Myasthenia gravis: the role of complement at the neuromuscular junction

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Generalized myasthenia gravis (gMG) is a rare autoimmune disorder characterized by skeletal muscle weakness caused by disrupted neurotransmission at the neuromuscular junction (NMJ). Approximately 74–88% of patients with gMG have acetylcholine receptor (AChR) autoantibodies. Complement plays an important role in innate and antibody-mediated immunity, and activation and amplification of complement results in the formation of membrane attack complexes (MACs), lipophilic proteins that damage cell membranes. The role of complement in gMG has been demonstrated in animal models and patients. Studies in animals lacking specific complement proteins have confirmed that MAC formation is required to induce experimental autoimmune MG (EAMG) and NMJ damage. Complement inhibition in EAMG models can prevent disease induction and reverse its progression. Patients with anti-AChR+ MG have autoantibodies and MACs present at NMJs. Damaged NMJs are associated with more severe disease, fewer AChRs, and MACs in synaptic debris. Current MG therapies do not target complement directly. Eculizumab is a humanized monoclonal antibody that inhibits cleavage of complement protein C5, preventing MAC formation. Eculizumab treatment improved symptoms compared with placebo in a phase II study in patients with refractory gMG. Direct complement inhibition could preserve NMJ physiology and muscle function in patients with anti-AChR+ gMG.

Keywords: complement; eculizumab; myasthenia gravis, neuromuscular junction; safety factor

Introduction

Myasthenia gravis (MG) is a rare autoimmune disorder resulting from impaired synaptic transmission at the neuromuscular junction (NMJ). MG is classified into different subtypes on the basis of the type of autoantibodies and the clinical manifestations of the disease, such as early or late onset, or the presence of generalized or ocular symptoms. Acetylcholine receptors (AChRs) are present in high numbers on the postsynaptic membrane of the NMJ (Fig. 1), and approximately 74–88% of patients with generalized myasthenia gravis (gMG) have autoantibodies to these receptors. There are three mechanisms by which AChR autoantibodies act to reduce synaptic transmission at the NMJ: blocking the binding of acetylcholine (ACh) to AChRs; accelerating the internalization and degradation of AChRs that are cross-linked by autoantibodies; and complement activation (Fig. 2). Complement activation damages the postsynaptic membrane at the NMJ, reducing the membrane surface area and the number of AChRs and voltage-gated sodium ion (Na+) channels (VGSCs). This leads to abnormal neuromuscular transmission and the characteristic muscle weakness associated with MG. This review discusses the role of complement in anti-AChR+ MG.

The neuromuscular junction

Overview

The NMJ is the site of synaptic transmission between motor neurons and muscle fibers (Fig. 1). Electrical signals called action potentials are conducted to skeletal muscles by motor neurons that enter the muscle tissue and divide into numerous branches to innervate individual...
Figure 1. Healthy neuromuscular junction. The normal function of the neuromuscular junction, with major components implicated in MG, is shown. The action potential at the presynaptic nerve terminal causes opening of voltage-dependent \( \text{Ca}^{2+} \) channels, triggering the release of acetylcholine and agrin into the synaptic cleft. Acetylcholine binds to AChRs, which promote sodium channel opening, which triggers muscle contraction. Agrin binds to the complex formed by LRP4 and MuSK, causing AChR clustering; this is required for maintenance of the postsynaptic structures of the neuromuscular junction. AChR, acetylcholine receptor; LRP4, low-density lipoprotein receptor–related protein 4; MG, myasthenia gravis; MuSK, muscle-specific kinase; VGCC, voltage-gated \( \text{Ca}^{2+} \) channel; VGSC, voltage-gated \( \text{Na}^+ \) channel. Adapted by permission from Ref. 2.
positively charged ions is called an endplate potential (EPP) and causes muscle fiber depolarization. The EPP activates the VGSCs in the junctional folds, creating a further influx of Na⁺ ions and the spread of the action potential along the muscle fiber, triggering muscle contraction. In between action potentials, single ACh-containing vesicles fuse spontaneously with the presynaptic membrane, giving rise to miniature endplate potentials (MEPPs). MEPPs can be used by researchers to assess changes in characteristics and function of the NMJ.

The safety factor
In the healthy NMJ, the amplitude of EPPs generated by synaptic transmission exceeds the threshold required to generate an action potential in the muscle. The ratio between the actual EPP and the threshold potential required to generate an action potential in the muscle is called the safety factor. If the EPP falls below the threshold level, the action potential will not be produced. The reduction in this neuromuscular transmission safety factor is the electrophysiological defect that results in MG symptoms and the electrophysiological findings seen in the clinical diagnostic laboratory.

