MYASTHENIA GRAVIS

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SUMMARY

Myasthenia gravis (MG) is an autoimmune condition of the neuromuscular junction characterised by fatiguable muscle weakness of the limbs, bulbar and ocular muscles. It is associated with the presence of nicotinic acetylcholine receptor antibodies, which act at the postsynaptic plate of the neuromuscular junction, interfering with normal synaptic function. About 15–20% of patients may have all the characteristic features without detectable antibodies in the serum, often causing some concern about the validity of the diagnosis. Because patients with seronegative myasthenia gravis (SNMG) respond to immunotherapy and have very similar clinical and pathological features to seropositive disease, it is suspected that this condition is also mediated by antibodies. Recent studies support this hypothesis, demonstrating antibodies to muscle specific receptor tyrosine kinase (MuSK) in 70% of seronegative patients. This protein is present at the postsynaptic membrane where it interacts with other proteins and growth factors which maintain the architecture of the neuromuscular junction. It is now possible to obtain a serological diagnosis in 95% of patients with myasthenia. However, further studies are required to elucidate the clinical characteristics and pathogenesis of MuSK-positive myasthenia gravis.

SEROPOSITIVE MYASTHENIA GRAVIS

Myasthenia gravis was probably first described by the physiologist Thomas Willis, who in 1672 wrote about 'a woman who temporarily lost her power of speech and became as mute as a fish'. The hallmarks of MG are those of muscular weakness and fatiguability. It has a generalised distribution in about 85% of patients and in the rest remains localised to the extraocular muscles. The generalised pattern will tend to affect the limbs in a proximal distribution but may also affect the diaphragm and the neck extensors.[1] The clinical severity of MG has been graded functionally and regionally by the Osserman scale: grade I involves focal disease (restricted to ocular muscles), grade II is generalised disease with mild (IIa) or moderate (IIb) sub varieties; grade III is severe generalised disease and grade IV represents a crisis with life-threatening impairment of respiration. However, the scale is not dynamic and often fails to detect clinically relevant changes that may be important in evaluating disability and in monitoring the effects of therapy. Electro-physiologically, the findings in MG are characterised by a 15% decrementing amplitude of the evoked muscle response to low frequency repetitive stimulation.[1]

The annual incidence of MG varies between 0·25 to 2·00 per 100,000 of the population. The frequency is bimodal in the younger population (below the age of 40), being biased towards female patients; the older population with MG (above the age of 60) is represented by male patients predominantly.

Simpson was the first to propose that MG might be an autoimmune condition, and it is now clear that MG is immune-mediated.[2] Toyka et al. showed that a circulating factor from serum of patients with MG could be injected and passively transferred into mice, reproducing the features of the disease in the animal model.[3] The mice showed reduced amplitudes of miniature endplate potentials and reduced numbers of acetylcholine receptors (AChRs) at the neuromuscular junctions. Both B- and T-lymphocyte reactivity play a role in the pathogenesis of MG. Antibodies are directed against the nicotinic AChR with deposition of immune complexes at the postsynaptic part of the neuromuscular junction. The antibodies induce accelerated degradation as well as the functional blockade of AChRs of skeletal muscle corresponding to a certain degree to the severity of MG.[4] Reduction of serum anti-AChR by plasma exchange was shown to result in a progressive improvement in strength, and when the antibody titres remained depressed the improvement was sustained.[5] Buckley et al. showed how in MG patients with thymoma the neoplastic tissue may be exporting mature long-lived T-cells which, in turn, may have an important role in supporting antibody production in the periphery.[6]

Different immunogenetic backgrounds are associated with the different forms of MG, and this is reflected by the various HLA linkages described. A strong and positive association exists between seropositive MG (SPMG) with antigens B8 and DR3, especially in female patients.[7] Different HLA associations are reported in patients with thymic hyperplasia when compared with patients with thymoma.[8] Further to this, there are different HLA associations in patients with generalised MG versus those presenting with ocular involvement only.[9] The HLA associations in SNMG (discussed in depth below) are not found to differ significantly to those in SPMG.[10]

The standard test for antibodies to AChRs is based on immunoprecipitation of ^{125}I-α-Bungarotoxin-labelled AChR extracted from human muscle cell lines.[5] Antibodies to the nicotinic AChR antibody are detected in approximately 80% of cases with generalised MG

