Myasthenia Gravis

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Abbreviations

ACh	Acetylcholine
AChE	Acetylcholinesterase
ACHE-I	Acetylcholinesterase inhibitor
AChR	Nicotinic acetylcholine receptor
AIRE	Autoimmune regulator gene
EAMG	Experimental autoimmune myasthenia gravis
ECD	Extracellular domain
EPP	End plate potential
HLA	Human leukocyte antigen
IFN	Interferon
IL	Interleukin
IVIg	Intravenous immunoglobulin
LNC	Lymph node cell
MAC	Membrane attack complex
MG	Myasthenia gravis
MHC	Major histocompatibility complex
MIR	Main immunogenic region
MuSK	Muscle-specific kinase
NMJ	Neuromuscular junction
PDE	Phosphodiesterase
RIPA	Radioimmunoprecipitation assays
SF-EMG	Single-fiber electromyography

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TEC	Thymic epithelial cell
VGSC	Voltage-gated sodium channels

Brief History

The first descriptions of patients with symptoms closely resembling those of MG date from the mid-seventeenth century, referring to patients with the "fatigable weakness." However, only in the late nineteenth century did a modern description of MG-like symptoms take place. At the same time, the term myasthenia gravis was coined (from the Greek words *mys* for muscle and *asthenia* for weakness, and the Latin *gravis* for severe). It took three to four more decades before in the 1930s treatment options became available. More specifically, the discovery that curare poisoning, which has similar symptoms, can be treated by cholinesterase inhibitors made them the first line of defense against MG. Around the same time, thymectomy was recognized as a potential therapy option since thymus pathology had long been associated with MG and thymectomy in some patients was followed by an improvement in their condition.

It was not until 1960 that the etiology of MG was hypothesized to be autoimmune by J. Simpson, followed by the addition of immunosuppressants in the arsenal against MG. Immunization of experimental animals with purified muscle nicotinic acetylcholine receptor (AChR), the target antigen in MG, gave symptoms similar to human MG. Finally, the antibody-mediated nature was unequivocally proven by direct induction of MG in experimental animals using transfer of pathogenic autoantibodies from MG patients.

Since then, the understanding of the etiology, pathogenic factors, and target antigens for MG has increased dramatically, making it one of the best characterized autoimmune conditions to date. Based on the accumulated knowledge, stringent diagnostic tests have been developed and several therapeutic options are currently available, each with its advantages and disadvantages. Furthermore, intensive research is currently under way to expand our understanding on the molecular basis of MG etiology and pathogenesis, followed by efforts to produce more sensitive diagnostic tools and more specific and free of side effects treatments for the disease.

Understanding MG Pathogenesis and Current Diagnostic and Treatment Options

Clinical Features of MG

MG Is an Autoimmune Disease Affecting the Muscles

MG is a prototype antibody-mediated autoimmune disease targeting the neuromuscular junction (NMJ) of skeletal muscles. Antibodies produced due to loss of

immunological tolerance of self-antigens (hence termed autoantibodies) are directed against proteins on the muscle postsynaptic membrane. This in turn impairs signal transduction from the nerve terminal, which leads to muscle weakness and muscle fatigability.

Muscle weakness is fluctuating, both on a daily basis but also on a larger scale, with periods of remission and relapses. It increases after strenuous activity and improves after a resting period, though generally it tends to worsen as the day progresses. The disease manifestation is highly variable, depending on the skeletal muscles affected, and can be life threatening when the respiratory muscles are involved. The symptoms also differ depending on the molecules targeted by the immune system, as will be discussed later. The muscles commonly affected fall into three separate groups: (1) extraocular and facial, (2) bulbar (they control swallowing, breathing, speech, and other functions of the throat), and (3) limb and axial muscles (located around the vertebral column). Accordingly, MG is termed ocular when only the extraocular muscles are affected and generalized when symptoms extend to other muscles. Moreover, patients are said to present with ocular MG when symptoms remain ocular for more than 2 years because most will have progressed to generalized MG by then. The heart muscle is under the control of the autonomic nervous system and, therefore, usually unaffected in MG, although some patients who also have a thymoma can present with cardiac disease. At disease onset, the extraocular muscles are targeted in about half of all the MG patients, without involvement of other muscle groups. In 20% of these patients, the weakness will remain purely ocular, but in the rest, it will eventually expand to facial, bulbar, and limb muscles, with most patients presenting generalized symptoms within 3 years. On the other hand, isolated bulbar and limb weakness at disease onset is relatively rare. The differences in symptom manifestation among these muscle groups are thought to occur due to their varied embryological origin. Overall, the susceptibility of the extraocular muscles is thought to be due to distinct attributes of the NMJ as discussed bellow.

In order for clinicians to be able to describe the type and severity of MG symptoms, several scoring systems have been developed. The Osserman classification has been used since 1958, which essentially separates MG into ocular and generalized, the later further divided into mild, moderate, and severe (Table 91.1). Currently, the MGFA system (developed by the MG Foundation of America) is also widely used, which takes into account the involvement of bulbar or limb weakness: all groups except from ocular weakness are divided into (a) and (b) depending on whether weakness affects predominantly the limb and/or axial muscles, or the bulbar and/or respiratory muscles, respectively (Table 91.2).

To address the need for a quantitative measure of patients' symptoms, outcome scoring systems have been devised. The best and most widely used is the quantitative MG score (QMG) originally developed in the 1980s. Since then, this first scoring system has been modified to include more graded items (raising their number from 8 to 13) and to replace items that could not be measured objectively. Each item is graded from 0 to 3 based on well-defined parameters, such as the number of seconds needed for ptosis or double vision to become evident.

Table 91.1 The Osserman classification

Group 1	Ocular MG
Group 2A	Mild generalized MG: ocular and limb but no bulbar symptoms
Group 2B	Moderate generalized MG: ocular, limb, and bulbar, but no myasthenic crises
Group 3	Acute fulminating generalized MG, marked bulbar involvement, and/or myasthenic crises
Group 4	Late severe generalized MG, marked bulbar involvement, and myasthenic crises

Table 91.2 The MGFA classification

Group I	Ocular muscle weakness
Group II (a + b)	Mild weakness affecting muscles other than ocular with or without concomitant ocular muscle involvement
Group III (a + b)	Moderate weakness affecting muscles other than ocular with or without concomitant ocular muscle involvement
Group IV (a + b)	Severe weakness affecting muscles other than ocular with or without concomitant ocular muscle involvement
Group V (a + b)	Requirement for intubation, with or without requirement for ventilation except for routine postoperative procedures[a]

[a]The use of a feeding tube without intubation

This gives a score ranging from 0 to 39, which can be used to accurately quantitate symptoms in MG patients. This is an extremely useful tool especially during clinical trials for the development of novel therapies, when an accurate and objective measure of symptom improvement (and consequently efficiency of therapy) is crucial.

MG Symptoms

Weakness of the extraocular muscles causes the typical symptoms of asymmetrical ptosis (involuntary lowering of the eyelids) and/or diplopia (double vision). The oropharyngeal muscles are commonly affected making it difficult for patients to chew and ingest (dysphagia) and may require alterations in their diet to facilitate food intake. Severe dysphagia can lead to weight loss, and in extreme cases, a nasogastric feeding tube has to be used. Pharyngeal muscle weakness can also lead to dysarthria (impaired speech), and the speech can become completely incomprehensible in severe cases or after exertion. When other facial muscles are involved, the patient has difficulty with facial expressions, such as smiling, which is reduced to the characteristic "myasthenic snarl" (or vertical laugh). Although the involvement of laryngeal muscles is not common, it is potentially life threatening since it can lead to breathing difficulties. If it becomes severe, breathing produces a characteristic stridor (high-pitched sound due to turbulent airflow in the airway) demanding immediate attention. The limb muscles are usually affected in a symmetrical pattern and most commonly the proximal ones.

This leads to difficulties with performing daily activities and walking. In addition, both extensor and flexor neck muscles can be affected, although the involvement of the extensor muscles gives the most marked symptom of dropped head, which is responsible for some of the rare cases when pain may become a problem for MG patients.

The most serious and life-threatening symptom in MG is related to a decreased ventilation capacity. As already mentioned, this can originate from paralysis of the laryngeal and vocal cord muscles leading to airway obstruction. More frequently though, breathing difficulties are caused by failing of the respiratory muscles (diaphragm and intercostals). A moderately decreased respiration is common in most patients. However, in severe cases, it can progress to a condition known as myasthenic crisis: respiratory failure, which constitutes a medical emergency and requires assisted ventilation to sustain the patient. During a myasthenic crisis, the decrease in ventilation is worsened by obstruction of the airways due to impaired coughing and palatal function. Myasthenic crises are usually rare in most patients but can be induced by circumstances such as infections, surgery, excessive dose, or withdrawal of cholinesterase inhibitors, as well as in the initial stages of prednisone treatment (a commonly used immunosuppressant).

Prevalence of Different Types of MG

MG has a prevalence of about 200–300 per million and is, therefore, classified as a rare condition. The incidence varies from 1.7 to 21 per million per year. These figures have been on the rise over the past decades and are expected to increase further, due to improvements in the diagnosis of the disease, especially in the elderly, reduced mortality rates of MG patients as a consequence of more effective therapies, and overall aging of the population. Although MG can develop at any age, it presents with two peaks for age of onset: an early onset between the second and fourth decade of life, with a predominance of females at a ratio of 7:4, and a second late-onset peak between the fifth and sixth decade with a male 3:2 predominance. The symptoms are the same in both early-onset and late-onset MG. Some clinicians consider that the disease is likely to be more severe in the late-onset patients, but this remains to be shown statistically.

Juvenile MG is not common and should not be confused with congenital myasthenic syndromes or transient neonatal MG. The congenital myasthenic syndromes are genetic disorders due to mutations that cause abnormal neuromuscular transmission. On the other hand, neonatal MG is a self-limiting disorder occurring in 10–15% of pregnancies of women with MG, caused by transfer of maternal autoantibodies through the placenta to the fetus. It develops within 2 days after birth and lasts up to 4 weeks. However, evidence suggests that in a few cases the autoantibodies may be responsible for the development of arthrogryposis multiplex congenital, a severe complication characterized by joint contractures, hypoplasia of the lungs, and other deformities due to the lack of movements in utero, which is potentially fatal for the infant because of breathing difficulties.

It should be noted that for women with MG, pregnancy is not contraindicated. It is very important though to monitor the pregnancy intensively and to cooperate

closely with the obstetrician (preferably together with a neurologist) since there is increased risk for abortion, prolonged gestation, premature labor, and other complications requiring intervention. In the mothers themselves, MG can remain unaffected, it can deteriorate (usually within the first trimester of pregnancy), or it can improve; the distribution of patients is approximately equal among the three possible outcomes.