A number of factors can affect the EPP, including the amount of ACh released by the synaptic terminals, the activity of acetylcholinesterase in the synaptic cleft, and the conduction properties and density of AChRs and VGSCs in the postsynaptic membrane. The folded structure of the postsynaptic membrane increases the density of AChRs lying directly below the ACh active-release zone of the presynaptic nerve terminal, as well as the density of VGSCs within the folds. VGSCs present within junctional folds, and the folds themselves, amplify the EPP. Consequently, a smaller amount of positive charge is required to trigger muscle cell contraction.

Figure 2. Pathophysiology of anti-AChR+ MG. The three major pathogenic mechanisms of AChR autoantibodies in MG include complement activation at the neuromuscular junction, which causes the formation of MACs on the muscle membrane and destruction of the typical folds in the motor endplate; antigenic modulation that results in the internalization and degradation of surface AChRs; and binding of AChR antibodies at the AChR ligand binding site, which could directly block ACh binding and, consequently, channel opening. ACh, acetylcholine; AChR, acetylcholine receptor; LRP4, low-density lipoprotein receptor–related protein 4; MAC, membrane attack complex; MG, myasthenia gravis; MuSK, muscle-specific kinase; VGCC, voltage-gated Ca²⁺ channel; VGSC, voltage-gated Na⁺ channel. Adapted by permission from Ref. 2.
than would be required if there were fewer or no folds. Changes in the number or activity of AChRs and VGSCs or alteration of folds in the membrane structure will decrease the efficiency of neuromuscular transmission.

Autoantibodies and complement at the NMJ

The majority of patients with MG have AChR autoantibodies. However, some patients who lack AChR autoantibodies may have autoantibodies against MuSK or, more rarely, LRP4 or agrin. In anti-AChR+ MG, antibodies bind to a variety of epitopes on AChRs and may block synaptic transmission, accelerate normal AChR internalization and turnover, or activate the complement cascade (Fig. 2). In anti-MuSK+ MG, which affects about 5% of patients with MG, the autoantibodies cannot bind to and activate complement. Instead, anti-MuSK antibodies disrupt MuSK signaling by blocking the interaction of agrin, LRP4, and MuSK. This reduces AChR density, lowering EPP amplitude and resulting in muscle weakness. Complement activation by AChR autoantibodies will be the focus of the remainder of this review.

The complement cascade

The complement system plays an important role in both innate and antibody-mediated immunity. It acts to defend the host against infection by recognizing and eliminating microorganisms and forms a link between innate and adaptive immunity by enhancing antibody responses. It is also involved in the removal of immune complexes and dead or modified self-cells. The complement cascade comprises more than 30 proteins found in the plasma and on cell surfaces, and its activation is sequential and carefully regulated. Initiation of the cascade is followed by amplification and culminates in formation of the membrane attack complex (MAC).

There are three distinct complement pathways: the classical pathway, the lectin pathway, and the alternative pathway, and each has a different initiation mechanism leading to the formation of a C3 convertase (Fig. 3). Following initiation, the complement cascade is amplified as C3 convertase cleaves C3 to form C3b, which then forms further surface-bound convertase complexes close to its site of generation. Terminal complement activation begins with C3b and a C3 convertase combining to form a C5 convertase. C5 convertases start the process of MAC formation by cleaving C5 to form C5b and C5a. C5b then combines with C6, C7, C8, and multiple copies of C9 to form the MAC. MACs are lipophilic protein complexes that insert into cell membranes, resulting in pore formation and eventually cell lysis of nonnucleated cells and damage to nucleated cells.

The classical complement pathway is activated in anti-AChR+ MG. C1q binds to the antigen/antibody complexes formed by immunoglobulin (Ig)G1 or IgG3 autoantibodies binding to AChRs. C1q activates C1r, which then cleaves C1s. C1s activates serine proteases that cleave C4 and C2 to form C4b2a, a C3 convertase, which cleaves C3 to C3a and C3b. C3b combines with C4b2a to form the C5 convertase (C4b2a3b), which splits C5 into C5a and C5b. Finally, as outlined already, C5b recruits C6, C7, C8, and multiple units of C9 to form MACs at the NMJ.

The lectin pathway is triggered by the binding of mannose-binding lectin (MBL) to carbohydrates on the surface of microbial pathogens (Fig. 3). It shares a common pathway with the classical pathway, except that the C1 complexes are replaced by MBL complexes that are activated by carbohydrates from pathogenic microorganisms.

Activation of the alternative pathway occurs spontaneously and continuously; this is called complement “tick-over” and is an important part of innate immunity. Pathogenic surfaces stimulate amplification of an alternative C3 convertase (C3bBb), produced transiently and spontaneously by the activity of complement factor B and properdin on C3b. Components C1, C2, and C4 are not involved in the alternative pathway; however, the alternative pathway, through a C3b- and C3-convertase–mediated amplification loop, contributes to both the classical and lectin pathways.

