Autoantibodies, neurotoxins and the nervous system

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Summary — Myasthenia gravis, the Lambert-Eaton myasthenic syndrome, and acquired neuromyotonia are three disorders of the neuromuscular junction or motor nerve that are caused by autoantibodies to ion channel proteins: acetylcholine receptors, voltage-gated calcium channels and voltage-gated potassium channels, respectively. The antibody titres can be measured using the relevant \textsuperscript{125}I-neurotoxins to label the extracted channels. Other disorders of the peripheral motor nerve are associated with antibodies to gangliosides. Sera with raised levels of anti-ganglioside antibodies have direct effects on the function of the distal motor nerve and motor nerve terminal. These conditions can be improved by therapies designed to reduce circulating antibodies. Antibodies that bind to neuronal surface antigens are proving to be of great clinical importance and interest in neurological disorders.

ion channels / neurotoxins / autoantibody / gangliosides / neuromuscular junction

Introduction

It is well known that muscle weakness in myasthenia gravis is due to autoantibodies against the nicotinic acetylcholine receptor at the neuromuscular junction. Two other conditions have now been defined in which serum antibodies to ion-channels are associated with abnormalities of neuromuscular transmission: antibodies to voltage-gated calcium channels in the Lambert-Eaton myasthenic syndrome, and antibodies to voltage-gated potassium channels in acquired neuromyotonia.

Recently, humoral factors have been identified in other disorders in which immune-mediated mechanisms are suspected. The Guillain-Barré syndrome (GBS) and Miller-Fisher syndrome (MFS) are acute polyneuropathies. Some cases of GBS have antibodies to ganglioside GM1, and the majority of MFS patients have IgG antibodies to GQ1b. In addition, IgM antibodies to these and other related polysialylated gangliosides are present in chronic neuropathies, particularly multifocal motor neuropathy (MMN) and neuropathies associated with IgM paraproteins. We have found that sera from each of these conditions, and some monoclonal anti-ganglioside antibodies derived from affected patients, produce marked effects on distal motor nerve function (mouse phrenic nerve/diaphragm preparation) both in acute \textit{in vitro} incubation and after injection intraperitoneally. Antibodies to GQ1b are associated with defects in neurotransmitter release, and antibodies to GM1 with failure of distal motor nerve conduction.

These observations add further diseases to the growing list of conditions that are due to, or associated with, autoantibody-mediated interference with the function of the nervous system. This chapter will review recent progress in defining the targets and mechanisms of action of these autoantibodies. The reader is referred to other more detailed reviews for the pathophysiology of these conditions (Wilcox \textit{et al.} 1993; Vincent \textit{et al.} 1994a; Willison, 1994; Hart \textit{et al.} 1995; Newsom-Davis and Lang, 1995).

Autoimmunity to ion channels at the neuromuscular junction

\textit{Myasthenia gravis and human AChR}

Myasthenia gravis is associated with antibodies to acetylcholine receptors (AChR) at the neuromuscular junction (see Vincent, 1980). These antibodies cause loss of AChR, reduced postsynaptic response to released ACh (ie reduced amplitudes of miniature endplate potentials (MEPPs) and endplate potentials (EPPs)), with consequent weakness and increased fatigability. Treatment involves anti-cholinesterase drugs to increase the efficacy of released ACh, thymectomy which results in clinical improvement in a proportion of patients, plasma exchange that temporarily reduces circulating antibodies, or long-term immunosuppressive treatment with prednisolone and azathioprine.

Anti-AChR antibodies are present in > 85% of patients with generalised weakness (Vincent and Newsom-Davis, 1985). The antibodies are measured by immunoprecipitation, using \textsuperscript{125}I-\alpha-bungarotoxin (from the venom of \textit{Bungarus multicinctus}) to label AChR extracted from human muscle or the rhabdomyosarcoma cell line, TE671 (Luther \textit{et al.} 1989). The anti-
bodies are high affinity and specific for the human AChR. They cause loss of functional AChR by a combination of antigenic modulation of AChR numbers and complement dependent lysis of the postsynaptic membrane; pharmacological blockade of ACh binding is probably not an important mechanism in the majority of patients (see Vincent, 1980).

The human AChR genes (α, β, γ, δ) have now been cloned, including the adult ε form and a new isoform of the α which contains a 25 amino acid insertion between α58 and 59 (Beeson et al., 1990). Both forms of the α subunit mRNA are expressed in TE671 cells, in human muscle, and in fetal muscle at various stages of development (Beeson et al., 1990; Morris et al., 1991; MacLennan et al., 1993). However, expression of cRNA for the longer isoform together with β, γ or ε, and δ subunit cRNAs, in Xenopus oocytes, does not lead to a functional acetylcholine receptor (Newland et al., in preparation). The role of this isoform, that is highly conserved in humans and present in approximately equal amounts with the shorter form, remains unexplained.

