

Anti-acetylcholine receptor antibodies

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SUMMARY Early suggestions that a humoral factor might be implicated in the disorder of neuromuscular transmission in myasthenia gravis have been confirmed by the detection of anti-AChR antibody in 85-90% of the patients with generalised disease and in 75% of cases with restricted ocular myasthenia. Plasma exchange reveals that serum anti-AChR usually has an inverse relationship to muscle strength and present evidence indicates that patients responding to thymectomy and immunosuppressive drug treatment usually show a consistent decline in serum anti-AChR titres. The antibody is heterogeneous and can lead to a loss of muscle AChR by several mechanisms. Anti-AChR is produced in the thymus in relatively small amounts. Anti-AChR antibody synthesis by thymic lymphocytes and pokeweed stimulated peripheral lymphocytes in culture provides a means of studying the effect of different lymphocyte populations *in vitro*. Analysis of clinical, immunological and HLA antigen characteristics in MG suggest that more than one mechanism may underlie the breakdown in tolerance to AChR, leading to the production of anti-AChR antibodies.

The prescience of Professor Iain Simpson's paper¹ in 1960 which formulated the hypothesis that myasthenia gravis (MG) was an autoimmune disorder has been amply confirmed. It is now known that antibody directed against 'end-plate protein' is present in the serum of patients with this disease, and that this antibody is directly implicated in the disturbance of acetylcholine receptor (AChR) function which underlies the disorder of neuromuscular transmission. The myasthenic illness that affects one in eight of babies born to MG mothers^{2,3} has also now been shown, as Simpson predicted, to be associated with placental transfer of maternal anti-AChR antibody,⁴ leading to transient disorder of neuromuscular transmission in the infant.

The idea that a circulating factor might be responsible for the muscle weakness of myasthenia goes back at least to the turn of the century. Campbell and Bramwell,⁵ whose account of the clinical features of MG can hardly be bettered, suggested that 'a toxin, probably of microbic origin' circulated in the blood and acted selectively on the lower motor neurone so as to modify its activity. The possibility that this was an 'autotoxic agent' was put forward by Buzzard⁶ in 1905. The similarity of the symptoms of MG to those of curare poisoning led

Mary Walker⁷ to try the effects of physostigmine, the anti-cholinesterase antidote to curare poisoning. Coincidentally, this was in the same year that Dale and Feldberg reported the chemical nature of neuromuscular transmission.⁸ The dramatic clinical improvement that Mary Walker observed in response to physostigmine is now well known. In the following year Lindsley⁹ confirmed the response to anti-cholinesterase treatment by electromyographic recording, demonstrating that the marked fluctuations in the single motor unit action potentials were restored to a uniform size after an injection of prostigmine.

Subsequently, animal experiments directed at demonstrating a circulating factor in MG gave rise to conflicting results. Wilson and Stoner¹⁰ maintained that MG sera contained a factor that blocked transmission in the frog neuromuscular preparation. However, Lammers and Van Spijk¹¹ could not confirm this either in the frog or in several other species, and further studies by Nastuk *et al*¹² indicated that Wilson and Stoner's finding could be explained by the cytolytic effect exerted by MG sera on frog's muscle. A few years later Parkes and McKinna¹³ studied the effect on muscle contraction in the rat of intraarterial injection of the crude globulin fraction of MG sera, and concluded that MG sera contained a blocking factor in the globulin

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fraction although they had no evidence that this was an antibody. In experiments similar to those of Parkes and McKinna, Namba and Grob¹⁴ obtained more variable results. Indeed, when they injected myasthenic serum, which had high levels of anti-striated muscle antibody binding activity, into other myasthenics it produced an increase in the amplitude of the evoked action potential in the hand, suggesting that the serum of MG patients might contain a substance which improved neuromuscular transmission in the disease.

Removal of circulating components provided an alternative strategy for investigating the presence of a causative humoral factor in MG. Haemodialysis in five MG patients¹⁵ caused an immediate but transient improvement. This procedure removes substances of low molecular weight and would not therefore have depleted the serum of immunoglobulins. These results thus tended to confirm the presence of a circulating factor, although the high dose of anticholinesterase treatment in some of the patients raises the possibility that haemodialysis was treating a state of partial cholinergic paralysis. More convincing evidence came from the study of Bergström's group¹⁶ on the effects of thoracic duct drainage, which depletes the individual of immunoglobulins and lymphocytes after they have passed through the lymphatic system. Clinical improvement always followed within 1–4 days of initiating drainage, and symptoms returned rapidly when the cell-free lymph was reinfused, implying that a humoral factor, possibly an immunoglobulin, might underlie the effect.

Antibodies to striated muscle

The first demonstration of a tissue specific antibody related to MG came in the same year that Simpson's hypothesis was published. Strauss and his colleagues¹⁷ showed the existence of antibodies which bound to the striations of skeletal muscle sections, and fixed complement in the presence of muscle extracts. These anti-striated muscle antibodies (anti-SM) were found in about 30% of all MG patients, and in virtually all of those with a thymoma. Anti-SM is also present, however, in about 0.2% of control patients¹⁸ and in 25% of thymoma cases without MG.¹⁹

Vetters²⁰ discriminated between antibodies reacting with the A and I bands and thought that only anti-A band antibody was specific to MG. However, recent analysis of staining patterns on glycerinated guinea pigs myofibrils by Peers *et al.*,²¹ has shown the presence of several

different specificities in MG sera and also in sera from some patients undergoing pencillamine treatment for rheumatoid arthritis who did not have any symptoms or clinical evidence of MG. It seems that, as is the case for anti-AChR (see below), anti-SM antibody may turn out to be a heterogeneous population. Although there is no reason to implicate anti-SM in the disturbance of neuromuscular transmission its ability to react with epithelial and myoid cells in the thymus²² has helped to emphasise the possible role of these cells in the aetiology of MG.