(seropositive MG or SPMG). In patients with pure ocular symptomatology, the antibodies are detected in 50% of cases.[1] Acetylcholinesterase inhibitors have been the mainstay of treatment since 1934 and patients are typically treated with pyridostigmine. However, this has a short half life and some patients fail to respond, so in the past two decades immunotherapies have been more widely adopted. Steroid therapy may produce remission but may also be associated with temporary relapse, so they are best given in hospital. The long-term complications of steroids are well known and it is important to aim for alternate day regimens and offer adequate protection against osteoporosis. Azathioprine has been shown to produce a 'steroid-sparing' effect by inducing remission. Some refractory patients may require intravenous immunoglobulin treatment or plasma exchange. Thymic hyperplasia is observed in 70–80% of patients and is associated with the presence of AChR antibody.[11] The goal of thymectomy is to induce remission in MG or an improvement such that immunosuppressive medication can be reduced.[1] Approximately 15% of myasthenia patients will have a thymoma. Surgical thymectomy in this group of patients will help to prevent spread of tumour. Expert opinion argues against surgery in patients over the age of 45 because the thymus is typically atrophic by this age.

SERONEGATIVE MYASTHENIA GRAVIS
Clinical features
Approximately 15–20% of patients with symptoms and signs of generalised MG will not have detectable antibodies to the AChR and are therefore classed as SNMG. It is a small, yet distinct and heterogenous group with clinical and electrophysiological features which are indistinguishable from those seen in SPMG.[12] Patients respond to the same therapeutic measures, but SNMG may be more difficult and challenging to treat. Physicians may be less confident about the role of potentially toxic long-term immunotherapies. Moreover, the thymus is often normal and hence thymectomy is often inappropriate in this subgroup of patients.[13, 14]

Immune mechanisms in SNMG
That SNMG is also immune-mediated was demonstrated early by the response that these patients showed to plasma exchange and immunosuppressive treatment.[13] Burges et al. also provided support for the hypothesis that SNMG was immune-mediated when they showed that passive transfer of serum from SNMG patients into mice caused defects in neuromuscular transmission.[15] Mossman et al. demonstrated that immunoglobulins from these patients caused a small but significant loss of endplate AChR from the muscle diaphragm, but this was insufficient to explain the decrement in twitch amplitude observed in neuromuscular transmission.[16] They concluded that these patients had an antibody that bound to determinants other than AChR and that also caused impairment of neurotransmission. Drachman et al.

observed that the numbers of the AChR were reduced in SNMG muscle.[17] They also showed that the motor endplate potential amplitudes and ^{125}I-α-Bungarotoxin binding was reduced in mice injected passively with immunoglobulin from SNMG patients, while other mice were shown to have increased sensitivity of neuromuscular transmission to D-tubocurarine without altering ^{125}I-α-Bungarotoxin binding. At the cellular level, SNMG behaves in a similar fashion to SPMG. T-cell responses are no different to those observed in seropositive patients in their ability to bind to acetylcholine-derived myasthogenic peptides in vitro.[18] Self-reactive antibody repertoires towards thymus antigens are similar in patients with SPMG and SNMG, thus suggesting that the two share common immunopathological features.[19] These early clinical and pathological observations pointed to indications that SNMG was another immune-mediated entity, but the site of action was still to be discovered.

The discovery of antibodies to MuSK
The first clues to the pathogenesis of SNMG were provided by Blaes et al. This group observed that the antibodies in SNMG sera were binding to a distinct antigen expressed on the muscle derived TE671 cells,[20] and this indicated that the target for SNMG antibodies was different to that in SPMG. This group also found that injection of SNMG sera into mice did not reduce the numbers of AChR, but increased the sensitivity of neuromuscular transmission to D-tubocurarine and reduced the amplitudes of MEPPs. Therefore, although the numbers were unaltered, the function of the receptor was affected. Sera and non-IgG fractions from SNMG patients reduced AChR function in TE671 cells without affecting AChR number, and the reduction in acetylcholine-induced currents was partly dependent on intracellular calcium. There was also the suggestion that inhibition of function in SNMG was due to AChR desensitisation secondary to an intra-cellular signalling mechanism that lead to AChR phosphorylation.[21, 22]