Pathogenic Mechanisms in MG

The autoantibodies of MG target, in the vast majority of patients, the AChR and, in a smaller fraction, the muscle-specific kinase (MuSK), both at the NMJ. The breakthrough in the identification of the AChR as the main auto-antigen in MG came in 1973 by the work of J. Lindstrom and J. Patrick, who immunized experimental animals with purified AChR, causing the emergence of symptoms similar to human MG. The same year, D. Drachman and colleagues found a reduction in the number of functional AChRs at the NMJ of MG patients, hypothesizing that this may account for the disease symptoms. Three decades later, the MuSK protein was also identified as an auto-antigen in MG by the work of A. Vincent and collaborators. For a better understanding of the pathogenic mechanisms that take place in MG, the NMJ and its principal components that are affected by the autoantibodies will be discussed first.

The NMJ and Nerve to Muscle Signal Transduction in Health

The NMJ is a highly specialized area dedicated to transmitting signals from the motor nerve to the muscle, leading to muscle membrane depolarization and ultimately muscle contraction. In more detail, arrival of the signal at the nerve terminal (presynaptic boutons) causes the release of the transmitter acetylcholine (ACh), which is stored in vesicles (Fig. 91.1). The amount of ACh released from a single vesicle is called a quantum (around 10,000 ACh molecules), while the total amount of vesicles released upon excitation of the nerve terminal is termed the quantal content (in humans, this is approximately 30). The ACh released travels across the synaptic cleft of the NMJ and binds to AChRs, which open upon ACh binding, allowing ion influx and depolarization of the muscle membrane (end plate potential, EPP). This causes activation of adjacent voltage-gated sodium channels (VGSCs), which open as well further depolarizing the muscle membrane. This leads to the generation of the action potential and its propagation along the muscle fiber, which in turn effects muscle contraction. Importantly, the EPP is much larger than the threshold required for activation of the VGSCs (in humans, it is almost three times greater). This is called the safety factor, and it accounts for the normally 100% efficiency of signal transmission from nerve to muscle, even after repeated stimulation. The ACh released into the synaptic cleft is eventually hydrolyzed by acetylcholinesterase (AChE), terminating its action, and the muscle membrane is repolarized by opening of voltage-gated potassium channels (VGCs).

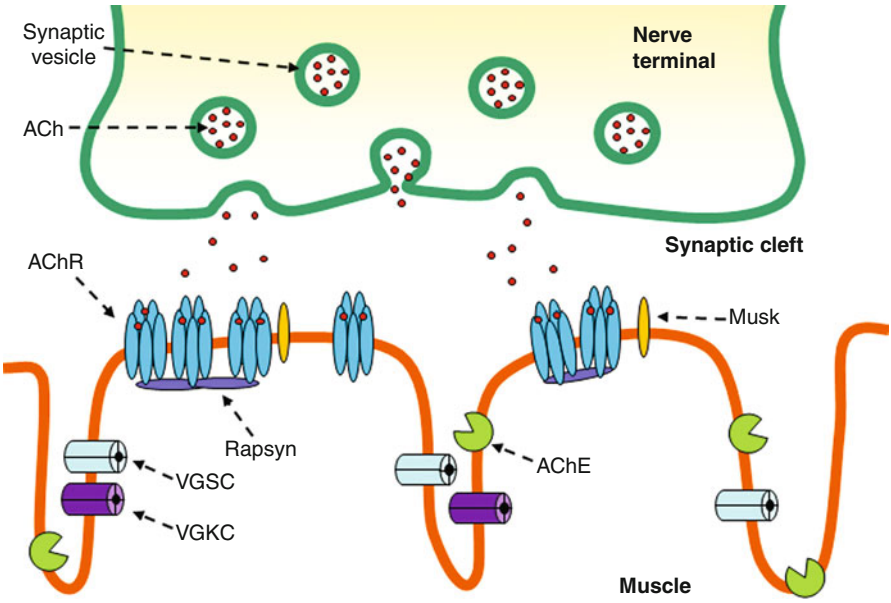

Fig. 91.1 The structure of the neuromuscular junction (NMJ) and the major components involved in neuromuscular transmission. The acetylcholine (*ACh*)-containing vesicles are located at the nerve terminal allowing rapid release of ACh upon stimulation. The released ACh binds to the AChRs causing the opening of the ion channel, depolarization of the muscle membrane (EPP), and subsequent activation of voltage-gated sodium channels (*VGSCs*) to initiate the action potential. Activation of the adjacent voltage-gated potassium channels (*VGKCs*) repolarizes the muscle membrane. Eventually, the ACh is hydrolyzed by acetylcholinesterase (*AChE*) and the signal is terminated. The muscle membrane has extensive folds with the AChRs densely packed at the top of the folds, via rapsyn. Muscle-specific kinase (MuSK) is a key player in agrin-induced AChR clustering and in the maintenance of NMJ structure

The AChR and MuSK Proteins

The nicotinic AChR of skeletal muscles is an integral membrane protein forming ligand-gated ion channels responsible for the generation of the EPP in response to nerve stimulation. It consists of five subunits that associate to form a ring on the muscle membrane, at the center of which lies the pore of the channel, allowing the passage of positive ions (Fig. 91.2a). The subunits forming the AChRs are called α1, β1, γ, δ, and ε, but the composition varies depending on the stage of development. In adult muscle, the composition is (α1)2β1εδ, while in embryonic or in denervated muscle, the subunit stoichiometry is (α1)2β1γδ, i.e., the γ subunit is substituted by the ε in innervated muscle.

The five subunits are homologous and share a basic structural pattern (Fig. 91.2b). The N-terminus of each subunit, about 200 amino acids long, forms a large globular extracellular domain (ECD), followed by four transmembrane domains (M1–M4) composed of α-helixes and a small C-terminal extracellular segment; between M3 and M4, there is a large mostly unstructured domain, the intracellular domain (ICD), highly variable in length and sequence among the

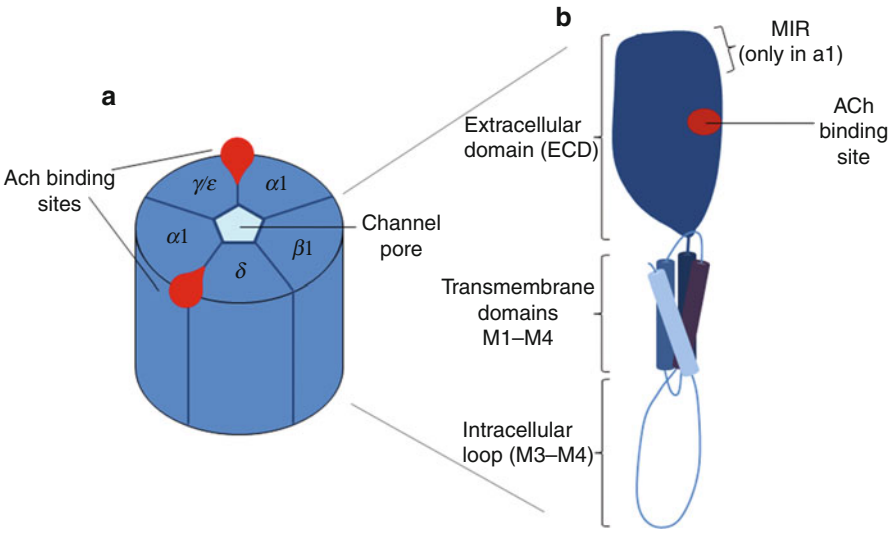

Fig. 91.2 The muscle nicotinic acetylcholine receptor (AChR). (**a**) The receptor is a transmembrane ion channel formed by five homologous subunits. Depending on whether the receptor is embryonic or adult, it contains a γ or ε subunit, respectively. Each of the ligand binding sites is located at the interface between an α1 subunit and the δ or γ/ε subunits. Binding of acetylcholine (ACh) causes opening of the central pore allowing the passage of ions. (**b**) Longitudinal view of an AChR subunit, with its ECD, four transmembrane domains, and the large M3–M4 cytoplasmic loop. The location of the main immunogenic region (MIR – only present on the α1 subunit) and the ACh binding site is shown

subunits. The ECD has the ligand binding site (formed at the interface between each α1 and its adjacent subunit); while being extracellular, it contains all the antigenic epitopes targeted by the pathogenic autoantibodies. Although antibodies to intracellular or transmembrane epitopes have been found, they are probably not clinically important since these epitopes are inaccessible in undamaged membranes.

The muscle membrane at the NMJ has a characteristic morphology with extensive folds and crevices. The AChRs are found normally clustered in high densities on the top of these folds (up to 12,000 receptors per μm^2), directly opposite the nerve synaptic boutons, and are almost absent from the rest of the muscle fiber. This clustering is achieved by interactions with additional muscle proteins and input from the adjacent nerve during development. In short, the proteoglycan agrin is synthesized and secreted by the nerve terminal. Agrin activates MuSK, a transmembrane protein found on the muscle membrane, which initiates a signaling cascade leading ultimately to AChR clustering, through the muscle cytoplasmic protein rapsyn. Rapsyn directly interacts with the ICD of the receptors and anchors them to the underlying cytoskeleton. Although this process takes place during myogenesis, MuSK is still expressed in the adult muscle where it is found localized at NMJs. Evidence strongly supports that MuSK continues to play a central role in the maintenance of the NMJ structure throughout life.

Anti-AChR Antibodies Are the Major Cause of Impaired Neuromuscular Transmission

In the vast majority of MG patients (~85%), the autoantibodies are directed against the AChR, in which case the disease is accordingly referred to as AChR-MG. Antibodies from the patient's circulation reach the NMJ, where they find their way in the synaptic cleft and onto their target epitopes. Binding of the anti-AChR antibodies to the receptors causes loss of functional receptors at the postsynaptic membrane and subsequent failure of signal transduction. The anti-AChR antibodies are of the IgG class; the vast majority belongs to the IgG1 and IgG3 isotypes (complement activating), while IgG2 and IgG4 isotype antibodies are scarce (weak and no complement activation, respectively). An interesting characteristic of MG is that, generally, the concentration of circulation antibodies (antibody titer) does not correlate well with the severity of the disease. In individual patients though, the antibody titer is related to the symptoms and measuring the titer is a useful tool in monitoring patients undergoing treatment. The poor correlation of antibody titer and disease severity among different patients can be attributed to several factors, such as differences in the relative ratios of IgG subclasses (indeed it is suggested that a correlation of the IgG1 antibodies alone with disease severity is possible), or the contribution of additional yet unidentified factors in the process.