The complement cascade is controlled by regulatory proteins that prevent complement from acting on intact host cells and so protects against inadvertent tissue injury. There are three major classes of these proteins: fluid-phase regulators, regulators attached to the surface of host cells, and those present as integral cell membrane receptors. The membrane surface regulators CD55 (also called decay-accelerating factor (DAF)) and CD59 (protection) act at different stages of the cascade to regulate complement activation. DAF (CD55) inhibits...
complement activation by accelerating the decay of C3 convertases.\textsuperscript{22} Protecstin (CD59), which is found on the surface of skeletal muscle cells, can oppose MAC formation by binding to C8. This results in inhibition of attachment of C9 subunits to the C5b–C8 complex, which is required for MAC pore formation to occur.\textsuperscript{23,24}

**The role of complement in MG**

The majority of evidence for the role of complement in MG has been established on the basis of data from a range of animal models, and this is reviewed in detail.\textsuperscript{25} Additional and confirmatory data that have been gained from patient studies are also presented.

**Animal models of MG**

Models of MG in rats and mice (experimental autoimmune MG (EAMG)) have been key to investigating the role that complement plays in the pathogenesis of MG.\textsuperscript{26} These well-established models share many immunopathological and clinical features with MG in humans, including the presence of anti-AChR antibodies in serum, deposition of IgG and complement at the NMJ, and the loss of muscle AChRs. Animals with EAMG develop muscle weakness and fatigue and have decremental responses to repetitive nerve stimulation.\textsuperscript{27}

EAMG models are classified as active or passive depending on how the condition is induced. Active EAMG can be induced by immunization either with purified AChR or, in rats, with a synthetic peptide corresponding to part of the α-subunit of the rat AChR. Affected animals show two clinically distinct disease phases—an acute transient phase and a chronic phase, starting approximately 7 and 28 days, respectively, after immunization. The chronic phase is similar to the clinical course of MG in humans. EAMG can also be induced in vivo by the passive transfer of antibodies derived from patients with MG or from AChR-immunized animals in the chronic phase of EAMG. Alternatively, administration of monoclonal antibodies directed...
against the α-subunit of AChR can be used to induce EAMG symptoms in rats within 24 hours.\textsuperscript{29}

**Evidence for the role of complement in EAMG**

Studies using EAMG models provide several lines of evidence for the role of complement in the pathogenesis of MG (key study outcomes are summarized in Table 1). Animals with active or passive EAMG have complement deposition at the NMJ, which is similar to that seen in patients with MG.\textsuperscript{30–32} Antibody colocalization studies and immunofluorescence imaging demonstrated the presence of IgG, C3, and MAC deposits at the NMJ in affected animals, and these deposits were associated with degradation of the postsynaptic membrane and debris in the synaptic space, loss of AChR from the endplate region, and a reduction in MEPP amplitude.\textsuperscript{30–32}

Treatment with cobra venom factor, an experimental tool used to deplete complement in laboratory animals, inhibits the induction of both active and passive EAMG in rats.\textsuperscript{33} In these studies, there was no reduction in AChR content in muscle, and neuromuscular transmission was not compromised, despite the high levels of antibody binding to muscle AChR, suggesting that complement is a critical factor in MG pathogenesis.

**EAMG studies in knockout animals**

Direct evidence for the role of complement in EAMG comes from studies involving animal strains with deficiencies in particular complement factors (shown below and summarized in Table 1).

Knockout mice deficient in either C3 or C4 had a significantly lower incidence of active EAMG than their wild-type litter mates.\textsuperscript{34} C3- and C4-deficient animals had IgG deposits at the NMJ, in common with wild-type mice, but, unlike in wild-type animals, C3 and MAC deposits were absent. This suggests that C3 and MAC play a critical role in destruction of the NMJ in EAMG, and that, on its own, IgG binding at the NMJ is insufficient to induce the loss of AChR and development of symptoms.\textsuperscript{34}

Similar findings have been observed in animals lacking C5 or C6. When AChR immunization was used to induce EAMG in two mouse strains differing only at the C5 gene locus, C5-deficient mice showed a significantly lower incidence of disease and death and reduced loss of muscle AChR compared with C5-sufficient animals, even though serum anti-AChR antibody levels were similar in both strains.\textsuperscript{35} IgG and C3, but not MAC deposits, were detected by immunofluorescence at the NMJ in C5-deficient mice.

C6-deficient rats were resistant to passive EAMG induction, whereas wild-type rats displayed symptoms of MG.\textsuperscript{36} Moreover, C6-deficient animals dosed with human C6 at the time of induction developed symptoms that resembled those observed in wild-type rats. Endplate destruction was significantly reduced in C6-deficient rats compared with wild-type rats. Although C3 deposits were present at the NMJ in both C6-deficient and wild-type animals, MAC deposits were only present in wild-type animals.\textsuperscript{36} These studies in C5- and C6-deficient animals indicate that MAC formation is required for endplate damage to occur at the NMJ, and this causes the development of muscle weakness and paralysis in EAMG.

