Tissue-specific antibodies in myasthenia gravis

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Myasthenia gravis (MG) is a disorder of the neuromuscular junction characterised by weakness which increases on effort and is improved by rest and anticholinesterase treatment. Thymic abnormality with increased numbers of germinal centres is common, thymoma occurs in about 15% of cases, and thymectomy is often beneficial in those without a tumour. The condition is commoner in women and typically becomes evident in early adult life. It can, however, present at any age, and there is a rare congenital form in which weakness dates from the neonatal period (see below). For a recent review see Drachman (1978).

The main features of the normal and myasthenic neuromuscular junction are shown in Fig. 1. In MG the presynaptic nerve terminals are essentially normal although there is often elongation of the endplate with changes in the number of terminal expansions. There are, however, marked postsynaptic changes which include simplification of the postsynaptic membrane and loss of the secondary folds (Santa et al., 1972). These are associated with a decrease in the total number of acetylcholine receptors, as detected by α-bungarotoxin binding (Fambrough et al., 1973), which are probably also reduced in number per unit area of postsynaptic membrane (Ito et al., 1978a).

These changes are responsible for the underlying physiological defect in MG—namely, a pronounced reduction in the sensitivity to acetylcholine (ACh). As a result the effect of each packet or quantum of ACh (the miniature endplate potential) is reduced (Elmqvist et al., 1964) and the effect of nerve impulse-evoked release of 50 or so packets (the endplate potential) is insufficient to activate the muscle (for a fuller description, see Ito et al., 1978b).

Autoantibodies in MG

The concept of MG as an autoimmune disease is not new. For instance, in 1905 Buzzard reported focal aggregations of lymphocytes in affected muscles and suggested the presence of an 'auto-toxic agent'. It was, however, not until 1960 that Simpson formulated a theory based on his observations of 440 cases. Reviewing the high incidence of MG in young females, the involvement of the thymus, and the

Fig. 1 Diagrammatic representation of sections through nerve terminal (NT) expansions in normal and MG endplate. Acetylcholine (small dots) released from the terminal in packets or quanta (large circles) interacts with postsynaptic acetylcholine receptors (AChRs) (filled squares). Trans-membrane ion channels open and the resulting depolarisation, the endplate potential or e.p.p., activates a self-propagating action potential which results in contraction. The spontaneous release of a single packet of ACh produces a small depolarisation called the miniature e.p.p. In MG (bottom) the quanta are normal in size but AChRs are reduced both in total number per endplate and in density per unit area of postsynaptic membrane. This results in a lower sensitivity to ACh with reduced amplitude of both the miniature e.p.p. and the e.p.p.
association of MG with other autoimmune diseases together with the phenomenon of transient neonatal MG in about 12% of the infants born to myasthenic mothers, he suggested that MG was an autoimmune disease caused by antibody to endplate protein and possibly resulting from an infection of the thymus. Although the next few years produced several reports of 'auto-antibodies' in MG (Strauss et al., 1960; Van der Geld et al., 1963; Downes et al., 1966) it was impossible at that time to identify antibodies binding to the endplate itself (for example, McFarlin et al., 1966).

The incidence of autoantibodies in 68 patients with MG seen by Dr J. Newsom-Davis at the National Hospital, Queen Square, London, is shown in Table 1. Although anti-striated muscle antibodies are present in a high proportion of patients they are not specific to MG. They are present in over 90% of patients with MG and thymoma, but also in 30% of patients with thymoma without MG (Oosterhuis et al., 1976). These antibodies, which are detected by immunofluorescent staining of muscle 'A' bands, also cross-react with thymic 'epithelial' cells (Van der Geld and Strauss, 1966) and react with the striations in the myoid cells of the turtle (Strauss et al., 1966). Although proof that immunofluorescent thymic epithelial cells are the same as those which show 'myoid' features in human thymus is lacking, epithelial cells with ultrastructural features of muscle have been described by Henry (1972).