The reduction of functional AChRs by the autoantibodies is effected by at least three mechanisms (Fig. 91.3). Firstly, being bivalent, antibodies of the IgG1, IgG2, and IgG3 subclasses can cross-link the AChRs causing their internalization in the muscle cell by endocytosis and subsequent degradation (a process called antigenic modulation). There is evidence suggesting that there is a compensatory mechanism, whereby the increased destruction of AChRs from the surface membrane causes an increase in the synthesis of new AChRs to replace the ones lost, but if this compensation is not sufficient, it will eventually result in a great reduction of mature receptors on the membrane. Importantly, not all antibodies cause antigenic modulation; if the location of the epitope does not allow cross-linking of adjacent molecules or if the antibodies belong to the IgG4 subclass (which are functionally monovalent), antigenic modulation is not possible.

The majority of anti-AChR antibodies are IgG1 and IgG3 which are very effective at activating the complement cascade. Therefore, when they bind to the receptors, they are concentrated in high densities at the NMJ, which induces complement activation. This leads to recruitment of the complement components and to severe damage of the postsynaptic membrane due to focal lysis by the membrane attack complex (MAC). In stained tissue sections of MG patients or experimental animals, it is possible to see IgG, C3 complement component, and MAC deposits at the NMJ. The damage made can be clearly seen when examining tissue from MG patients or experimental animals under the microscope, where it is evident that the characteristic architecture of the NMJ has deteriorated and the extensive synaptic folds have been significantly reduced and in some cases are almost completely invisible. The direct reduction in total surface area is coupled to indirect loss of AChRs and associated proteins. This is particularly important because many of these proteins (such as MuSK, Dok-7, rapsyn, and utrophin) are

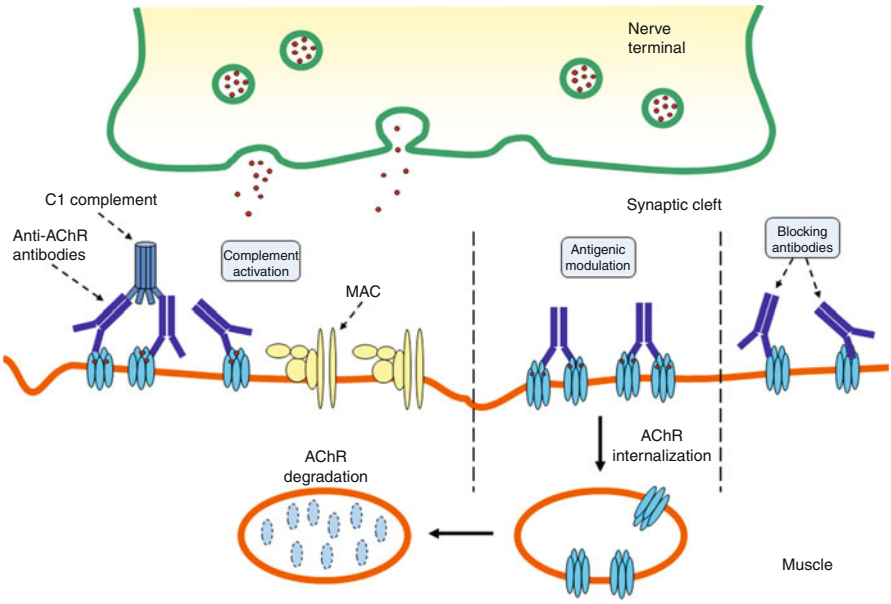

Fig. 91.3 The three major ways of anti-AChR action at the neuromuscular junction. Complement activation leads to the formation of membrane attack complexes (MACs) on the muscle membrane and destruction of the typical junctional folds (*left*); cross-linking of the acetylcholine receptors (AChRs) by the autoantibodies causes antigenic modulation, receptor internalization, and subsequent degradation (*center*); and blocking antibodies directly inhibit acetylcholine binding to its binding site on the AChRs (*right*). Notice the overall change of the characteristic morphology of the neuromuscular junction

crucial for maintaining the structure of the NMJ and their loss results in significant reduction in the repair rate, further aggravating the disease symptoms.

A fraction of the circulating anti-AChR antibodies binds in the region of the ligand binding site on the receptor, thus directly blocking ACh binding and channel opening. These antibodies may be particularly significant because, although they may represent a small fraction of the total antibodies in a patient, they cause direct receptor blockage, which could precipitate acute symptoms.

The immune response in MG is polyclonal; the antibodies produced have multiple target epitopes on the different AChR subunits. Moreover, there is considerable epitope spreading so that over periods of time additional epitopes become targeted. All the pathogenic antibodies seem to bind to epitopes on the AChR subunit ECDs, which are exposed extracellularly. However, it appears that some regions of the ECD are more immunogenic than others. The majority of patients have antibodies directed against the $\alpha 1$ subunit, while detectable antibodies against the δ subunit are atypical. Moreover, more than half of the antibodies are directed against the so-called main immunogenic region (MIR), a group of overlapping epitopes located on the ECD of the $\alpha 1$ subunit, whose central core lies between amino acids 67–76.

The reduction of functional AChRs from the NMJ results in a decrease in the EPP achieved by each ACh release. Due to the safety factor of neuromuscular transmission, this does not become immediately apparent, as the EPP generated is still greater than the threshold for activation of the VGSCs. However, repeated stimulation of the same muscle causes a reduction in the quantal release of ACh. Thus, the EPP will eventually decrease to such an extent that it does not exceed the threshold and the action potential is not initiated.

This effect does not have an equal impact on all muscles, since the specific NMJ characteristics of each muscle influence the outcome. For example, it is thought that NMJs with bigger quantal contents and more prominent synaptic folds, such as those of fast-twitch fibers, are less susceptible to the decrease of EPP and, thus, less likely to develop symptoms of MG. The extraocular muscles share a number of features that make them especially susceptible. They are always under nerve stimulation, and so they are very liable to fatigue. Additionally, they do not have very pronounced synaptic folds, generating a smaller EPP, and thus have a smaller safety factor even in healthy individuals. That is why these muscles are very commonly affected and are usually among the first to show signs of fatigability in MG patients.

Pathogenic Antibodies Against Additional Targets on the Muscle Membrane

Approximately 15% of MG patients with generalized MG do not have detectable antibodies against the AChR. From them, about 40% (i.e., 6% of all the MG patients) have autoantibodies against the MuSK protein (termed MuSK-MG). MuSK-MG patients, with rare exceptions, do not have anti-AChR circulating antibodies. Interestingly, in contrast to AChR-MG, anti-MuSK antibody titer appears to correlate to disease severity. Binding of the antibodies to MuSK results in muscle abnormalities such as atrophy and fatty replacement, although the mechanism by which they exert their pathogenic role remains unclear. Anti-MuSK antibodies mainly belong to the IgG4 subclass and are, therefore, unable to activate compliment. Small levels of IgG1 have been reported in some cases, but they are not common and they are not thought to be the primary cause of MuSK-MG pathology. Furthermore, IgG4 antibodies can undergo a dynamic process called Fab arm exchange, the exchange of the fragment antigen binding (Fab) domains between two different antibodies. This renders them bispecific and functionally monovalent (each antibody can recognize two different unrelated epitopes and cannot cross-link identical antigens). It is thus unlikely that their main mode of action is antigenic modulation. Indeed, complement deposits, significant structural damage, or loss of AChRs is not seen at the NMJs of most MuSK-MG patients. On the other hand, a reduction in the EPP of MuSK-MG NMJs has been shown. It is possible that anti-MuSK antibodies prevent MuSK from functioning normally in the regeneration of the NMJ.

MuSK-MG shows a different pattern of muscle susceptibility from AChR-MG, with more frequent involvement of bulbar and cranial muscles, while limb weakness is less severe. These patients, therefore, commonly experience ptosis and diplopia, while they are at high risk for presenting with myasthenic crises.

Additionally, there is a strong predominance of females, while the age of onset in usually under 40 years of age. The thymus of MuSK-MG patients is usually normal, and thymectomy does not improve the clinical outcome.

Interestingly, MuSK-MG shows a distinctive ethnic and geographical distribution, with a higher incidence, up to 49%, in southern Europe (around the Mediterranean) and blacks in the USA and South Africa and a lower incidence, about 23%, in northern Europe (Scandinavia) and individuals of Chinese ancestry. A broader and more detailed analysis is required to establish whether this is due to genetic or environmental factors. Overall, the MuSK-MG patients comprise the most uniform and distinct group from all the MG forms in terms of clinical presentation.

The patients who do not have detectable anti-AChR or anti-MuSK antibodies are termed seronegative (SN-MG) and account for about 10% of all MG patients. These patients present disease symptoms similar to AChR-MG, accompanied by some forms of thymic hyperplasia. Recently, it was shown that about half of these patients actually have anti-AChR antibodies that were previously undetectable. It appears that they are low-affinity antibodies that need to "see" their target antigen at high densities to bind, and sensitive cell-based assays had to be used for their detection. They belong to the IgG1 subclass and are capable of activating the classical complement pathway.

The remaining 4–5% of MG patients is still seronegative, since no specific antigen has been found to be responsible for the development of the disease. Interestingly, some of these patients benefit from therapies aiming at the removal of circulating antibodies, such as plasmapheresis, suggesting the existence of antibodies recognizing unidentified protein targets in the NMJ. It is also hypothesized that especially in low-titer patients, most of the autoantibodies are localized at the NMJs, leaving few circulating antibodies available for detection. New, more sensitive assays or the discovery of novel antigen targets should shed light as to the nature of the antibodies in the rest of the seronegative patients.

Nonpathogenic MG-Associated Autoantibodies Are Present in Many Patients

In addition to the anti-AChR and anti-MuSK antibodies which are the main factors responsible for the pathogenicity of MG, other autoantibodies are also frequently present, especially associated with thymic abnormalities. These are not always restricted to MG since they can be detected in other autoimmune disorders as well. Some of the antigens targeted are intracellular, so the corresponding autoantibodies are probably insignificant with respect to the pathogenicity. Nonetheless, they often pose as useful diagnostic tools, correlating well with disease severity and the coexistence of other pathologies such as thymic anomalies.

The most commonly found such autoantibodies are directed against titin and the ryanodine receptor (RyR), both expressed in the muscle fibers. Titin is found intracellularly, and it is a structural component of muscle fibers, responsible for giving elasticity. Anti-titin antibodies, which belong mainly to the IgG1 subclass, are found in most patients with thymoma of all age groups (70–90%), but are also

present in about half of the late-onset MG patients without thymoma. They are generally absent from early-onset non-thymomatous AChR-MG and from all MuSK-MG patients. The production of the anti-titin antibodies is probably not caused by the destruction of the muscle fibers after autoimmune attack, since they are not found in early-onset MG, MuSK-MG, or in patients suffering from other conditions which cause muscle damage.

The RyR is an intracellular membrane protein found in the sarcoplasmic reticulum, which is responsible for calcium release. Antibodies against RyR belong to the IgG1 and IgG3 subclasses, and they are detected in over 50% of patients with thymoma, as well as in some cases of late-onset non-thymomatous MG (30–40%). They are absent from early-onset MG without thymoma and from MuSK-MG patients.

