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Introducing Autoimmunity at the Synapse by a Novel Animal Model of Experimental Autoimmune Myasthenia Gravis

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Abstract—The neuromuscular junction (NMJ) is a peripheral synapse between motor neurons and skeletal muscle fibers that controls muscle contraction. The NMJ is the target of various disorders including myasthenia gravis (MG), an autoimmune disease in which auto-antibodies (auto-Abs) attack the synapse, and thus cause muscle weakness in patients. There are multiple auto-Abs in the MG patient sera, but not all the Abs are proven to be pathogenic, which increases the difficulties in clinical diagnoses and treatments. To establish the causative roles of auto-Abs in MG pathogenesis, the experimental autoimmune MG (EAMG) induced by the active immunization of auto-antigens (auto-Ags) or the passive transfer of auto-Abs is required. These models simulate many features of the human disease. To date, there are three kinds of EAMG models reported, of which AChR-EAMG and MuSK-EAMG are well characterized, while the recent LRP4-EAMG is much less studied. Here, we report a current summary of LRP4-EAMG and its pathogenic mechanisms. The features of LRP4-EAMG are more similar to those of AChR-EAMG, indicating a similar clinical treatment for LRP4- and AChR-positive MG patients, compared to MuSK-positive MG patients.

Key words: neuromuscular junction, synapse, myasthenia gravis, experimental autoimmune myasthenia gravis, LRP4.

Contents

INTRODUCTION

The NMJ is a peripheral synapse between motoneurons and skeletal muscle fibers that controls muscle contraction (Sanes and Lichtman, 2001; Wu et al., 2010; Shen et al., 2015). The presynaptic release of the neurotransmitter acetylcholine (ACh) activates ACh receptors (AChR) on muscle fibers. The assembly of high-density postsynaptic AChR cluster (approximately 12,000 molecules per μm^2) is regulated by the agrin-LRP4-MuSK complex (Matthews-Bellinger and Salpeter, 1983; Shen et al., 2015). Neuronal agrin from motor nerve terminals binds to its co-receptor LRP4 and activates the tyrosine kinase receptor MuSK (Weatherbee et al., 2006; Kim et al., 2008; Zhang et al., 2008). Activation of the latter triggers a cascade of events that culminate in the clustering of AChRs. The disruption of agrin signaling causes neuromuscular disorders including myasthenia gravis (MG).

MG is the most common NMJ disorder, affecting 40–800 per million people with an incidence of 4–12 per million people annually (Conti-Fine et al., 2006; Gilhus and Verschuuren, 2015; Gilhus et al., 2016). MG patients exhibit characteristic fatiguing weakness of voluntary ocular, bulbar, and limb muscles. In more severe cases, patients develop weight loss, dysarthria, dysphagia, and

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Abbreviations: Abs, antibodies; ACh, acetylcholine; AChR, ACh receptors; ALS, amyotrophic lateral sclerosis; auto-Abs, auto-antibodies; CMAPs, compound muscle action potentials; EAMG, experimental autoimmune MG; ECD, extracellular domain; ENU, N-ethyl-N-nitrosourea; Ig, immunoglobulin; mEPPs, miniature end-plate potentials; MG, myasthenia gravis; NMJ, neuromuscular junction.

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even respiratory difficulties (Gilhus et al., 2016). The name "myasthenia gravis" was created by fusing the Greek terms for muscle weakness (myasthenia) and severe (gravis) (Keesey, 2002). MG is an autoimmune disease caused by the association of pathogenic antibodies (Abs) against postsynaptic membrane proteins at NMJs. Thus, MG meets these criteria of an autoimmune disorder: (1) Abs are present at the NMJ; (2) immunization of animals with auto-antigens (auto-Ags) reproduces the disease symptoms; (3) auto-Abs immune globulin (Ig) from patients or experimental animals causes MG symptoms when transferred to naive animals; and (4) removing Abs by plasma exchange alleviates the symptoms (Conti-Fine et al., 2006; Gilhus et al., 2016). AChR auto-Abs can be detected in ~80% of MG patients, and MuSK Abs are seen in ~8% (Gilhus and Verschuuren, 2015; Gilhus et al., 2016). The remaining ~12% are double-seronegative (anti-AChR or anti-MuSK negative), which is difficult to diagnose in the clinic.

LRP4 is a member of the low-density lipoprotein receptor family. Its function in the NMJ was first discovered accidentally by Weatherbee et al., who performed an N-ethyl-N-nitrosourea (ENU) mutagenesis screen in mice for a bone study (Weatherbee et al., 2006). The homogenous mutants ($LRP4^{mitt}$ and $LRP4^{mte}$ mice) died at birth with similar NMJ deficits as in the MuSK knockout mice (DeChiara et al., 1996; Weatherbee et al., 2006). The idea that LRP4 acted as the missing link between agrin and MuSK was further proven by Mei and Burden's lab (Kim et al., 2008; Zhang et al., 2008). LRP4 interacts with agrin as well as MuSK (Kim et al., 2008; Zhang et al., 2008; Zong et al., 2012) to stimulate AChR clustering in cultured muscle cells (Kim et al., 2008; Zhang et al., 2008) and promote NMJ formation and maintenance in vivo (Wu et al., 2012; Barik et al., 2014) (Fig. 1).