Recombinant AChR subunits can be used to stimulate and clone AChR-specific T cells from the peripheral blood or thymus of MG patients (Ong et al., 1991; Willcox et al., 1993; Harcourt et al., 1993). T cells recognise short peptide sequences in the groove of specific alleles of the polymorphic HLA class II molecules; these peptide sequences, and the HLA class II molecules that present them to the T cells, are being defined so that specific immunotherapy can be developed (eg Nicolle et al., 1994).

**Anti-AChR antibodies and fetal arthrogryposis**

MG frequently presents during child-bearing years, and a proportion of babies born to MG mothers suffer from a transient form of MG, neonatal MG, due to placental transfer of maternal anti-AChR antibodies (Papazian, 1992). A much smaller number of MG mothers have pregnancies complicated by recurrent fetal abnormalities, which can end in fetal or neonatal death (for a review of the literature, see Dinger and Prager 1993). Antibodies specific for fetal AChR could be involved in causing fetal and neonatal problems (Vernet-der Garabedian et al., 1994). Recently, we have studied an asymptomatic mother with a history of recurrent fetal arthrogryposis. Serum from this woman inhibited fetal AChR function in TE671 cells, and in Xenopus oocytes injected with cRNAs for fetal AChR, but had no effect on oocytes injected with cRNAs for adult AChR (Vincent et al., 1995). Thus the presence of antibodies inhibiting fetal AChR function can cause severe fetal abnormalities in the absence of maternal symptoms. Fetal AChR is replaced by the adult form by about 33 weeks gestation in humans (Hesselmans et al., 1993). Therefore, fetal AChR would be most important early in development, at which time experimental inhibition of AChR function leads to arthrogryposis (Drachman and Coloumbe, 1962).

**Seronegative myasthenia gravis (SNMG)**

In about 15% of MG patients anti-AChR antibodies are not detectable by the immunoprecipitation method. SNMG patients respond to therapeutic measures that are known to reduce serum antibody levels, and clearly have an antibody-mediated disease (Mossman et al., 1986). Injection of SNMG plasma or Ig into mice for 7–15 days reduces MEPP amplitudes in all cases, and changes the number of packets of ACh released in some (Burges et al., 1994). We reported that SNMG plasma, and IgM containing fractions from some patients, reduced the carbachol-stimulated 22Na+ flux into TE671 cells, without altering the number of 125I-α-BuTx binding sites or associating with antibody-bound to the AChR. These observations suggested the action of an antibody that reduces AChR function without binding directly to AChR (Yamamoto et al., 1991). Interestingly, 22Na+ flux was also reduced by lectins, that cross-link membrane glycoproteins and can stimulate their function (Lin and Levitan, 1991) and by cholera toxin and the β2 adrenergic receptor agonist, salbutamol, that raise internal cAMP by activating Gs (Li et al., in preparation). Moreover, in whole cell clamp experiments the inhibition of ACh-induced currents by two of three SNMG plasmas was less with Ca2+ free internal solutions (Barrett-Jolley et al., 1994). Taken together these studies suggest that SNMG plasmas and probably some anti-AChR positive plasmas (Yamamoto et al., 1991) contain antibodies that induce an increase in cAMP by binding to a cell surface receptor. This would lead to activation of a (Ca2+ modulated) cAMP-dependent kinase and phosphorylation of the AChR. There are potential cAMP-dependent phosphorylation sites on the δ and ε human AChR subunits (Beeson et al., 1993), and phosphorylation is known to reduce AChR function (Mulle et al., 1988; Huganir and Greengard, 1990) by increasing the rate of desensitisation.

Another ligand that increases intracellular cAMP, and for which there are receptors at the neuromuscular junction (Popper and Micevych, 1989), is calcitonin-gene related peptide (CGRP). This peptide reduced MEPP amplitudes at the mouse diaphragm endplates in a reversible, dose-dependent manner. Plasma from seronegative MG patients also reduces MEPP amplitudes, with a similar time course and reversibility, sug-
sugest that SNMG plasma may contain antibodies or other factors that act in a way similar to CGRP (Li et al., in preparation).

These preliminary studies should stimulate further work on factors in MG plasma that modulate AChR function. Importantly, they indicate the potential of autoantibodies to act indirectly via activation of second messenger systems, as well as by direct pharmacological block (as in the case of arthrogryposis described above), and by antibody-induced modulation of receptor numbers (as in typical MG).

