myasthenia gravis. Clin Immunol. 2008;128:172–80.
68. Sheng JR, Muthusamy T, Prabhakar BS, Meriggioli MN. GM-CSF-induced regulatory T cells selectively inhibit anti-acetylcholine receptor-specific immune responses in experimental myasthenia gravis. J Neuroimmunol. 2011;240-241:65–73.
69. Rowin J, Thiruppathi M, Arhebamen E, Sheng J, Prabhakar BS, Meriggioli MN. Granulocyte macrophage colony-stimulating factor treatment of a patient in myasthenic crisis: effects on regulatory T cells. Muscle Nerve. 2012;46:449–53.
70. Monson NL, Cravens PD, Frohman EM, Hawker K, Racke MK. Effect of rituximab on the peripheral blood and cerebrospinal fluid B cells in patients with primary progressive multiple sclerosis. Arch Neurol. 2005;62:258–64.
71. Robeson KR, Kumar A, Keung B, DiCapua DB, Grodinsky E, Patwa HS, et al. Durability of the rituximab response in acetylcholine receptor autoantibody-positive myasthenia gravis. JAMA Neurol. 2017;74:60–6.
72. Diaz-Manera J, Martinez-Hernandez E, Querol L, Klooster R, Rojas-García R, Suárez-Calvet X, et al. Long-lasting treatment effect of rituximab in MuSK myasthenia. Neurology. 2012;78:189–93.
73. Baek WS, Bashey A, Sheean GL. Complete remission induced by rituximab in refractory, seronegative, muscle-specific, kinase-positive myasthenia gravis. J Neurol Neurosurg Psychiatry. 2007;78:771.
74. Hain B, Jordan K, Deschauer M, Zierz S. Successful treatment of MuSK antibody-positive myasthenia gravis with rituximab. Muscle Nerve. 2006;33:575–80.
75. Lisak RP, Ragheb S. The role of B cell-activating factor in autoimmune myasthenia gravis. Ann N Y Acad Sci. 2012;1274:60–7.
76. Kim JY, Yang Y, Moon JS, Lee EY, So SH, Lee HS, et al. Serum BAFF expression in patients with myasthenia gravis. J Neuroimmunol. 2008;199:151–4.
77. Berrih-Aknin S, Ragheb S, Le Panse R, Lisak RP. Ectopic germinal centers, BAFF and anti-B-cell therapy in myasthenia gravis. Autoimmun Rev. 2013;12:885–93.
78. Avidan N, Le Panse R, Harbo HF, Bernasconi P, Poulas K, Ginzburg E, et al. VAV1 and BAFF, via NFkappaB pathway, are genetic risk factors for myasthenia gravis. Ann Clin Transl Neurol. 2014;1:329–39.
79. Ibtehaj N, Huda R. High-dose BAFF receptor specific mAb-siRNA conjugate generates Fas-expressing B cells in lymph nodes and high-affinity serum autoantibody in a myasthenia mouse model. Clin Immunol. 2017;176:122–30.
80. Bongioanni P, Ricciardi R, Pellegrino D, Romano MR. T-cell tumor necrosis factor-alpha receptor binding in myasthenic patients. J Neuroimmunol. 1999;93:203–7.
81. Li H, Shi FD, Bai X, Huang Y, Diab A, He B, et al. Cytokine and chemokine mRNA expressing cells in muscle tissues of experimental autoimmune myasthenia gravis. J Neurol Sci. 1998;161:40–6.
82. Rowin J, Meriggioli MN, Tuzun E, Leurgans S, Christadoss P. Etanercept treatment in corticosteroid-dependent myasthenia gravis. Neurology. 2004;63:2390–2.
83. Tuzun E, Meriggioli MN, Rowin J, Yang H, Christadoss P. Myasthenia gravis patients with low

