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The role of complement in experimental autoimmune myasthenia gravis

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Abstract

Complement plays an important role in the pathophysiology of experimental autoimmune myasthenia gravis (EAMG). The deposition of IgG at the neuromuscular junction, followed by the activation and observance of C3 at the site, and finally the insertion of the membrane attack complex, which results in the destruction of the plasma membrane at the neuromuscular junction. Animal models' of complement-deficient components show the importance of the mediated lysis in EAMG. These events have regulators that allow for the limitation in the cascade and the ability of the cell to inhibit complement at many places along the pathway. The complement regulatory proteins have many roles in reducing the activation of the complement cascade and the inflammatory pathways. Mice deficient in complement regulatory proteins, decay accelerating factor and CD59, demonstrate a significant increase in the destruction at the neuromuscular junction. Inhibition of complement-mediated lysis is an attractive therapeutic in MG.

Keywords

complement; complement regulators; myasthenia gravis; C5; autoimmunity

Introduction

Myasthenia gravis (MG) is an antibody-mediated autoimmune disease that targets the neuromuscular junction (NMJ). The preponderance of experimental data indicates that the deleterious effects of AChR autoantibody are mediated through activation of complement in animal models. Among this evidence, it has been observed that: C3 activation fragments, C9, and other components of the membrane attack complex (MAC) are readily detectable at motor endplates in animals with EAMG;^{1–3} depletion of C3 by cobra venom factor protects rats against induction of EAMG;⁴⁵ and administration of complement inhibitors (anti-C6, anti-C5, soluble CR1, or rEV576)^{6–9} protects in passively induced and active EAMG. The data, taken together, strongly support the notion that complement activation in particular assembly of the MAC at the NMJ leads to destruction of post-synaptic structure, compromising neuromuscular transmission.

In human MG, there is strong evidence for complement as the primary pathogenic mechanism. This derives from identification of C3 and MAC deposition at NMJ of

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Conflicts of interest

The authors declare no conflicts of interest.

patients.^{2, 3, 10} NMJ consistently demonstrate a simplified structure consistent with complement injury.¹¹ Depletion of serum complement components, C3 and C4, is observed in patients,¹² terminal components of complement are present in MG patient sera,¹³ and sera induces complement-mediated lysis of cultured myotubes.¹⁴ The role of complement in MG associated with antibodies directed towards the muscle specific kinase or LRP-4 has not been fully defined and is not discussed in this review.

Complement

Complement is a major defense in the innate immune system and is recognized as augmenting the adaptive immune response. The complement system is made up of over 30 proteins with the function to eliminate invading pathogens, prevent infection, and clear altered host cells. The initiation of complement can occur by three separate mechanisms: classical, alternative, and lectin pathways. Although the activation of the pathways differs, all intersect at the C3 level, and progress to the membrane attack complex (Fig. 1). In MG, the classical pathway is involved by the initiation of the antigen/antibody complex being recognized by C1q. The process progresses to the membrane attack complex (MAC) insertion into the postsynaptic membrane of the NMJ.

The initiation of the classical pathway by the binding of the antibody (IgG or IgM) to the antigen (AChR) is recognized by C1q (Fig. 1). The C1q forms a pentameric complex with the C1r2C1s2 serine protease tetramer. The conformational change of C1q binding to the cell surface allows C1r to activate through auto-cleavage.¹⁵ Active C1r can then cleave C1s. The zymogen reaction continues by C1s proteolytic activation of C4 to C4a (peptide anaphylatoxin), and C4b which attaches to the cell surface and binds C2. C2 also becomes cleaved by C1s to form the C3 convertase (C4b2a). The C3 convertase cleaves C3 forming C3a (peptide anaphylatoxin) and C3b, which binds the C3 convertase to become the C5 convertase (C4b2a3b). The activation of C5 by C5 convertase to form C5a and C5b initiates formation of the membrane attack complex by recruitment of C6, C7, C8, and C9.

Complement-deficient EAMG models

Animal models are efficient means of analyzing the effect of the complement system in MG. Animals can be induced in a passive form of EAMG with an antibody to the AChR or actively by administering the antigen. By these methods, the roles of the particular components of the complement system can be discriminated by producing NMJ damage and resultant weakness. Components to the complement system have been genetically deleted from mice and rats to discern the nature of the classical pathway and the relevance to the NMJ injury and antibody production.