Lambert-Eaton myasthenic syndrome and antibodies to voltage-gated calcium channels (VGCC)

The Lambert-Eaton myasthenic syndrome (LEMS) is another condition in which the patient complains of muscle weakness. Patients present at any age, but about 60% have, or develop subsequently, a small cell lung carcinoma (O’Neill et al., 1988). This strong association places LEMS in the rare but important group of ‘paraneoplastic’ disorders (see Posner and Furneaux, 1990). In LEMS, antibodies induce loss of VGCC on the presynaptic motor nerve terminal, mainly by modulation of VGCC numbers rather than a direct action, leading to a marked reduction in the quantal content of the endplate potential (Lang et al., 1986). VGCCs are present on SCLC cells, and LEMS serum or IgG reduces the number of functioning VGCC on cultured SCLC cell lines (Roberts et al., 1985; Johnston et al., 1994).

In the last few years several groups have used 125I-ω-conotoxin (GVIA) to label VGCCs for immunoprecipitation assays. This toxin, from the marine snail Conus geographus, is thought to be specific for N-type voltage-gated calcium channels. However, the titres obtained were quite low, and only about 30% of LEMS sera gave clearly positive results in our hands (Leys et al., 1991), even though all LEMS sera reduce the K+-stimulated 45Ca2+ flux of a cultured SCLC cell line (MB) derived from a LEMS patient (Johnston et al., 1994). ω-Aga-toxin (IVA) from the funnel-web spider, Agelenopsis aperta, which is specific for P-type channels, reduces quantal release at the mammalian neuromuscular junction (Uchitel et al., 1992), and is a much more effective inhibitor of SCLC VGCCs, but it has not yet successfully been used to label solubilised VGCC. However, recently an immunoprecipitation assay for LEMS patients, positive in > 85% of cases (Motomura et al., 1995), has been established employing VGCC extracted from human cerebellum and labelled with 125I-ω-conotoxin (MVIIIC) from another

Fig 1. Antibodies to voltage-gated calcium channels detected in LEMS patients by immunoprecipitation of calcium channels extracted from human cerebellar cortex and labelled with 125I-Conus magus MVIIIC toxin. Three groups of LEMS patients (small cell lung cancer; no cancer detected; cancer status uncertain) have greater than 80% positivity for the antibody; none of the control sera (healthy; small cell lung cancer without LEMS, other neurological disorders, myasthenia gravis, rheumatoid arthritis or systemic lupus erythematosus) showed values > 20 pM. Taken from Motomura et al (1995) with permission of the publisher.

Neuromyotonia (NM)

This rare condition is hereditary or can be acquired. The patients complain of painful muscle cramps and twitching, and spontaneous motor unit potentials, occurring in doublets, triplets or multiplets at high intraburst frequencies; it can be detected by electromyography. The origin of the muscle twitching is in the nerve, since it is abolished by curare but persists after proximal peripheral nerve block (Isaacs, 1961).

In some cases, at least, the acquired form is antibody mediated. Plasma exchange reduces the number of neuromyotonic discharges (Bady et al., 1991; Sinha et al., 1991). Injection of NM IgG into mice causes resistance to d-tubocurarine (Sinha et al., 1991) and an increase in the number of quanta of ACh released (Shil-
lito et al. submitted). These results are similar to those found when low doses of the K⁺ channel blockers TEA and 3,4-diaminopyridine are applied, suggesting that the antibodies are directed against voltage-gated potassium channels (VGKC).

In experiments analogous to those employed in MG and LEMS, we have shown that VGKC extracted from human frontal cortex and labelled with the VGKC-specific snake toxin, [35S]-α-dendrotoxin (Harvey and Anderson, 1991), are immunoprecipitated by some NM sera. Moreover, NM sera precipitated α-dendrotoxin-labelled K⁺ channels expressed in Xenopus oocytes (Hart et al, 1994). Thus it seems likely that antibodies to VGKCs involved in the repolarisation of the motor nerve terminal may cause, or contribute to, the enhanced motor nerve excitability that characterises the disease.

Autoimmunity to gangliosides at the neuromuscular junction

Gangliosides are sialylated glycolipids that are prevalent in the nervous system, where they are concentrated at particular sites, and thought to modulate a wide range of neuronal functions (Hanun and Bell, 1989; Hakamori, 1990; Thomas and Brewer, 1990; Testamenti and Riboni, 1993). Antibodies to gangliosides and other glycolipids are found in several peripheral nerve diseases (Latov, 1990; Pestronk, 1991) but until recently their pathogenic role was uncertain (Willison, 1994).

