Published in final edited form as:

Ann N Y Acad Sci. 2008 ; 1132: 220–224. doi:10.1196/annals.1405.037.

Extraocular Muscle Susceptibility to Myasthenia Gravis Unique Immunological Environment?

Jindrich Soltys, Bendi Gong, Henry J. Kaminski, Yuefang Zhou, and Linda L. Kusner
Department of Neurology and Psychiatry, Saint Louis University, St. Louis, Missouri, USA

Abstract

Extraocular muscle (EOM) is susceptible to neuromuscular junction disorders, in particular, myasthenia gravis (MG). While EOM physiological characteristics and the ocular motor system requirements contribute to the propensity of ocular motor deficits observed among patients with MG, the authors propose that EOM have immunological features that place the muscles at risk for immune attack. Genomic profiling studies have demonstrated that genes associated with the immune response are differentially expressed in EOM, with particular differences in both classical and alternative complement-mediated immune response pathways. Intrinsic complement regulators are expressed at lower levels at rodent EOM neuromuscular junctions, which would put them at risk for the complement-mediated injury that occurs in MG. In fact, systemic C inhibition in experimental autoimmune MG (EAMG) induced by administration of acetylcholine receptor (AChR) antibodies or immunization with AChR will eliminate complement deposition at junctions of other skeletal muscle, but not EOM. Also, EOM junctions have greater injury in active and passive EAMG by several measures, suggesting that the lack of complement inhibition puts the EOM at risk. Among ocular myasthenia patients, serum AChR antibody levels are low, which would support the concept that EOM junctions are more susceptible to antibody injury than are other junctions. These observations suggest that complement inhibitory therapies may prove to be particularly effective in treatment of ocular myasthenia.

Keywords

complement; myasthenia gravis; ocular muscles

Introduction

It is well appreciated that extraocular muscle (EOM) is susceptible to neuromuscular transmission disorders, regardless of whether transmission is compromised by an autoimmune disease, toxin, or genetic defect, which suggests that inherent physiological reasons form the basis for their involvement.^{1,2} While EOM physiological characteristics and the ocular motor system's requirements contribute to the propensity of ocular motor deficits observed among patients with myasthenia gravis (MG), there is mounting evidence that the EOM, and orbital tissue in general, have immunological features that place the muscles at risk for immune attack.^{3–8} This review will first briefly review role of complement in MG and experimental autoimmune MG (EAMG) and then provide support for why we think EOM susceptibility to MG is driven in part by a deficiency of intrinsic

Address for correspondence: Henry J. Kaminski, Department of Neurology & Psychiatry, Saint Louis University, 1438 South Grand Avenue, St. Louis, MO 63104. HKaminsk@slu.edu.

Conflicts of Interest

The authors declare no conflicts of interest.

complement-inhibitory proteins. With the advent of complement-inhibitor therapies coming to clinical use, the ocular manifestations of MG may be particularly useful for therapeutic evaluation.

Complement Mechanisms in the Pathogenesis of Experimental and Human Myasthenia Gravis

Complement serves to protect the host from pathogens by cell lysis of pathogens, opsonization with complement fragments, and attraction of inflammatory cells.⁹⁻¹⁰ Complement activation involves a series of about 30 plasma and membrane proteins participating in classical, alternative, and lectin pathways, and for MG, the classical pathway is relevant because of its activation by complement-fixing IgG antibodies that target the acetylcholine receptor (AChR) (Fig. 1). The activation by antibody ultimately leads to formation of the membrane attack complex (MAC), a multimeric protein complex that produces cell lysis and, in MG, produces focal damage of the postsynaptic surface of the muscle, which ultimately compromises neuromuscular transmission.¹¹

To protect host tissues from spontaneous autologous complement activation, host tissues possess a system of cell-associated and serum regulatory proteins.¹²⁻¹³ During spontaneous or antibody-initiated activation of complement, complement components condense on biological membranes; therefore, host cells have developed cell-associated complement regulators, which include decay-accelerating factor (DAF, or CD55), the membrane cofactor protein (MCP, or CD46), and the membrane inhibitor of reactive cell lysis (MIRL, or CD59).¹⁴ These proteins increase the decay of autologous C3 convertases that assemble on cell membranes; enhance cleavage of uncomplexed, autologous, cell-bound complement components¹⁵; and inhibit the assembly of MAC.¹⁶⁻²⁰ In contrast to humans, mice have a rodent-specific membrane regulator, termed Crry, which has activity similar to human MCP, but also has overlapping functions with DAF.²¹

