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The emerging diversity of neuromuscular junction disorders

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Research advances over the last 30 years have shown that key transmembrane proteins at the neuromuscular junction are vulnerable to antibody-mediated autoimmune attack These targets are acetylcholine receptors (AChRs) and muscle specific kinase (MuSK) in myasthenia gravis, voltage-gated calcium channels (VGCCs) in the Lambert-Eaton myasthenic syndrome (LEMS), and voltage-gated potassium channels (VGKCs) in neuromyotonia. In parallel with these immunological advances, mutations identified in genes encoding pre-synaptic, synaptic and post-synaptic proteins that are crucial to neuromuscular transmission have revealed a similar diversity of congenital myasthenic syndromes (CMS). These discoveries have had a major impact on diagnosis and management.

Key words: Myasthenia gravis, myasthenic syndromes, congenital myasthenia

Introduction

The Mediterranean Society of Myology witnesses at its meetings the many advances that have occurred in the understanding of muscle disease. These advances have been paralleled by the remarkable diversity of disorders of the neuromuscular junction that has emerged over recent years. Crucial to these developments have been the basic science discoveries involving neurophysiology, biophysics, immunology and molecular biology. Crucial too have been the insights provided by the actions of predator neurotoxins such as α-bungarotoxin (BuTx), α-dendrotoxin (Dtx) and ω-conotoxin (CgTx). It is not the place here to describe the well-known and often devastating clinical consequences of the actions of these toxins at the neuromuscular junction. But the availability of these purified toxins, together with advances in molecular genetics, have played a key role in revealing the pathological processes that underlie human disorders of neuromuscular transmission, which are the focus of this brief historical review.

Myasthenia Gravis (MG)

For many years it had been recognized that MG could exist in several forms, namely as a congenital or familial condition, as an acquired disorder affecting individuals of all ages from about one year onwards, and as a transient disorder affecting babies born to MG mothers. This last observation was one of the clinical clues that suggested to Iain Simpson that MG might be an autoimmune disease in which antibodies were directed to the 'end-plate protein'. This proved to be an accurate prediction, although it took more than a decade before his hypothesis was validated. This was achieved by the discovery that rabbits immunised with electric organ acetylcholine receptors (AChRs), purified using α-BuTx, developed an MG-like disorder, had circulating AChR antibodies and responded to acetylcholinesterase inhibitors (1).

AChR antibodies were then detected in human MG sera (2, 3). Initial uncertainty as to whether the antibodies were protective rather than disease-causing was resolved by the passive transfer of the disorder to mice by injection of MG plasma or immunoglobulins (4) and by the demonstration that plasma exchange (plasmapheresis) that reduces the level of circulating antibodies could induce a striking clinical improvement lasting several weeks (5, 6). Pathological studies showed that AChR loss was caused to varying degrees by complement-mediated lysis, cross-linking and consequent down-regulation, and blocking of the ACh binding site. Figure 1 is a cartoon of the neuromuscular junction based on what was known in 1977. The inset illustrates the five subunit structure of the adult AChR, the ε-subunit being located between the two α-subunits. This subunit replaces the γ subunit at about 33 week's gestation in man.

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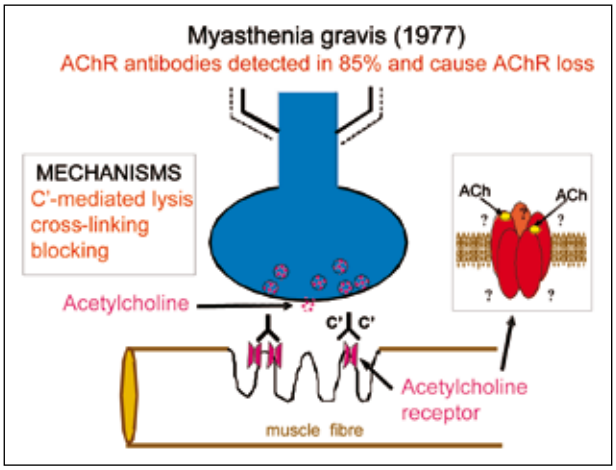

Figure 1. Myasthenia gravis (1977).