plasma IL-6 and IFN-gamma benefit from etanercept treatment. J Autoimmun. 2005;24:261–8.
84. Christadoss P, Goluszko E. Treatment of experimental autoimmune myasthenia gravis with recombinant human tumor necrosis factor receptor Fc protein. J Neuroimmunol. 2002;122:186–90.
85. Fee DB, Kasarskis EJ. Myasthenia gravis associated with etanercept therapy. Muscle Nerve. 2009;39:866–70.
86. Strober J, Cowan MJ, Horn BN. Allogeneic hematopoietic cell transplantation for refractory myasthenia gravis. Arch Neurol. 2009;66:659–61.
87. Bryant A, Atkins H, Pringle CE, Allan D, Anstee G, Bence-Bruckler I, et al. Myasthenia gravis treated with autologous hematopoietic stem cell transplantation. JAMA Neurol. 2016;73:652–8.
88. Hakansson I, Sandstedt A, Lundin F, Askmark H, Pirskanen R, Carlson K, et al. Successful autologous haematopoietic stem cell transplantation for refractory myasthenia gravis—a case report. Neuromuscul Disord. 2017;27:90–3.
89. Conti-Fine BM, Milani M, Kaminski HJ. Myasthenia gravis: past, present, and future. J Clin Invest. 2006;116:2843–54.
90. Christadoss P. C5 gene influences the development of murine myasthenia gravis. J Immunol. 1988;140:2589–92.
91. Kusner LL, Satija N, Cheng G, Kaminski HJ. Targeting therapy to the neuromuscular junction: proof of concept. Muscle Nerve. 2014;49:749–56.
92. Tuzun E, Scott BG, Goluszko E, Higgs S, Christadoss P. Genetic evidence for involvement of classical complement pathway in induction of experimental autoimmune myasthenia gravis. J Immunol. 2003;171:3847–54.
93. Biesecker G, Gomez CM. Inhibition of acute passive transfer experimental autoimmune myasthenia gravis with Fab antibody to complement C6. J Immunol. 1989;142:2654–9.
94. Piddlesden SJ, Jiang S, Levin JL, Vincent A, Morgan BP. Soluble complement receptor 1 (sCR1) protects against experimental autoimmune myasthenia gravis. J Neuroimmunol. 1996;71:173–7.
95. Morgan BP, Harris CL. Complement, a target for therapy in inflammatory and degenerative diseases. Nat Rev Drug Discov. 2015;14:857–77.
96. Zhou Y, Gong B, Lin F, Rother RP, Medof ME, Kaminski HJ. Anti-C5 antibody treatment ameliorates weakness in experimentally acquired myasthenia gravis. J Immunol. 2007;179:8562–7.
97. Howard JF Jr, Utsugisawa K, Benatar M, Murai H, Barohn RJ, Illa I, et al. Safety and efficacy of eculizumab in anti-acetylcholine receptor antibody-positive refractory generalised myasthenia gravis (REGAIN): a phase 3, randomised, double-blind, placebo-controlled, multicentre study. Lancet Neurol. 2017;16(12):976–86.
98. Fredslund F, Laursen NS, Roversi P, Jenner L, Oliveira CL, Pedersen JS, et al. Structure of and influence of a tick complement inhibitor on human complement component 5. Nat Immunol. 2008;9:753–60.
99. Soltys J, Kusner LL, Young A, Richmonds C, Hatala D, Gong B, et al. Novel complement inhibitor limits severity of experimentally myasthenia gravis. Ann Neurol. 2009;65:67–75.
100. Subias M, Tortajada A, Gastoldi S, Galbusera M, López-Perrote A, Lopez Lde J, et al. A novel antibody against human factor B that blocks formation of the C3bB proconvertase and inhibits complement activation in disease models. J Immunol. 2014;193:5567–75.
101. Huda R, Tuzun E, Christadoss P. Complement C2 siRNA mediated therapy of myasthenia gravis in mice. J Autoimmun. 2013;42:94–104.
102. Song C, Xu Z, Miao J, Xu J, Wu X, Zhang F, et al. Protective effect of scFv-DAF fusion protein on the complement attack to acetylcholine receptor: a possible option for treatment of myasthenia gravis. Muscle Nerve. 2012;45:668–75.
103. Brenner T, Hamra-Amitay Y, Evron T, Boneva N, Seidman S, Soreq H. The role of readthrough acetylcholinesterase in the pathophysiology of myasthenia gravis. FASEB J. 2003;17:214–22.
104. Argov Z, McKee D, Agus S, Brawer S, Shlomowitz N, Yoseph OB, et al. Treatment of human myasthenia gravis with oral antisense suppression of acetylcholinesterase. Neurology. 2007;69:699–700.
105. Luo J, Lindstrom J. AChR-specific immunosuppressive therapy of myasthenia gravis. Biochem Pharmacol. 2015;97:609–19.
106. Bartfeld D, Fuchs S. Specific immunosuppression of experimental autoimmune myasthenia gravis by denatured acetylcholine receptor. Proc Natl Acad Sci U S A. 1978;75:4006–10.
107. Ma CG, Zhang GX, Xiao BG, Link J, Olsson T, Link H. Suppression of experimental autoimmune myasthenia gravis by nasal administration of acetylcholine receptor. J Neuroimmunol. 1995;58:51–60.
108. Okumura S, McIntosh K, Drachman DB. Oral administration of acetylcholine receptor: effects on experimental myasthenia gravis. Ann Neurol. 1994;36:704–13.
109. Wang ZY, Qiao J, Link H. Suppression of experimental autoimmune myasthenia gravis by oral administration of acetylcholine receptor. J Neuroimmunol. 1993;44:209–14.
110. Luo J, Kuryatov A, Lindstrom JM. Specific immunotherapy of experimental myasthenia gravis by a novel mechanism. Ann Neurol. 2010;67:441–51.
111. Luo J, Lindstrom J. Antigen-specific immunotherapeutic vaccine for experimental autoimmune myasthenia gravis. J Immunol. 2014;193:5044–55.
112. Araga S, Blalock JE. Use of complementary peptides and their antibodies in B-cell-mediated autoimmune disease: prevention of experimental autoimmune myasthenia gravis with a peptide vaccine. ImmunoMethods. 1994;5:130–5.

113. Araga S, LeBoeuf RD, Blalock JE. Prevention of experimental autoimmune myasthenia gravis by manipulation of the immune network with a complementary peptide for the acetylcholine receptor. Proc Natl Acad Sci U S A. 1993;90:8747–51.
114. Araga S, Xu L, Nakashima K, Villain M, Blalock JE. A peptide vaccine that prevents experimental autoimmune myasthenia gravis by specifically blocking T cell help. FASEB J. 2000;14:185–96.
115. Galin FS, Chrisman CL, Cook JR Jr, Xu L, Jackson PL, Noerager BD, et al. Possible therapeutic vaccines for canine myasthenia gravis: implications for the human disease and associated fatigue. Brain Behav Immun. 2007;21:323–31.
116. Shelton GD, Lindstrom JM. Spontaneous remission in canine myasthenia gravis: implications for assessing human MG therapies. Neurology. 2001;57:2139–41.
117. Roopenian DC, Akilesh S. FcRn: the neonatal Fc receptor comes of age. Nat Rev Immunol. 2007;7:715–25.
118. Liu L, Garcia AM, Santoro H, Zhang Y, McDonnell K, Dumont J, et al. Amelioration of experimental autoimmune myasthenia gravis in rats by neonatal FcR blockade. J Immunol. 2007;178:5390–8.
119. UCB. UCB First Three Months Interim Report 2017: UCB with a good start into 2017; 2017.
120. Argenx. ARGX-113 [online]. http://www.argen-x.com/en-GB/content/argx-113/22/. Accessed 8 May 2017.
121. Thiruppathi M, Sheng JR, Li L, Prabhakar BS, Meriggioli MN. Recombinant IgG2a Fc (M045) multimers effectively suppress experimental autoimmune myasthenia gravis. J Autoimmun. 2014;52:64–73.
122. Somnier FE, Langvad E. Plasma exchange with selective immunoadsorption of anti-acetylcholine receptor antibodies. J Neuroimmunol. 1989;22:123–7.
123. Ptak J. Changes of plasma proteins after immunoadsorption using Ig-Adsopak columns in patients with myasthenia gravis. Transfus Apher Sci. 2004;30:125–9.
124. Antozzi C, Berta E, Confalonieri P, Zuffi M, Cornelio F, Mantegazza R. Protein-A immunoadsorption in immunosuppression-resistant myasthenia gravis. Lancet. 1994;343:124.
125. Grob D, Simpson D, Mitsumoto H, Hoch B, Mokhtarian F, Bender A, et al. Treatment of myasthenia gravis by immunoadsorption of plasma. Neurology. 1995;45:338–44.
126. Lagoumintzis G, Zisimopoulou P, Kordas G, Lazaridis K, Poulas K, Tzartos SJ. Recent approaches to the development of antigen-specific immunotherapies for myasthenia gravis. Autoimmunity. 2010;43:436–45.
127. Lagoumintzis G, Zisimopoulou P, Trakas N, Grapsa E, Poulas K, Tzartos SJ. Scale up and safety parameters of antigen specific immunoadsorption of human anti-acetylcholine receptor antibodies. J Neuroimmunol. 2014;267:1–6.
128. Lazaridis K, Evaggelakou P, Bentenidi E, Sideri A, Grapsa E, Tzartos SJ. Specific adsorbents for myasthenia gravis autoantibodies using mutants of the muscle nicotinic acetylcholine receptor extracellular domains. J Neuroimmunol. 2015;278:19–25.