It is possible that the absence of C5a may have contributed to the lower incidence of EAMG observed in C5-deficient mice than in wild-type animals.\textsuperscript{35} However, a study comparing C5a gene knockout and wild-type mice showed that both types of mice were equally susceptible to the development of EAMG by AChR immunization.\textsuperscript{37} Assessments of muscle strength were similar in the two groups, and both groups showed similar levels of serum anti-AChR antibodies and amounts of IgG, C3, and MAC deposited at the NMJ. This suggests that C5a is not involved in the development of EAMG.\textsuperscript{37}

Similarly, studies with C4 gene knockout mice raised the possibility that the lectin complement pathway could play a role in EAMG pathogenesis, as C3-convertase C4bC2a is activated by both the classical and lectin pathways. MBL gene knockout mice did not demonstrate resistance to EAMG induction by AChR immunization, but showed similar IgG and complement accumulation at the NMJ to that of wild-type mice.\textsuperscript{38} Serum MBL levels in patients with anti-AChR\textsuperscript{+} MG were similar to those in healthy individuals, suggesting that lectin pathway components were not overutilized in MG. These findings indicate that the lectin pathway does not have a role in MG pathogenesis.\textsuperscript{38}

**Complement regulators in EAMG**

Further evidence for the role of complement in MG pathogenesis comes from evaluating animals deficient in particular complement regulators (Table 1). Several studies have demonstrated that
## Table 1. Evidence from EAMG models of the role of complement in MG pathogenesis

| Study | Summary of findings | Details |
|-------|---------------------|---------|
| **General findings**<br> Sahashi *et al.*<sup>32</sup> | IgG, C3, and MAC components are present at damaged motor endplates in active and passive EAMG animal models exhibiting muscle weakness and paralysis. | • Complement-mediated injury to the junctional folds, including deposition of IgG and C3 on terminal expansions of junctional folds and on debris in the synaptic cleft in an active EAMG rat model<br> • AChR deficiency associated with reduced MEPP amplitude |
| Engel *et al.*<sup>30</sup> | | • Complement-mediated injury to the junctional folds, including deposition of IgG and C3 on terminal expansions of junctional folds and opsonization of the postsynaptic region in a passive EAMG rat model |
| Graus *et al.*<sup>31</sup> | | • Showed C3 and C5b-9 MAC at the NMJ and loss of AChR from the endplate region in passive EAMG rat model |
| **Complement factor–deficient animal models**<br> Christadoss *et al.*<sup>35</sup> | MAC formation is required for endplate damage at the NMJ and the development of muscle weakness and paralysis. | • In an active EAMG model, C5-deficient mice showed a significantly lower incidence of clinical disease and death and had reduced loss of muscle AChRs compared with C5-sufficient mice.<br> • C5-deficient mice had IgG and C3, but not MAC deposits, at the NMJ. |
| Tüzün *et al.*<sup>34</sup> | | • C3-deficient mice and C4-deficient mice were resistant to active EAMG induction.<br> • C3-deficient mice and C4-deficient mice had IgG deposits at the NMJ, but no C3 or MAC deposits. |
| Chamberlain-Banoub *et al.*<sup>36</sup> | | • C6-deficient rats were resistant to passive EAMG induction.<br> • Endplate destruction was reduced markedly compared with C6-sufficient mice.<br> • C3 deposits, but no MAC deposits, were observed at the NMJ.<br> • C6-deficient rats dosed with human C6 at the time of induction developed EAMG with MAC deposition at the NMJ. |
| Qi *et al.*<sup>37</sup> | C5a is not involved in EAMG development. | • C5a receptor gene knockout mice were not resistant to active EAMG induction. |
| Li *et al.*<sup>38</sup> | The lectin complement pathway is not involved in EAMG pathogenesis. | • MBL-knockout mice were not resistant to active EAMG induction. |
| **Complement regulator–deficient animal models**<br> Lin *et al.*<sup>39</sup> | DAF1 and CD59 are involved in EAMG pathogenesis and are required for a healthy NMJ. | • DAF1-deficient mice were susceptible to passive EAMG induction.<br> • EAMG was more severe than in WT controls. |
| Morgan *et al.*<sup>40</sup> | | • In a minimal-dose passive EAMG model, Daf1 knockout mice developed mild symptoms, whereas WT mice were symptom free.<br> • DAF1-deficient mice showed evidence of AChR loss at endplates.<br> • Cd59a knockout mice also exhibited mild symptoms, with some muscle inflammation but undamaged endplates.<br> • Cd59a and Daf1 double knockout mice had severe paralytic disease with marked muscle inflammation and loss of endplates.<br> • Inhibition of MAC assembly reversed clinical disease. |
The role of complement in myasthenia gravis