Last year investigators at the Max Planck Institute, Germany and the Institute of Molecular Medicine, Oxford showed that the optical density values for IgG binding to human TE671 cell lines corresponded to a MuSK, thus indicating that the previously identified cell surface antigen on these cell lines might be MuSK.[23] Muscle specific kinase is a receptor tyrosine kinase selectively expressed in skeletal muscle and localised at the neuromuscular junction (see Figure 1). Phosphorylation of MuSK leads to recruitment of a phophotyrosine binding domain containing protein that stimulates phophorylation and clustering of AChRs. Muscle specific kinase is expressed at low levels in proliferating myoblasts. In the embryo, it is expressed specifically in the early myotomes and developing muscle. Muscle specific kinase is then dramatically downregulated in mature muscle, where it remains prominent at the neuromuscular junction. Expression of MuSK is induced throughout the adult
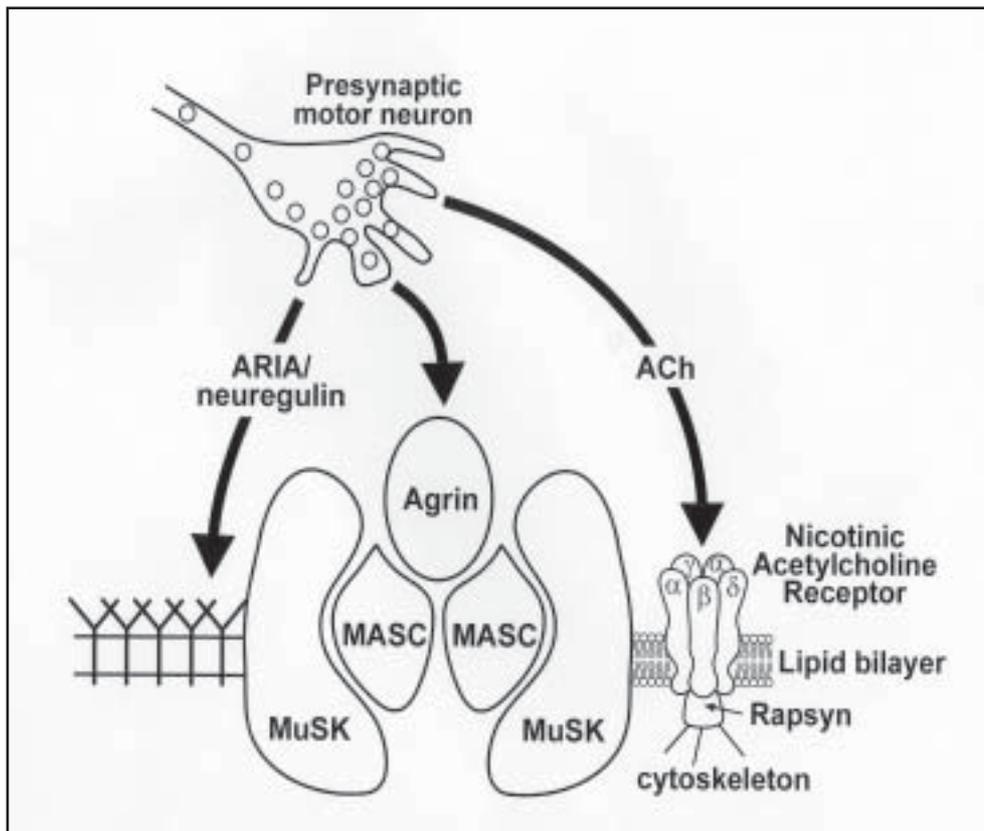
FIGURE 1
A representative cartoon of the neuromuscular junction (see text).

MuSK interacts with agrin, a nerve-derived protein, via MASC (Myotube- Associated-Specificity Component). This interaction activates a clustering process of the AChRs, a process that requires phosphorylation and dimerisation of MuSK. MuSK also interacts with Rapsyn at the junction and together they provide a scaffolding effect, which is important in the maintenance of the architecture as a whole. Muscle specific kinase will also interact with the dystrophin-utrophin glycoprotein complex at the same site (not shown here). Neuregulin (or ARIA) is an extracellular signal, concentrated at synaptic sites, that activates synapse-specific transcription, in turn activating AChR gene expression in cultured muscle cells.

myofibre after denervation, block of electrical activity or physical immobilisation. In humans, MuSK maps to chromosome 9q31·3–32 which overlaps with the region reported to contain the Fukuyama muscular dystrophy mutation.[24]

Data from Hoch et al.[23] indicate that the antibodies to MuSK are directed against the extracellular N-terminal domain of MuSK. These authors also reported that these antibodies had an inhibitory effect on agrin-induced clustering of AChRs – an interaction that depends on the N-terminal domain of MuSK. It was postulated that antibodies bind to MuSK in a manner that prevents its interaction with MASC (Myotube–associated specificity component, a hypothetical agrin-binding component), thus interfering with the agrin/MuSK/AChR clustering pathway in myotubes with the potential to alter MuSK function at the neuromuscular junction. A limited number of biopsies indicate that antibodies to MuSK not only interfere with MuSK function but also alter the numbers and distribution of AChRs. The binding of IgG to MuSK at the postsynaptic membrane activates complement and,

in fact, reports confirm the presence of complement deposition at the motor endplates of SNMG patients.[23]