Clinical Trial Design for Myasthenia Gravis

21

Radwa Aly, Gary R. Cutter, and Henry J. Kaminski

Introduction

During the last century, the evidence base of treatment for patients with myasthenia gravis (MG) has been derived largely from retrospective case series, open-label studies, and expert opinions. More recently, evaluation of therapeutics for MG has become much more rigorous using randomized clinical trial methodologies, and several phase III trials have been successfully undertaken. Advances in immunotherapeutics and the biological basis of MG have accelerated the number of drugs under development for MG leading to a greater necessity to optimize approaches for evaluation of new treatments. Clinical trials are rigorously constructed experiments designed to restrict confounding variables beyond the influence of the therapy under evaluation while attempting to mimic standard medical practice. There is limited value to a trial that may be so controlled that it could not be reproduced in a typical outpatient or inpatient setting. Internationally accepted guidelines have been established for clinical trial performance and reporting [1]. This chapter will provide a broad overview of clinical trial development for MG based on general principles and experience from completed trials.

Study Rationale

The heart of clinical trial development lies with the question, "what compelling reason justifies the expenditure of time, effort, and money as well as the exposure of subjects to harm including potential for death?" Present therapies (see Chap. 11) have significant adverse effects and upward of 30% of patients are treatment-resistant with current therapies. MG is not the nearly uniformly lethal condition that it once was, but death may occur with myasthenic crisis, which one third of patients still experience [2, 3]. Need exists for improved therapeutics with an ultimate goal for a cure. Further, advances in understanding the mechanisms of autoimmune disorders are leading to therapies that more rationally target MG pathology providing a clearer rationale for specific trial performance.

Trials must have the expectation to improve present therapies based on improved efficacy or reduced adverse effects. Given the effective-

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H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*, Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6_21

ness of prednisone but its numerous complications, a focus of clinical trials has been to evaluate immunosuppressive agents for their corticosteroid-sparing effect while achieving improvement in clinical manifestations. Despite excellent biological and clinical rationale, the failure of trials of mycophenolate, tacrolimus, and methotrexate for generalized MG [4–7] suggests a need to more precisely determine optimal outcome measures and trial design.

Trial Design

The four phases of clinical trials—phases I, II, III, and IV—present their own unique processes and challenges for patient identification, enrollment, and retention. Phase I trials are the first investigation of a potential new drug to determine the mechanism of action and often pharmacokinetic properties and safety. This phase is most commonly conducted in a small number of patients, can be conducted in healthy patients with the target disease, and can last several months. Phase II and III trials focus on the safety and then effectiveness of the drug and are conducted on patients with the target disease. Phase II trials are meant to determine the short-term side effects and identify safety risks associated with the investigational drug as well as providing some information on dose and efficacy either through a biomarker or clinical endpoint. These trials can last several months to 2 years. During phase III trials, the obvious side effects are known, and the drug is compared against a placebo or drug designed for the target disease currently on the market. Phase III trials are designed to provide more complete safety information on a larger population to uncover rarer negative effects to balance against efficacy in assessing benefit versus risk. Phase III trials can last up to 4 years or longer as in some cancer trials. Phase IV studies are post-marketing trials to examine longer-term safety issues and sometime durability of the treatment effects and usually last several years in duration.

Phase I

Phase I studies focus on dose finding and schedules of administration in healthy humans based on pharmacokinetic and pharmacodynamic properties. They examine characteristics of response as well as the immediate safety of a treatment. At times phase I trials are only performed with subjects with the disease of interest because of the potential toxicity of the agent not being justified for testing in a healthy individual. Most studies are based on the assumption that the more agent, the greater the response. Often a number of animal studies have been used to inform dose ranging in humans and assume some direct generalization from animal to man. Some new considerations for these designs have been so-called adaptive designs. A few newer designs adjust the dose based on response as well as toxicity and side effects, such as continual reassessment method or CRM. Many past efforts have evolved examining dose response, but newer agents may have varied effects at differing doses. They challenge the assumption that more is better on the efficacy side of the equation, which requires many of the dose escalation designs to be reconsidered. Without the monotonic assumptions on dose, there may be substantial increases in the sample size even in these early studies. To date for MG, therapeutics have all gone through phase I to III evaluations in other disorders prior to evaluation for MG.

Phase II

Phase II studies serve as proof-of-concept evaluations for preliminary safety and estimates of treatment efficacy. Phase II trials offer greater flexibility in design and especially newer designs than do phase III trials. Phase II trials have multiple goals and even an exploratory component to them compared to phase III where the central focus is on demonstrating predefined effectiveness and safety. Phase II trials can be designed to define the best dosage, estimate efficacy to plan a phase III trial, identify the presumed safest dose, examine the responsiveness of various endpoints,

or identify target populations most likely to respond. Phase II designs may use biomarkers as outcomes or purported surrogate outcomes, where phase III designs usually have as the primary outcome a clinical measure. In MG, phase II trials have nearly all had straightforward designs as randomized, double-blind controlled investigations comparing active drug versus placebo [5, 7–13]. Some trials have compared treatments [14–17]. Safety is an important consideration for all of the trials but of primary importance for phase I and II trials.

