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THE COMPLEMENT SYSTEM IN Refractory Myasthenia Gravis

JAMES F. HOWARD, JR, MD
Neuromuscular Disorders Section | Department of Neurology | The University of North Carolina at Chapel Hill | Chapel Hill, NC

The complement system fends off invasive pathogens, extracts circulating immune complexes, and communicates between the innate and acquired immune systems.[1] In addition to these positive effects, the complement system can also start a proteolytic cascade that is able to generate several potent inflammatory effectors, such as anaphylatoxins C3a and C5a (**Figure**).[2,3] Soluble and membrane-bound regulators, such as CD59 or CD55, maintain balance. Failure to maintain the delicate equilibrium between activation and inhibition and the resultant excessive or inadequate complement activation plays a critical role in many acute and chronic disease processes.

Complement Is Strongly Involved in MG Pathology

Overactivation of the complement system significantly contributes to the pathogenesis of various acute and chronic diseases. Complement-associated diseases can be inflammatory (eg, Crohn's disease, rheumatoid arthritis, vasculitis), neurodegenerative (eg, Alzheimer's disease, age-related macular degeneration), or autoimmune (eg, systemic lupus erythematosus, multiple sclerosis, psoriasis, paroxysmal nocturnal hemoglobinuria, myasthenia gravis [MG]).[1]

The complement system is strongly involved in the pathogenesis of MG.[4] Normally, the neurotransmitter acetylcholine (ACh) traverses the synaptic space to bind to acetylcholine receptors (AChR) located on the postsynaptic membrane of the muscle fiber of the neuromuscular junction (NMJ). In MG, neuromuscular blockade occurs by one of several mechanisms. There is antibody binding to one of several epitopes of the AChR complex that sterically hinders, by conformational change, the binding of the neurotransmitter, acetylcholine, to its binding site. In addition, there are antibodies that directly bind to the binding site of acetylcholine. Cross-linking of bound antibody to the receptor complex initiates an accelerated internalization and degradation of the normal turnover of the receptor resulting in a net loss in receptor density in the postjunctional membrane. Finally, there is focal lysis of the postjunctional membrane by the terminal component of complement. Each of these mechanisms contributes to a reduction in neuromuscular transmission.

Complement was first recognized as playing a role in the destruction of the AChR complex with an electron microscopic demonstration of membrane attack complex (MAC) and complement debris, as well as a morphologic simplification of the postjunctional membrane.[5] Immune complexes were found to be more abundant at the postsynaptic membrane in the less severely affected MG patients than in the more severely affected ones.[5] The researchers observed that a linear correlation was shown between the length of the postsynaptic membrane binding immune complexes and the amplitude of the miniature endplate potential. The less intense reaction for immune complexes in the more severely affected MG patients can be ascribed to the lesser quantity of AChRs remaining at their endplates. These findings provide unmistakable proof of a destructive autoimmune reaction involving the postsynaptic membrane in MG.

T-cells Working With B-cells

This aforementioned reaction is supported by the fact that MG is a T-cell-dependent B-cell-mediated disease,

DISCLOSURES: J.F. Howard, Jr. is on the board of directors for the American Board of Electrodiagnostic Medicine; is on the medical/scientific advisory board for the Myasthenia Gravis Foundation of America; and the Medical Advisory committee of the Muscular Dystrophy Association (MDA). He provides consulting services for Alexion Pharmaceuticals, Alpha Cancer Technologies Inc, Argenx BVBA and Toleranzia; and has received research support from Alexion Pharmaceuticals, UCB Pharmaceuticals, CDC, NIH/NIAMSNINDS, and MDA.

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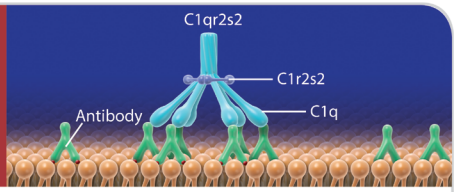
The complement system is composed of more than 30 soluble proteins that, when activated, collaborate in a catalytic cascade.[2]

Three pathways activate the complement system (the classical complement pathway, the alternative complement pathway, and the lectin pathway), all leading to local inflammation and tissue injury via formation of the membrane attack complex (MAC)[2]

Proximal Complement

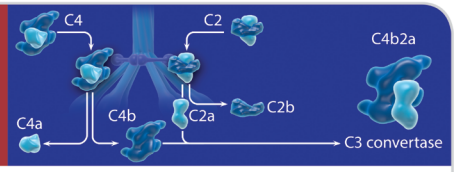
Classical Pathway[1]



- Antibody binds with antigen to initiate complement cascade
- C1q binds to antibody and activates C1s