MuSK in the maintenance of the architecture of the neuromuscular junction

To understand the role of MuSK, one must grasp the concepts of the stages involved in pre- and postsynaptic differentiation.[25] Developing muscle fibres undergo a complex differentiation program, and signals from the muscle regulate differentiation of the presynaptic terminal. Two signalling pathways are involved in mediating postsynaptic differentiation. The signal for one pathway is agrin, a synaptic basal lamina protein which redistributes AChRs to synaptic sites. The signal for the other pathway is also associated with the synaptic basal lamina but stimulates expression of the AChR genes in myofibre nuclei near the synaptic site. Formation of the neuromuscular junction requires a series of reciprocal inductive interactions between the motor neuron and the muscle cell that culminate in the precise juxtaposition of a highly specialised presynaptic nerve terminal with a complex postsynaptic endplate on the muscle surface.

This is only possible when the interactions between agrin and MuSK come into play.[25, 26] Agrin is a nerve-derived protein and is synthesised by motor neurons and deposited in the extracellular matrix of the neuromuscular junction. Muscle specific kinase is a component of the agrin-receptor complex and mice lacking agrin or MuSK lack neuromuscular synapses. Muscle specific kinase activation signals cascades that are important in synapse formation, including organisation of the postsynaptic membrane, synapse-specific transcription and presynaptic differentiation.[26] It appears that it has a role in the maintenance of the architecture of the postsynaptic membrane and a scaffolding effect whereby agrin induces MuSK to activate AChR clustering through a synapse-specific cytoplasmic protein rapsyn.[27] Rapsyn-MuSK interactions are mediated by the ectodomain of MuSK. Rapsyn is necessary not only for its structural role but is involved in MuSK-signaling AChR phosphorylation. This requires the ectodomain of MuSK. Dimerisation of MuSK, induced by agrin, also has an important scaffolding effect for other postsynaptic proteins, namely the dystrophin/utrophin glycoprotein complex. The MuSK ectodomain is not only responsible in mediating ligand binding and receptor dimerisation, but also recruits neuromuscular junction components to a MuSK-based scaffold.

WHAT DOES THE FUTURE HOLD?

The discovery of antibodies to MuSK is important in helping to define this group of SNMG even further and substantially helps in the diagnosis and clinical management of these patients. Using simple ELISA techniques it should now be possible to detect AChR antibodies in 95% of patients who have clinical evidence of MG. However, many questions remain. Our knowledge of the complex nature of the architecture and electrophysiology of the neuromuscular junction continues to expand, and we now have clear evidence that MuSK is a target antigen for immune-mediated myasthenia. However, further laboratory research is required to characterise the exact function of this extracellular receptor protein, its interactions with other proteins and growth factors and the precise mechanisms of pathogenesis. In the clinic, the availiability of this test will also allow us to define the clinical and epidemiological characteristics of MG more precisely. We will now also be able to define the prognosis of MuSK-positive MG more clearly. Finally, it should be possible to design trials for this group of patients, who often prove to be somewhat more difficult and challenging to treat.