The titer of either anti-titin or anti-RyR autoantibodies correlates well with the severity of MG. Furthermore, their presence is indicative of a reduced chance for a beneficial outcome of thymectomy.

The importance of rapsyn in the formation of AChR clusters at the NMJ has been discussed. Antibodies against rapsyn have been found in about 15% of all MG patients, but they are also present in other autoimmune diseases and so there is no specific association with MG. Up to 36% of MG patients have been reported to have anti-AChE antibodies. Binding of these antibodies to AChE could affect its normal function to hydrolyze ACh in the synaptic cleft. On the other hand, these antibodies have also been detected in other autoimmune disorders as well as healthy individuals, so their pathogenicity is unclear.

Several other autoantibodies have been reported in MG patients. However, these are also sometimes found in patients of other autoimmune disorders, while a clear correlation between the presence or titer of these antibodies and a specific pathology is missing. It is conceivable that the breakdown of self-tolerance eventually leads to the emergence of many autoantibodies of varying specificities. However, not all of these antibodies play a role in the development of symptoms, either due to the lack of access, such as intracellular proteins, or due to the distribution of their target antigens, as in the case of sparsely distributed proteins that do not cause the concentration of a critical density of antibodies to have a marked effect.

The Thymus Is Involved in the Pathogenesis of MG

The thymus is a major organ involved in the establishment of tolerance of the immune system to self-antigens and is mostly composed of thymocytes and thymic epithelial cells (TECs). Thymic anomalies, in the form of thymic hyperplasia, or to a lesser extent thymoma, are quite common in MG patients. These pathologies are only observed in AChR-MG, while for seronegative MG, the information available is inconclusive probably due to the heterogeneous population in this group. In MuSK-MG, thymic abnormalities are minimal but can sometimes present with thymic atrophy. Corticosteroid treatment can alter thymic pathology, by depleting immature thymocytes, blurring the interpretation of the observations and should always be taken into account.

Thymic lympho-follicular hyperplasia is found in 60–70% of MG patients, especially early-onset females. Sex hormones are thought to be contributing to

the prevalence of thymic hyperplasia in these individuals. Germinal centers, which are mostly absent from the thymuses of healthy adults, develop ectopically, indicating an active immune response. AChR expression, autoreactive T cells (against AChR), and B cells producing anti-AChR antibodies are found in these germinal centers, often in close proximity to thymic myoid cells. Furthermore, there appears to be a correlation between the existence of thymic hyperplasia and elevated anti-AChR antibody titer.

It is not clear what causes thymic hyperplasia. The inflammatory environment that has been described in the MG thymus could be responsible. In addition, TECs constitutively overexpress components of the p38 and ERK1/2 MAPK signaling cascade. An imbalance in this critical pathway, which is involved in both transcriptional and posttranscriptional events, may initiate the pathologic and morphologic changes in the thymus.

Thymoma (tumor originating from the TECs of the thymus but also containing thymocytes) is found in about 10% of all MG patients (paraneoplastic MG), although it is more common in patients over the age of 40. On the other hand, up to half of the patients with thymoma also present with MG. MG-associated thymomas share a number of common characteristics: the generation and maturation in the tumor of potentially autoreactive T cells, enrichment of the tumor with autoreactive T cells restricted to specific human leukocyte antigen (HLA) isotypes, reduced expression of the major histocompatibility complex (MHC) class II genes, export of mature T cells to the periphery, and coexisting response against several auto-antigens, including the AChR, titin, RyR, neuronal antigens, and cytokines. Interestingly, patients with thymoma have antibodies against interleukin-12 (IL-12) and interferon-α (IFN-α), and their titer correlates with disease severity. Paraneoplastic MG is equally prevalent in men and women and is generally associated with more severe clinical symptoms. The thymoma itself is usually benign, but in up to 10% of the patients, it can become metastatic, mostly in the chest cavity.

All of the above show a strong association of thymic anomalies and MG, but it is not clear whether these are a causative factor or a consequence of the disease. Currently, it is thought that the thymus has a role in the initiation of autoantibody production in MG. Several lines of evidence support this. Animals with spontaneous disease development commonly have thymic anomalies, while in experimental animals with induced MG after AChR immunization, the thymus appears healthy, so the autoantibodies are not responsible for these anomalies. Furthermore, transfer of thymic cells from MG patients to experimental animals leads to anti-AChR production and subsequent AChR loss at the NMJs suggesting that they are sufficient for triggering an anti-AChR autoimmune response. Thymectomy can be beneficial and is often used as a therapeutic approach, but it is more effective when performed in early stages of the disease. It seems that the pathogenic autoreactive lymphocytes originate in the thymus and later, during the course of the disease, they are disseminated in the periphery.

Thymic stromal cells (dendritic cells and TECs) have an important role in MG pathogenesis. Lymphocytes mature and differentiate in the thymus, with the concomitant removal of autoreactive cells, by means of negative selection,

a process mainly controlled by interaction with the dendritic and the TECs. Several studies have identified potential factors responsible for the deregulation of antigen presentation and the escape of autoreactive T cells. The most promising candidate is the transcription factor autoimmune regulator (AIRE). AIRE is expressed in the thymus and is responsible for driving the promiscuous transcription in medullary TECs of organ-specific proteins so that they can be presented as self-antigens. More specifically for MG, AIRE has been shown to control the transcription of the CHRNA1 gene (the gene encoding for the α1 subunit of the AChR) in human TECs. Although a link between inefficient AIRE expression and a decreased expression of CHRNA1 in the medullary TECs followed by failure to sustain tolerance against the AChR is apparent, this remains to be shown in practice and is the focus of ongoing research.

The TECs produce cytokines that are important in T-cell maturation such as IL-1 and IL-6. Both these cytokines have been shown to be overexpressed by MG TECs either spontaneously or after stimulation. The CXCL3 chemokine, which is a known factor attracting B cells, has also been found to be produced in higher amounts from the TECs of MG patients, and this could be a contributing factor to the recruitment of B cells toward the ectopic germinal centers of the hyperplastic MG thymus.

MG Is a T-Cell-Dependent Disease

The NMJ of MG muscle presents with very little if any cellular infiltration, so T cells are not directly involved in tissue damage processes. However, input from autoreactive T cells is required for stimulation of anti-AChR antibody production. These autoreactive T cells are key players in the presentation of antigen to B cells, driving production of the pathogenic anti-AChR antibodies. T cells also contribute to MG development by releasing pro-inflammatory cytokines. The degree of contribution of T cells in MuSK-MG is not clear and requires further investigation.

Several cytokines have been implicated in the pathogenesis of MG. Antigen presentation to T cells can elicit a Th1 or Th2 response; the former is generally regarded as pro-inflammatory and the later anti-inflammatory. A third recently identified response is Th17, important in antimicrobial immunity in mucosal and epithelial barriers, but with crucial implications in a variety of autoimmune diseases. Studies performed in animal models of the disease have been extremely useful in illuminating the involvement of various cytokines. However, it is sometimes difficult to discern a clear-cut effect of specific cytokines mainly due to the varying genetic background of the experimental animals used, and such results need to be interpreted with caution with respect to human disease. IL-12 and IFN-γ have been found to facilitate experimental MG development. IL-12, a cytokine produced by dendritic cells and macrophages, is responsible for activating T cells and contributes toward a Th1 response. When IL-12 is not present, a Th2 immune response is favored and complement activating antibodies against AChR are diminished. On the other hand, blocking IFN-γ signaling results in increased resistance to experimental MG development. IL-18 is a pro-inflammatory cytokine also thought to promote MG development since blocking IL-18 results in resistance to disease

manifestation and increased expression of the immunosuppressive cytokine tumor growth factor-β (TGF-β). TGF-β is an important stimulator of regulatory T cells (Treg), also known as suppressor T cells. These cells are very important in maintaining immune homeostasis and self-tolerance by controlling the immune response, and they have become the center of attention in recent years. It appears that Treg cells from the thymus of MG patients, although present in good numbers, are not very effective at suppressing the autoreactive CD4+ T cells. Although the exact mechanisms of action are largely unknown, some evidence suggests that the FoxP3 protein, a Treg-specific transcription factor, is downregulated in these cells.

The Th2 cytokines IL-5, IL-6, and IL-10 are also thought to contribute to susceptibility to experimental MG, while IL-4 appears to play a protective role. Indeed, IL-6 can inhibit the generation of Treg cells in response to TGF-β. On the other hand, blocking the Th2 pathway and the production of Th2 cells in experimental animals lead to increased susceptibility to the disease, compared to normal animals but even to animals with inhibition of the Th1 response. This evidence strongly supports the notion that an intricate interplay between the two responses is responsible for conferring the necessary breakdown of self-tolerance.

Peripheral lymphocytes from MG patients have been found to react in vitro to AChR-derived peptides presented in an MHC class II context, by increased proliferation and cytokine production, suggesting their prior sensitization to AChR. Interestingly, IL-4 and IFN-γ are both expressed by the stimulated T cells, supporting the notion that both Th1 and Th2 responses contribute to autoimmunity. In some patients, TGF-β expression, which is protective in animal studies, is elevated following thymectomy.

Expression of Cytokines and Their Receptors Are Often Deregulated in MG

In addition to the CXCL3 chemokine described previously in the context of thymic abnormalities, several other chemokines as well as their receptors are subject to altered expression in MG patients compared to healthy individuals. The CXCR3 receptor and its ligand CXCL10, as well as the chemokines CXCL13, CCL21, and CCL5, have been found upregulated in the thymus of MG patients. Furthermore, the chemokine receptor CXCR3 is upregulated only in CD4+ T cells, while CCR1 is upregulated in both CD4+ T and CD8+ T cells but its levels decrease after treatment.

B-cell-activating factor (BAFF) is a cytokine belonging to the TNF family. BAFF plays an important role in the differentiation and proliferation of B lymphocytes, which are the cells mainly expressing the BAFF receptors BAFF-R, BCMA, and TACI. BAFF has been shown to be elevated in MG patients. The levels of serum BAFF do not correlate with disease severity, although there is a correlation with seropositivity for anti-AChR, so that patients with higher BAFF levels are more likely to have anti-AChR antibodies.

A detailed presentation of all these signaling pathways is quite complex and beyond the scope of this chapter. The aforementioned examples, though, illustrate the extensive deregulation of the chemical balance of the immune system that takes place in the autoimmune setting of MG.

Genetic Factors Contribute to the Development of MG

It has been difficult to prove a link of genetic factors with MG predisposition, but there have been several lines of evidence hinting in that direction. Firstly, although it is rare to find more than one members of a single family with the disease, the rate is much larger than that of the whole population (2–4%). Similarly, the percentage of monozygotic twins with MG is much higher than that observed in dizygotic ones. These results indicate the existence of contributing genetic factors in the development of MG.

