

Review articles

Neuromuscular junction channelopathies : a brief overview

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Summary

The neuromuscular junction lacks the protection of the blood-nerve barrier and is vulnerable to antibody-mediated disorders. In myasthenia gravis (MG), 85% of patients have IgG antibodies to acetylcholine receptors (AChRs). About half the remaining patients have IgG antibodies to Muscle Specific Kinase (MuSK), an AChR-associated transmembrane post-synaptic protein concerned in AChR aggregation. Bulbar weakness is typically predominant in this form of MG, and females are more often affected.

The Lambert-Eaton Myasthenic Syndrome (LEMS) can occur in a paraneoplastic form (P-LEMS) usually with small cell lung cancer, or in a non-paraneoplastic form (NP-LEMS). In both, IgG antibodies to nerve terminal voltage-gated calcium channels (VGCCs), detectable in over 90% of patients, lead to VGCC loss and impaired quantal release of transmitter and may be implicated in the occasionally associated cerebellar ataxia.

Neuromyotonia (NMT) and Cramp-Fasciculation syndrome (C-FS) are manifestations of peripheral nerve hyperexcitability and share some clinical and electromyographic features. Antibodies to voltage-gated potassium channels (VGKCs) are present in about 40% of NMT patients, but less frequently in C-FS, and appear to cause loss of functional VGKCs. They may also be implicated in the *Maladie de Morvan* and limbic encephalitis that can associate with NMT. The antibodies described here provide valuable aids to diagnosis and management.

The Congenital Myasthenic Syndromes are a group of genetically determined heterogeneous disorders, usually recessively inherited. The commonest mutation sites appear to be the acetylcholine receptor ϵ -subunit and rapsyn.

(Key words : myasthenic syndromes ; MUSK ; autoimmune channelopathies ; neuromyotonia.

Introduction

The neuromuscular synapse is well characterised. It lies outside the blood-nerve barrier and is rich in ion channels and other channel-associated proteins concerned with neuromuscular transmission. Understanding the pathological processes that can affect neuromuscular transmission is important both for diagnosis and for management. Excep-

tional research advances have been made in the field since the discoveries in the early 1970s that myasthenia gravis (MG) was an antibody-mediated disease. Those seminal observations were followed some eight years later by the demonstration that the Lambert-Eaton Myasthenic Syndrome (LEMS) was similarly autoantibody-mediated and – more recently – that peripheral nerve hyperexcitability syndromes (e.g. neuromyotonia or Isaacs' syndrome) can also be antibody-mediated. Furthermore, there have been comparable advances in the understanding of Congenital (inherited) Myasthenic Syndromes (CMS), which have proved to be a highly heterogeneous group of disorders in which mutations can affect presynaptic, synaptic and post-synaptic proteins involved in neuromuscular transmission.

Figure 1 illustrates the principal targets for autoantibodies and the proteins identified as sites of mutations in CMS.

Neuromuscular transmission

Invasion of the nerve terminal by the nerve impulse leads to the brief opening of P/Q-type voltage-gated calcium channels (VGCCs) and a local influx of calcium ions, triggering the release of about 30 vesicles (quanta), each vesicle containing about 10,000 molecules of acetylcholine (ACh). Binding of ACh to the two recognition sites on the α -

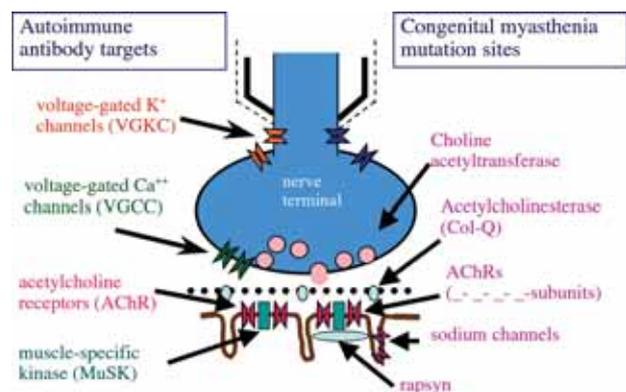


FIG. 1. — Sites of autoimmune and inherited neuromuscular junction disorders.

subunit of the acetylcholine receptors (AChRs) causes brief channel opening, the influx of small cations (mainly Na^+) and the production of an endplate potential. In normal muscle, this triggers the muscle action potential that propagates along the muscle fibre membrane and leads to muscle contraction.

Disorders of the neuromuscular junction can be due to (1) mutations of key proteins (causing congenital myasthenic syndromes) (2) autoantibodies that target the extracellular domains of ion channels or channel-related transmembrane proteins (3) toxic causes e.g. Botulinum toxin or snake toxins such as α -Bungarotoxin (which will not be discussed further here).