Table 1. Continued

| Study         | Summary of findings | Details                                                                 |
|--------------|--------------------|-------------------------------------------------------------------------|
| Kaminski et al.41 |                   | • In a standard passive EAMG protocol, Daf1 knockout mice were          |
|              |                    | weaker than Cd59a knockout and WT mice, with greater NMJ                |
| Soltys et al.42 |                   | • Mice deficient in CRRY showed similar EAMG severity and               |
|              |                    | complement deposition at the NMJ as did WT animals.                    |

Abbreviations: AChR, acetylcholine receptor; CRRY, complement receptor 1–related gene/protein y; DAF1, decay acceleration factor 1; EAMG, experimental autoimmune myasthenia gravis; IgG, immunoglobulin G; MAC, membrane attack complex; MBL, mannose-binding lectin; MEPP, miniature endplate potential; MG, myasthenia gravis; NMJ, neuromuscular junction; WT, wild-type.

DAF1 deficiency in mice increases the susceptibility to EAMG induction.39–41 C3 and C5 convertases are required for C3 and C5 activation and the subsequent formation of MAC; DAF1 in mice (DAF in humans) increases the breakdown of these enzymes.16 In DAF1-deficient mice, the severity of EAMG was increased compared with wild-type mice; moreover, deficient mice showed greater C3 deposition at NMJs, larger reductions in endplate AChR levels, and increased junctional damage compared with control animals.39–41 However, not all complement regulators have such a major effect on EAMG outcome. Knockout mice deficient in complement receptor 1–related gene/protein y (CRRY) showed similar EAMG severity and complement deposition at the NMJ to wild-type animals.42 Similarly, mice deficient in CD59 (which inhibits MAC formation by preventing C9 from binding to the C5b-8 complex16) showed either minimal or no increase in disease susceptibility with EAMG induction compared with wild-type mice.40,41,43 By contrast, mice with both DAF1 and CD59 deficiency developed severe disease; these double-deficient animals showed marked damage to the motor endplate region, with a loss of structure and AChRs, and MAC deposits were detected at the NMJ.40,41

Complement inhibition in EAMG

The central role of complement in EAMG would be further supported if therapies targeting complement effectively inhibited EAMG induction or improved symptoms in animals with EAMG. Several approaches for blocking the complement cascade have been studied in EAMG (these are outlined below and summarized in Table 2).

Administration of the anti-C1q antibody in mice significantly reduced the incidence of EAMG induced by immunization compared with control antibody and ameliorated clinical signs of disease when administered following EAMG induction.44 Reductions in C3 and MAC deposits were observed at the NMJ, and anti-C1q antibody also reduced IL-6 levels, suggesting that C1q has an effect on cellular immunity. However, anti-C1q antibody was also associated with the development of renal C3 and IgG deposits and large increases in the levels of circulating immune complexes, suggesting that antibody treatment also affected the ability of C1q to clear immune complexes.44

Inhibition of C2 using small interfering RNA (siRNA) significantly improved muscle strength and survival in mice with established EAMG.45 C3 and MAC deposits at the NMJ were reduced and muscle AChR levels were increased in mice that received C2 siRNA compared with animals that received only control siRNA. Serum C3 levels were similar in the two groups, suggesting that the alternative complement pathway was unaffected.

Use of an anti-C6 antibody to inhibit MAC formation was effective in preventing EAMG in rats when administered before the passive transfer of AChR antibodies.46 The accumulation of MAC components C6 and C9 at the NMJ was inhibited, but serum levels of C3 and C5 were not affected, suggesting that the effects of anti-C6 antibody occurred solely via blocking the formation of MAC.

Pretreatment with anti-C5 antibody prevented the development of EAMG by passive transfer and reduced muscle weakness when it was administered after EAMG induction.47 Rats treated with
Table 2. Studies of potential complement targets to treat MG

| Study                        | Target | Therapeutic molecule                          | Outcomes                                                                                                                                                                                                                                                                 |
|------------------------------|--------|-----------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| **Classical pathway**        |        |                                               |                                                                                                                                                                                                                                                                          |
| Tüzün et al.44               | C1q    | Anti-C1q antibody                              | • Increased resistance to active EAMG induction compared with control antibody  
• Improved clinical signs of diseases when administered after EAMG induction  
• Reduced C3 and MAC deposits at the NMJ  
• IL-6 levels were reduced in response to AChR challenge.  
• The ability of C1q to clear immune complexes was compromised. |
| Huda et al.45                | C2     | siRNA                                         | • Muscle strength and survival were improved in mice with established EAMG.  
• C3 and MAC deposits at the NMJ were reduced.  
• Increased AChR levels at the NMJ  
• The alternative complement pathway was unaffected. |
| **Terminal pathway**         |        |                                               |                                                                                                                                                                                                                                                                          |
| Biesecker and Gomez46        | C6     | Anti-C6 antibody                               | • Prevented induction of passive EAMG  
• Did not affect serum levels of C3 or C5  
• Inhibited accumulation of MAC components C6 and C9 at the NMJ |
| Zhou et al.47                | C5     | Anti-C5 antibody                               | • Inhibited induction of passive EAMG  
• Reduced deposition of C9, but not C3, at the NMJ  
• Prevented NMJ destruction |
| Soltys et al.48              | C5     | rEV576 (recombinant C5 inhibitor)              | • Reduced muscle weakness and disease severity and improved survival in passive EAMG  
• In active EAMG, severely affected rats saw improvements in symptoms, and mildly affected rats had a reversal of weight loss.  
• Reduced deposition of C9 at the NMJ |
| **Complement regulators**    |        |                                               |                                                                                                                                                                                                                                                                          |
| Piddlesden et al.49          | Classical and alternate pathways | sCR1                                         | • Reduced symptom severity and weight loss after passively induced EAMG in rats |
| Hepburn et al.51             | Convertases (cleave C3 and C4 during complement activation) | CRRY regulator coupled to rat IgG2a Fc | • Prevented weight loss and clinical disease in passively induced EAMG rat model |
| Kusner et al.50              | NMJ    | DAF-regulator protein coupled to a single-chain antibody to AChR α-subunit | • Significantly less severe muscle weakness and lower MAC deposition at the NMJ compared with control rats |