Anti-GM1 antibodies

Multifocal motor neuropathy

MMN is a chronic peripheral neuropathy that responds to immunosuppressive therapy including intravenous immunoglobulin, indicating a role for humoral immune factors in its pathogenesis. Clinical electrophysiology demonstrates focal motor conduction block and/or reduced motor conduction velocity with dispersion of muscle action potentials, without any abnormality of neuromuscular transmission (Lewis et al, 1982; Summer, 1994). IgM antibodies to GM1 ganglioside are found in about 60% of MMN patients, and monoclonal anti-GM1 antibodies have been cloned from the peripheral blood cells of three affected individuals (Willison et al, 1994a). Several papers have investigated a possible pathogenic role of these antibodies on motor nerve conduction (Arasaki et al, 1993; Uncini et al, 1993).

We have used the nerve-muscle preparation to look for an action on motor nerve conduction by examining the effects on nerve-evoked muscle contraction and endplate potentials (EPPs). We applied serum or plasma (diluted in Krebs' solution) to the mouse phrenic nerve/hemidiaphragm preparation and measured: a) the nerve stimulus required to evoke muscle contraction; and b) the endplate potentials (EPPs) in individual muscle fibres. For the latter experiments, the muscle was first paralysed with 1 μM α-conotoxin to prevent muscle contraction (Hong and Chang, 1989). Serum and plasma from MMN patients
Table 1. Targets for autoantibodies at the distal motor nerve and neuromuscular junction.

<table>
<thead>
<tr>
<th>Target</th>
<th>Symptoms</th>
<th>Disorder</th>
<th>Antigen for assay</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>K⁺ channel</td>
<td>Nerve-evoked muscle spasms</td>
<td>Neuromyotonia</td>
<td>¹²⁵I-α-DendroTx-VGKC</td>
<td>? loss of VGKC</td>
</tr>
<tr>
<td>GM1 ? nodes of Ranvier</td>
<td>Weakness</td>
<td>MMN Some GBS</td>
<td>GM1 ganglioside</td>
<td>? block of Na⁺ channels</td>
</tr>
<tr>
<td>Ca²⁺ channel</td>
<td>Weakness</td>
<td>Lambert Eaton syndrome</td>
<td>¹²⁵I-α-ConoTx-VGCC</td>
<td>Loss of VGCC</td>
</tr>
<tr>
<td>GQ1b ? presynaptic active zones</td>
<td>Weakness</td>
<td>Miller-Fisher syndrome Some sensory neuropathies</td>
<td>GQ1b ganglioside</td>
<td>? interference with release mechanism</td>
</tr>
<tr>
<td>AChR</td>
<td>Weakness</td>
<td>Myasthenia gravis</td>
<td>¹²⁵I-α-BuTx-ACh</td>
<td>Loss of AChRs</td>
</tr>
</tbody>
</table>

produced similar effects namely: a) a progressive increase in the stimulus required for nerve-evoked muscle contraction, sometimes resulting in complete block of neuromuscular transmission (fig 2a); and b) a decrease in the EPP amplitude followed by complete loss of nerve-evoked EPP generation (fig 2b). At this stage, depolarisation of the nerve terminal, by a raised external K⁺ concentration, increased MEPP frequency to a normal extent, indicating that there was no alteration in neurotransmitter release (Vincent et al. 1994; Roberts et al. 1995).

These changes were seen in 66% anti-GM1 positive sera from MMN patients, and indicate that serum antibodies in this disorder directly block nerve conduction since similar effects were seen with two monoclonal anti-GM1 antibodies (Willison et al. 1994b). GM1 ganglioside is concentrated at nodes of Ranvier as well as being present in peripheral nerve myelin (Corbo et al. 1993), and the motor endplate (Schluep and Steck, 1988). Focal application of tetrodotoxin to the distal nodes and heminodes of the mouse phrenic nerve-diaphragm preparation, to block voltage-gated sodium channels, produces a reduction in EPP amplitude or complete block of EPPs depending on the number of nodes affected (Konishi, 1985). Thus it is possible that antibodies binding to GM1 at the distal nodes of Ranvier interfere with, or modulate, sodium channel function.

**Guillain-Barré syndrome (GBS)**

This is a form of acute polyneuropathy which commonly occurs 10–14 days after acute bacterial or viral infections. A particularly severe form of the disease with prominent axonal involvement is sometimes found after infection with specific strains of *Campylobacter jejuni* (Yuki et al. 1993). GBS is associated with IgG, IgA or IgM antibodies to GM1 in around 20% of cases and with antibodies to related gangliosides in others (Ilyas et al. 1988).