Lectin Pathway

Complement Initiation



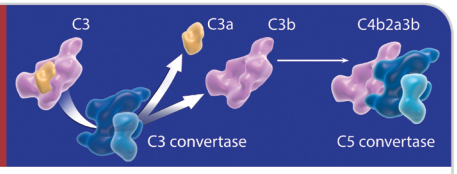
- C1s cleaves C4 and C2
- C4b and C2a** combine
- C3 convertase is formed

Alternative Pathway*

Antibody binding activates the classical complement cascade, which initiates a localized attack by 1) formation of the C5a peptide, a potent proinflammatory chemotaxin, and 2) the MAC, which penetrates cell membranes and causes cell lysis.[2]

C3 Amplification

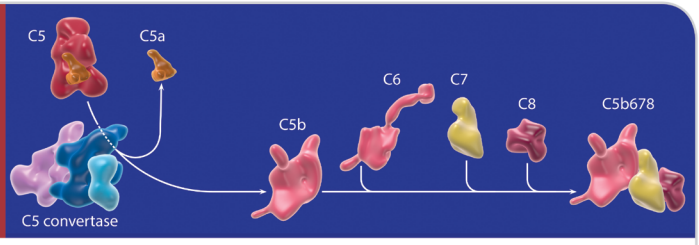
Cascade of amplification occurs: >10^9 C3 molecules can be activated in 15 minutes[3]



- C3 convertase hydrolyzes C3 molecules to C3b and C3a
- C3b and C4b2a combine to form C4b2a3b (C5 convertase)

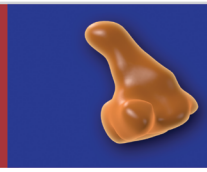
Terminal Complement

C5 is cleaved



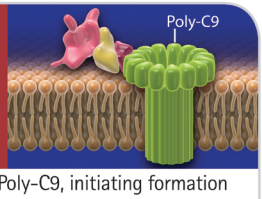
- C5 convertase cleaves C5 into C5a and C5b
- C5b binds C6 and C7 to make C5b67
- C5b67 combines with C8 to make C5b678

C5a



- C5a is a robust anaphylatoxin that stimulates the release of proinflammatory mediators from basophils, eosinophils, neutrophils, macrophages, and mast cells
  - This drives the migration of these cells to the site of injury, and increases endothelial activation

C5b initiates the MAC



- C5b678 binds to Poly-C9, initiating formation of the MAC
- The MAC creates pores in cellular membranes, ultimately resulting in tissue injury, cell lysis, and apoptotic necrosis
- Sublytic levels of C5b-9 also contribute to inflammation

* In the alternative pathway, C3 convertase is C3bBb, and C5 convertase is C3bBbC3b.
** Some researchers conform to the convention that the "b" fragment of C2 is the larger active fragment, and the "a" fragment is smaller; however, here C2a is the larger active fragment of C2.

meaning that CD4+ T-helper cells and T-regulatory cells stimulate and enable the proliferation and differentiation of B-cells into AChR antibody-producing plasma cells.[6]

AChR and MuSK: Two Autoantigens Associated With MG

The hallmarks of MG are varied and include multiple antibodies. The target autoantigen in seropositive MG is the AChR located on the postsynaptic NMJ membrane.[7] Muscle weakness develops as a result of faulty transmission between motor nerves and muscle tissue due to defective functioning or reduction of AChRs at the NMJ site. Muscle-specific kinase (MuSK), another autoantigen, is a receptor tyrosine kinase required for the formation and maintenance of the NMJ. Anti-MuSK antibodies are the most common autoantibodies found in those MG patients who do not demonstrate antibodies to the AChR, or are otherwise Ab-seronegative. While about 80% of MG patients are AChR-Ab-seropositive, the current thinking is that AChR-Ab-seronegative MG affects as many as 20% of patients with MG.[8] However, one study revealed that 70% of AChR-Ab-seronegative patients with MG had serum anti-MuSK autoantibodies.[8] MuSK antibodies are only rarely found in AChR-AB-positive patients. These findings imply that since 2 immunologically distinct forms of the disease exist, identifying MuSK antibodies will aid in diagnosis and disease management. For example, the clinical phenotype of MuSK myasthenia is different than seropositive MG. In the United States, the MuSK patient tends to be female, have less ocular involvement, and more oro-facial involvement with muscular atrophy compared to the seropositive patient. The AChR-Abs are isotypes IgG1 and IgG3, whereas MuSK-Abs are IgG4 and are not considered to bind complement. Additional autoantibodies have been associated with MG, including antibodies to LRP4 (whose phenotype is yet to be established), agrin, cortactin, titin, and ryanodine. Titin and ryanodine have been proposed as markers for more severe MG in the elderly population.