Studies of complement depletion were the first approaches to assess the necessity of complement to the pathology of EAMG. Cobra venom was injected during the acute phase of the active EAMG in rats and before the passive transfer of antibodies to the AChR. In each experimental design, the lack of complement resolved the NMJ pathology that was seen in the complement sufficient groups.⁴ Direct evidence for the involvement of the classical complement pathway in EAMG derives from the identification of IgG, C3, and MAC deposition at the neuromuscular junctions of both EAMG animals and MG patients.^{2, 3, 10, 16, 17}

Mice deficient in C3 and C4 demonstrate reduced clinical scores compared to their wild-type littermates during the course of active EAMG, with C3-knockout mice showing no signs of weakness.¹⁸ The total IgG and AChR specific IgG levels were equivalent in the C4-deficient animals compared to their wild-type littermates, whereas, C3-deficient animals showed a reduction in total IgG and AChR specific IgG. Both C3-and C4-deficient animals

demonstrate reduced AChR IgG2b levels. Reduced B cell expression was also seen in non-immunized C3- and C4- deficient mice, which remained low in the EAMG state. Therefore, the state of the immune system is altered in the complement deficient animals. Despite the presence of AChR-specific antibodies neuromuscular junctions show an absence of C3 or MAC deposits when EAMG was induced in C3-deficient mice. Complement-deficient EAMG animals showed a preservation of AChR density at the neuromuscular junction.

A later component of the complement cascade, C5, also is involved in the development of EAMG. C5 cleavage by the C5 convertase results in C5a, a potent anaphylatoxin, and C5b, an initiator of MAC assembly. The influence of the C5 on EAMG susceptibility was analyzed in C5-sufficient and C5-deficient mice in an active model.¹⁹ During the time course of EAMG, both strains had comparable levels of serum AChR specific antibodies. C5-deficient mice demonstrated reduced incidence of disease compared to the C5-sufficient mice. Therefore, C5 appeared to be critical in EAMG pathogenesis, likely through activation of the terminal C5b-C9 sequence required for neuromuscular junction destruction. As a potential for therapeutic target, blocking of C5 does not impair function of early activated complement components.⁷

The final components of the MAC involve C5b recruiting C6-C7-C8, which then accumulates C9, which forms the pore of the complex. A study on C6-deficient rats demonstrated that the lack of one of these components to the MAC causes resistance to passively induced EAMG.¹⁷ The study also looked at the ability to restore the MAC complex and its destruction to the NMJ resulting in weakness by addition of C6 to a C6 deficient EAMG animal. Therefore, all components in the classical pathway must be in place for MAC deposition.

Therapeutic targets in EAMG

Since complement deficient animals demonstrated a resistance to EAMG, therapeutic agents that target the classical pathway of the complement system may allow improved outcome in MG. The classical pathway has many components that could be potential targets for inhibition of MAC and the reduction in the damage done to the NMJ in EAMG.

The target for therapeutic effect has focused on the C5 component. An antibody to C5 was used in a passive study on rats.⁷ The rats were given injections of anti-C5 or a control IgG four hours before being induced by AChR specific antibody. The IgG control and untreated rats showed weakness in 24 hours with untreated decreasing in strength over the time course. The rats treated with anti-C5 demonstrated only mild weakness on day 3, which resolved by day 5. Complement deposition at the NMJ was assessed by immunohistochemistry to C9. The C9 is relatively absent at the NMJ of the anti-C5 treated group compared to the untreated and control IgG treated animals. The anti-C5 treated animal showed preserved synaptic folds of the NMJ, whereas, the untreated animals demonstrated reduced synaptic folds of the neuromuscular junction and increase in cell membrane debris.

A complement inhibitor has been discovered in the saliva of the soft tick. The compound was found to bind and modify C5, inhibiting its interaction with C5 convertase.²⁰ To determine the effect of the complement inhibitor, labeled rEV576, a passive model of EAMG was employed in rats.⁶ Treatment with rEV576 demonstrated significantly less weakness compared to the untreated group. As part of the same study, the active model of EAMG was produced in Lewis rats. After weakness was observed, treatment was initiated with rEV576 or vehicle. The rats in the control group declined rapidly over the course of twelve days, whereas, the treated group improved. C9 deposition at the NMJ of the treated group was significantly less than the vehicle treated group.

Cell surface complement regulators

The complement cascade has regulators, which are responsible for limiting damage of spontaneously activated complement. For the classical pathway of the complement cascade, decay accelerating protein (Daf, CD55), membrane co-factor protein (MCP, CD46), CR1-related gene/protein Y (Crry), and CD59 are critical inhibitors of activation. These regulators have been found on the surface of most myofibers and concentrated at the NMJ.^{21, 22} The CD59 inhibits the membrane attack complex formation and decay accelerating factor accelerates the decay of C3 convertase and C5 convertase. Both have been investigated in EAMG (see below).