Abbreviations: AChR, acetylcholine receptor; CRRY, complement receptor 1–related gene/protein γ; DAF, decay acceleration factor; EAMG, experimental autoimmune myasthenia gravis; IgG2a Fc, immunoglobulin G2a crystallizable fragment; IL-6, interleukin 6; MAC, membrane attack complex; MG, myasthenia gravis; NMJ, neuromuscular junction; sCR1, soluble recombinant form of human complement receptor 1; siRNA, small interfering RNA.

the recombinant C5 inhibitor rEV576 developed less muscle weakness and less severe disease and survived for longer than untreated rats after passive EAMG transfer.48 Disease severity was also moderated when rEV576 was administered after active EAMG induction.48

Complement regulators have also been evaluated as potential therapeutic targets and agents.
Administration of the soluble recombinant form of human complement receptor 1 (sCR1), which inhibits both the classical and alternate complement pathways, was shown to reduce symptom severity and weight loss in passively induced EAMG. Rats treated with the DAF regulator protein coupled to a single-chain antibody to the AChR α-subunit designed to deliver therapy to the NMJ showed significantly less muscle weakness and lower MAC deposition at the NMJ than control animals. Treatment with the CRRY regulator coupled to rat IgG2a crystallizable fragments (the Fc region of the antibody) to prolong circulating half-life prevented the induction of EAMG and significantly decreased the deposition of C3 and C9 at the NMJ compared with untreated rats.

**Evidence for the role of complement in human studies**

Studies in patients with MG from the late 1950s first suggested a role for complement in the pathogenesis of MG. Changes in the levels of various complement proteins in the serum of patients with MG suggested that complement components were being utilized in the pathogenic process and were being bound to muscle. In subsequent decades, electron microscopy and immunohistochemistry studies of the NMJ from patients with MG showed that IgG antibodies and complement components C3 and C9 localized to the postsynaptic membrane and on fragments of degenerating junctional folds present in the synaptic space (Table 3).

**Complement inhibition in patients with refractory MG**

Current treatment options for MG include acetylcholinesterase inhibitors, long-term therapy with corticosteroids or steroid-sparing immunosuppressive treatments (ISTs), and short-term immunomodulatory therapies, such as plasma exchange, therapeutic apheresis, or intravenous immunoglobulin (IVIg) (Fig. 4). IVIg preparations comprise pooled donor IgG and exert their therapeutic effects via multiple mechanisms. These include neutralization of autoantibodies by idiotypic antibodies, neutralization of T or B cell-activated cytokines, and complement inhibition; the latter may be the predominant mechanism in complement-fixing antibody-mediated autoimmune diseases. In patients with dermatomyositis, an autoimmune inflammatory myopathy, it has been shown that infused Ig rapidly forms complexes with C3b and reduces MAC deposition by reducing the production of C5 convertase; this is associated with rapid clinical improvements. This mechanism has not been evaluated in MG, although increased C3 utilization has been demonstrated in sera from patients with MG. However, IVIg also has a range of other complex interactions with the complement system. Current treatment options, with the exception of IVIg, do not target complement directly and therefore do not target the principal pathway that leads to the destruction of the NMJ and reductions in AChRs and VGSCs.

Approximately 10–15% of patients do not respond adequately to currently available therapies for MG, or they experience intolerable side effects on IST, so are considered to have refractory MG. Inhibition of the complement cascade has now been studied in patients with refractory MG. The complement component C5 is an attractive target for inhibition of the complement cascade. Preventing cleavage of C5 stops the production of the proinflammatory and prothrombotic C5a and C5b molecules, which are important for inflammatory cell chemotaxis and activation of the MAC. Furthermore, complement pathway blockade at C5 will not impede the key immunoprotective and immunoregulatory functions of the proximal cascade. Functions of the proximal cascade that would be preserved include initiation and amplification that are essential for C3b-mediated opsonization, the C3a inflammatory response, and immune complex clearance.