Phase III

Phase III trials are considered pivotal in that their goal is to alter practice, are generally directed at so-called clinically meaningful endpoints, and are usually large multicenter trials. Trials have generally been designed to demonstrate superiority of therapy compared to placebo. Superiority trials aim to demonstrate that one treatment is better than another. Non-inferiority trials attempt to show that one treatment is not worse than another, and equivalence trials attempt to show that a treatment is neither worse nor better than a standard treatment. For MG, investigations of mycophenolate, azathioprine, and methotrexate [6] were designed as superiority trials with placebo controls, but there was no evidence of a difference between groups. MGTX compared thymectomy plus prednisone versus prednisone alone and demonstrated the superiority of the surgical arm; however, if the outcomes were equivalent, common practice would have changed since thymectomy could no longer be justified.

Randomization

Randomization is a key requirement for treatment trials and serves to control for potential confounding factors by completely unbiased allocation of treatments to participants. These confounding factors range from the obvious such as gender and age to much more challenging factors, which include genetic makeup and environment. As medicine enters the era of whole genome sequencing, even more information will be available to define an individual's phenotype, and methods will be needed to incorporate such considerations into trial assessment beyond the bucket or basket and umbrella approaches of today. The bucket design tests the effect of one or more drugs on one or more single mutations in a variety of diseases versus the umbrella designs which test the impact of different drugs on different mutations in a single disease. This becomes additionally complicated when one considers ongoing advances in the understanding of the microbiome of humans and its potential influence on disease and alteration by therapeutics.

Entrance Criteria

Trials must consider a host of decisions that impact both the generalizability of results and the likelihood of successfully performing an investigation. There is often a trade-off between generalizability where the more heterogeneous the sample, the wider the results can be applied and the desire for homogeneity in patients to minimize extraneous variability thereby making it easier to see treatment differences. This dilemma is often faced in designing the specifications of the study population.

Diagnostic Specificity

Assuring that subjects have the diagnosis of MG is a critical first step in trial design. All subjects must have typical clinical manifestations of MG, including objective weakness, and studies to date have required elevations of serum autoantibodies, essentially always the acetylcholine receptor (AChR) binding antibody, as entrance criteria. Recent phase II studies have included a mix of AChR antibody and muscle-specific kinase (MuSK) antibody-positive subjects. Both these tests are highly specific for MG and simple to obtain, which is the rationale for assuring diagnostic specificity. Because of variability in performance of AChR antibody assays, the

thymectomy plus prednisone versus prednisone alone (MGTX) trial used a higher cutoff level than set by commercial labs to allow entrance into the study. In contrast, repetitive stimulation and single fiber EMG require specialized personnel to perform and have unknown reliability dependent on examiner skill making them a challenge to use for diagnostic confirmation for a trial.

Investigations of ocular myasthenia face a particular challenge in that upward of 50% of patients with the disease do not have AChR antibodies detected by the standard radioimmunoassay. Trials have thus far relied on identification of a characteristic clinical presentation and exclusion of other diagnoses as an entrance requirement with the addition of at least one confirmatory test including a positive response to a cholinesterase inhibitor, elevation of serum AChR antibody, decremental response to repetitive stimulation, or abnormal single fiber EMG [18]. Therefore, ocular myasthenia trials would be expected to have a greater number of inclusion criteria than generalized MG trials.

Therapeutic Target Decisions

As described in several chapters of this text, MG is a heterogeneous disorder with differences based on autoantibody status, age of onset, association with thymoma, and clinical presentation (ocular vs. generalized) [19–21]. Genetic factors associated with pathogenesis and treatment response are beginning to be defined [22–25]. The critical aspect for trial design is that these subtypes are likely to have a differential response to therapeutics. For example, the primary effector mechanism of AChR antibodies is through activation of complement, while MuSK antibodies induce disease mediated by IgG4, which does not activate complement [26]; thereby eliminating MuSK patients from trials of complement inhibition may be appropriate. The thymus of MuSK antibody patients does not show the characteristic hyperplasia of early-onset AChR antibody-positive patients, which again brings into question thymectomy as a treatment for this group as well. Patients with ocular manifestations may remain with ocular symptoms or develop generalized weakness. These distinctions suggest variations in pathophysiology from patients with initially generalized disease as well as a need for weakness-specific outcome assessments, i.e., double vision and ptosis vs. generalized weakness. Persistent symptoms among patients with double vision may be a function of the requirements of the ocular motor system to maintain precise ocular muscle alignment even in the face of a largely suppressed immune attack [27]. Statistical designs often assume a common response to therapy, and thus potential differences in response to patient subtypes should be considered, and those likely not to respond or those who likely will respond differently may be eliminated via inclusion or exclusion criteria.

Another aspect of target engagement relates to the expected duration for a biological response. An appreciation of the expected biological effect of a drug is required to determine study duration. The failure of trials of mycophenolate and tacrolimus for generalized MG was at least partially related to the trials being too short for a meaningful reduction of circulating lymphocytes to occur as well as not adequately dealing with the durability of prednisone treatment [4, 7, 28–30]. The expected mechanism of action of an intervention is critical for trial design and is a review criterion for NIH in assessment of clinical trials for funding.

Age and Gender

Clinical trials for MG typically restrict enrollment to individuals over the age of 18 years with a variable older age cutoff. No restrictions have been made based on gender. However, gender and age may be factors in underlying pathophysiology (see Chap. 3) [21]. At present there is no compelling evidence that existing therapeutics have the potential for a differential effect based on gender. However, given the differential occurrence between men and women over the life span, it may simply be that sufficient sized trials have yet to elucidate the differences in response.

Clinical subtypes of MG are characterized and grouped into early- and late-onset with a poorly defined dividing line of 45–60. Thymic pathology observed among patients with MG differs among early- and late-onset patients, which suggests fundamental differences in pathogenesis and therefore potential difference in response to therapeutics. Adverse effect profiles are likely to differ based on age and gender. This must be considered carefully in the design of MG trials.