Congenital (inherited) myasthenic syndromes

Recent reviews have described the clinical and genetic features of the congenital myasthenic syndromes (e.g. Engel *et al.*, 2003). Of these rare disorders, the congenital AChR deficiency syndrome has, in our experience, been the commonest and is the only one to be discussed here. In the majority of cases, it is due either to mutations in the endplate protein Rapsyn (Ohno *et al.*, 2002 ; Burke *et al.*, 2003 ; Muller *et al.*, 2003) which is responsible for initiating and maintaining the integrity of the post-synaptic structures at the neuromuscular junction, or to mutations in the ϵ -subunit of the AChR. Both are inherited as autosomal recessive disorders. With regard to mutations in the AChR ϵ -subunit, many different sites have been identified within extracellular, transmembrane or intracellular domains of the protein. It might be asked how survival is possible with nul mutations affecting a subunit of the AChR. The answer lies with the fact that the fetal AChR γ -subunit is synthesised at low levels in man and appears able to substitute for the absent ϵ -subunit, although with less efficiency.

Mutations in the AChR ϵ -subunit are evident at birth with ophthalmoplegia, bulbar and generalised muscle weakness. Rapsyn mutations, by contrast, can have either an early onset or a late onset phenotype (Burke *et al.*, 2003 ; Burke *et al.*, 2004). In the early onset form of Rapsyn mutations, arthrogryposis is commonly present at birth and there may be frequent life-threatening exacerbations requiring hospital admission ; in the late onset form, symptoms may first become evident in adolescence or in adult life and can easily be mistaken for seronegative autoimmune myasthenia gravis.

Diagnosis of a disorder of neuromuscular transmission can be made from the electromyographic changes that are evident in both forms of CMS, characterised by increased decrement at slow rates of stimulation and increased jitter with blocking. Both groups also show a positive response to intravenous Edrophonium. But definitive diagnosis is

dependent on DNA screening for genes that are known to be mutated in these disorders, and is available at special centres.

Patients respond well to anticholinesterase medication with pyridostigmine, especially those with rapsyn mutations.

Myasthenia gravis

It is well-known that the majority of MG patients have IgG antibodies to AChRs. These lead to AChR loss by three mechanisms : complement-mediated lysis, cross-linking of neighbouring AChRs by the divalent antibodies causing down-regulation, and pharmacological block where the antibody binds to the ACh recognition site on the α -subunits (Fig. 1, inset). These antibodies are present in about 85% of patients with generalized MG but in only about 50% of restricted ocular MG.

Several lines of evidence have indicated that pathogenic antibodies are likely to be present even in those who are seronegative for AChR antibodies. First, an 'experiment of Nature' : seronegative mothers can give birth to babies who have transient neonatal MG, strongly implying the placental transfer of maternal antibodies. Second, seronegative plasmas can transmit a disorder of neuromuscular transmission when injected into mice (Mossman *et al.*, 1986). In addition, patients with seronegative MG can improve considerably following plasmapheresis.

MuSK antibodies

A potential target for antibodies in seronegative MG is MuSK (Muscle Specific Kinase), a member of the receptor specific kinase family. MuSK is a transmembrane protein with an immunoglobulin-like extracellular domain. It is known to play a key role in aggregating AChRs at the neuromuscular junction during development. Antibodies to MuSK were detected by ELISA in about 70% of severely affected seronegative MG patients (Hoch *et al.*, 2001), confirmed by Scuderi *et al.*, (2002) using a different assay. These antibodies were not detected in a range of other neurological disorders nor, interestingly, were they detected in restricted ocular MG patients. The antibodies are now detected using an immunoprecipitation assay. Using this assay, in a clinically more representative patient population, MuSK antibodies were detected in just under half those who were seronegative for AChR antibodies (Vincent *et al.*, 2003). Whether these antibodies are the cause of weakness in those who harbour them is uncertain, but they are unquestionably valuable in diagnosis. The target for the putative antibodies in patients who are seronegative for AChR antibodies and for MuSK antibodies is uncertain.

The clinical features are still currently being documented, but certain features are emerging

Table 1

Immunopathogenesis of Myasthenia Gravis: an update

Antibody	AChR	AChR	AChR	MuSK	'Seroneg'
Thymus	hyperplasia	thymoma	normal	normal	?hyperplasia
Onset age	<45Y	any	>45Y	any	any
Sex (M:F)	1:3	1:1	2:1	1:4	1:1

(Vincent *et al.*, 2003 ; Evoli *et al.*, 2003 ; Sanders *et al.*, 2003). The age of onset can be from early childhood to old age. Our youngest case presented with the subacute onset of bulbar weakness at the age of 2 years. Patients are more often female, in a female : male ratio of about 4:1. Bulbar weakness is typically dominant, and diaphragm weakness may also be relatively severe compared to weakness in the limbs. There may also be generalized weakness and ocular muscle weakness, though as mentioned above myasthenia restricted to eye muscles (ocular myasthenia) does not seem to associate with MuSK antibodies.