One of the reasons it was hard to identify such genetic factors is the heterogeneity of the disease itself. Therefore, it is crucial to distinguish among groups of patients that form distinct entities in terms of pathophysiology. Commonly used criteria for this grouping are the target auto-antigen, age of onset, thymic pathology, female/male predominance, and of course ethnic origin. These are not always easily identifiable though, and the task of acquiring a homogeneous group of sufficient size to perform an analysis is quite daunting.

HLA Alleles Are Commonly Associated with Increased Risk for MG

The recognition of antigens by T cells requires their association with HLA proteins, which are coded for by the MHC group of genes. MHC class I are expressed in all the types of nucleated cells in the body, while MHC class II are expressed on the surface of antigen-presenting cells such as dendritic cells, monocytes, macrophages, and B cells.

Several HLA haplotypes have been associated with MG. In Caucasian populations, the class I HLA-A1 and HLA-B8 and the class II HLA-DR3 are highly frequent (up to 60%) in early-onset MG with thymic hyperplasia but not in other forms of the disease. This extended A1-B8-DR3 haplotype is called the 8.1 haplotype, and because these alleles are linked in disequilibrium, it is hard to distinguish which of the three is mostly linked to this form of MG. On the other hand, the class II allele HLA-DR7 has a negative association with thymus hyperplasia MG (i.e., it is a protective factor). However, the DR7 allele is positively associated with non-thymomatous late-onset MG in a French cohort. Late-onset MG was also associated with the B7 and DR2 alleles. Finally, increased risk for MuSK-MG was found to be linked to the class II HLA-DR14-DQ5 allele. Different associations have been revealed in cohorts of other ethnic groups; in Chinese and Japanese populations, an association of DR9 with mild ocular MG was found.

Polymorphisms in the CHRNA1 and PTPN22 Genes Are MG Predisposing Factors

Early-onset MG with thymic hyperplasia has been associated with a polymorphism of the CHRNA1 gene promoter. This is a single-nucleotide polymorphism (SNP) that disrupts the recognition sequence at the binding site of the IRF8 transcription factor. This leads to decreased levels of CHRNA1 expression in TECs, which can be detrimental in the process of self-tolerance that requires sufficient expression of the self-antigens by TECs.

Another strong association of the early-onset non-thymomatous MG patients without anti-titin antibodies has been found for a polymorphism of the PTPN22 gene. The PTPN22 gene product is a protein phosphatase involved in the signaling downstream of the T-cell antigen receptor leading to IL-2 production. The polymorphism is a mutation that causes disruption of the signaling cascade and leads to decreased IL-2 production. This, however, has been linked to the development of autoimmune diseases, providing a functional link between the identified polymorphism and MG pathology.

Diagnosis of MG

The diagnosis of MG is not always straight forward, since many different disorders can have a similar symptom manifestation. MG itself is relatively rare, and it has a heterogeneous presentation with varying degrees in the involvement and severity of affected muscles. Neurologists, who are the normally first to come into contact with the patient, need to be well trained to identify the critical symptoms for MG, but further confirmation is required in the form of tests to confer a final diagnosis. These diagnostic tests are discussed next.

Electrodiagnostic Tests

In electrophysiological tests, a peripheral motor nerve is stimulated repetitively (repetitive nerve stimulation or RNS) at a low rate of three stimulations per second. Then, the total action potential generated by the corresponding muscle is recorded using surface electrodes. Typically, in MG, there is a decrease in the action potential from the first stimulation of at least 10%, due to failure of an increasing number of NMJs to become activated, as opposed to the absence of an effect seen in healthy individuals. This test effectively simulates muscle exertion, and the decrease in the action potential mirrors the muscle fatigability. Therefore, the sensitivity of the test depends on the degree of weakness spreading; although in generalized MG it is positive up to 70%, it becomes less useful in cases of ocular or MuSK-MG.

A more sophisticated electrophysiological test than RNS is single-fiber electromyography (SF-EMG), which can record action potentials generated by individual muscle fibers. When two fibers innervated by the same motor unit are monitored, the variability in the interval between the two action potentials upon the same stimulation is called jitter and it is very sensitive to transmission anomalies. In MG, jitter times are increased and can even result in complete blocking of the second action potential. The percentage of jitter increase and the percentage of blocking of the second action potential are both taken into account to assist in diagnosis. It is most commonly performed in the extensor digitorum communis muscle and, when required, in facial muscles. It is the most sensitive means of diagnosis to date with more than 95% positivity and very useful to diagnose ocular and MuSK-MG when performed in cranial muscles, but like RNS, it is prone to false-positive results due to similar results from other NMJ disorders. A useful

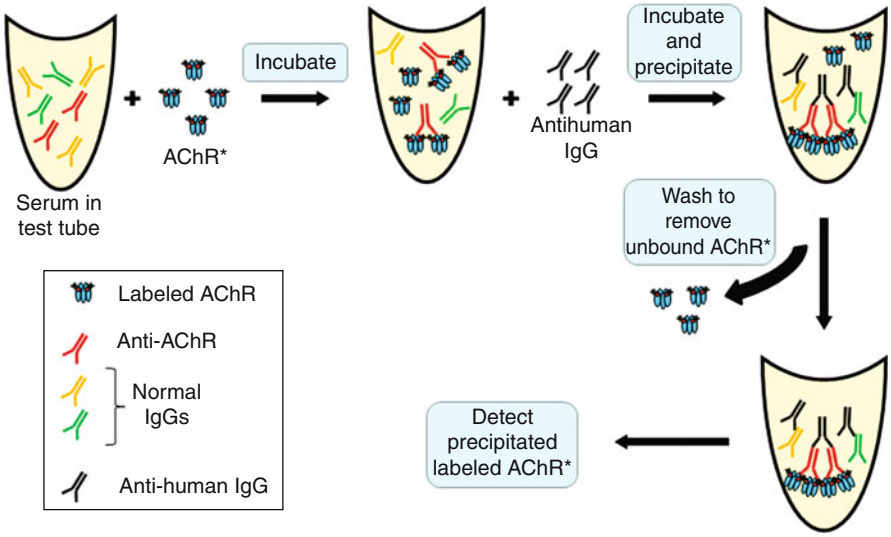

Fig. 91.4 Schematic representation of the RIPA assay for the detection of anti-AChR autoantibodies from MG patients' sera. The serum of each patient is mixed in a test tube with I^{125}-labeled human acetylcholine receptor (*AChR**) and incubated to allow the anti-AChR antibodies to bind to the AChRs. Then antihuman IgG is added, and the whole complex is precipitated with centrifugation. The pellet is washed and measured for radioactivity, which is proportional to the precipitated AChRs and thus to the anti-AChR antibodies present in the serum

indicator is the measurement of fiber density (the local concentration of muscle fibers), which should be normal in MG. Also, it is a time-consuming and difficult to perform procedure with the requirement of expert personnel and the appropriate equipment.

Serological Tests

The circulating antibodies against several antigenic targets in MG can be detected using radioimmunoprecipitation assays (RIPA) and other immunoassays. In these, a small volume of patient serum is mixed with labeled antigen and allowed to form complexes between the labeled antigen and any existing autoantibodies. These complexes are then precipitated using a second antibody with specificity for human IgG, and the relative amount of labeled ligand corresponding to the amount of autoantibody present is detected (Fig. 91.4). In fact, using known standards, the antibody titer of the patient can be quantified.

Anti-AChR antibodies are detected using I^{125}-labeled human AChR isolated form cultured cells. A mixture of γ- and ε-containing AChRs are used to ensure detection of antibodies against the fetal form of the receptor as well. In the most commonly used assay, the AChR is labeled indirectly through complex formation with I^{125}-labeled α-bungarotoxin, a small antagonist protein directed at the ligand binding site of the receptor. Thus, with this RIPA, the blocking antibodies are

probably undetectable. Other assays are available, such as enzyme-linked immunosorbent assays (ELISA), which do not require AChR labeling, but they are not as widely used.

Anti-MuSK antibodies are similarly detected using RIPA by means of I^{125}-labeled human recombinant MuSK expressed in mammalian cell lines.

Detection of the autoantibodies is very sensitive, and, most importantly, it is highly specific with up to 99% specificity for the anti-AChRs. However, up to 10% of all MG patients are seronegative with no detectable antibodies against AChR or MuSK and require alternative diagnosis. Furthermore, the level of detected autoantibodies can be affected by therapies like immunosuppressive drugs (both AChR-MG and MuSK-MG) and thymectomy (only in AChR-MG). On the other hand, it is possible for a patient who is initially negative in the serological test due to undetectable levels of autoantibodies to become positive later on (seroconversion), so a second test should be performed after a period of a few months.

Serological tests are also performed for the detection of anti-titin and anti-RyR receptors. As already mentioned, these antibodies have not been shown yet to have a pathogenic role, but their presence is highly associated with the presence of thymoma (95% of thymoma patients are positive for anti-titin, and 73%, for anti-RyR antibodies), while they are rare in early-onset non-thymomatous MG. Thus, they are useful diagnostically as prognostic markers in determining thymic pathologies, characterizing the type of MG, and allowing for the implementation of better-suited therapies. Of course, for the definitive diagnosis of thymic anomalies and especially thymomas, the chest imaging techniques of mediastinal computerized tomography and magnetic resonance are available and should be used.

Serological tests for the detection of anti-AChR and anti-MuSK antibodies are not only of diagnostic value but are also useful tools in the follow-up of the patients. Antibody titer does not correlate with disease severity in the population, but in individual patients, a reduction of the antibody titer does correlate with improvement of the symptoms. These tests are, therefore, used for the evaluation of therapies such as thymectomy, plasmapheresis, and immunosuppressive drugs.

Pharmacological Tests

The AChE enzyme is responsible for terminating the action of ACh at the NMJ, so its inhibition prolongs the lifetime of ACh. This can be used for diagnostic purposes since administration of an AChE inhibitor (ACHE-I) will rapidly increase the availability of ACh and alleviate the symptoms of fatigability. For the diagnosis to be accurate, weak muscles must be used, such as the extraocular muscles, the fatigue of which is easily observed and can be measured quantitatively. Furthermore, the patient's subjective opinion should not be taken into account, and a placebo test before the actual ACHE-I administration will increase the validity of the observations.

The most commonly used ACHE-I is edrophonium chloride (Tensilon). It is administered by intravenous injection, and its effects are apparent within a few seconds, lasting up to 10 min. Neostigmine, which has been reported to have fewer side effects, can be used alternatively, keeping in mind that its effects take longer to develop (15–30 min).