Disease Severity

A critical consideration is the level of weakness for trial entrance. Therapeutic trials for MG have been performed almost exclusively on generalized MG patients with MGFA classifications of II–IV. No investigations beyond retrospective evaluation of myasthenic crisis (MGFA classification V) have been performed with the exception of evaluations of IV Ig or plasma exchange, and none of these were performed in a randomized, controlled fashion [14, 31–33]. Given the uniform clinical agreement of plasma exchange's efficacy for severe MG, it is unlikely a trial can be performed in an ethical fashion from the practicing physician's perspective [34]. From the societal perspective, which is often grounding the FDA's perspective, exceptional efforts may be needed to design ethical trials [35]. Outcome measures, such as the Quantitative MG (QMG) score and MG Composite, are now also used to set a level of weakness for study entrance with the MG Foundation of America Clinical Research Standards Committee setting a QMG score of 12 or greater being used generally as the minimal severity of disease for a clinical trial [36]. Designs need to consider floor effects as well as ceiling effects when selecting patient populations. The critical importance of disease severity is illustrated by the negative results of a trial of tacrolimus, which entered subjects in minimal manifestation status, a population that likely would be difficult to demonstrate a treatment effect [28]. Further, if too stringent criteria for weakness are used, the trial may have excessive regression toward the mean as a result of the high hurdle to qualify, thereby overaccentuating the benefits. Whether there are fundamental differences to be expected in treatment response based on severity of weakness, as assessed by QMG or other measures, is an important question to consider in design. Further, the outcome scales are nonlinear ordinal scales, and, therefore, a three-point improvement in QMG from 20 to 17 versus 3 to 0 has different clinical and biological significance.

Exclusion Criteria

Exclusions for MG trials are those typical for any disease based on significant coexistent medical or psychiatric disorders for safety considerations, competency for informed consent, and likelihood that the participant can follow the instructions of the trial. MG trials typically have excluded individuals with history of thymoma. The basis for this exclusion presumably lies with concerns of an immunosuppressive agent leading to recurrence of tumor and the observation that thymoma-associated MG patients have greater weakness and are more likely to be treatment-resistant [37]. This exclusion criterion may needlessly eliminate a subgroup of patients for involvement in many therapeutic trials, when the decision to exclude is based on convention rather than a specific biological risk. In the clinical setting, treatment approaches for thymoma-associated MG are often identical beyond the obligatory indication for thymoma resection and monitoring for tumor recurrence.

Outcome Measures

Objective outcome measures are of critical importance for all trials, and the last decade has seen efforts to more precisely develop outcome measures specific for MG (see Chap. 19) [38–40]. For MG trials, a grading of weakness severity and a reduction of cumulative corticosteroid exposure have been used as primary outcome measures [36, 41]. The QMG, manual muscle testing, and modified Besinger's score are

examples of simple ordinal scales that have been used [12, 16, 42–47] with the QMG being the most extensively studied and used in clinical trials [30, 44, 46, 47]. A three-point reduction in the QMG has been considered to be clinically significant [47].

In the last 15 years, the FDA has placed greater emphasis on patient-reported outcomes and is further refining expectations of definitions of a positive clinical response [48]. An analysis of outcome measures of a trial of mycophenolate mofetil for generalized MG revealed that the MG-ADL could serve as a reliable substitute for the Quantitative MG score and be easier to administer [49]. A prospective study suggested that a two-point reduction on the MG-ADL was clinically significant [38]. A phase III trial of eculizumab for treatment-resistant MG used the MG-ADL scale as the primary outcome measure, and while multiple secondary outcome measures, including the QMG and MG Composite, were significantly improved in the treatment group, the MG-ADL showed no statistical difference between treatment and control despite all secondary outcome measures being significantly different [50]. At the time of this writing, the results have not appeared in a peer-reviewed format, and therefore, explanations for this discrepancy are not immediately apparent.

A Task Force of the MG Foundation of America with international representation has recommended the MG Composite as the preferred quantitative measure for assessment of changes in subject response for generalized MG. The MG Composite is a mix of examination and patient-reported measures and is easily administered [40, 51, 52]. The scale has thus far not been used as a primary outcome measure in a clinical trial.

Reduction of corticosteroid treatment has been used as a primary outcome measure in several trials. The principle that underlies the use of steroid sparing as a primary outcome measure is its importance as a safety measure. The adverse effects of corticosteroids are so severe that therapies limiting their use would be beneficial for patient care [53]. Investigations of azathioprine and mycophenolate assessed the difference in corticosteroid dose at each assessment time over the course of the study [4, 54]. Steroid sparing was demonstrated in the azathioprine study but not until the 18-month time point, while the 36-week-long mycophenolate study was negative. In contrast, the MGTX trial used a measure of prednisone dose over the 3-year study, an integrated assessment, which reflects the cumulative exposure of prednisone. MGTX also assessed the QMG score over time to assure and found subjects to be on a lower cumulative dose of prednisone but also had improved cumulative MG scores [44]. An investigation of methotrexate for corticosteroid-sparing effect also used the area under the dose time curve as a primary outcome measure [6]. Comparison of these studies emphasizes the need for appropriate study duration and expected action of the therapeutic in study design [55].

Trial Duration

There is no reproducible process that reliably predicts the duration of a clinical trial. However, it is important to understand that all trials for MG have lasted longer than originally anticipated for reasons such as the variation in the durations of specific phases, rates of enrollment, or unanticipated events. In the MGTX trial [56], wide variations in the regulatory process were evident in start-up.

A major determinant of trial duration is the ability to enroll subjects. Recruitment rates for recent multicenter trials have varied from less than one to at best two subjects per month [6, 30, 44], and all studies initially overestimated the ability to identify and enroll patients for the trials. Inclusion criteria for some investigations, in particular the MGTX study, lead to a reliance on the incident rate of the disease, which for MG is extremely low, thereby extending the duration of recruitment.

The regulatory burden for trials is large and adds to trial duration. Over the past 20 plus years, a steady increase in the rigor and requirements of trials has occurred. In part, these

increased efforts have been in response to poor practices. Good clinical practice (GCP) is an international ethical and scientific standard for design and performance and reporting of clinical trials that involve human subjects [57]. A thorough discussion of GCP is beyond the scope of this review but involves items ranging from traceable data input to source documentation to investigator training and competence in protocol compliance. GCP increases the cost and complexity of trials, and there has been little to no measurable impact on the quality of the outcomes or the information [58]. There is no class I evidence that all of the GCPs improve drug discovery or treatment outcomes.