Considering its critical role in the NMJ (Kim et al., 2008; Zhang et al., 2008; Wu et al., 2012; Barik et al., 2014), its large extracellular domain, and the interaction with MuSK, LRP4 was proposed to be the ideal auto-Ags in double-seronegative patients (Shen et al., 2015). Indeed, in 2011, three groups independently identified LRP4 auto-Abs in double-seronegative MG patients (Higuchi et al., 2011; Pevzner et al., 2011; Zhang et al., 2011a). The occurrence of LRP4 + MG varies by different ethnicity and country of origin (Table 1) (Higuchi et al., 2011; Pevzner et al., 2011; Zhang et al., 2011a; Cossins et al., 2012; Zouvelou et al., 2013; Zisimopoulou et al., 2014; Marino et al., 2015; Hong et al., 2017; Li et al., 2017). It seems to be more prevalent in Western populations (~17%) than in Asian populations (~3.5%). A critical issue is whether the auto-Abs are pathogenic. Notice that in addition to AChR, MuSK, and LRP4 Abs, there are approximately 10 more Abs identified in MG patients, such as Abs against agrin (Gasperi et al., 2014; Zhang et al., 2014; Cordts et al., 2017), titin (Aarli, 2001), cortactin (Gallardo et al., 2014; Cortes-Vicente et al., 2016), myosin (Mohan et al., 1994), fast troponin (Mohan et al., 1994), ryanodine receptor (Baggi et al., 1998; Shelton et al., 2001), and myofibrillary proteins (Romi et al., 2005). However, not all of these Abs

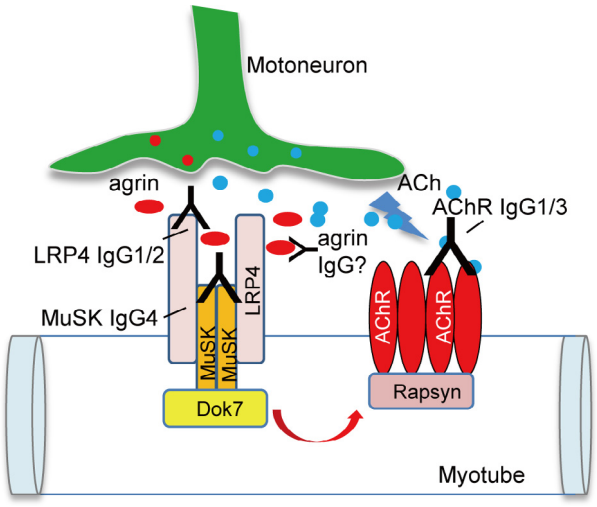

Fig. 1. Autoantibodies targeting agrin signaling in the NMJ. Auto-Abs against AChR, MuSK, and LRP4 induce MG-like symptoms in antigen active immunization or antibody passive transfer MG models.

are pathogenic and directly trigger NMJ pathology. To definitively prove its causative role in the disease progress, and not a mere association between the Ab and the disease, the establishment of an EAMG model is necessary. We will summarize the three EAMG models (AChR-EAMG, MuSK-EAMG, LRP4-EAMG) (Fig. 1), especially the LRP4-EAMG (Table 2), with the hopes of a better understanding of pathogenic mechanisms and potential distinct treatment.

AChR-EAMG

AChR-EAMG can be induced in many mammals including guinea pigs, rats, and mice (Conti-Fine et al., 2006). In 1973, the first AChR-EAMG model was established by Patrick and Lindstrom, which halted the fierce debate about the pathogenesis of MG through the 1960s (Patrick and Lindstrom, 1973). They immunized rabbits with AChR proteins from Electrophorus electricus emulsified in complete Freund's adjuvant, which developed MG-like symptoms with flaccid paralysis and impaired electromyographs. Edrophonium or neostigmine effectively reduced paralysis and fatigue in the immunized rabbits (Patrick and Lindstrom, 1973). Many subsequent studies demonstrated that anti-AChR impaired the NMJ structure and function. These findings promote the application of immunosuppressants for treating MG in the clinic.

To conclusively prove antibodies are pathogenic, passively transfer of auto-Abs is the most direct evidence, as it bypasses the possible interference of inflammation from Ag boosting. In 1975, it was first reported that features of MG could be passively transferred into experimental animals (Toyka et al., 1975). Female BDF11 mice were intraperitoneally injected daily with immunoglobulin (Ig) from an MG patient, followed by a single injection of cyclophosphamide to suppress active immune responses to the human protein (Toyka et al., 1975). Acute fatiguing weakness developed 1–3 weeks after the first auto-Ab injec-