**Clinical trials of eculizumab in refractory generalized MG**

Eculizumab is a humanized monoclonal antibody that binds to C5 and prevents the cleavage of C5 to C5a and C5b. It has been used for more than 10 years to treat paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS). The efficacy and safety of eculizumab for the treatment of MG have been evaluated in two clinical trials in patients with refractory anti-AChR+ gMG. A phase II pilot study of eculizumab showed clinically meaningful improvements in patients with severe, refractory anti-AChR+ gMG. Six of seven
### Table 3. Evidence from patients with MG for the role of complement in MG pathogenesis

| Study            | Summary of findings                                                                 | Details                                                                                       |
|------------------|--------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|
| Nastuk et al.52  | Complement component utilization is altered in patients with MG.                   | • Serum complement activity fluctuates over an abnormally wide range in patients with MG.     |
|                  |                                                                                      | • Fluctuations in complement activity are correlated with exacerbation and remission of MG symptoms. |
| Casali et al.55  | Patients with MG have elevated quantities of immune complexes (bound to C1q).        | • Provides preliminary evidence that consumption of C4 is linked to increases in circulating immune complexes in patients with MG. |
| Strauss et al.56 | A component of serum from patients with MG binds to muscle and also binds complement. | • Fluorescein-conjugated myasthenic globulin fraction, from the serum of patients with MG, binds to human muscle in vitro. |
|                  |                                                                                      | • Human muscle that has been treated with serum from patients with MG and then with guinea pig complement binds fluorescein-conjugated rabbit anti-guinea pig complement. |
| Rash et al.60    | Patients with MG have altered NMJ structure and function, associated with the presence of immune complexes and complement components at the motor endplate. | • Junctional folds on motor endplates of a patient with a recent diagnosis of MG are covered with a “fuzzy coat” (particle aggregates seen in electron microscopy). |
|                  |                                                                                      | • Muscle fibers from patients with MG had decreased sensitivity to ACh compared with muscle fibers from healthy individuals. |
|                  |                                                                                      | • Muscle fibers from patients with chronic MG were less sensitive to ACh than fibers from the patient with a recent diagnosis of MG. |
|                  |                                                                                      | • Patients with chronic MG had NMJs with grossly altered or absent junctional folds.         |
|                  |                                                                                      | • Provides evidence that membrane remnants contain AChRs in the synaptic cleft that are covered with a “fuzzy coat.” |
| Engel et al.7    | Patients with MG have altered NMJ structure and function, associated with the presence of immune complexes and complement components at the motor endplate. | • IgG and C3 localized to the NMJ in patients with MG, compared with no IgG or C3 being detected in control individuals who did not have MG. |
|                  |                                                                                      | • More severely affected patients have fewer AChRs than less severely affected patients.       |
|                  |                                                                                      | • Motor endplate destruction in patients with MG is associated with reduced MEPP amplitude.    |
| Sahashi et al.57 |                                                                                      | • C9, the terminal and lytic complement component, is found at the motor endplate and on debris in the synaptic cleft of patients with MG. |
|                  |                                                                                      | • No C9 was detected at the motor endplates of control individuals.                           |
| Nakano et al.59  | All patients with MG (n = 30) tested positive for MAC deposits at their motor endplates. | • All patients with MG (n = 30) tested positive for MAC deposits at their motor endplates.     |
|                  |                                                                                      | • 100% of endplates tested positive for MAC in patients with MG.                              |
|                  |                                                                                      | • By comparison, T cells were detected in 8 of 30 patients with MG and only at 2.4% of endplates. |
| Howard et al.71  | A phase II study treating patients with refractory gMG with a complement-inhibiting therapy resulted in clinically meaningful improvements in symptoms. | • A phase II study compared eculizumab MAb with placebo in patients with refractory gMG.     |
|                  |                                                                                      | • Rapid and sustained decreases in QMG scores were observed in patients treated with eculizumab compared with placebo. |
|                  |                                                                                      | • Eculizumab therapy was associated with greater improvements in ADL scores than placebo.     |

Abbreviations: ACh, acetylcholine; AChR, acetylcholine receptor; ADL, activities of daily living; gMG, generalized myasthenia gravis; IgG, immunoglobulin G; MAb, monoclonal antibody; MAC, membrane attack complex; MEPP, miniature endplate potential; MG, myasthenia gravis; NMJ, neuromuscular junction; QMG, quantitative myasthenia gravis test.
Role of complement in myasthenia gravis