The sensitivity of ACHE-I diagnosis is quite high with 90% positive responses. However, there is a risk for severe muscarinic side effects in the form of bradycardia (slow heart rate below 60 bpm) and bronchospasm (constriction of the bronchioles leading to breathing difficulties), so they are only performed when the clinical status of the patient is known, an electrocardiogram has been performed, and atropine is available in case of emergency. Other common side effects include sweating, lacrimation, nausea, and diarrhea. To minimize the adverse effects, a small starting dose is given and increasing doses can follow after 60-s intervals until the maximum dose is administered, unless positive results have been observed sooner, in which case, the test is considered positive and it is terminated. The cholinergic side effects of neostigmine are fewer. Importantly, the ACHE-I test can fail in the case of MuSK-MG when improvement of symptoms is less common, while severe side effects such as cramps, generalized fasciculations, and even worsening of the weakness can precipitate. On the other hand, there is the possibility of a false-positive diagnosis. This has been reported in cases of congenital myasthenic syndromes, the Lambert-Eaton myasthenic syndrome, amyotrophic lateral sclerosis, and the Guillain-Barré syndrome.

Overall, the test or combination of tests to be used depends highly on the clinical condition of the individual. In patients with mild symptoms not requiring immediate treatment, the serological tests can be used which are highly specific and a positive result confirms the presence of MG without the need for further testing. Among the electrodiagnostic methods, the RNS is more relevant for generalized MG, while the increased sensitivity of SF-EMG makes it invaluable for ocular and MuSK-MG. Pharmacological tests may be preferred in cases with severe symptoms when an immediate diagnosis is needed in order to administer treatment.

MG Therapy

The major course of action against MG, like many autoimmune disorders, is immunomodulation or immunosuppression. These therapeutic approaches are largely nonspecific and are accompanied by a variety of potentially serious side effects. Other therapies include the use of ACHE-Is, or the use of therapeutic devices that remove the pathogenic autoantibodies from the patients' circulation (Fig. 91.5). The later are not selective, also removing useful plasma components. MG patients have a highly variable clinical presentation of the disease and respond differently to the various treatments. Combinations of drugs allow the administration of lower doses to minimize the adverse effects, especially useful in the case of steroids. The experienced clinician should evaluate these parameters and establish a therapeutic regime to best suit each patient.

ACHE-Is

As already discussed above, ACHE-Is block the activity of the AChE, increasing the lifetime of the released ACh at the NMJ and increasing the safety factor. This

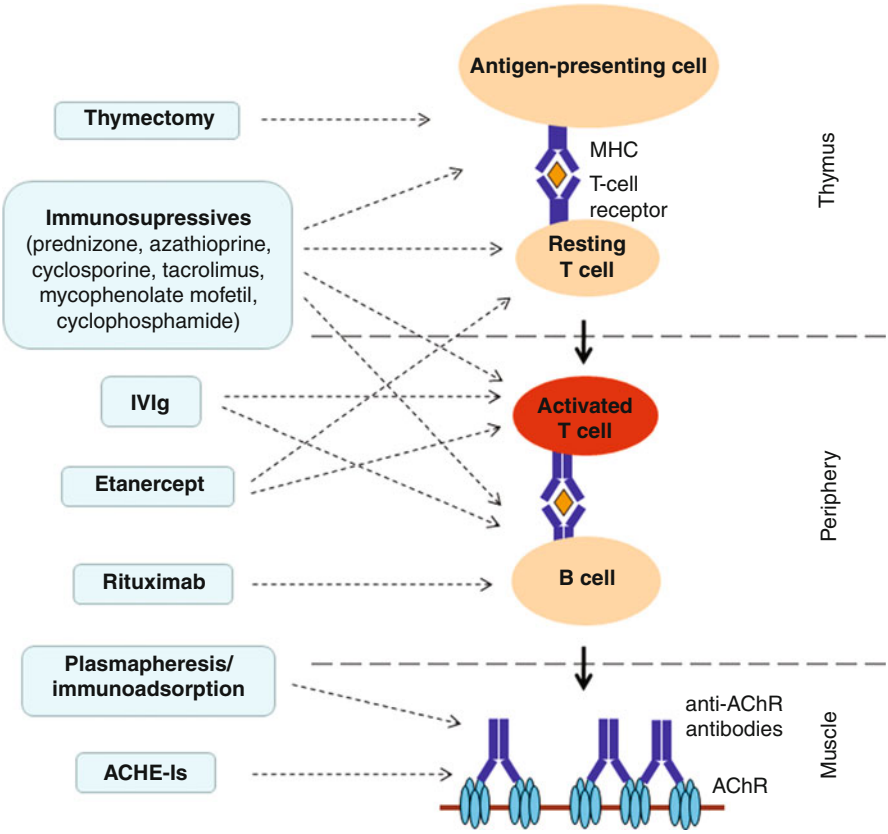

Fig. 91.5 Overview of the therapeutic options for the treatment of MG. Thymectomy may be beneficial in patients with thymic abnormalities, which are commonly present in MG patients. The use of immunosuppressives is the most commonly used approach especially for long-term management of MG. They act mostly by altering the chemokines being secreted by immune cells favoring an anti-inflammatory environment. The mode of action of intravenous immunoglobulins (IVIg) is not fully known yet, but may involve inhibition of complement activation, favoring an anti-inflammatory profile and inhibition of antibody production. Etanercept targets the T cells by inhibiting normal signaling through Fas, and Rituximab is a monoclonal antibody specifically targeting the B cells via their membrane protein CD20. Plasmapheresis and immunoadsorption remove the pathogenic autoantibodies from the patient's circulation providing temporary relief, and acetylcholinesterase inhibitors (ACHE-Is) increase the half-life of acetylcholine minimizing the disease symptoms (Modified from a figure made by Dr S. Berrih-Aknin)

leads to an immediate improvement of the symptoms, which, in addition to helping the diagnosis, is beneficial in terms of symptom management. However, ACHE-Is only alleviate the symptoms (symptomatic therapy) and have no added effect on the actual underlying pathology, i.e., the autoimmune attack on the NMJ. The most widely used agents are pyridostigmine bromide (Mestinon) and neostigmine bromide (Prostigmin). They are administered orally; their action begins about 30 min

after ingestion and can last for several hours. The doses are usually given thrice a day, although the clinical presentation in every patient can shape the dosage and timing to better suit them: individuals with dysphagia may take the drug before the meal; when disease symptoms are more pronounced later in the day, the timing of administration can be adjusted accordingly. Generally, ACHE-Is are the treatment of choice for ocular and mild MG, when immunosuppressive drugs are contraindicated or as an adjunctive therapy to immunosuppression.

Common adverse effects are due to increased muscarinic activity and include abdominal pain, vomiting, diarrhea, bradycardia, and lacrimation, or due to nicotinic toxicity including cramping and fasciculations (twitching of the muscles). Pyridostigmine is usually better tolerated than neostigmine with fewer gastrointestinal side effects. Furthermore, the side effects are dependent on the dose administered, so a careful dosage planning is required to effect maximal alleviation of the symptoms with minimal side effects.

Corticosteroids

Corticosteroids, such as prednisone, are usually the first treatment of choice in patients with mild symptoms especially those with moderate responsiveness to ACHE-Is. Their exact mode of action in MG is not known, but they have an immunomodulatory effect and can alter the production of cytokines and lymphocyte function.

The treatment is long term, lasting for several years. Corticosteroids are initiated at high doses until a marked effect is observed, normally weeks or a couple of months after treatment initiation. Some studies have shown improvement of the condition in 70–90% of patients treated with prednisone. Subsequently, the dose is decreased gradually to the lower beneficial limit for the patient. This decrease is performed with long intervals (up to several months) between each step, to ensure that the dose is not lowered too much too rapidly, as this might cause relapse and even myasthenic crisis. To assure the patients' safety, careful examination of their clinical status before each dose decrease is required to ensure the absence of symptoms.

Corticosteroids have various important side effects including hypertension, weight gain, potassium loss, obesity, gastric ulceration, skin friability, glaucoma, and osteoporosis to name but a few. Often, diet changes are recommended to minimize some of these untoward effects, such as low-fat, low-sugar diet, supplements of potassium and calcium, and antacids to prevent ulcerations. Unfortunately, up to one third of MG patients undergo a worsening of their symptoms at the beginning of their treatment with corticosteroids. Rarely, this can even lead to the requirement for intubation. In some cases, plasmapheresis (see below) may be performed before corticosteroid administration to minimize the possibility of weakness exacerbation. Alternatively, some clinicians suggest starting the treatment at low doses and gradually increasing them, although this significantly delays the clinical improvement, so it is used for patients with mild symptoms and may not alter the probability of an exacerbation. Due to these adverse effects, which are dose related, corticosteroids are sometimes used in combination with additional therapies such as thymectomy or immunosuppression to lower the administered dose.

Nonsteroid Immunosuppressants

The most commonly used immunosuppressants in MG are azathioprine, cyclosporine, tacrolimus, mycophenolate, and cyclophosphamide. They are used in the tapering phase of corticosteroids, or in patients not responsive to corticosteroids, but also as steroid-sparing agents, to reduce the dose of steroids required for long-term treatment.

Azathioprine is the second most widely used pharmaceutical after corticosteroids to treat MG. It is taken orally, and it is a purine analogue, which hinders the development of T and B cells through its metabolite 6-mercaptopurine by inhibiting DNA synthesis. Clinical studies showed it is beneficial in up to 90% of the patients. Currently, azathioprine is used in cases where corticosteroid treatment alone is not sufficient to control the symptoms, or as a steroid-sparing agent. However, the response to azathioprine is relatively slow since clinical improvement begins from 3 to 6 months after treatment initiation and maximal effect is observed 2–3 years later. It is well tolerated for long-term treatment regimes, but it can have adverse effects with gastrointestinal and hematological implications such as vomiting, nausea, anemia, leucopenia, and thrombocytopenia. Liver function, in particular, is continually monitored throughout the treatment as well as red, white, and platelet cell numbers. In the rare event of an allergic reaction, azathioprine has to be ceased immediately. Importantly, azathioprine is potentially teratogenic.

Cyclosporine was first used for immunosuppression during transplantations. Its effects on MG are due to induced changes in IL-2, IL-2 receptor, and IFN-γ production, which cause a reduction in CD4+ T-cell function. Cyclosporine binds to the cytoplasmic protein cyclophilin, and their complex acts by blocking calcineurin, a protein phosphatase involved in the upregulation of IL-2 production during T-cell activation. Benefits take several months to appear (2–9 months). Patients in a cyclosporine regime can experience kidney toxicity or hypertension, warranting termination of the treatment. Additionally, it has been suggested that due to the changes in the production of certain cytokines like TGF-β, it increases the risk for malignancy. Other adverse effects due to cyclosporine treatment include fever, vomiting, diarrhea, and hyperlipidemia.