**Table 1: Incidence of autoantibodies in 68 patients with myasthenia gravis**

<table>
<thead>
<tr>
<th>Antibody Type</th>
<th>Incidence</th>
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<tbody>
<tr>
<td>Striated muscle</td>
<td>45%</td>
</tr>
<tr>
<td>Antinuclear factor</td>
<td>22%</td>
</tr>
<tr>
<td>Gastric parietal cell</td>
<td>15%</td>
</tr>
<tr>
<td>Other</td>
<td>21%</td>
</tr>
<tr>
<td>None</td>
<td>46%</td>
</tr>
<tr>
<td>Anti-acetylcholine receptor</td>
<td>89%</td>
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Alpha-bungarotoxin and acetylcholine receptors

The advances in the last few years in understanding the pathogenesis of MG derive from the discovery of a snake toxin that binds specifically and almost irreversibly to nicotinic acetylcholine receptors (AChR) (see Lee, 1972). This polypeptide, α-bungarotoxin (α-BuTx), can be labelled radioactively with little loss of activity and has been used to demonstrate the number and distribution of AChRs in muscle, to assay the AChR during extraction and purification, and to label AChR in crude extracts of muscle for assay of anti-AChR antibodies.

The first extraction and purification of acetylcholine receptors from the electric organ of *Torpedo* and the electric eel occurred in the early 70s. In 1973, Patrick and Lindstrom reported that rabbits injected with purified eel AChR developed paralysis and EMG evidence of neuromuscular block similar to that found in MG. This condition was subsequently termed experimental autoimmune myasthenia gravis (EAMG). Examination of muscles from rabbits immunised against *Torpedo* AChR showed that the miniature endplate potentials were very small and the number of α-BuTx-binding sites was reduced (Green et al., 1975). Antibodies binding to AChR were present in the serum, and it was subsequently shown that rat anti-AChR sera could passively transfer the condition to normal animals (Lindstrom et al., 1976). Moreover, serum containing anti-AChR antibodies blocked ACh sensitivity of neuromuscular preparations in vitro (Green et al., 1975). These observations suggested that anti-AChR antibodies formed against foreign AChR were capable of cross-reacting with the recipient animals' own AChRs at the neuromuscular junction, and stimulated the search for antibodies directed against the endplate AChRs in MG.

**Anti-AChR antibodies in MG**

The first demonstration of antibodies to AChR in MG were somewhat indirect since they relied on the inhibition by sera of the binding of α-BuTx to solubilised or intact muscle preparations (Almon et al., 1974; Bender et al., 1975). However, an IgG was shown to be responsible (Almon and Appel, 1975) and complement fixation in the presence of *Torpedo* AChR was found (Aharanov et al., 1975). Subsequently anti-AChR antibodies were demonstrated in over 85% of MG sera by an immunoprecipitation technique using human muscle extract (Lindstrom et al., 1976). Fig. 2 shows the basis for this assay, which has now been used by several groups with minor modifications (for example, Monnier and Fulpius, 1977; Lefvert et al., 1978). Results with this assay on 68 MG patients are shown in Fig. 3 and compared with 55 controls including patients with other neurological or autoimmune disease. Control subjects, either diseased or normal, have a mean antibody titre of 0·03 ± 0·1 (± SD) nmol of α-BuTx binding sites precipitated per litre of serum. About 85% of MG patients have values above the statistical limits of the control levels.

Because of its specificity, this assay is being used increasingly in the diagnosis of MG. There is, however, one particular group of patients in 25% of whom values lie within the control range. In these patients the disease is restricted to the ocular muscles. Another important feature is the poor cor-
relation between disease activity and antibody levels: some patients with severe disease have low amounts of antibody and yet high amounts are often found in patients whose symptoms have remitted.