Extensive data support the proposition that the classical complement cascade is the primary mechanism that mediates AChR loss at the neuromuscular junction (NMJ) in EAMG and MG:²² 1) Complement fragments, including MAC, are found at patient and EAMG animal post-synaptic surfaces.²³⁻²⁶ 2) Complement depletion or inhibition limits the severity of EAMG produced by AChR antibody or immunization with AChR.²⁷⁻³¹ 3) Mice deficient of C5 or C4 have less severe EAMG,³²⁻³³ while mice deficient in cell-surface complement inhibitors develop more weakness with EAMG.³¹⁻³⁴⁻³⁵

It is important to appreciate that human MG is more complicated than its animal models. Two other mechanisms may compromise neuromuscular transmission in human MG. AChR antibody may bind the AChR at the binding site for acetylcholine producing a block of activation. Such antibodies could be of profound clinical impact.³⁶ In EAMG, antibodies that bind the receptor at its ligand binding site lead to profound and rapid weakness.³⁷ Antibody may cross-link two AChR, resulting in accelerated endocytosis and degradation of the AChR.³⁸⁻³⁹ IgG from most MG patients has been shown to accelerate the degradation rate of the AChR *in vivo* and in cultured muscle cells.³⁸

In a series of experiments, we have investigated the importance of complement regulator proteins in EAMG. Mice deficient in DAF, when administered AChR antibodies, develop profound weakness, while wild-type mice show no obvious signs of weakness.³⁵ Additional studies confirmed the original report and demonstrated that DAF had greater role in “protection” of the NMJ from complement than did CD59.³⁴ Mice with a deficiency of both DAF and CD59 develop such severe weakness, even at reduced doses of AChR antibody administration, that euthanasia is required, while CD59-deficient mice develop much milder

weakness compared to the DAF-deficient mice. Morgan and colleagues have confirmed the protective effect of complement regulatory proteins in EAMG.³¹ The significance of complement regulators in EAMG produced by administrations of purified AChR has been evaluated in one investigation and an exacerbation of the disease was not evident in the CD59-deficient mice.⁴⁰

Complement Hypothesis for Extraocular Muscle Susceptibility to Myasthenia Gravis

Genomic profiling and serial analysis of gene expression demonstrated that EOM represents a distinct muscle allotype with differential expression of numerous genes, including those associated with the immune response.^{41–44} In particular, classical and alternative complement component genes are differentially expressed. EOM expresses higher levels of negative regulators of the alternative pathway of complement activation, complement factor H (CFH), and related protein (CFHR), while being deficient in DAF.⁶ The identification of low levels of DAF expression in EOM led to the hypothesis that EOM could be particularly susceptible to the complement-mediated injury produced by EAMG, and by extension MG.

It should be appreciated that complement regulatory proteins are concentrated at the NMJ of skeletal muscle,⁴⁵ but at lower levels in murine EOM compared to diaphragm NMJ.⁶ We produced EAMG with antibody directed against AChR and found that the complement regulatory gene expression was downregulated significantly and limited to no upregulation of complement regulators was observed at the NMJ of EOM at a time of expected maximal disease induction.⁶ These observations, coupled with studies of complement regulator-deficient mice, support the postulation that EOM NMJ are inherently more susceptible than other NMJ.

We have indirect data that support low levels of intrinsic complement inhibitors contributing to EOM susceptibility. Systemic complement inhibition in rodents with EAMG induced by AChR antibodies or immunization with AChR will eliminate complement deposition at junctions of skeletal muscle, but not at EOM.⁷ We treated mice with an antibody directed against the C5 component of complement and induced EAMG by use of antibody directed against the AChR (Fig. 2).³⁰ In the same animal, complement deposition is found at the EOM NMJ, but not at diaphragm junctions.³⁰ We also investigated complement deposition and NMJ damage in active EAMG and found greater complement component deposition at EOM NMJ than diaphragm NMJ and a greater degree of ultrastructural injury.