It soon became clear that AChR antibodies could only be detected in about 85% of patients with generalised MG. Nevertheless, clinical evidence strongly suggested the presence of pathological antibodies in these 'seronegative' patients. Plasma exchange improved the patient's strength, neonatal MG could affect babies born to mothers seronegative for AChR antibodies and seronegative plasmas or IgG injected into mice could induce an MG-like disorder of neuromuscular transmission (7, 8). Studies in the last few years have implicated antibodies to Muscle Specific Kinase (MuSK) in many of these patients

Antibodies to Muscle Specific Kinase (MuSK)

MuSK is a postsynaptic transmembrane protein at the neuromuscular junction with an extracellular Ig-like domain. It plays a key role during muscle development. Agrin, released by in-growing motor nerve terminals, leads *via* an intermediary protein to the activation of MuSK and subsequently to phosphorylation of rapsyn, thereby triggering AChR clustering and the formation of a neuromuscular junction. Hoch et al. (9) using an ELISA assay and rat MuSK as antigen detected MuSK antibodies in many patients with MG who were seronegative for AChR antibodies, confirmed by Scuderi et al. (10) using an alternative experimental approach. MuSK antibodies were not detected in healthy controls, in other neurological disorders or in MG patients with restricted ocular MG or whose serum harboured AChR antibodies. Further studies showed that MuSK antibodies could be detected in about 40% of MG patients ('Musk MG') who were seronegative for AChR antibodies (11).

The extracellular domain of MuSK can be 'seen' by circulating antibodies. Passive immunisation of mice with IgG (7) that was subsequently found to be MuSK positive, and active immunisation of rabbits with rat MuSK (12) can both induce a myasthenic disorder, suggesting that MuSK antibodies may be the effector mechanism in those harbouring them. Babies born to mothers with Musk MG can exhibit transient myasthenia with a similar distribution of muscle weakness.

Clinically, MuSK MG patients show some characteristic features that help to distinguish them from AChR MG. Bulbar weakness and sometimes respiratory weakness are often dominant, and tongue wasting may be present (11, 13-15). Onset can be at any age from about one year onwards. Females are much more often affected than males (4:1). Thymoma does not seem to associate with MuSK MG and studies of the thymus show that the changes do not differ significantly from healthy thymus, in striking contrast to the changes of hyperplasia seen in early onset MG (16, 17). The response to anticholinesterase medication (e.g. pyridostigmine) is often weak and sometimes absent. Electromyography shows typical changes of MG.

Immunopathogenesis update

Table 1 is an update of the immunopathogenesis of generalised MG. The prevalence figures are approximations. Recent evidence shows that the prevalence of late onset MG is progressively increasing in contrast to early onset disease where the prevalence appears stable (18, 19).

Many of those cases shown in the Table as 'seronegative' for both AChR and MuSK antibodies may in fact have low affinity AChR antibodies. Consistent with that is the observation that the thymus in these patients can show mild thymic hyperplasia (16). Recognizing these different subgroups is important because they influence the response to treatment.

Table 1. Immunopathogeneis of generalised MG.

Antibody	AChR			'Seronegative'	Musk
Prevalence ~%	10	30	45	8	7
Thymus	Thymoma	Hyperplasia	Involuted	Mild hyperplasia	Normal
Onset age ~yrs	Any	< 45	> 45	Any	> 1
Gender (M:F)	1:1	1:3	2:1	? 1:2	1:4

Neonatal MG

Neonatal MG affects about 1 in 8 of babies born to MG mothers. There may be fetal akinesia, and evidence of weakness at birth that responds to anticholinesterase medication. It is caused by the placental transfer of maternal AChR antibodies and is typically transient, recovering completely within 3 months.

In rare cases, however, neonatal MG can associate with Arthrogryposis Multiplex Congenita, oesophageal atresia, hydramnios and fetal death (20, 21). This appears to occur when the maternal AChR antibodies target the fetal form of AChR (α_2, β, γ, δ). The fetal form persists until about the 33rd week of gestation when the γ subunit is replaced by an ε-subunit (see Fig. 1 inset). In exceptional cases, the mother herself may exhibit no manifestations of MG, presumably because her antibodies are mainly or exclusively targeting fetal AChR, thus sparing her own 'adult' AChRs.

Neuromyotonia, limbic encephalopathy, thymoma and MG

It has been known for many years that thymoma can associate with other autoimmune diseases besides MG (for example, red cell aplasia). Neuromyotonia (NMT) or Isaacs' syndrome has also been observed to associate with thymoma or MG. NMT is characterized by hyperexcitability of motor nerves, causing myokymia and fasciculations, and characteristic EMG changes of doublet or multiplet motor unit ('myokymic') discharges, spontaneous neuromyotonic burst discharges and after discharges (22). The spontaneous discharges continue during sleep and general anaesthesia. Patients may also experience sensory symptoms that appear to arise from a similar hyperexcitability of sensory nerves. The autoimmune associations with neuromyotonia suggested that it too might have an autoimmune origin (22).