In addition to GCP, other regulatory requirements and trial activities all conspire to increase trial duration. For example, the time for MGTX study centers to obtain full regulatory approval to recruitment was approximately 10 months for US sites and for non-US sites 13.5 months [56]. The difference related to non-US sites needing Federal Wide Assurance certification and State Department clearance along with ethics reviews, which can be more involved with surgical trials. An investigation of slightly more than 10,000 trials from 1999 to 2005 in a variety of phases found that procedural frequency had grown by nearly 9% annually and the mean number of inclusion criteria escalated threefold [59]. Burden of work for study sites increased by 10%. Investigations of phase III and phase IV protocols identified increases in study endpoints, procedures, and inclusion/exclusion criteria, while subject randomization actually decreased. There is a need to make trials as simple as possible without reducing the key processes for successful trial completion. While there is a call for more pragmatic trials, they often become much more complicated when the regulatory requirements and ethics committee considerations begin to encroach on the concept of pragmatic designs. It is then the role of the investigators, sponsor, and coordinating center to anticipate and minimize these potential challenges when designing the study.

Statistical Considerations

There are two major philosophies of statistical analyses to trial designs: the classical or frequentist and the Bayesian approach. Frequentist approaches utilize p-values to summarize how likely an outcome is from a conceptualization, so how often by chance would we observe the result obtained, if we repeated the same trial numerous times? Bayesian methods are models that take prior knowledge and based on an experiment update that knowledge yielding a degree of belief in a result. The two approaches do differ. The former is useful for setting up a straw man and finding results that refute it, whereas the latter is superior for estimation making optimal use of all available data. For example, a clinician considers a certain complex of symptoms, variable ptosis, double vision, and generalized weakness and determines that there is a high likelihood of myasthenia gravis. She then orders an AChR antibody test. This is a Bayesian interpretation of the analyses of the physical examination. She believes that there is a high probability of MG.

Bayesian approaches have intuitively familiar interpretations and in fact are a more natural interpretation. Bayesian methods do have another benefit. An interpretation that a result has a trend toward significance in a situation that the observed p-value is not significant is not appropriate within the frequentist hypothesis-testing paradigm. From a frequentist perspective, being close is one of the many outcomes that can occur by chance with a frequency that is not rare enough to be considered more likely to have occurred by the null hypothesis. The result is either rejection or failure to reject area and thus the outcome is binary. However, the concept of trending toward significance has more meaning in a Bayesian context.

The Bayesian approach uses information in a way that enables one to use prior information to make an assessment and then updates that assessment with each new piece of data. In terms of clinical trials, the idea of incorporating prior information into the final analysis seems natural. The fundamental question is whether one believes a trial is designed to find the best estimate of the

treatment effect (the Bayesian approach) or whether the expectation is an *independent* demonstration of the effect of a treatment garnered from the prior work (the Frequentist approach). This perspective leads to greatly different views on the value of Bayesian statistics in phase III trials, whereas there is much less controversy regarding their use in phase II trials.

Adaptive designs are now under consideration for trials at several phases. Adaptive designs include (1) adapting on allocations (how subjects are allocated to treatments), (2) sampling rule (adapting how many subjects are used in each stage), (3) stopping rule (adapting when to stop the trial, e.g., for efficacy, harm, or futility), and (4) decision rule (adapting to how the next steps move forward). Such designs may save resources and time, if there are unequivocal signs that a treatment is not effective.

There are challenges with the decision rules and the implementation of the changes. Adaptive designs could seamlessly move from phase I through phase II and onto phase III. The same information could be obtained without the expected time loss between phase II and phase III. A portion of the data is monitored as it is being generated and allows for design adjustment typically by sample size re-estimation and/or stop a trial arm and continue. This process would eliminate noninformative or poorly performing doses and remove the interval between phase II and phase III. However, the design commits investigators in advance to the predefined corrections from the phase II trial. When a classical phase II approach is used followed by a phase III trial, the similar adjustments may be made, but with a time lag to work through the alterations, which often can be as much as 1–2 years. Hence, there must be better understanding of endpoints, recruitment patterns, and expected treatment responses. The knowledge gained from a typical phase II trial on other outcome assessments would not be available. Further, the decision rules must be completely and clearly spelled out in advance. Decisions to add subjects may be required if initial assumptions were incorrect, and this could lead to increased sponsor costs, investigator frustration, and even lower subject participation. Recruitment motivation may diminish if signals of safety or lack of efficacy are observed. Nevertheless, there are the advantages of altering design when initial assumptions are wrong, for example, the event rate in the control group differs greatly from expected and limiting unrealistic expectations for trial outcomes. Logistical decisions must be made as to when to make adaptations and critically who sees results and makes decisions. A problem for MG trials is that rates of events are not necessarily constant in time and there, if review of data is taken too early during follow-up, the assessment may not accurately represent the study period. However, a delay in adaptation decisions to obtain longer-term outcomes creates its own challenge with delayed enrollment and compromises the next enrollment period.

Conclusion

The design of clinical trials involves a multidisciplinary approach encompassing the disease and its characteristics, the drug or treatment and its putative mechanism of action, the inclusion and exclusions criteria, the endpoints, the logistics and duration of the study, and the ability to recruit and complete the trial in a reasonable time frame. The type of design is wide ranging and involves what has been done in the past as well as what might be more optimal. No design is perfect and all designs involve trade-offs. The rigor required in clinical trials today is increasing, but the payoff from high caliber clinical trials may well be worth the effort.

References

1. CONSORT. Transparent reporting of trials. http://www.consort-statement.org/. Accessed 30 Sept 2014.
2. Alshekhlee A, Miles JD, Katirji B, Preston DC, Kaminski HJ. Incidence and mortality rates of myasthenia gravis and myasthenic crisis in US hospitals. Neurology. 2009;72(18):1548–54.
3. Luchanok U, Kaminski HJ. Natural history of myasthenia gravis. In: Engel AG, editor. Myasthenia gravis and myasthenic disorders. New York: Oxford University Press; 2012. p. 90–107.

