The two faces of MuSK antibody pathogenicity and their cause and consequences in myasthenia gravis

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Appendix

English summary

Myasthenia gravis (MG) is a neuromuscular autoimmune disease caused by antibodies against muscle-specific kinase (MuSK) in about 5% of MG patients. MuSK MG patients suffer from fatigable skeletal muscle weakness of mainly bulbar muscles and have a high risk of experiencing a respiratory crisis. MuSK is a critical protein in forming and actively maintaining the neuromuscular junction (NMJ). Disruption of its function by antibodies impairs neurotransmission at the NMJ. MuSK antibodies are predominantly of the IgG4 subclass and therefore have the ability to swap half-molecules with other IgG4s (Fab-arm exchange), resulting in monovalent-antigen binding. MuSK antibodies have different characteristics depending on their (species-specific) subclass or isotype. **Chapter 1** describes the lessons we learned from prior MuSK antibody research by placing these different antibody characteristics in the appropriate context, thus helping us to understand the mechanisms underlying MuSK MG and the importance of understanding the source of antibodies that was used.

One of the hypotheses regarding the cause of the IgG4 response against MuSK is addressed in **chapter 2** by investigating the serum immunoglobulin composition in MuSK MG patients (IgG4 autoimmune disease) compared to acetylcholine receptor (AChR) MG patients (IgG1/3 autoimmune disease) and healthy donors. This study shows serum IgG4 is enriched in MuSK MG patients compared to the other groups, but for most MuSK MG patients their serum IgG4 titers fall within the normal range. MuSK MG patients do not seem to have a general predisposition to generate IgG4 responses. The predominance of IgG4 MuSK antibodies thus has a different cause.

Monoclonal antibodies are great tools to systematically investigate the consequence of specific antibody characteristics. **Chapter 3** describes the isolation of monoclonal MuSK antibodies from MuSK MG patients. Six B-cell receptor sequences were determined from single MuSK-reactive B-cell cultures and produced recombinantly as monoclonal antibodies. Monovalent anti-MuSK Fab fragments are antagonists of MuSK signaling, recapitulating the effects of MuSK MG patient-purified IgG4. In contrast, bivalent anti-MuSK IgG are agonists of MuSK signaling, likely by forcing the dimerization of two MuSK molecules independent of agrin. Valency of these monoclonal MuSK antibodies thus dictates their consequences on *in vitro* MuSK signaling.

Chapter 4 translates the valency-dependent *in vitro* effects of MuSK antibodies to *in vivo* pathogenicity. Upon passive transfer, stable monoclonal monovalent MuSK antibodies cause rapid onset fatigable muscle weakness characterized by complete loss of muscle strength, weight loss and postsynaptic loss of AChR receptors, independent of antibody clone. In contrast, the pathogenicity of the bivalent equivalents was clone-dependent and less severe: one clone was not pathogenic, while the other clone caused slower onset muscle weakness and weight loss. These differential effects are likely caused by the opposing valency-dependent effects on MuSK signaling described in chapter 3. Functional monovalency thus amplifies the pathogenicity of monoclonal MuSK antibodies, indicating that class-switch to IgG4 MuSK antibodies may be a critical step in developing MuSK MG symptoms.

To further understand the pathomechanisms underlying MuSK MG, **chapter 5** explores the consequences of MuSK antibodies with different functional characteristics on the NMJ. Antibody valency influenced the consequences on agrin-induced MuSK activation, Dok7 binding to MuSK and NMJ gene expression. In addition, monovalent MuSK antibodies binding the frizzled domain of MuSK did not inhibit agrin-induced MuSK activation, while Ig-like 1 domain binders do. Bivalent MuSK antibodies affected the kinetics of Dok7 degradation differently, possibly depending on binding epitope between and within structural domains of MuSK. The consequences and pathogenicity of MuSK antibodies thus at least depend on a combination of valency and epitope. The pathophysiology underlying MuSK MG in individual patients thus likely depends on the unique composition of their MuSK antibody pool.

To broaden the view on potential consequences of antibodies in MG beyond the NMJ, **chapter 6** gives a comprehensive overview of the timing and localization of gene expression of MG-related genes. This *in silico* analysis using existing expression databases revealed that MuSK and seven other genes affected in (congenital) myasthenia gravis are expressed in different locations outside skeletal muscles. Since the co-expression of these genes in these locations is limited, it is unlikely they converge on the same functional pathway as in skeletal muscles. However, these locations are at risk for non-muscular (side-)effects of autoantibodies and new therapeutics targeting these proteins.