Further studies showed that the clinical and electromyographic abnormalities improved following plasma exchange (23), implicating a serum antibody. Neuronal voltage-gated potassium channels (VGKCs) were identified as a likely target since their down-regulation would prolong depolarisation, increasing the quantal release of transmitter and causing repetitive firing. Injection of NMT IgG into mice reproduced the electrophysiological abnormalities, and rat dorsal root ganglion cells exposed to NMT IgG in culture showed repetitive firing following a step depolarisation, in contrast to controls (24). An assay based on the radio-labelled snake toxin ^{125}I-α-dendrotoxin detected VGKC antibodies in about 40% of patients with NMT (25). Moreover, immunostaining of peripheral nerves shows that NMT IgG labels VGKCs at juxta-paranodes, co-extensive with experinmentally raised rabbit antisera to VGKCs (26). Interestingly, these antibodies can be detected in some patients with the cramp-fasciculation syndrome, indicating that NMT and CFS lie on the same spectrum (25). Thus antibody-mediated autoimmunitry needs to be added to the known causes of peripheral nerve hyperexcitability (Table 2).

Table 2. Principal causes of peripheral nerve hyperexcitability.

– Axonal damage (e.g. compression, radiation)
– Envenomation (Green Mamba, timber rattle snake)
– Inherited neuropathies (e.g. PMP22 in Charcot-Marie-Tooth disease)
– Ion channel mutations (e.g. Kv1.1 in Episodic ataxia and myokymia)
– VGKC antibody-mediated

An unexpected development has been the recognition that VGKC antibodies are implicated in limbic encephalopathy, and also in Morvan's syndrome that had long been an unexplained disorder (27, 28). Buckley et al. (29) described a patient with thymoma and longstanding AChR antibody positive MG who developed limbic encephalopathy late in her illness. At this point, for the first time, VGKC antibodies became detectable, declining in parallel with recovery of her encephalitis in response to immunosuppressive therapy. The likely involvement of VGKC antibodies in limbic encephalitis and Morvan's syndrome is now increasingly recognized and has been reviewed elsewhere (30) although the issue of whether the antibodies are the effector mechanism in these conditions is unresolved.

Lambert-Eaton Myasthenic Syndrome (LEMS)

The myasthenic disorder that can associate with lung cancer was first characterised electromyographically by Lambert and colleagues (31). With Elmqvist (32), Lambert later showed that LEMS was a presynaptic disorder in which the quantal release of transmitter was strikingly reduced. In man, 30 or more quanta are released by each nerve impulse, but in LEMS the number may be fewer than 10. Clinically these patients have proximal weakness that first affects their gait, augmentation of strength during the first few seconds of a maximal effort, and post-tetanic potentiation. Importantly, they may also have autonomic disturbances: dry mouth constipation and erectile failure in males.

The commonest underlying tumour is the smoking-associated small cell lung cancer (SCLC). LEMS can precede the appearance of the underlying SCLC by at least 2 years and occasionally for as long as 5 years (33). It soon became clear that not all patients with LEMS were harbouring a neoplasm. Many patients followed for 5 years

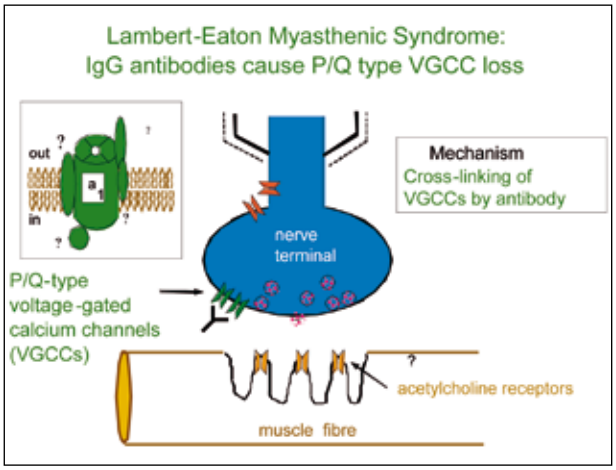

Figure 2. Lambert-Eaton Myasthenic Syndrome: IgG antibodies cause P/Q type VGCC loss.

or more and who were non-smokers failed to develop a tumour. Moreover these 'non-paraneoplastic' patients had a markedly increased association with other autoimmune diseases, notably thyroid disease and vitiligo. This non-paraneoplastic form of LEMS can affect children and presents with the features of a myopathy including a pronounced lumbar lordosis. Enquiry may reveal autonomic symptoms that provide a clue to the real nature of the disorder.

The association with other autoimmune disorders suggested a possible autoimmune pathogenesis, confirmed by the improvement that followed plasmapheresis (34) and the successful passive transfer of the pathophysiological (35) and morphological changes (36) of LEMS to mice. Antibodies to P/Q-type voltage-gated calcium channels (VGCCs) were detected in over 90% of patients (37), whether or nor they had an associated cancer, in a radioimmunoassay using the iodinated cone snail toxin (^{125}I-ω-conotoxin) that is specific for this channel sub-type (Fig. 2). Moreover these antibodies block post-ganglionic cholinergic and adrenergic VGCCs, providing an explanation for the observed autonomic changes (38).