Table 1 provides an update on the immunopathogenesis of MG.

Patients often do not benefit substantially from pyridostigmine or other anticholinesterase medications. But many patients respond well to prednisone although in some of them additional immunosuppressive medications such as azathioprine are required. Short-term improvement, lasting 3-5 weeks, can follow plasmapheresis or a course of intravenous immunoglobulin, but should only be considered in those with severe weakness. In our experience, thymectomy is not helpful, and as shown in Table 1, the thymus does not show the changes of hyperplasia observed in young onset AChR antibody positive patients, nor does thymoma appear to associate with MuSK antibody positive MG.

Lambert-Eaton myasthenic syndrome

CLINICAL FEATURES

This disorder exists in two forms : paraneoplastic (P-LEMS) and non-paraneoplastic (NP-LEMS). The prevalence of the two forms is similar but their incidence differs because survival of P-LEMS is much shorter. The age of onset of P-LEMS is typically over the age of 40 years. NP-LEMS can occur at any age from early childhood to extreme old age. There is an underlying small cell lung cancer in almost all patients with P-LEMS, the remainder usually having a lymphoma. NP-LEMS associates with other autoimmune diseases, notably vitiligo and thyroid disease. Furthermore, in contrast to P-LEMS, there is strong linkage with the HLA A1-B8-DR3 haplotype (Wirtz *et al.*, 2001)

The great majority of patients first complain of difficulty in walking. Other symptoms are upper

limb weakness, and autonomic deficits including dry mouth, constipation and erectile failure in males. Examination reveals augmentation of strength during the first few seconds of a maximum voluntary effort (a physical sign that is easily overlooked), and depressed tendon reflexes that can often show pronounced post-tetanic potentiation following 10 secs of maximum voluntary contraction of the muscle being tested.

Electromyographic studies will show a reduced amplitude of the compound muscle action potential measured using surface electrodes which enhances by 100% or more (post-tetanic potentiation) following 15 seconds maximum voluntary contraction of the muscle being tested. Stimulation at 40 Hz will have a similar action but is painful and usually unnecessary.

VOLTAGE-GATED CALCIUM CHANNEL ANTIBODIES

The physiological defect in LEMS is a reduced quantal release of transmitter from the presynaptic nerve terminal, typically only 10 or fewer. The clinical response to plasmapheresis and the transfer of the physiological and morphological defects to mice injected with LEMS plasma or IgG strongly suggested that the disorder was likely to be caused by autoantibodies (Lang *et al.*, 1981 ; Lang *et al.*, 1983 ; Fukunaga *et al.*, 1983). Further physiological and morphological studies (Lang *et al.*, 1987 ; Fukuoka *et al.*, 1987) showed that the effect was likely to be mediated by antibodies to the P/Q-type voltage-gated calcium channels (VGCCs) at motor nerve terminals, on which transmitter release depends (Fig. 1).

Anti-P/Q-type VGCC antibodies can be detected in over 90% of P-LEMS and NP-LEMS patients in an immunoprecipitation assay in which VGCCs are radiolabelled with a cone snail toxin (ω -conotoxin MVIIC) that is specific for channels of the P/Q type (Motomura *et al.*, 1995 ; Lennon *et al.*, 1995). The antibodies are specific for LEMS, and the assay thus provides an important diagnostic test for LEMS and is now generally available.

In P-LEMS, the antibodies appear to be provoked by VGCCs expressed in the membrane of small cell lung cancer cells (Roberts *et al.*, 1985), but the site(s) of the antigenic stimulus in NP-LEMS is unknown.

Occasional patients with LEMS also have cerebellar ataxia, and this sometimes dominates the

clinical picture so that the presence of LEMS is overlooked. These patients may have raised a raised titre of antibodies to P/Q-type VGCCs in their cerebrospinal fluid as well as in their serum. P-type VGCCs are present in cerebellar Purkinje cells and granule cells, thus raising the question of whether the antibodies in LEMS might in some cases exert a central action. Pinto *et al.*, (1998), using an *in vitro* approach, showed that incubating rat Purkinje cells overnight in LEMS IgG led to a substantial loss of P-type VGCCs compared to incubation in control human IgG. A similar effect was observed in cerebellar granule cells. This finding, that appears to implicate these antibodies in the cerebellar ataxia, is supported by the report that there is a substantial loss of P/Q-type VGCCs in post-mortem studies of patients dying with LEMS-associated cerebellar ataxia, and that the proportion of channels with antibody bound is greatly increased compared with controls (Fukuda *et al.*, 2003). Thus it appears that anti-P/Q-type VGCC antibodies may be implicated in the cerebellar ataxia sometimes seen in association with LEMS.