Tacrolimus (FK-506 or fujimycin) is a macrolide lactone derived from *Streptomyces tsukubaensis* with immunosuppressive activity similar to cyclosporine due to its inhibition of calcineurin. Tacrolimus has been used in transplantations and other autoimmune disorders, and there is now some evidence suggesting that it can also be beneficial in MG. Like cyclosporine, an increased likelihood of malignancy is recognized. However, it is more potent than cyclosporine and with possibly lower toxicity. Side effects associated with tacrolimus include cardiac damage, hypertension, blurred vision, liver and kidney toxicity, and hyperglycemia.

Mycophenolate mofetil is another drug initially used in transplantation surgery, which acts at the level of purine synthesis. It selectively blocks inosine monophosphate dehydrogenase of activated T and B cells, inhibiting their growth. The effects are evident after about 3–6 months of treatment, and it has the

advantage of being very well tolerated due to its selectivity for T and B cells. Adverse effects include nausea, diarrhea, and abdominal pain, but they are usually mild. Nonetheless, patients need to be monitored for normal cell blood counts to avoid anemia and leucopenia. Its minimal organ toxicity has lead to its increased use in MG therapy as a steroid-sparing drug, but further clinical trials are needed to provide a better assessment of its therapeutic value.

In patients with poor response to other therapeutic approaches, cyclophosphamide has proven useful. It is relatively more fast acting than some of the agents mentioned above, with clinical improvement emerging within weeks after treatment initiation. Most patients are responsive to cyclophosphamide, and about half of them are asymptomatic within a year of treatment. However, it is commonly accompanied by numerous severe side effects, such as alopecia in 75%, leucopenia in 35%, and nausea in 25% of patients, and, less commonly, vomiting, anorexia, and skin discoloration. Long-term administration of cyclophosphamide has been associated with increased risk for bladder and lymphoreticular malignancies. Therefore, its use is limited and monitoring of the patients' health is critical. Recent studies suggest that pulsed intravenous administration rather than daily oral ingestion may help to hinder these side effects.

Thymectomy

The high frequency of thymic anomalies in MG made the thymus a primary target for early therapeutic attempts. Initially, in the 1920s, it was observed that some patients who underwent thymectomy (surgical removal of the thymus) achieved a significant improvement of symptoms and even remission, without further medication. However, it is still not clear in which cases thymectomy can be beneficial, and a large fully controlled clinical study addressing the issue is missing. This is largely due to the different forms of MG, the low incidence of MG, the variable times after diagnosis when thymectomy may be performed, the coexistence of other treatments, and the long period of time required for the clinical improvement to set in (up to years). During the past few years, a large multicenter trial has been initiated and organized by the efforts of the late Newsom-Davis. Over 80 centers are involved, from North America, South America, Europe, South Africa, Asia, and Australia, and have already begun recruitment of patients.

Generally, it is thought that thymectomy is most beneficial in patients with thymic hyperplasia, but not thymoma, and when it is performed early in the course of the disease. Patients with ocular MG or MuSK-MG may not benefit from this procedure. It is the most invasive approach, and the surgical procedure is accompanied with increased risks such as infection and myasthenic crisis. Thus, short-term perioperative treatments, such as plasmapheresis and IVIg (described below), are commonly used.

Intravenous Immunoglobulin (IVIg)

Immunoglobulins (Ig) are isolated from human plasmas of a large pool of healthy donors and administered by intravenous injection. The usual course of treatment

involves daily doses over 5 days. Symptom improvement is quite rapid taking place within 3–4 days, although in some cases it can take up to 2 weeks, but they are short lived. IVIg is especially useful in exacerbations of the disease particularly in myasthenic crises, or in preparation for surgery, although long-term treatments with IVIg have been reported.

IVIg function is complex and not fully understood. It is thought to have its effects through alterations in cytokine production favoring an anti-inflammatory profile, inhibition of complement activation, idiotypic-anti-idiotypic interactions resulting in neutralization of autoantibodies, reduction of antibody production, and transient lymphopenia. IVIg treatment is well tolerated, but occasionally, adverse effects in the form of headache, nausea, vomiting, fever, myalgia, and renal failure may develop. Additionally, IVIg treatment has a high cost which poses a problem especially for long-term treatment regimes, while the fact that it is obtained from human plasma means that it can be hard to obtain sufficient quantities to supply an ever-growing global demand.

Plasmapheresis and Related Procedures

Plasmapheresis (plasma exchange) is used in MG similarly to IVIg, i.e., for an immediate but temporary improvement of muscle weakness. In plasmapheresis, large veins of the patient are catheterized and the blood is continuously circulated through the plasmapheresis unit back into the patient. Inside the unit, the blood is separated into its cell and plasma components using continuous centrifugation. The plasma, which contains the patient's IgGs including the pathogenic autoantibodies, is discarded, while the cellular component is returned to the patient. In order to compensate for the volume of liquid and some of the soluble factors lost with the discarded plasma, the cells are mixed with saline, albumin, or plasma protein fraction before they are reintroduced into the circulation. Other blood products are avoided to limit the risk of disease transmission, but infections and allergic reactions cannot be ruled out.

Like IVIg, plasmapheresis is usually performed for short-term treatment during symptom exacerbations, or preoperatively, although it can be used more frequently in some patients who are refractory to all other treatments. In an average plasmapheresis session, about 2 l of plasma are replaced and usually treatment requires five or six sessions performed over a total of 10–15 days. The exact regime used depends mostly on the response of the individual in terms of muscle strength improvement and absence of side effects. Increased strength can be seen after the second session, and the improvement lasts in most cases for a couple of months after treatment cessation. For long-term treatment, a single session is performed once every 1 or 2 months.

Side effects are usually associated with catheterization and hemodynamic shifts, such as hypotension, thrombosis, embolism, sepsis, and infection. Also, several useful plasma components and protective antibodies are removed from the patient in addition to the autoantibodies, requiring costly replacements. The long-term effects and possible complications of this are not fully known. During each session,

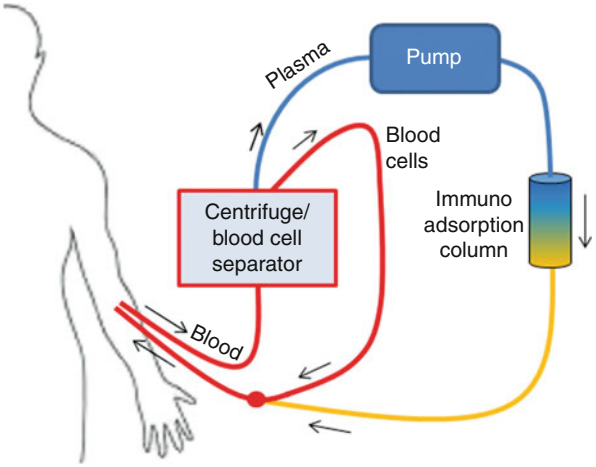

Fig. 91.6 Therapeutic immunoadsorption for the removal of autoantibodies from patients' plasma. The patient's blood is separated by continuous centrifugation into plasma and blood cells. The plasma is then passed through a column containing the adsorption matrix. Depending on the material used, different populations of antibodies can be retained, increasing the specificity of this method (see text for more details). One of the devices under development uses immobilized extracellular domains of the different acetylcholine receptor subunits aiming at selective depletion of the anti-AChR antibodies. The "cleared" plasma devoid of the pathogenic agents is mixed again with the blood cells and returned into the patient

which can last for several hours, the patients are monitored by a clinician. The patient needs to visit a clinic with a specialized unit for the procedure, so it may be difficult to perform outside of major urban areas.

An alternative to plasmapheresis is double-filtration plasmapheresis (DFP), in which after the separation of blood into blood cells and plasma, a filter is used to further fractionate plasma components according to their size. The fraction with the larger sized components contains the immunoglobulins and is the one discarded. Therefore, smaller components such as albumin are not removed from the circulation alleviating the need for their replacement. Indeed, in cases where DFP has been used, the frequency of allergic reactions has been decreased.

Another procedure related to plasmapheresis is immunoadsorption, the extracorporeal removal of immunoglobulins from the patient's blood (Fig. 91.6). This is achieved by passing the plasma fraction through a column containing a matrix capable of binding IgGs, therefore, effecting their selective depletion from the blood without the loss of additional plasma components. There is still considerable lack of selectivity though since all IgGs are removed including protective antibodies and not only the pathogenic autoantibodies. IgG-binding matrixes explored so far are a tryptophan-linked polyvinyl alcohol resin (TR350) and columns with immobilized staphylococcal protein A or sheep antihuman IgG (Adsopak 200). However, they both remove nonspecifically all IgGs. Further attempts focused on

the use of more specific antibody-removing devices with a variety of adsorbents: polypeptide segments from amino acids 183–200 of the α1 subunit ECD from the AChR of Torpedo californica (Medisorba MG-50) or amino acids 67–76 from the human α1 subunit (hMIR10-CB) immobilized on cellulose particles. These adsorbents remove only a small fraction of anti-AChR antibodies, i.e., the ones targeted against these specific epitopes. Efforts are in progress to achieve the selective removal of the majority of the autoantibodies by the use of much larger recombinant domains of human AChR (see below).

Rituximab and Etanercept

Rituximab and etanercept are two agents not frequently used in the systematic treatment of MG. They are used to treat other autoimmune diseases, and preliminary results from small-scale clinical trials have shown that they may be beneficial in MG as well. However, more extensive trials are awaited to assess their impact on MG management.

Rituximab is a human-mouse chimeric antibody against the surface antigen CD20, consisting of the human IgG1 constant regions and the variable domains of a mouse anti-CD20 monoclonal antibody. CD20 is almost exclusively found on the surface of all B cells, except for stem cells and plasma cells. It, therefore, causes selective depletion of B cells in all stages of their differentiation. It acts by antibody-mediated and complement-mediated B-cell destruction as well as by inducing phospholipase C–driven apoptosis. Rituximab has a reported in vivo half-life of 3 weeks. Clinical improvement is seen after 1 month of treatment, and minor side effects have been reported to date, such as nausea, vomiting, headache, anemia, cardiac arrhythmia, and leucopenia. Rituximab has been used in individual selected cases of MG with a promising potential, but no controlled clinical trials have been performed to date.

Etanercept is a recombinant fusion protein, which inhibits the TNF-α pathway. The use of a TNF-α pathway blocker is based on the findings that blood mononuclear cells of MG patients express higher levels of TNF-α, while T cells express more TNF-α receptors. Etanercept consists of the soluble TNF receptor 2 fused to the Fc region of IgG1. When introduced into the circulation, it acts as a decoy receptor binding TNF-α, blocking its normal function and hindering the inflammatory response. It has been tested successfully in a limited number of MG patients, although in a few patients the disease was exacerbated. Larger clinical trials will follow to obtain more reliable results.