Howard

Figure 4. Currently available therapies for anti-AChR+ MG. Available therapies for anti-AChR+ MG do not target complement activation. MG treatment can restore function of the neuromuscular junction by increasing the levels of available ACh (acetylcholinesterase inhibitors), which improves signal transduction, or by reducing the concentration of autoantibodies (immunosuppressive therapies and short-term immunomodulation), which alleviates the pathogenic mechanisms shown in Figure 2. These conventional therapies do not directly target complement activation. AChR, acetylcholine receptor; LRP4, low-density lipoprotein receptor–related protein 4; MAC, membrane attack complex; MG, myasthenia gravis; MuSK, muscle-specific kinase. Adapted by permission from Ref. 2.

patients (86%) treated with eculizumab showed a clinically meaningful improvement in severity compared with four of seven patients (57%) receiving placebo. Decreases in quantitative myasthenia gravis (QMG) total score were observed as early as 1 week after starting eculizumab and were maintained throughout the treatment period. Moreover, four eculizumab-treated patients (57%) showed an 8-point improvement in total QMG score from baseline compared with one placebo-treated patient (one of seven; 14%). Improvements in activities of daily living (ADL) were also reported, measured by patient self-reporting using the myasthenia gravis–specific (MG)-ADL scale. When receiving eculizumab, 9 of 13 patients (69%) achieved clinical improvement in ADL (defined as a ≥2-point improvement in MG-ADL score), compared with 3 of 13 patients (23%) receiving placebo. The safety profile of eculizumab in this phase II study in patients with refractory gMG was consistent with the safety profile and extensive clinical experience of eculizumab use in PNH and aHUS.

The safety and efficacy of eculizumab in refractory gMG have also been investigated in the phase III REGAIN (eculizumab for refractory generalized myasthenia gravis) study (ClinicalTrials.gov Identifier: NCT01997229).

Complement-mediated damage in MG lowers the safety factor at the NMJ

Reductions in the safety factor at the NMJ have been demonstrated in intercostal muscle preparations from patients with MG. At the motor end-plate, AChR loss decreased the EPP, while VGSC loss increased the depolarization threshold needed to generate an action potential. It was concluded that 59% of the reduction in safety factor in these samples from patients was from AChR loss and 40% was from VGSC loss.
Variations in NMJ characteristics between different types of muscle alter the safety factor for neurotransmission and are likely to affect a muscle’s susceptibility to MG. The extrinsic ocular muscles (EOMs) are prone to developing muscle weakness in patients with MG and are often the first muscles affected. These muscles show less pronounced synaptic folds than other types of skeletal muscle, lowering their safety factor while the high firing rate increases the susceptibility of EOMs to fatigue. Furthermore, reduced expression of complement regulators in EOMs increases the likelihood of complement-mediated damage, which will lower the safety factor further by decreasing AChR and VGSC density. Conversely, NMJs in fast-twitch skeletal muscle fibers show increased ACh release, greater postsynaptic folding, and increased postsynaptic sensitivity to ACh, increasing their safety factor and reducing their susceptibility to MG compared with slow-twitch fibers. This highlights that motor endplate morphology is of central importance in anti-AChR+ MG disease mechanisms.

Another consequence of the presence of a safety factor for neurotransmission at the NMJ is that there can be marked changes to the NMJ of apparently healthy individuals without obvious symptoms. One measure from an animal model of MG suggested that as many as 60% of AChRs had to be lost before clinical symptoms became apparent; however, 75% loss of AChRs was fatal. This suggests that relatively modest recovery of AChR numbers could lead to a marked improvement in clinical symptoms.

Summary

Maintaining AChR density at the NMJ is critical for effective neuromuscular transmission and muscle function. In anti-AChR+ MG, the number of functional AChRs is markedly reduced. While this may be caused by autoantibody-mediated acceleration of AChR internalization or by autoantibodies blocking the binding of ACh to AChRs, complement destruction at the NMJ is considered to be the most important pathologic mechanism in affected patients.

Support for the role of complement in MG ranges from preclinical studies to advanced-stage clinical studies. Evidence from EAMG animal models suggests that induction of the classical complement pathway and particularly formation of the MAC are required for induction of EAMG symptoms. Inhibiting formation of the MAC improves EAMG symptoms. Patients with anti-AChR+ MG experience muscle weakness because of disrupted neuromuscular transmission at the NMJ. MACs are associated with damaged motor endplates in patients with MG. Clinical studies in patients with refractory gMG have now shown that inhibiting the cleavage of C5 by treating with eculizumab leads to significant, often rapid, improvement in symptoms.

Current immunosuppressant treatments target autoantibody production but can take months to have an effect. Immunomodulatory therapies, such as IVIg and plasma exchange, have a more rapid effect than immunosuppressants but cannot be used continuously. These conventional treatments, with the exception of IVIg (which exerts therapeutic effects via multiple mechanisms), do not directly target complement (Fig. 4). Direct complement inhibition could preserve normal endplate physiology and preserve muscle function in patients with MG.

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Competing interests

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Role of complement in myasthenia gravis

Howard

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