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Antibodies to Low Density Lipoprotein Receptor-Related Protein 4 in Seronegative Myasthenia Gravis

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Myasthenia gravis (MG) is a disorder of the neuromuscular junction (NMJ) characterized by muscle weakness that worsens with effort and improves with rest. The electrodiagnostic correlate is decrementing compound muscle action potential amplitudes to slow rates of repetitive nerve stimulation. Although rare forms of myasthenia result from heritable mutations in individual proteins comprising the NMJ, the congenital myasthenic syndromes,¹ the vast majority of cases of MG are autoimmune in origin. For 90% of patients with generalized autoimmune MG (ie, involving more than the ocular muscles), the disorder results from circulating autoantibodies to the nicotinic acetylcholine receptor (AChR), which is the NMJ postsynaptic neurotransmitter receptor. The remaining 10% of patients have been referred to as having seronegative (autoimmune) MG. About a decade ago, Hoch and coworkers² identified antibodies to a second postsynaptic NMJ membrane protein, muscle-specific kinase (MuSK) present in about 40% of seronegative patients. Subsequent studies^{3–6} in rodents have provided data supporting a pathogenic role for MuSK antibodies in this subgroup of patients. It is the remaining 60% of seronegative patients (now referred to as patients with double-seronegative MG) who are the subject of the study of antibodies to low density lipoprotein receptor-related protein 4 (LRP4) by Zhang et al⁷ published in this issue of the *Archives*.

Unlike AChR, the autoimmune targets in seronegative MG (MuSK and LRP4) are not directly involved in synaptic transmission across the NMJ. Rather, both proteins play a major role in the development of this synapse. The NMJ synapse begins to form when an axon growth cone of a developing motor neuron encounters a developing myotube and begins to secrete agrin, a glycoprotein with a laminin-binding domain that anchors it to the extracellular matrix. The secreted agrin induces dense clustering of the AChRs in the postsynaptic end-plate membrane; prior to this step, the AChRs are diffusely dispersed throughout the surface of the developing myotube. The clustering of AChRs is the crucial step in the elaboration of the complex structure of the mature NMJ, including the pretzel-like topographic profile of the end-plate membrane and its marked folding and specialization at the ultrastructural level, along with anchoring and specialization of the presynaptic motor nerve terminal.

It has been known for nearly 2 decades that the agrin-induced AChR clustering and the subsequent elaboration of the mature NMJ require the presence of MuSK. However, extensive work failed to demonstrate direct binding of agrin to MuSK, leading to the postulation of a third compound (referred to as MASC, the myotube-associated specificity component) involved in the interaction.⁸ What followed was a decade-long search for this

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holy grail of NMJ developmental biology, culminating in the discovery by 2 independent groups,^{9,10} one of which included Dr Zhang and his coauthors, of the role of LRP4 in agrin and MuSK binding and subsequent NMJ formation.

Zhang et al¹⁰ have now carried out what might be considered the next logical step in the analysis of double-seronegative MG, a search for autoantibodies to LRP4. They studied serum samples from 217 well-defined patients with MG from 2 large MG clinical centers, one in Greece and one in the United States, along with appropriate control serum samples, and they identified LRP4 antibodies in 9.2% of 120 double-seronegative patients compared with 1 of 36 patients with MuSK antibodies and 0 of 61 patients with AChR antibodies. Their results differ from 2 recently published studies^{11,12} of seronegative patients with MG. One of these studies, which involved 300 patients from Japan who tested negative for AChR antibodies, found that 3% of these patients had antibodies to LRP4.¹¹ (However, one-third of these patients who tested positive for antibodies to LRP4 were also positive for MuSK antibodies.) In the other study¹² of a much smaller number of double-seronegative patients from Germany, 8 of 15 patients had serum samples that tested positive. Because each study used different LRP4 antibody assays and likely had different levels of precision in the diagnosis of MG, the differences in the results between the 3 studies^{11,12} might be purely technical rather than related to differing genetic or environmental factors in the 3 populations.

These observations define a new subgroup of patients with MG and beg the question of whether the LRP4 antibodies are the pathogenic agents in these patients or whether they are simply biological markers for the disease. For AChR antibodies and, more recently, MuSK antibodies,^{3–6} the pathogenic potential of the antibodies has been confirmed by the use of animal models in which the antibodies, induced by either active immunization or passive immunization, produce experimental MG.

In the case of the MuSK antibodies in patients and in the animal models, and perhaps similarly for LRP4 antibodies, the attack is upon the mature NMJ. In this structure, both MuSK and LRP4 are present, but very little is understood concerning their function in the mature synapse, in contrast with their crucial roles in the developing synapse. But, at least for MuSK, observations in human and animal disease provide evidence supporting the hypothesis that this protein also has an important role to play in the function of the adult synapse. These observations in seronegative MG demonstrate once more the usefulness of the study of spontaneous (human) diseases in advancing our knowledge of basic neuroscience.

The collection of information on LRP4 antibody-positive MG has to be considered in its infancy. In comparison, our knowledge of MuSK antibody-positive MG, now 10 years after the identification of MuSK antibodies in patients with seronegative MG, includes the results of clinical studies indicating that the disease differs considerably from AChR antibody-positive MG, especially in the relatively focal muscle involvement, in the frequent occurrence of wasting in the affected muscles, and in the absence of thymic abnormalities. Also, as already noted, the development of animal models of MuSK antibody-positive MG has demonstrated the pathogenic role of MuSK antibodies in the human disease. We await similar studies of this exciting new form of MG.

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