Preliminary experiments suggest that serum from anti-GM1 ganglioside antibody positive GBS patients
alters nerve conduction in the phrenic nerve/hemidiaphragm preparation in a manner that is similar to that found with IgM anti-GM1 positive sera (Vincent et al., 1994b; manuscript in preparation).

**Miller-Fisher syndrome (MFS)**

Miller-Fisher syndrome is an acute disorder characterised by weakness of ocular muscles, ataxia and loss of reflexes (Fisher, 1956; Berlit and Rakicky, 1992), generally considered to be a variant of GBS. Both often follow gastrointestinal or respiratory infections. Serum IgG antibodies to GQ1b ganglioside are present in over 90% of MFS cases (Chiba et al., 1992; Willison et al., 1993), and are thought to be part of the immune response to the infectious agent, which may share structurally-related epitopes for antibody binding. Since botulinum toxin binds to polysialogangliosides and produces muscle paralysis by inhibiting ACh release at the neuromuscular junction (Dolly et al., 1994; Van der Koot and Molgo, 1994), we investigated the effect of MFS sera in vitro (see Willison and Kennedy, 1993). Each of three MFS sera produced a highly significant increase in MEPP frequency within the first 30 min of incubation at room temperature, followed by a gradual decline in MEPP frequency to zero by about 3 h. At this time nerve stimulation failed to evoke any muscle contraction, although direct muscle stimulation was still effective (fig 3; Roberts et al., 1994). Thus, at room temperature, MFS sera produce a complete block of neuromuscular transmission in the mouse phrenic nerve/diaphragm preparation.

The complete loss of MEPPs suggests that these sera affect neurotransmitter release, rather than block conduction of the nerve impulse. Further studies are in progress to define the nature of this effect.

**Conclusions**

The neuromuscular junction has proved to be a fruitful area in which to investigate the effect of sera from patients with acquired peripheral nerve or muscle disorders. In MG, LEMS and NM, the similarity between the effects of serum, plasma or Ig preparations and those observed in the presence of specific neurotoxins, has led to the development of antibody assays employing these neurotoxins as labels for the target antigen. In MMN and GBS, anti-GM1 antibodies appear to block nerve conduction in a manner similar to that found with tetrodotoxin. In MFS, sera containing anti-GQ1b antibody reduce both nerve-evoked and spontaneous neurotransmitter release, reminiscent of the effects of botulinum toxin (see Dolly et al., 1994). The mechanisms of action of anti-ganglioside antibodies in these situations require further study, which should also provide information about the role of gangliosides in normal neuronal function.

Although the central nervous system (CNS) and peripheral nerve are thought to be protected from circulating immune factors by the blood-brain and blood-nerve barrier, these studies clearly indicate that protection of peripheral nerve is far from complete. Moreover, there is increasing evidence for the association of autoantibodies with central nervous system diseases such as the stiff man syndrome (antibodies to glutamic acid decarboxylase; Solimena et al., 1988), Rasmussen's encephalomyelitis (antibodies to glutamate receptors; Rogers et al., 1994), and paraneoplastic syndromes in which antibodies to tumour antigens cross-react with CNS antigens (Posner and Fremeaux, 1990). Although the pathogenic role of the antibodies has not yet been established, it seems likely that some of these conditions will prove to be further examples of antibody-mediated neurological disorders.

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**References**


Druchman DB, Coulombre AJ (1962) Experimental club foot and arthrogryposis multiplex congenital. Lanec 2, 523


Hong SJ, Chang CC (1989) Use of geogaphatanin II (α-conotoxin) for the study of neuromuscular transmission in muscles. Br J Pharmacol 93, 1933-1940


Nicolle M, Nag B, Sharma SD, Wilcox N, Vincent A, Ferguson DIP, Newsom-Davis J (1994) Specific tolerance to an acetylcholine receptor epitope induced in vitro in myasthenia gravis CD4+ lymphocytes by soluble major histocompatibility complex class II peptide complexes. J Clin Invest 93, 1361-1369


Autoantibodies to glutamate receptor GluR3 in Rasmussen’s encephalitis. Science 265, 648–651
Van der Kloo W, Molgo J (1994) Quantal acetylcholine release at the vertebrate neuromuscular junction. Physiol Rev 74, 899–991
Vincent A (1980) Immunology of acetylcholine receptors in relation to myasthenia gravis. Physiol Rev 60, 756–824
Vincent, A Newsom-Davis J (1985) Acetylcholine receptor antibody as a diagnostic test for myasthenia gravis: results in 153 valuated cases and 2967 diagnostic assays. J Neurol Neurosurg Psychiatry 48, 1246–1252