Experimental MG

Experimental autoimmune MG (EAMG) is the animal model for MG. It serves as an invaluable tool in both the understanding of MG pathophysiology and the development of novel therapies for humans. EAMG can be induced by two different methods. The most direct is passive transfer EAMG, where autoantibodies derived

Table 91.3 EAMG symptom grading

Grade 0	Absence of EAMG symptoms
Grade 1	Mild symptoms: decreased activity, weakened grip, and fatigability
Grade 2	Moderate symptoms: weakness, decreased body weight, hunched posture, tremor, and discharge from the eyes
Grade 3	Severe symptoms: generalized severe weakness, flaccid paralysis, extreme decrease in body weight, and moribund
Grade 4	Death

from MG patients or monoclonal anti-AChR antibodies are injected into the experimental animals. Due to the cross-reactivity of the human anti-AChR antibodies with the animal AChRs, the injected antibodies bind to the receptors at the NMJ causing the development of MG-like symptoms (weakness and fatigability). The symptoms are transient, since, if the animal survives, after turnover of the injected antibodies they subside. Additionally, instead of antibodies, it is possible to use lymphocytes isolated from MG patients. This, however, does not necessarily induce the immune response actually present in human MG involving changes in cytokine profile. A more physiologically relevant method for EAMG induction is the immunization of the animals with AChR derived from the electric organ of Torpedo californica or from mammalian skeletal muscles. The produced antibodies against the injected AChR cross-react with the AChR of the experimental animal, causing the development of MG symptoms.

EAMG can be induced in many species including primates. The animals most commonly used today are young female rats and mice. In rats, EAMG induction is relatively easy, and in addition to the acute phase, it also has a chronic phase resembling the human disease, but without thymic involvement. On the other hand, disease induction in mice is more difficult, but the large number of mouse strains with specific gene deletions which allow intricate analyses on pathogenesis to be performed make it an attractive option. For instance, the SCID mouse strain (standing for severe combined immunodeficiency) has a mutation that impairs development of T and B lymphocytes allowing the transplantation of lymphocytes or thymic tissue from MG patients. Interestingly, different strains of mice have different degrees of susceptibility to EAMG, undoubtedly due to their different genetic background.

New therapies are usually first tested in the acute phase, and, subject to a positive outcome, they are tested in the chronic phase of EAMG. To assess the effect of a given pharmaceutical or procedure on the disease, the symptoms are graded according to their severity. The most commonly used grading system has five scores ranging from 0 to 4 (Table 91.3). Additional tests may be used to fully describe the diseased animals, such as determination of their anti-AChR antibody titer, visualization of structural anomalies at the NMJ due to complement-mediated damage, measurement of the total AChR content of the muscle, and determination of changes in the levels of cytokines involved in MG.

Outlook

Future Perspectives

Improved Diagnosis Promises Higher Sensitivity Levels

Major improvements in diagnostic methods have taken place in the area of serological tests. As already mentioned, a small but important fraction of MG patients remain seronegative, i.e., have no detectable antibodies against any of the known auto-antigens. Current efforts are concentrated toward identifying other potential targets, or increasing the detection efficiency for the known antigens. Recent advances have shown that up to 50% of these patients have antibodies against the AChR, albeit at low levels. These antibodies were detected using a cell-based technique. Specifically, the cells used express on their surface AChR molecules, which are clustered by the concomitant expression of the protein rapsyn. This leads to the formation of receptor clusters similar to those seen on the surface of muscle cells. Serum from MG patients is incubated with these cells allowing for any autoantibodies to bind to the receptor clusters. Subsequently, the bound autoantibodies are detected using a second antihuman antibody fluorescently labeled. It appears that these low-affinity antibodies need to "see" clustered AChRs to bind efficiently. Antibodies from patients with very low titers can also be detected using a modified RIPA technique. In the conventional RIPA, there is a limitation as to the starting volume of serum that can be used due to increased background noise. In the new method, the anti-AChR antibodies are first semi-purified from the rest of the serum and concentrated. This allows the use of large starting volumes of serum to increase the total amount of anti-AChRs, thus rendering them detectable, overcoming the limitation of the conventional RIPA.

Furthermore, efforts are made to develop safer diagnostic assays that rely on fluorescence rather than isotope detection. Some preliminary efforts have shown them to be equally sensitive and correlate well with the regular RIPA results, but further evaluation is required before they can be widely adopted as a diagnostic test.

Novel Therapeutic Approaches

Most of the therapeutic strategies currently under development aim at diminishing the autoimmune response, while bypassing generalized immunosuppression, which is responsible for the majority of adverse effects seen in long-term treatment. Several ongoing projects are under way aiming to develop novel improved therapies for MG. Although most of these are still at the stage of animal trials, they offer interesting ideas and potentially highly valuable therapeutic strategies for the future.

Using a rat EAMG model, it has been found that several phosphodiesterases (PDEs) are expressed at higher levels, including PDE1-4 and PDE7 which are expressed mainly in lymph node cells (LNCs) and in the muscle. PDEs are important regulators of intracellular signaling. They catalyze the hydrolysis of the second messengers cAMP and cGMP, which are involved in a variety of functions, including immune responses. PDE inhibitors have already been investigated as potential therapeutic agents for other autoimmune disorders. Their possible use in

MG treatment has been evaluated in EAMG models where the PDE inhibitor pentoxifylline (PTX) was found to inhibit disease progression. The clinical presentation of the experimental animals was significantly improved. Examination of the cytokine levels showed that PTX caused a significant change in the production of both Th1 and Th2 cytokines by LNCs including TNF-α, IL-10, IL-12, and IL-18. Furthermore, PTX was shown to increase the effects observed by low doses of corticosteroids in EAMG. The fact that PTX is a pharmaceutical already in use for treatment in humans with few side effects make it a prime candidate for the initiation of clinical trials for the treatment of MG as well.

The initiation of the immune response depends on the way the antigen is presented to the effector cells of the immune system. In EAMG, mucosal administration of AChR or peptide fragments can induce tolerance. Various mechanisms are involved; lower antigen doses appear to stimulate cells that produce the immunosuppressive cytokines TGF-β, IL-4, and IL-10, while larger doses induce anergy or deletion of antigen-specific T cells. Overall, the mechanism of mucosal tolerization is complex and the results depend highly on the administered dose, the size and conformation of the antigen, and the route of administration (e.g., oral versus nasal).

The elimination of AChR-specific CD4+ T cells would be beneficial as it would decrease the stimulation toward B cells for anti-AChR production. In vitro experiments have used antigen-presenting cells engineered to express the AChR but also the Fas ligand, a membrane protein that binds to Fas on T cells and induces apoptosis. These cells were capable of inducing apoptosis only in the anti-AChR CD4+ T cells. However, limited studies have been performed in vivo using this method to date.

Blocking the antigen presentation process can also hinder the response to a particular antigen. This has been attempted using peptides resembling AChR fragments but with substitution of certain amino acids termed altered peptide ligands (APLs), which compete with the AChR peptides for antigen presentation. The APLs presented in a MHC class II context can cause differential response of the corresponding CD4+ T cells ranging from partial activation to complete inhibition. Studies with APLs in EAMG models showed that they could reduce the severity of symptoms in the affected animals, as well as their anti-AChR antibody titers. The pro-inflammatory Th1 cytokines were downregulated, while the Th2 (anti-inflammatory) and Th3 (associated with Treg cells) responses were upregulated. A possible limitation of this method is the high variety of antigenic epitopes in MG against which the APLs would have to compete to confer total blocking of MG-related antigen presentation.

T-cell vaccination is another way to inhibit the autoimmune response, and it refers to the induction or direct administration of antibodies against the T-cell receptor binding site. Blocking this binding site will interfere with normal activation of the CD4+ T cells by antigen-presenting cells. In fact, T-cell vaccination has been used in clinical trials for other autoimmune disorders and has shown promising results in EAMG animals.

An interesting approach is the use of protective antibodies, i.e., antibodies that recognize the AChR epitopes but do not cause disease, thus competing with the pathogenic autoantibodies. To this end, antibody fragments are commonly used,

which are monovalent (i.e., they cannot cross-link antigens to cause antigenic modulation) and lack the complement binding domains to prevent complement activation. In order to avoid the potential immunogenicity of rodent-derived antibodies, the antibody fragments must be human or humanized: the former are produced by immunization of humanized mice (mice with human Ig genes) or by phage-antibody display technology, while the later are made by transferring the complementarity-determining regions of a protective animal antibody into a human antibody scaffold. This approach of course leads to short-term treatment since autoantibody production remains unaltered, and when the protective antibodies are cleared from the circulation, their pathogenic effect will resume. Most efforts are, therefore, focused on increasing the half-life of the protective antibodies.

The most direct method for immediate relief of symptoms is the removal of the causative agent, i.e., the autoantibodies. Plasmapheresis and related methods offer this option, albeit with no or little selectivity as previously discussed. Therefore, efforts are currently made to improve this procedure by highly increasing its specificity so as to remove only the pathogenic antibodies from the circulation. The explored technique involves the use of a column with extracellular domains (ECDs) from AChR or MuSK proteins immobilized onto a sepharose matrix as specific ligands for the autoantibodies, as opposed to the nonselective ligands used today for immunoadsorption. A major limitation for this approach in the past was the inadequate amount of available ECDs to be used in a large-scale clinical application. The advancement of biotechnology allowed the expression of high levels of the ECDs with near-native conformation in yeast or insect cell systems that can act as high-affinity specific adsorbents. The main drawback at present seems to be the fact that, for a significant proportion of the AChR-MG patients, not all anti-AChR antibodies are bound. Nonetheless, the anti-AChR antibodies from many patients bind efficiently to the adsorbent, while in the case of anti-MuSK antibodies, they all bind to the adsorbent. Additionally, it is possible that even a partial removal of the autoantibodies could be highly beneficial for the patient. Preliminary results from in vitro experiments and from EAMG animals are promising: the method appears to be fast and safe. It could, thus, provide an invaluable tool in cases of emergency such as myasthenic crises, as well as for the long-term management of MG in patients unresponsive to other treatments. Most efforts currently are made toward increasing the binding efficiency of the adsorbent and addressing all the necessary safety aspects. Furthermore, the possibility of whole-blood immunoadsorption, where the patient's blood is directly passed through the column without prior need for separation of the plasma, is explored. Clinical trials are in preparation for the near future.

Conclusions

MG is arguably the best characterized antibody-mediated autoimmune disease, with well-defined antigenic targets and pathology. Furthermore, the development of many animal models of the disease allows the investigation of the triggering mechanisms, which remain largely unknown to date. Importantly, these animal models aid in the

straggle for the discovery of novel therapies with improved specificity and minimized side effects. These discoveries will shed light and provide invaluable therapeutic solutions not only for MG but also for a variety of related disorders.

Further Reading

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