VGCCs that are known to be expressed by SCLC cells appear to be the provoking factor in paraneoplastic LEMS because LEMS IgG significantly reduces K+ stimulated Ca++ influx into cultured SCLC cells (39). Interestingly, non-paraneoplastic LEMS IgG acts similarly but the triggering factor for the disorder in these patients in unknown.

Congenital Myasthenic Syndromes (CMS)

Although Congenital Myasthenic Syndromes are the rarest of the myasthenic disorders affecting man (estimated at up to 3 per million), they have nevertheless shown the greatest emerging diversity. They arise from mutations affecting crucial presynaptic, synaptic or postsynaptic proteins at the neuromuscular junction on which synaptic formation and function depend. The majority are recessively inherited. They have been the subject of recent reviews (40, 41).

Although many of these disorders can present as fetal akinesia or in the perinatal period with hypotonia, feeding or breathing difficulties, ptosis, ophthalmoplegia, and sometimes arthrogryposis, some only become first evident during adolescence or even adult life, for example the Slow Channel syndrome (42), thus making the diagnosis especially challenging. Others have a limb-girdle pattern that can be mistaken for a myopathy.

Figure 3 shows the proteins that are currently known to be mutated in CMS. However, as the figure makes clear, some gene targets remain to be identified.

The commonest site for mutations in CMS is the ε-subunit of the AChR, giving rise to a congenital AChR deficiency syndrome in most instances. Deletions or single nucleotide substitutions typically result in complete loss of function of the subunit. However, in man the fetal γ-subunit has the capacity to substitute for the ε-subunit, though resulting in less efficient neuromuscular transmission. Mutations in rapsyn (43), which plays a key role in AChR clustering during development, are another relatively frequent cause of CMS and can occur as an early-onset or late-onset phenotype (Table 3) (44).

Table 3. Distinct phenotypes associated with Rapsyn mutations.

Early Onset (85%)
– Birth or infancy in most patients – Foetal movements often reduced; arthrogryposis in 70% – Episodic apnoeas/crises
– Ophthalmoplegia rare
Late Onset (15%) – Onset adolescence/adulthood in ~15% – Mimics 'seronegative' MG
Both groups
– EMG: typical of myasthenia – Respond to AChE inhibitors

An interesting recent discovery has been the demonstration of mutations in Dok-7 (45), a post-synaptic protein (Fig. 3) that, like MuSK, is crucial for AChR clustering (46). These result in a CMS with a limb-girdle pattern of weakness. The clinical features, summarised in Table 4, have recently been described (47-49).

The principal differential diagnosis for Dok-7 mutations is Limb-girdle CMS with tubular aggregates in which Dok-7 mutations were not found (48). A distinguishing clinical feature in this disorder is the strongly positive re-

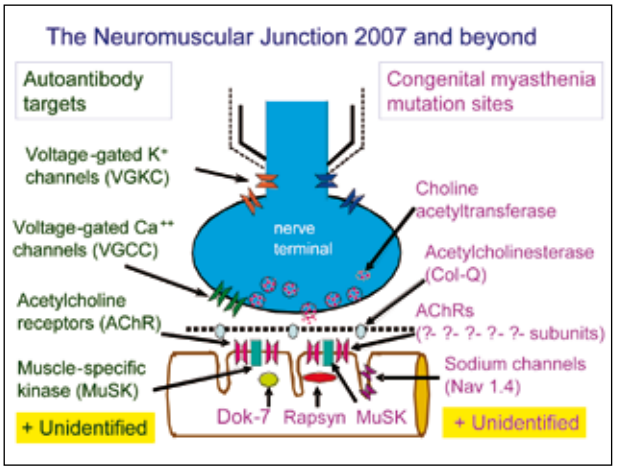

Figure 3. The Neuromuscular Junction 2007 and beyond.

sponse to acetylcholinesterase inhibitors (Edrophonium, pyridostigmine) in contrast to the transient or absent response in Dok-7 synaptopathy.

Table 4. Clinical features of Dok-7 neuromuscular junction synaptopathy.

– Normal motor milestones, then increasing difficulty walking
– Proximal > distal weakness; ptosis, eye movements normal
– Weak or absent response to cholinesterase inhibitors
– EMG: abnormal decrement and single fibre studies
– Small simplified NMJs; normal AChRs and AChE

Mutations are not always identified in patients thought to have CMS on clinical grounds. Thus although at least 10 genes have now been identified as sites of mutations that can cause CMS, there are others yet to be identified.

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