**Significance of anti-AChR antibodies in MG**

Despite the fact that anti-AChR antibody appears to be specific to MG there was some initial scepticism about its significance owing partly to the discrepancies noted above and also to the apparent lack of an inhibitor effect of serum on neuromuscular preparations (for example, Albuquerque et al., 1976). The latter objection was overcome when it was shown that daily injections of MG immunoglobulins into mice for several days resulted in weakness, reduced m.e.p.ps, and reduced α-BuTx binding to the endplates—the main features of MG (Toyka et al., 1977). In a different approach it was also shown that plasma exchange, by removing anti-AChR antibody, produced a clinical remission (Pinching et al., 1976) which was associated with a fall in anti-AChR antibody. More important, subsequent deterioration, which occurred after variable intervals, was preceded by a rise in antibody levels (Newsom-Davis et al., 1978). In babies with transient neonatal MG (thought to be caused by placental transfer of a serum factor) anti-AChR is present and declines with a half life of about 10 days.

**Mechanisms of anti-AChR attack on the neuromuscular junction in MG**

The mechanisms outlined in Table 2 and shown in Fig. 4 are those for which there is some evidence in MG and which may account for the reduction in AChRs which underlies the defect of neuromuscular transmission. Cellular attack on the endplate by specifically sensitised cells is probably very infrequent although antibody-dependent attack may occur occasionally (K. Toyka, personal communication). Both IgG, C3 (Engel et al., 1977), and C9 (A. G. Engel, personal communication) have been demonstrated histochemically at the MG neuromuscular junction but the extent of complement-dependent lysis which occurs is a matter of speculation. It has been suggested that complement is responsible for the release of fragments of AChR-rich postsynaptic membrane into the synaptic cleft (Engel et al., 1977). If this is the case it would be interesting to know the final fate of such fragments.

The AChR at the normal neuromuscular junction is degraded and resynthesised at a rate of about 5% per day (Berg and Hall, 1975). One mechanism by which the number of AChRs can be reduced in MG is termed AChR modulation. MG sera were found
Table 2  Mechanisms of anti-acetylcholine receptor antibody attack on the neuromuscular junction

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>EAMG</th>
<th>MG</th>
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<tbody>
<tr>
<td>(1) Antibody-dependent cellular attack</td>
<td>Early stage in rats</td>
<td>Very infrequent</td>
</tr>
<tr>
<td>(2) Complement-dependent lysis of the postsynaptic membrane (PSM)</td>
<td>C3 present on PSM</td>
<td>C3 and C9 present on PSM</td>
</tr>
<tr>
<td>(3) Antibody-induced increase in degradation of endplate AChRs</td>
<td>Shown in culture</td>
<td>Shown in passive transfer</td>
</tr>
<tr>
<td>(4) Direct block of AChR function</td>
<td>Shown in vitro</td>
<td>Infrequent in vitro</td>
</tr>
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(Albuquerque et al., 1976). However, in one recent report reversible reduction in miniature e.p.p. amplitude was found in rat diaphragm exposed to five out of seven Japanese MG sera (Shibuya et al., 1978).

Interestingly, all four mechanisms seem to be operative in the experimental disease EAMG in contrast to the situation in MG (Table 2). For instance, an antibody-dependent cellular attack on the endplate occurs during the early (acute) phase found in rats, and block of neuromuscular function by EAMG sera has been reported in several instances (see Lindstrom, 1979). Therefore one might conclude from these observations that the antigenic specificity and IgG subclass of antibodies against purified AChR, which cross-react with the experimental animals' own muscle AChR, are not necessarily the same as those which bind to the human AChR in MG.

Heterogeneity of anti-AChR

The mechanisms discussed above may not occur in all MG patients to the same extent. Certainly both antibody-dependent cellular attack and direct 'pharmacological' block of receptor activity appear to be infrequent. Thus different mechanisms may be operative in different patients. This together with the poor correlation between disease activity and anti-AChR antibody levels (Fig. 3) suggests that the anti-AChR antibody is not homogeneous. It is clear from the work on EAMG that the AChR has many antigenic sites, and the proportion of antibodies raised against the purified, Triton-extracted, AChR that actually bind to the recipient animal's own muscle AChR is in the region of 1%. In the case of the human disease the nature of the antigenic stimulus is not known (see below), but it is reasonable to suppose that there are several potentially antigenic determinants on the intact membrane-bound receptor. Indeed, there is now some evidence from a number of studies that the anti-AChR antibodies as detected against human or rat solubilised AChR include several antigenic specificities (for example, Lindstrom et al., 1978; Vincent and Newsom-Davis, 1979a) and different IgG subclasses (Lefvert and Bergström, 1978).
Tissue-specific antibodies in myasthenia gravis