4. Sanders DB, Hart IK, Mantegazza R, Shukla SS, Siddiqi ZA, De Baets MH, et al. An international, phase III, randomized trial of mycophenolate mofetil in myasthenia gravis. Neurology. 2008;71(6):400–6.
5. Heckmann JM, Rawoot A, Bateman K, Renison R, Badri M. A single-blinded trial of methotrexate versus azathioprine as steroid-sparing agents in generalized myasthenia gravis. BMC Neurol. 2011;11:97.
6. Pasnoor M, He J, Herbelin L, Burns TM, Nations S, Bril V, et al. A randomized controlled trial of methotrexate for patients with generalized myasthenia gravis. Neurology. 2016;87(1):57–64.
7. Yoshikawa H, Kiuchi T, Saida T, Takamori M. Randomised, double-blind, placebo-controlled study of tacrolimus in myasthenia gravis. J Neurol Neurosurg Psychiatry. 2011;82(9):970–7.
8. Tindall RS, Rollins JA, Phillips JT, Greenlee RG, Wells L, Belendiuk G. Preliminary results of a double-blind, randomized, placebo-controlled trial of cyclosporine in myasthenia gravis. N Engl J Med. 1987;316(12):719–24.
9. Lindberg C, Andersen O, Lefvert AK. Treatment of myasthenia gravis with methylprednisolone pulse: a double blind study. Acta Neurol Scand. 1998;97(6):370–3.
10. De Feo LG, Schottlender J, Martelli NA, Molfino NA. Use of intravenous pulsed cyclophosphamide in severe, generalized myasthenia gravis. Muscle Nerve. 2002;26(1):31–6.
11. Meriggioli MN, Rowin J, Richman JG, Leurgans S. Mycophenolate mofetil for myasthenia gravis: a double-blind, placebo-controlled pilot study. Ann N Y Acad Sci. 2003;998:494–9.
12. Zinman L, Ng E, Bril V. IV immunoglobulin in patients with myasthenia gravis: a randomized controlled trial. Neurology. 2007;68(11):837–41.
13. Howard JF Jr, Barohn RJ, Cutter GR, Freimer M, Juel VC, Mozaffar T, et al. A randomized, double-blind, placebo-controlled phase II study of eculizumab in patients with refractory generalized myasthenia gravis. Muscle Nerve. 2013;48(1):76–84.
14. Barth D, Nabavi Nouri M, Ng E, Nwe P, Bril V. Comparison of IVIg and PLEX in patients with myasthenia gravis. Neurology. 2011;76(23):2017–23.
15. Trikha I, Singh S, Goyal V, Shukla G, Bhasin R, Behari M. Comparative efficacy of low dose, daily versus alternate day plasma exchange in severe myasthenia gravis: a randomised trial. J Neurol. 2007;254(8):989–95.
16. Kohler W, Bucka C, Klingel R. A randomized and controlled study comparing immunoadsorption and plasma exchange in myasthenic crisis. J Clin Apher. 2011;26(6):347–55.
17. Gajdos P, Tranchant C, Clair B, Bolgert F, Eymard B, Stojkovic T, et al. Treatment of myasthenia gravis exacerbation with intravenous immunoglobulin: a randomized double-blind clinical trial. Arch Neurol. 2005;62(11):1689–93.
18. Benatar M, McDermott MP, Sanders DB, Wolfe GI, Barohn RJ, Nowak RJ, et al. Efficacy of prednisone for the treatment of ocular myasthenia (EPITOME): a randomized, controlled trial. Muscle Nerve. 2016;53(3):363–9.
19. Compston DAS, Vincent A, Newsom-Davis J, Batchelor JR. Clinical, pathological, HLA antigen, and immunological evidence for disease heterogeneity in myasthenia gravis. Brain. 1980;103:579–601.
20. Berrih-Aknin S, Le Panse R. Myasthenia gravis and autoantibodies: pathophysiology of the different subtypes. Rev Med Intern. 2014;35(7):413–20.
21. Avidan N, Le Panse R, Berrih-Aknin S, Miller A. Genetic basis of myasthenia gravis—a comprehensive review. J Autoimmun. 2014;52C:146–53.
22. Xie Y, Meng Y, Li HF, Hong Y, Sun L, Zhu X, et al. GR gene polymorphism is associated with intersubject variability in response to glucocorticoids in patients with myasthenia gravis. Eur J Neurol. 2016;23(8):1372–9.
23. Renton AE, Pliner HA, Provenzano C, Evoli A, Ricciardi R, Nalls MA, et al. A genome-wide association study of myasthenia gravis. JAMA Neurol. 2015;72(4):396–404.
24. Avidan N, Le Panse R, Berrih-Aknin S, Miller A. Genetic basis of myasthenia gravis—a comprehensive review. J Autoimmun. 2014;52:146–53.
25. Xie Y, Li H-F, Sun L, Kusner LL, Wang S, Meng Y, et al. The role of osteopontin and its gene on glucocorticoid response in myasthenia gravis. Front Neurol. 2017;8:230.
26. Phillips WD, Vincent A. Pathogenesis of myasthenia gravis: update on disease types, models, and mechanisms. F1000Res. 2016;5. https://doi.org/10.12688/f1000research.8206.1.
27. Soltys J, Gong B, Kaminski HJ, Zhou Y, Kusner LL. Extraocular muscle susceptibility to myasthenia gravis: unique immunological environment? Ann N Y Acad Sci. 2008;1132:220–4.
28. Benatar M, Sanders D. The importance of studying history: lessons learnt from a trial of tacrolimus in myasthenia gravis. J Neurol Neurosurg Psychiatry. 2011;82(9):945.
29. Hirano M. Two strikes against mycophenolate mofetil therapy for myasthenia gravis. Curr Neurol Neurosci Rep. 2009;9(1):57–8.
30. Muscle Study Group. A trial of mycophenolate mofetil with prednisone as initial immunotherapy in myasthenia gravis. Neurology. 2008;71(6):394–9.
31. Gajdos P, Chevret S, Toyka K. Plasma exchange for myasthenia gravis. Cochrane Database Syst Rev. 2002;4:CD002275.
32. Gajdos P, Chevret S, Toyka K. Intravenous immunoglobulin for myasthenia gravis. Cochrane Database Syst Rev. 2008;1:CD002277.
33. Cortese I, Chaudhry V, So YT, Cantor F, Cornblath DR, Rae-Grant A. Evidence-based guideline update: plasmapheresis in neurologic disorders: report of the Therapeutics and Technology Assessment