REFERENCES

1   Drachman DB. Myasthenia gravis. *N Engl J Med* 1994; **330:**1797-810.
2   Simpson JA. Myasthenia gravis: a new hypothesis. *Scott Med J* 1960; **5:**419-39.
3   Toyka KV, Brachman DB, Pestronk A *et al.* Myasthenia gravis: passive Transfer from man to mouse. *Science* 1975; **190:**397-9.
4   Drachman DB, Adams RN, Josifek LF *et al.* Functional activities of autoantibodies to acetylcholine receptors and the clinical severity of myasthenia gravis. *N Engl J Med* 1982; **307:**769-74.
5   Newsom-Davis J, Pinching AJ, Vincent A *et al.* Function of circulating antibody to acetylcholine receptor in myasthenia gravis: investigation by plasma exchange. *Neurology* 1978; **28:**266-72.
6   Buckley C, Douek D, Newsom-Davis J *et al.* Mature, long-lived CD4+ and CD8+ T cells are generated by the thymoma in myasthenia gravis. *Ann Neurol* 2001; **50(1):**64-72.
7   Matej H, Nowakowska B, Kalamarz M *et al.* HLA antigens and susceptibility to myasthenia gravis. *Arch Immunol Ther Exp (Warsz)* 1987; **35:**795-801.
8   Spurkland A, Gilhus NE, Ronningen KS *et al.* Myasthenia gravis patients with thymus hyperplasia and myasthenia gravis patients with thymoma display different HLA associations. *Tissue Antigens* 1991; **37:**90-3.
9   Kida K, Hayashi M, Yamada I *et al.* Heterogeneity in myasthenia gravis: HLA phenotypes and autoantibody responses in ocular and generalized types. *Ann Neurol* 1987; **21:**274-8.
10 Evoli A, Batocchi AP, Lo Monaco M *et al.* Clinical heterogeneity of seronegative myasthenia gravis. *Neuromuscul Disord* 1996; **6:**155-61.
11 Matsui M, Wada H, Ohta M *et al.* Potential role of thymoma and other mediastinal tumours in the pathogenesis of myasthenia gravis. *J Neuroimmunol* 1993; **44:**171-6.
12 Newsom-Davis J, Willcox N, Schluep M *et al.* Immunological heterogeneity and cellular mechanisms in myasthenia gravis. *Ann NY Acad Sci* 1987; **505:**12-26.
13 Birmanns B, Brenner T, Abramsky O *et al.* Seronegative myasthenia gravis: clinical features, response to therapy and synthesis of acetylcholine receptor antibodies *in vitro*. *J Neurol Sci* 1991; **102:**184-9.
14 Verma PK, Oger JJ. Seronegative generalized myasthenia gravis: low frequency of thymic pathology. *Neurology* 1992; **42:**586-9.
15 Burges J, Vincent A, Molenaar PC *et al.* Passive transfer of seronegative myasthenia gravis to mice. *Muscle & Nerve* 1994; **17:**1393-400.
16 Mossman S, Vincent A, Newsom-Davis J. Myasthenia gravis without acetylcholine–receptor antibody: a distinct disease entity. *Lancet* 1986; **1:**116-9.
17 Drachman DB, de Silva S, Ramsay D *et al.* Humoral pathogenesis of myasthenia gravis. *Ann NY Acad Sci* 1987; **505:**90-105.
18 Karni A, Zisman E, Katz-Levy Y *et al.* Reactivity of T cells from seronegative patients with myasthenia gravis to T cell epitopes of the human acetylcholine receptor. *Neurology* 1997; **48:**1638-42.
19 Sharshar T, Lacroix-Desmazes S, Mouthon L *et al.* Selective impairment of serum antibody repertoires toward muscle and thymus antigens in patients with seronegative and seropositive myasthenia gravis. *Eur J Immunol* 1998; **28:**2344-54.
20 Blaes F, Beeson D, Plested P *et al.* IgG from 'seronegative' myasthenia gravis patients binds to a muscle cell line, TE671, but not to human acetylcholine receptor. *Ann Neurol* 2000; **47:**504-10.
21 Barrett-Jolley R, Byrne N, Vincent A *et al.* Plasma from patients with seronegative myasthenia gravis inhibit nAChR

responses in the TE671/RD cell line. *Pflugers Arch* 1994; **428:**492-8.

22 Li Z, Forester N, Vincent A. Modulation of acetyl-choline receptor function in TE671 (rhabdomyosarcoma) cells by non-AChR ligands: possible relevance to seronegative myasthenia gravis. *J Neuroimmunol* 1996; **64:**179-83.

23 Hoch W, McConville J, Helms S *et al*. Autoantibodies to the receptor tyrosine kinase MuSK in patients with myasthenia gravis without acetylcholine receptor antibodies. *Nat Med* 2001; **7:**365-8.

24 Valenzuela DM, Stitt TN, DiStefano PS *et al*. Receptor tyrosine kinase specific for the skeletal muscle lineage: expression in embryonic muscle, at the neuromuscular junction, and after injury. *Neuron* 1995; **15:**573-84.

25 Hopf C, Hoch W. Dimerisation of the muscle-specific kinase induces tyrosine phophorylation of acetylcholine receptors and their aggregation on the surface of myotubes. *J Biol Chem* 1998; **273:**6467-73.

26 Bowen DC, Park JS, Bodine S *et al*. Localisation and regulation of MuSK at the neuromuscular junction. *Dev Biol* 1998; **199:**309-19.

27 Apel ED, Glass DJ, Moscoso LM *et al*. Rapsyn is required for MuSK signaling and recruits synaptic components to a MuSK – containing scaffold. *Neuron* 1997; **18:**623-35.

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