Fig. 5 Anti-AChR antibody reactivity with AChR from different muscles. Columns 1, 2, and 3 show the reactivity of five different sera with 125I-α-BuTx-labelled crude extracts of denervated leg muscle (b), human extraocular muscle and mouse muscle expressed as a percentage of the reactivity against a standard leg muscle preparation (ordinate). Column 4 shows the inhibition of α-BuTx binding to leg muscle preparation (a) by each serum, expressed as the number of sites inhibited per litre of serum as a percentage of the number of sites precipitated per litre of serum. Abscissa: absolute values for anti-AChR antibody (nmol/l) measured against leg muscle (a) extract.

Results from the binding of five different sera to three different muscle extracts are compared in Fig. 5. The absolute value of the anti-AChR antibody as detected against leg muscle extract (a) are given along the abscissa. It is worth mentioning that all the patients had severe generalised disease except the one with the highest anti-AChR titre, in whom weakness was barely detectable. The columns give the reactivity of the sera against three different extracts as a percentage of the stated values. There was very little difference in the titre when a different leg muscle extract (leg b) was used, but the degree of cross-reactivity with the AChR in human ocular muscle extract was quite variable. Similarly, one of the sera precipitated AChR extracted from mouse muscle to the extent of 40% of anti-human muscle values, but the others much less so. The final column indicates the proportion of antibodies that appear to be directed against the α-BuTx binding site on the human receptor. The reactivity with this site on the receptor also varies considerably between sera and is not related to the total antibodies detected by immunoprecipitation.

One way of approaching the problem of the number of antigenic sites recognised by anti-AChR antibodies is to look at the size of soluble antigen-antibody complexes. Antibody-AChR complexes formed in 20-50 fold excess of antibody were analysed by gel-filtration on Sepharose 4B. Reproducible differences in the gel-filtration profiles for different sera were found, indicating that the number of antibodies which can bind to each AChR molecule differs (Vincent and Newsom-Davis, 1979a). Another approach is to examine the isoelectric point of either the antibodies or the complex of antibody and antigen. Using a one to one ratio of antibody to 125I-α-BuTx-AChR, most sera gave multiple ill-defined peaks of radioactivity when isoelectric focusing was performed between pH 3-5 and 9-0 (Fig. 6). Only a few sera gave one or two well-defined peaks suggesting the presence of a limited number of anti-AChR antibodies.

Fig. 6 Isoelectric focusing of antibody-125I-α-BuTx-AChR complexes formed at a ratio of <1 (B) and 1:1 (C, D, E). Most sera give ill-defined peaks (e.g., D and E) but one (C) gave two well-marked peaks of antibody-AChR complexes.

The antigenic stimulus in MG

Since there appears to be heterogeneity of anti-
AChR antibodies in most patients it seems unlikely that these antibodies arise as the result of the uncontrolled proliferation of a single B cell clone. They probably result from a breakdown in tolerance either to normal muscle AChR or to an altered AChR-like protein. It has been suggested, for instance, that viral modification of membrane proteins may be responsible (Datta and Schwartz, 1974), and there have been some recent attempts to implicate virus infection in MG (for example, Tindall et al., 1978), although in some studies the level of immunity to virus was not abnormal in MG patients (Smith et al., 1978).