Subcommittee of the American Academy of Neurology. Neurology. 2011;76(3):294–300.
34. Kaminski HJ, Cutter G, Ruff R. Practice parameters and focusing research: plasma exchange for myasthenia gravis. Muscle Nerve. 2011;43(5):625–6.
35. Patra K, Cree BA, Katz E, Pulkstenis E, Dmitrienko A. Statistical considerations for an adaptive design for serious rare disorder. Ther Innov Regul Sci. 2015;50(3):375–84.
36. Benatar M, Sanders DB, Burns TM, Cutter GR, Guptill JT, Baggi F, et al. Recommendations for myasthenia gravis clinical trials. Muscle Nerve. 2012;45(6):909–17.
37. Romi F. Thymoma in myasthenia gravis: from diagnosis to treatment. Autoimmune Dis. 2011;2011:474512.
38. Muppidi S, Wolfe GI, Conaway M, Burns TM, Mg C, MG Composite and MG-QOL15 Study Group. MG-ADL: still a relevant outcome measure. Muscle Nerve. 2011;44(5):727–31.
39. Burns TM, Grouse CK, Conaway MR, Sanders DB. Construct and concurrent validation of the MG-QOL15 in the practice setting. Muscle Nerve. 2010;41(2):219–26.
40. Burns TM, Conaway M, Sanders DB. The MG composite: a valid and reliable outcome measure for myasthenia gravis. Neurology. 2010;74(18):1434–40.
41. Alshaikh J, Kusner LL, Kaminski HJ. Clinical trials of myasthenia gravis. In: Mineo TC, editor. Novel challenges in myasthenia gravis. Hauppage: Nova Biomedical; 2015. p. 573–86.
42. Jaretzki A III, Barohn RJ, Ernstoff RM, Kaminski HJ, Keesey JC, Penn AS, et al. Myasthenia gravis: recommendations for clinical research standards. Task Force of the Medical Scientific Advisory Board of the Myasthenia Gravis Foundation of America. Neurology. 2000;55(1):16–23.
43. Besinger VA, Toyka KV, Homberg M, Heininger K, Hohlfeld R, Fetch-Moghadam A. Myasthenia gravis: long-term correlation of binding and bungarotoxin blocking antibodies against acetylcholine receptors with changes in disease severity. Neurology. 1983;33:1316–21.
44. Wolfe GI, Kaminski HJ, Aban IB, Minisman G, Kuo HC, Marx A, et al. Randomized trial of thymectomy in myasthenia gravis. N Engl J Med. 2016;375(6):511–22.
45. Sanders DB, Tucker-Lipscomb B, Massey JM. A simple manual muscle test for myasthenia gravis: validation and comparison with the QMG score. Ann N Y Acad Sci. 2003;998:440–4.
46. Bedlack RS, Simel DL, Bosworth H, Samsa G, Tucker-Lipscomb B, Sanders DB. Quantitative myasthenia gravis score: assessment of responsiveness and longitudinal validity. Neurology. 2005;64(11):1968–70.
47. Barohn RJ, McIntire D, Herbelin L, Wolfe GI, Nations S, Bryan WW. Reliability testing of the quantitative myasthenia gravis score. Ann N Y Acad Sci. 1998;841:769–72.
48. McLeod LD, Coon CD, Martin SA, Fehnel SE, Hays RD. Interpreting patient-reported outcome results: US FDA guidance and emerging methods. Expert Rev Pharmacoecon Outcomes Res. 2011;11(2):163–9.
49. Wolfe GI, Barohn RJ, Sanders DB, McDermott MP, Muscle Study Group. Comparison of outcome measures from a trial of mycophenolate mofetil in myasthenia gravis. Muscle Nerve. 2008;38(5):1429–33.
50. Corporation A. http://news.alexionpharma.com/press-release/product-news/alexion-announces-topline-results-phase-3-regain-study-eculizumab-soliris.
51. Farrugia ME, Carmichael C, Cupka BJ, Warder J, Brennan KM, Burns TM. The modified rankin scale to assess disability in myasthenia gravis: comparing with other tools. Muscle Nerve. 2014;50(4):501–7.
52. Burns TM, Conaway MR, Cutter GR, Sanders DB. Construction of an efficient evaluative instrument for myasthenia gravis: the MG composite. Muscle Nerve. 2008;38(6):1553–62.
53. Hoffmann S, Kohler S, Ziegler A, Meisel A. Glucocorticoids in myasthenia gravis—if, when, how, and how much? Acta Neurol Scand. 2014;130(4):211–21.
54. Palace J, Newsom-Davis J, Lecky B. A randomized double-blind trial of prednisolone alone or with azathioprine in myasthenia gravis. Myasthenia Gravis Study Group. Neurology. 1998;50(6):1778–83.
55. Sanders DB, Siddiqi ZA. Lessons from two trials of mycophenolate mofetil in myasthenia gravis. Ann N Y Acad Sci. 2008;1132:249–53.
56. Aban IB, Wolfe GI, Cutter GR, Kaminski HJ, Jaretzki A III, Minisman G, et al. The MGTX experience: challenges in planning and executing an international, multicenter clinical trial. J Neuroimmunol. 2008;201-202:80–4.
57. Food and Drug Administration. Good clinical practice 2017 [9 May 2017]. https://www.fda.gov/scienceresearch/specialtopics/runningclinicaltrials/.
58. Duley L, Antman K, Arena J, Avezum A, Blumenthal M, Bosch J, et al. Specific barriers to the conduct of randomized trials. Clin Trials. 2008;5(1):40–8.
59. Getz KA, Wenger J, Campo RA, Seguine ES, Kaitin KI. Assessing the impact of protocol design changes on clinical trial performance. Am J Ther. 2008;15(5):450–7.

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H. J. Kaminski, L. L. Kusner (eds.), *Myasthenia Gravis and Related Disorders*,
Current Clinical Neurology, https://doi.org/10.1007/978-3-319-73585-6