Activation of MAC

As discussed, the binding of complement factors to the pathogenic AChR autoantibody induces formation of MAC, which ultimately leads to the destruction of the NMJ. The assembly of this attack complex requires the participation of a number of complement proteins. C5 is split by C5 convertase into C5a and C5b.[9] C5a increases the permeability of blood vessels and attracts inflammatory cells by chemotaxis. Newly activated C5b binds to other complement components ultimately forming the C5b-6-7-8 complex. The C5b-6-7-8 complex subsequently binds to C9 and acts as a catalyst in the polymerization of C9 to form the attack complex. MAC forms a ring structure creating a pore through the lipid bilayer of the muscle membrane leading to movement of ions and water across the membrane, ultimately culminating in cell lysis.[10]

As a key trigger of inflammatory processes, complement activation may be a factor in autoimmune conditions such as neuromyelitis optica spectrum disorder and MG.[11] Complement activation leads to the assembly of the MAC at the motor endplate, which is the principle process underlying destruction of the postsynaptic membrane.

In the late 1970s, cobra venom factor, which blocks complement, was found to prevent induction of experimentally-acquired MG (EAMG).[12,13] Administration of anti-C6 Ab to Wistar Furth rats inhibited the muscle weakness, electrophysiologic abnormalities, and loss of AChR associated with EAMG, depending on the dose used.[14] Administration of soluble C3b receptor, a C3/C5 convertase inhibitor, was shown to protect rats against the induction of EAMG.[15] It binds C4b and C3b and accelerates the decay of C3 and C5 convertases. Thus, the complement cascade is blocked at an early stage, inhibiting anaphylatoxin release and MAC formation. In that study, the C3b receptor significantly reduced the weight loss and severity of clinical symptoms, and treated animals could recover normal muscle function.

In a more recent study, administration of a mouse anti-mouse C5 monoclonal Ab protected CD59-deficient mice from passive EAMG in the absence of CD59, the intrinsic regulator that protects self-cells against endogenous C5b-mediated injury and works to inhibit the MAC.[16]

Conventional Treatment Strategies and the Complexity of Treating Refractory Disease

Most patients with MG are successfully managed with a variety of agents. The acetylcholinesterase inhibitors, such as pyridostigmine, modulate neuromuscular transmission by prolonging ACh activity.[17] The corticosteroids provide general immunosuppression. Azathioprine is a purine analog that inhibits T-cell and B-cell production.[17] Mycophenolate mofetil inhibits guanosine nucleotide synthesis and selectively inhibits activated T-cells.[17] Cyclosporine and tacrolimus block T-cell activation and growth.[17] Immunomodulation, another current therapeutic strategy for MG management, includes thymectomy, plasma exchange, intravenous immunoglobulin (IVIg), and immunoadsorption.[17]

However, despite this largesse of therapies, an estimated 10% to 15% of patients with MG suffer from treatment-refractory disease.[18] A patient is deemed "refractory" if he or she: (a) fails to respond to otherwise adequate doses and durations of conventional immunosuppressive

treatments; (b) experiences intolerable side effects due to the treatments; (c) has comorbidities that preclude the use of conventional therapy; or (d) requires repeated rescue with short-term IVIg or plasma exchange treatments.[19] When patients are considered to be refractory, more aggressive treatment or treatment specifically directed to the underlying pathogenesis of the disease is warranted to prevent life-threatening crises, attempt restoration of strength, and improve quality of life.

Rituximab is an IgG1 kappa monoclonal Ab that depletes B-cells by binding to their CD20 molecule and initiating complement-dependent cytolysis or Ab-dependent cell-mediated cytotoxicity.[18] One study of rituximab in patients with refractory MG revealed a longer-lasting treatment effect of rituximab in MuSK Ab-positive patients and hypothesized that this may be due to differences in the pathophysiology of this form of the disease, which is largely mediated by the IgG4 immunoglobulin subclass.[20]

High-dose cyclophosphamide is another option in refractory MG. It is a pro-drug that is converted in the liver to intermediates that are ultimately transformed to a nitrogen mustard alkylating agent.[18] A study of patients with severe MG demonstrated that intravenous pulses of cyclophosphamide allowed reductions of systemic corticosteroid usage without muscle strength deterioration or drug-related adverse reactions.[21] Drachman and colleagues proposed "rebooting" the immune system with high-dose cyclophosphamide, which effectively eliminates the immune system while leaving the hematopoietic precursors intact.[19]

More information on current treatment standards from the Myasthenia Gravis Foundation of America can be found at www.myasthenia.org.

Not all patients will respond to these conventional treatment strategies, so alternative approaches must be considered for these refractory patients. Most conventional immunosuppresive treatment options for MG do not target complement. Since complement activation is integral in the pathophysiology of MG and destruction of the NMJ, it is of interest to consider complement as a potential target for the future. ■

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