Whatever the stimulus, any theory of the aetiology of MG must take into account the role of the thymus. Thymic hyperplasia with germinal centre formation occurs in 65% of patients and about 15% of the thymus glands removed have a thymoma. In the thymus gland there are cells, probably epithelial in origin, which react with anti-striated muscle antibody (Van der Geld and Strauss, 1966) and muscle-like cells are found in some adult human glands (Henry, 1972). Moreover, cultured thymic epithelial cells develop morphological features of muscle cells (Wekerle et al., 1975) and physiological and biochemical evidence of acetylcholine receptors (Kao and Drachman, 1977b). These findings strongly suggest that AChR may be present on muscle-like cells in the myasthenic thymus. Attempts to demonstrate the presence of AChR in the adult gland, however, have not been entirely convincing, and the presence of the antigen in the human thymus remains a controversial issue (Engel et al., 1977; Nicholson and Appel, 1977). One finding which tends to support it, nevertheless, is that the thymus often contains substantial amounts of anti-AChR, and cultured thymic lymphocytes spontaneously synthesise anti-AChR in culture (Vincent et al., 1978a). This suggests that antigen is localised in the thymus though it does not indicate how it might have arrived there.

One interesting observation is that lymphocytes derived from glands in which a thymoma was present have not been found to synthesise anti-AChR antibody in culture (Vincent et al., 1979). This is surprising, since patients with thymoma usually have high titres of antibody. Thymoma cases, however, do not benefit in general from thymectomy and their anti-AChR antibody titres tend to remain static after the operation (Fig. 7). In contrast, patients with hyperplastic glands tend to improve after the operation and this is associated with a fall in anti-AChR (Vincent et al., 1979). Perhaps, therefore, the site of the antigen localisation and antibody formation is different in thymoma cases. Other differences between thymoma and non-thymoma cases are summarised in Table 3.

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![Figure 7](http://jcp.bmj.com/)  
**Fig. 7**  
Fall in anti-AChR titres associated with various treatments for MG. Thymectomy for thymoma and hyperplasia; azathioprine therapy; prednisone therapy; withdrawal of penicillamine in penicillamine-induced MG; and fall in infant anti-AChR levels in one case of neonatal MG due to placental transfer of maternal antibody. Number of patients in brackets. (Reproduced from Plasmapheresis and the Immunobiology of Myasthenia Gravis, edited by P. C. Dau, 1979, by permission of the publisher, Houghton Mifflin, Boston.)

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**Myasthenia associated with D-penicillamine treatment**

A form of MG develops in a small proportion of patients undergoing treatment with D-penicillamine for rheumatoid arthritis or other diseases. More than 20 such cases have been reported (see Bucknall, 1977) and anti-AChR antibodies have been present in the

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**Table 3**  
Distinctive features of thymoma cases

<table>
<thead>
<tr>
<th>Feature</th>
<th>Source</th>
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<tbody>
<tr>
<td>HLA-B8 low incidence</td>
<td>Oosterhuis et al., 1976</td>
</tr>
<tr>
<td>Anti-striated muscle antibody positive &gt; 95%</td>
<td>Oosterhuis et al., 1976</td>
</tr>
<tr>
<td>Anti-AChR antibody levels high</td>
<td>Vincent et al., 1979</td>
</tr>
<tr>
<td>Normal number of B cells in thymus</td>
<td>Lisak et al., 1976</td>
</tr>
<tr>
<td>No anti-AChR antibody synthesis by thymic lymphocytes</td>
<td>Vincent et al., 1979</td>
</tr>
<tr>
<td>Little change in anti-AChR levels after thymectomy</td>
<td>Newsom-Davis et al., 1979</td>
</tr>
<tr>
<td>Good response to immunosuppression</td>
<td></td>
</tr>
</tbody>
</table>
few tested (for example, Vincent et al., 1978b). Of particular interest was the fact that anti-AChR antibody levels dropped rapidly after stopping penicillamine treatment in three cases reported recently (Vincent et al., 1978b). The rate of fall of anti-AChR was 50% in 45 to 60 days. Attempts to reproduce this condition in experimental animals by treatment with penicillamine has so far been unsuccessful (see Russell and Lindstrom, 1978), but the temporary nature of the myasthenia gravis suggests that the drug has a direct reversible effect on the immune system.