Thus far, there are no data in humans that support the “complement hypothesis” for differential involvement of EOM in human MG. Ocular myasthenia patients have low to absent serum levels of AChR antibody,^{46–48} and if human EOM junctions had low levels of complement inhibitory proteins, then one would expect them to be differentially injured by MG. However, if physiological reasons make the EOM more susceptible to neuromuscular transmission failure and, therefore, low levels of AChR antibodies could induce minimal injury, which still could lead to neuromuscular transmission failure that would not occur at other skeletal muscle junctions.⁸ Several autoimmune diseases, such as Graves ophthalmopathy and orbital myositis, specifically involve the tissues within the bony orbit.⁵ Such long-standing clinical observations suggest that there could be basic differences in the immunological properties of the cellular components of the orbit compared to other tissues.

Implications of Treatment of Myasthenia Gravis

If the complement hypothesis were correct, complement inhibitor treatment may be particularly beneficial for the ocular manifestations of human MG. Visual disability produced by MG can be difficult to treat. The initial treatment of corticosteroids has significant adverse effects.⁸ Antibody against the C5 component of complement (eculizumab) has proven to be safe and effective in chronic treatment of paroxysmal nocturnal hemoglobinuria⁴⁹ and could prove to be a particularly good agent for severe MG. Treatment trials of complement inhibitors for MG that evaluate ocular muscle function would provide a proof of concept for the complement hypothesis of EOM susceptibility.

Acknowledgments

Supported by National Institutes of Health Grants EY014837 and EY015306.

References

- Engel, A. The Myasthenic Syndromes. Oxford University Press; Oxford: 1999.
- Kaminski, HJ. Myasthenia Gravis and Related Disorders. Humana Press; Totowa, New Jersey: 2003.
- Smith TJ. Insights into the role of fibroblasts in human autoimmune diseases. Clin Exp Immunol. 2005; 141:388–397. [PubMed: 16045727]
- Prabhakar BS, Bahn RS, Smith TJ. Current perspective on the pathogenesis of Graves' disease and ophthalmopathy. Endocr Rev. 2003; 24:802–835. [PubMed: 14671007]
- Kaminski HJ, et al. Susceptibility of ocular tissues to autoimmune diseases. Ann NY Acad Sci. 2003; 998:362–374. [PubMed: 14592898]
- Kaminski HJ, et al. Complement regulators in extraocular muscle and experimental autoimmune myasthenia gravis. Exp Neurol. 2004; 189:333–342. [PubMed: 15380483]
- Kaminski, H., et al. The complement hypothesis to explain preferential involvement of extraocular muscle by myasthenia gravis. In: Leigh, R.; Devereaux, M., editors. Advances in Understanding Mechanisms and Treatment of Infantile Forms of Nystagmus. Oxford University Press; New York: 2008.
- Kusner LL, Puwanant A, Kaminski HJ. Ocular myasthenia: diagnosis, treatment, and pathogenesis. Neurologist. 2006; 12:231–239. [PubMed: 16990734]
- Kohl J. Self, non-self, and danger: a complementary view. Adv Exp Med Biol. 2006; 586:71–94. [PubMed: 16893066]
- Walport MJ. Complement. N Engl J Med. 2001; 344:1058–1066. [PubMed: 11287977]
- Conti-Fine BM, Milani M, Kaminski HJ. Myasthenia gravis: past, present, and future. J Clin Invest. 2006; 116:2843–2854. [PubMed: 17080188]
- Janeway, C., et al. Innate immunity. In: Janeway, C., et al., editors. Immunobiology The Immune System in Health and Disease. Garland Publishing; New York: 2001. p. 35-91.
- Miwa T, Song WC. Membrane complement regulatory proteins: insight from animal studies and relevance to human diseases. Int Immunopharmacol. 2001; 1:445–459. [PubMed: 11367529]
- Medof ME, Kinoshita T, Nussenzweig V. Inhibition of complement activation on the surface of cells after incorporation of decay-accelerating factor (DAF) into their membranes. J Exp Med. 1984; 160:1558–1578. [PubMed: 6238120]
- Seya T, Turner J, Atkinson J. Purification and characterization of a membrane protein (gp45–70) that is a cofactor for cleavage of C3b and C4b. J Exp Med. 1986; 163:837–855. [PubMed: 3950547]
- Davies A, et al. CD59, an LY-6-like protein expressed in human lymphoid cells, regulates the action of the complement membrane attack complex on homologous cells. J Exp Med. 1989; 170:637–654. [PubMed: 2475570]