Neuromyotonia, Cramp-Fasciculation syndrome and Maladie de Morvan

CLINICAL FEATURES

These disorders are manifestations of neuronal hyperexcitability that can involve peripheral motor and sensory nerves, and apparently also neurones of the autonomic nervous system and central nervous system (CNS). A simplified classification of these disorders together with the clinical features and electromyographic findings are given in Table 2. The Maladie de Morvan (Morvan, 1890) has striking similarities to Limbic Encephalitis.

VOLTAGE-GATED POTASSIUM CHANNEL ANTIBODIES

The association of neuromyotonia (NMT) with thymoma, myasthenia gravis, other autoimmune

disorders and sometimes with lung cancer suggested to us that the disorder might be autoimmune. A clear-cut and highly significant reduction in neuromyotonic discharges recorded electromyographically following a 5 day course of plasmapheresis supported the hypothesis (Sinha *et al.*, 1991). It raised the possibility that the putative antibodies were targeting motor nerve voltage-gated potassium channels (VGKCs) since their down-regulation would interfere with nerve repolarisation, prolonging the depolarisation of the nerve terminal and leading to repetitive firing. Further evidence was provided by the transfer of the pathophysiological changes to mice injected with NMT IgG (Sinha *et al.*, 1991 ; Shillito *et al.*, 1995). Moreover, NMT IgG applied overnight to rat dorsal root ganglion cells *in vitro* led to repetitive firing, an effect that was mimicked by the application of 4-aminopyridine that is known to block VGKCs (Shillito *et al.*, 1995).

Serum antibodies to VGKCs can be detected in many patients with NMT by an immunoprecipitation assay in which a component of the venom of the Green Mamba (α -dendrotoxin) specific for the relevant subtypes of VGKCs (Kv1.1, 1.2 and 1.6) is radiolabelled. About 40% of patients overall harbour these antibodies, but the incidence is higher in those in whom a thymoma is also present (Hart *et al.*, 1997 ; Hart *et al.*, 2002).

Evidence that Cramp-Fasciculation syndrome may, like NMT, be due to antibody-mediated nerve hyperexcitability comes from the association of both disorders with myasthenia gravis, with other autoimmune disorders and with thymoma. Furthermore, antibodies to VGKCs are found in some patients with Cramp-Fasciculation syndrome, though less frequently than in NMT, especially when a thymoma is also present (Hart *et al.*, 2002)

MALADIE DE MORVAN AND LIMBIC ENCEPHALITIS

Although this paper principally concerns ion channelopathies at the neuromuscular junction, it is

Table 2

Classification of peripheral nerve and central nervous system hyperexcitability synd

Disorder	Clinical features	EMG findings
Cramp-Fasciculation	cramps fasciculations	single motor unit discharges after-discharges
Neuromyotonia or Isaacs' syndrome	As above <i>plus</i> myokymia, pseudomyotonia, muscle hypertrophy, paraesthesias	As above <i>plus</i> myokymic discharges prolonged motor unit discharges
Maladie de Morvan or Limbic encephalitis	As above <i>plus</i> pruritis autonomic changes insomnia confusion hallucinations memory loss	Changes of neuromyotonia may be present

clinically highly relevant that NMT can sometimes associate with CNS abnormalities. This association was first observed by (Morvan, 1890) who described 'chorée fibrillaire' in a patient who then developed autonomic changes and features consistent with encephalitis. Morvan's account of 'chorée fibrillaire' in his patient strongly resembles Isaacs' syndrome or NMT. Interestingly, patients with NMT may sometimes have increased anxiety, hallucinations and impaired sleep, but without features sufficient to diagnose encephalitis. VGKC antibodies were reported in the serum and cerebrospinal fluid of a patient with clinical features of the Maladie de Morvan who also had dysautonomia and cardiac arrhythmia (Liguori *et al.*, 2001).

The CNS abnormalities described by Morvan strongly resemble limbic encephalitis. Buckley *et al.*, (2001) described two patients with limbic encephalitis and autonomic features who had high titres of VGKC antibodies but no clinical evidence of NMT. The first patient also had MG (with AChR antibodies) and a thymoma; the encephalitis and associated raised levels of VGKC antibodies developed at the time of tumour recurrence. It responded to plasma exchange. Recovery was spontaneous in the second case, and in both the recovery was accompanied by a decline in VGKC antibodies.

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