Absence of anti-AChR in congenital MG

Weakness is detectable at birth in about 1% of MG patients. It is relatively stable and does not respond well to immunosuppressive measures, although anticholinesterase treatment is usually beneficial. This congenital form of the disease does not appear to be associated with anti-AChR antibodies (Vincent and Newsom-Davis, 1979b) and is probably due to a congenital defect at the neuromuscular junction (Cull-Candy et al., in preparation). A further form of infantile myasthenia (see Fenichel, 1978) is characterised by severe respiratory and feeding difficulties neonatally or during infection. This form tends to remit spontaneously and serological investigations have not yet been reported.

Discussion

There seems to be good evidence that the anti-AChR antibodies measured by immunoprecipitation of extracted muscle bear a causal relationship to the defect at the neuromuscular junction. That is not to say that the assay measures all the antibodies present, and possibly some patients have antibodies to other functionally important endplate structures in addition to or instead of those binding to the AChR. Indeed, there are some patients (less than 5%) with typical generalised MG and hyperplastic thymus glands who have no detectable antibodies by any of the methods available at present. Some new form of endplate solubilisation may be helpful in assaying for antibodies in these patients. In most cases, however, the anti-AChR antibody assay described can be used to follow the progress of the disease and is a useful adjunct to clinical assessment during various forms of treatment.

The strong inverse relationship between anti-AChR levels and clinical state during and after plasma exchange (see Newsom-Davis et al., 1978) is not so easy to demonstrate during longer-term therapeutic procedures, and there have been no detailed correlations as yet. Fig. 7 shows mean anti-AChR levels from a number of patients undergoing various forms of treatment. There is substantial variation between the response of different patients, but in general a decline in antibody is associated with clinical improvement (see, for instance, Newsom-Davis et al., 1979). Clearly the mean rate of fall of antibody varies with different forms of treatment. It should be interesting to look at the effect of immunosuppression on the synthesis of anti-AChR antibody by cultured lymphocytes. The spontaneous synthesis of antibody by MG thymic lymphocytes in culture (Fig. 8a) and the pokeweed-stimulated synthesis by MG peripheral lymphocytes (Fig. 8b) (Clarke et al., 1979) should provide a suitable in-vitro system for this sort of purpose.

One puzzling feature of MG is the pronounced ocular symptoms shown by many patients. Ocular symptoms often present first even in cases that progress to generalised weakness. Some patients have disease restricted to ocular muscles, and this group tends to have low or control antibody levels as detected by the standard assay. On the other hand, there are a few patients in whom ocular signs are
slight or absent. One explanation for these phenomena could be that the antigenic nature of the extraocular muscle AChR is different from that of limb muscle, with the result that involvement of eye muscles depends on the reactivity of anti-AChR antibodies with determinants that are not shared with limb muscle AChR. With this in mind, the reactivity of MG sera with AChR preparations from both leg and eye muscle was compared (Vincent and Newsom-Davis, 1979b). Most sera showed higher reactivity with leg muscle AChR but about 10% reacted preferentially with AChR in ocular muscle extracts (for example, see Fig. 5). However, there was no clear correlation between the results and the degree of eye involvement, and the incidence of extraocular weakness is probably related at least partly to the nature of these muscles, which have a high proportion of slow fibres (Hess and Pilar, 1963).

One of the problems for the future is to establish the relationship between genetic factors, the antigenic stimulus, and the source of antibody diversity in this disease. Wekerle and Ketelsen (1977) think there are two potential stages at which genetic factors could play a part—firstly, the development of muscle-like cells bearing AChR in the thymus, and, secondly, the ability to mount an autoimmune response against the AChR. The association of HLA-B8 with young female MG patients (Oosterhuis et al., 1976) might be related to the first, since the thymus glands from these patients usually contain an excess of B-lymphocytes (Lisak et al., 1976) and synthesise anti-AChR antibody in culture (Vincent et al., 1978a). This suggests that the hyperplastic thymus contains a source of antigenic stimulus and contrasts with the findings in thymomatous glands (see Table 3). On the other hand, the spectrum of antibodies produced in each patient might be related to the presence of particular immune response genes, and there is preliminary evidence of a significant correlation between the presence of HLA-DRW2 and low titres of anti-AChR antibody against various AChR preparations (Compston et al., in preparation).

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