17. Holguin MH, et al. Isolation and characterization of a membrane protein from normal human erythrocytes that inhibits reactive lysis of the erythrocytes of paroxysmal nocturnal hemoglobinuria. *J Clin Invest.* 1989; 84:7–17. [PubMed: 2738160]
18. Harada R, et al. Purification of 1F5 antigen that prevents complement attack on homologous cell membranes. *J Immunol.* 1990; 144:1823–1828. [PubMed: 2307842]
19. Medof ME, et al. Decay accelerating factor of complement is anchored to cells by a C-terminal glycolipid. *Biochemistry.* 1986; 25:6740–6747. [PubMed: 2432921]
20. Davitz M, Low M, Nussenzweig V. Release of decay-accelerating factor (DAF) from the cell membrane by phosphatidylinositol-specific phospholipase C (PI-PLC). *J Exp Med.* 1986; 163:1150–1161. [PubMed: 2422313]
21. Lin F, et al. Tissue distribution of products of the mouse decay-accelerating factor (DAF) genes. Exploitation of a Daf1 knock-out mouse and site-specific monoclonal antibodies. *Immunology.* 2001; 104:215–225. [PubMed: 11683962]
22. Kusner LL, Kaminski HJ, Soltys J. Effect of complement and its regulation on myasthenia gravis pathogenesis. *Expert Rev Clin Immunol.* 2008; 4:43–52. [PubMed: 20477586]
23. Strauss AJL, et al. Immunofluorescence demonstration of a muscle binding, complement fixing serum globulin fraction in myasthenia gravis. *Proc Soc Exp Biol.* 1960; 105:177–184. [PubMed: 13727889]
24. Sahashi K, et al. Ultrastructural localization of immune complexes (IgG and C3) at the end-plate in experimental autoimmune myasthenia gravis. *J Neuropathol Exp Neurol.* 1978; 37:212–223. [PubMed: 147324]
25. Sahashi K, et al. Ultrastructural localization of the terminal and lytic ninth complement component (C9) at the motor end-plate in myasthenia gravis. *J Neuropathol Exp Neurol.* 1980; 39:160–172. [PubMed: 7373347]
26. Nakano S, Engel AG. Myasthenia gravis: quantitative immunocytochemical analysis of inflammatory cells and detection of complement membrane attack complex at the end-plate in 30 patients. *Neurology.* 1993; 43:1167–1172. [PubMed: 8170563]
27. Lennon VA, et al. Role of complement in the pathogenesis of experimental autoimmune myasthenia gravis. *J Exp Med.* 1978; 147:973–983. [PubMed: 206648]
28. Biesecker G, Gomez CM. Inhibition of acute passive transfer experimental autoimmune myasthenia gravis with Fab antibody to complement C6. *J Immunol.* 1989; 142:2654–2659. [PubMed: 2703710]
29. Piddlesden SJ, et al. Soluble complement receptor 1 (sCR1) protects against experimental autoimmune myasthenia gravis. *J Neuroimmunol.* 1996; 71:173–177. [PubMed: 8982117]
30. Zhou Y, et al. Anti-c5 antibody treatment ameliorates weakness in experimentally acquired myasthenia gravis. *J Immunol.* 2007; 179:8562–8567. [PubMed: 18056404]
31. Morgan BP, et al. The membrane attack pathway of complement drives pathology in passively induced experimental autoimmune myasthenia gravis in mice. *Clin Exp Immunol.* 2006; 146:294–302. [PubMed: 17034582]
32. Christadoss P. C5 gene influences the development of murine myasthenia gravis. *J Immunol.* 1988; 140:2589–2592. [PubMed: 3356901]
33. Tuzun E, et al. Genetic evidence for involvement of classical complement pathway in induction of experimental autoimmune myasthenia gravis. *J Immunol.* 2003; 171:3847–3854. [PubMed: 14500686]
34. Kaminski HJ, et al. Deficiency of decay accelerating factor and CD59 leads to crisis in experimental myasthenia. *Exp Neurol.* 2006; 202:287–293. [PubMed: 16859686]
35. Lin F, et al. Enhanced susceptibility to experimental autoimmune myasthenia gravis in the absence of decay-accelerating factor protection. *J Clin Invest.* 2002; 110:1269–1274. [PubMed: 12417565]
36. Whiting PJ, Cooper J, Lindstrom JM. Antibodies in sera from patients with myasthenia gravis do not bind to nicotinic acetylcholine receptors from human brain. *J Neuroimmunol.* 1987; 16:205–213. [PubMed: 3624454]
37. Gomez CM, Richman DP. Anti-acetylcholine receptor antibodies directed against the alpha-bungarotoxin binding site induce a unique form of experimental myasthenia. *Proc Natl Acad Sci USA.* 1983; 80:4089–4093. [PubMed: 6575398]