Levels of complement regulators vary among muscles

The levels of complement regulators have been evaluated in diaphragm and extraocular muscle and found to change in response to passive EAMG induction.²² The transcript levels of *Daf1*, *Crry*, and *CD59* were determined in the extraocular muscle and the diaphragm of C57BL/6 mice under normal and passive EAMG conditions. Under the normal condition, all three complement regulators' transcripts were lower in extraocular muscle compared to diaphragm. When comparing normal muscle to passive EAMG induced muscle, extraocular muscle had a reduction of the level of transcripts of the complement regulators during disease state. However, the diaphragm did not show any changes in complement regulators due to EAMG. In assessing the protein level, immunohistochemistry showed an increase in *Daf1* expression at the NMJ of extraocular under EAMG conditions, whereas, EAMG induced showed an increase in *Crry* at the NMJ of the diaphragm. The study demonstrates a dynamic expression of complement regulators and the decrease expression may affect the susceptibility of muscles to complement mediated diseases.

The complement regulators have been assessed in the background of genetic modification of specific complement regulator expression.²³ The transcript levels of *Daf*, *CD59a*, and *CD59b* did not show any increases to compensate for the loss of the other complement regulator in genetically modified mice. However, the genetic knockout of both the *Daf* and *CD59ab* showed an increase in the *Crry* transcript levels. These results suggest that the modification of one of the complement regulators can affect the expression of other complement regulators. Also, disease and tissue type influence complement regulator levels.

C9 deposition increases in complement regulator knockout mice with passive EAMG

To determine the effect of EAMG on complement regulator knockouts, wild-type mice and mice deficient in *Daf1*, *CD59ab*, or both *Daf1* and *CD59ab*, were induced by McAb3 (gift of Dr. Vanda Lennon).²³ After 24 hours, the *Daf1*^{-/-} *CD59ab*^{-/-} mice required euthanasia due to the severity of weakness. The levels of MAC deposition was much greater in *CD59ab* deficient mice compare to wildtype and *Daf1*^{-/-} mice. The nature of MAC deposits differed among the *Daf1*^{-/-} *CD59ab*^{-/-} mice having deposits internalized in the myofiber. Ultrastructural analysis of the NMJ of the *Daf1*^{-/-} *CD59ab*^{-/-} animals demonstrated extensive damage at the NMJ as well as myofibers that were necrotic. Comparing only *CD59ab* deficient mice to the *Daf1*^{-/-} mice, MAC deposition was much greater at the NMJ of the *CD59ab*^{-/-} mice at 24 and 60 hours of EAMG induction. However, the *Daf1*^{-/-} mice appeared weaker. Both complement regulator-knockout animals had much more severe weakness and MAC deposition than wild-type mice. These and other studies²⁴⁻²⁶ signal the critical importance of complement regulation confined to the NMJ and point to the possibility that enhancing complement inhibition at the NMJ may be a successful form of therapy.

Increasing complement inhibitors to the NMJ as a therapeutic

In light of the complement regulator studies, we have developed a therapeutic approach to concentrate complement inhibitors to the NMJ. In a preliminary study, we produced a compound consisting of the Daf regulatory region conjugated to a single chain antibody that binds the acetylcholine receptor. The construct was found to bind the AChR and demonstrated *in vivo* complement inhibitory function.²⁷ In unpublished studies, we have demonstrated *in vitro* efficacy and importantly have not found evidence of compromise of neuromuscular transmission by the compound.

Conclusion

We have briefly summarized work performed in rodents that support a therapeutic benefit for EAMG produced by complement inhibition. The extensive studies of complement and its regulators in EAMG have led to consideration that therapies could be targeted against the complement system for human MG, and in fact, a phase 2 trial of eculizumab, which targets the C5 component, for generalized MG was recently completed. It is clear that complement inhibition is a fruitful path for therapeutic evaluation; however, greater elucidation of the complement mediated pathology of MG is required. The differential susceptibility and response of muscle groups will be critical to define further in order to determine whether complement inhibition may be of greater benefit to patients with ocular myasthenia compared to generalized MG. Thus far, the C5 step of complement activation has been a focus for inhibition, but MAC assembly may be more important. Finally, limiting the adverse effects of chronic complement inhibition will be critical to enhance the treatment benefit; one approach would be to develop agents that specifically concentrate to the NMJ.

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Figure 1.

Complement cascade. The three pathways of complement are initiated by different mechanisms and intersect at the C3 level. The classical pathway is involved in the pathology of EAMG, initiated by antibody–antigen complex. The final steps in the cascade are focused on the formation of the membrane attack complex, with C5b–C8 accumulation on the plasma membrane and C9 recruitment to form the pore, which causes the destruction of the nerve–muscle communication.