38. Drachman DB, et al. Myasthenic antibodies cross-link acetylcholine receptors to accelerate degradation. *N Engl J Med.* 1978; 298:1116–1122. [PubMed: 643030]
39. Lindstrom J, Einarson B. Antigenic modulation and receptor loss in experimental autoimmune myasthenia gravis. *Muscle Nerve.* 1979; 2:173–179. [PubMed: 503104]
40. Tuzun E, et al. Complement regulator CD59 deficiency fails to augment susceptibility to actively induced experimental autoimmune myasthenia gravis. *J Neuroimmunol.* 2006; 181:29–33. [PubMed: 17056125]
41. Porter JD, et al. Extraocular muscle is defined by a fundamentally distinct gene expression profile. *Proc Natl Acad Sci USA.* 2001; 98:12062–12067. [PubMed: 11572940]
42. Cheng G, Porter JD. Transcriptional profile of rat extraocular muscle by serial analysis of gene expression. *Invest Ophthalmol Vis Sci.* 2002; 43:1048–1058. [PubMed: 11923246]
43. Fischer MD, et al. Expression profiling reveals metabolic and structural components of extraocular muscles. *Physiol Genom.* 2002; 9:71–84.
44. Fischer MD, et al. Definition of the unique human extraocular muscle allotype by expression profiling. *Physiol Genom.* 2005; 22:283–291.
45. Navenot JM, et al. Expression of CD59, a regulator of the membrane attack complex of complement, on human skeletal muscle fibers. *Muscle Nerve.* 1997; 20:92–96. [PubMed: 8995588]
46. Howard FJ, et al. Clinical correlations of antibodies that bind, block, or modulate human acetylcholine receptors in myasthenia gravis. *Ann NY Acad Sci.* 1987; 505:526–538. [PubMed: 3479935]
47. Limburg PC, et al. Anti-acetylcholine receptor antibodies in myasthenia gravis. I Relation to clinical parameters in 250 patients. *J Neurol Sci.* 1983; 58:357–370. [PubMed: 6842264]
48. Lennon V. Serologic profile of myasthenia gravis and distinction from Lambert-Eaton myasthenic syndrome. *Neurology.* 1997; 48(Suppl 5):S23–27.
49. Hillmen P, et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *N Engl J Med.* 2006; 355:1233–1243. [PubMed: 16990386]

FIGURE 1.

Complement activation can be initiated via three pathways—classical, alternative, and lectin. For MG, the classical pathway is most critical given its initiation by antigen–antibody complex. Activation of the lectin pathway is initiated by recognition of mannose-binding lectin (MBL) and ficolins which recognize mannose on bacteria. The alternative pathway is initiated by polysaccharides on microbial surfaces. Each pathway follows a unique course as shown in the figure. After the C5 step, the cascade is similar for all three pathways. The ultimate formation of the membrane attack complex (MAC) induces direct cell injury. All pathways are regulated by soluble (C1 inhibitor, C4bp, factor H, vitronectin, clasterin) and cell membrane–bound (CR1, DAF, MCP, CD59) proteins.

FIGURE 2.

Panels **A–C** are muscle sections from rats with EAMG produced by infusion of anti-AChR antibody, while Panels **D–F** are muscle sections from EAMG rats treated with anti-C5 antibody. Note the reduction in C9 deposition.