Anti-acetylcholine receptor antibodies

The existence of antibodies that react with the 'end-plate protein' as first suggested by Simpson was demonstrated in 1973 when Almon, Andrew and Appel²³ showed that serum globulins from MG patients could inhibit α -bungarotoxin binding to solubilised rat acetylcholine receptors. The success of this and future assays lay in the use of α -bungarotoxin (α -BuTx) as a specific, and essentially irreversible, label for AChR.²⁴ α -BuTx is a polypeptide from the venom of *Bungarus multicinctus* which blocks the action of acetylcholine on the postsynaptic AChRs both *in vivo* and *in vitro*. Its action is essentially irreversible, and radioactively labelled toxin binds almost exclusively to the end-plate region of normal muscle where the AChR is localised. In denervated muscle, when AChRs appear over the entire surface of the muscle fibre, α -BuTx binding is also found throughout the muscle fibres.²⁵ This specificity, together with the fact that α -BuTx binding is retained by solubilised AChRs, has made it an invaluable tool in all aspects of AChR and myasthenia gravis research.

The original paper of Almon *et al* was followed by a more detailed account where it was shown that the factor inhibiting α -BuTx binding was an immunoglobulin and that α -BuTx-labelled AChR with bound IgG could be precipitated by anti-human IgG from crude detergent extracts of rat denervated muscle.²⁶ At the same time Bender and his colleagues,²⁷ by using a sandwich technique consisting of α -BuTx, anti- α -BuTx antibodies and peroxidase-labelled anti-rabbit IgG, were able to show a similar inhibition by MG sera of α -BuTx binding to frozen sections of human endplates. Another early demonstration of anti-AChR antibodies was that of Aharanov *et al.*,²⁸ who found complement fixation by MG sera in the presence of purified Torpedo AChR in 85% of patients.

In an attempt to assess the best means of demonstrating anti-AChR antibodies Mittag,

Kornfeld, Tormay and Woo²⁹ compared four different techniques and found that immunoprecipitation of α -BuTx-labelled AChR was the most effective. This technique, as described by Lindstrom,³⁰ has now been used by many other workers and is established as the simplest and most quantitative measurement of anti-AChR antibodies currently available.

Muscle membranes are extracted in detergent, usually Triton X-100, and the solubilised proteins are exposed to a saturating concentration of 125-I- α -BuTx. Serum or IgG is added and any complexes of IgG and AChR formed over a two to 16 hour period are precipitated by addition of antihuman IgG. The presence of AChR in the precipitate can be determined by counting the I-125- α -BuTx bound to it. Control incubations are usually formed by adding an excess of a competitive inhibitor of 125-I- α -BuTx binding before the radioactive toxin is added. This controls for non-specific trapping of radioactivity in the IgG-anti-IgG pellet. The results are usually expressed in terms of the number of α -BuTx binding sites precipitated per litre of serum.

The first detailed assessment of anti-AChR antibodies in MG patients and controls was published by Lindstrom and his colleagues.³¹ They found values between 0 and 844 nmoles of α -BuTx binding sites precipitated per litre of serum in 71 MG patients. Control values were all below 0.6 nmoles/litre. There was however no particular correlation between anti-AChR antibody values and the severity of the disease and no significant correlation of antibody titre with age, sex, steroid therapy or duration of symptoms. Similar findings have been reported by others,³²⁻³⁵ though the range of anti-AChR titres, and the values found in control patients tend to reflect variations in the experimental technique. Several studies have, for instance, used partially purified human³⁶ or rat denervated muscle extracts.³⁷ The use of rat AChR, rather than human, tends to result in a high proportion of negative values,³⁷ and in general, it appears that any attempt to purify the AChR preparation tends to result in a loss of antigenicity as shown by reduced anti-AChR titres.³⁶

Antibodies in MG sera binding to AChR in the rat diaphragm have also been demonstrated by indirect immunofluorescence, using fluorescein isothiocyanate-labelled anti-human Ig.³⁸ The end-plate was located by tetramethylrhodamine isothiocyanate-labelled anti- α -BuTx. As with the immunoprecipitation method, positive results were confined to MG patients, but the proportion of positive results was low (12 to 57 patients). In

some cases the negative results could be attributed to interference from anti-SM antibody.

Although across individuals there is no strong correlation between anti-AChR antibody titre and clinical state (fig 1), it has been consistently found that patients whose symptoms are confined to extraocular muscles tend to have low antibody titres, substantially lower than in many cases who are in clinical remission.^{31 33 35} This suggests that the antibodies in these patients may be different in nature, and we now have some evidence for this (see below).

The highest percentage of positive values in MG patients has been obtained by the immunoprecipitation assay described above, but there may be patients in whom this assay is inadequate because their anti-AChR antibodies react only with the α -BuTx binding site which is unavailable in the routine assay where α -BuTx is already bound. This situation is not normally important because antibodies interacting with the α -BuTx binding site are usually a proportion, and often a very small one, of the total antibody population in the patient's serum³⁴ (fig 2). However, it has been claimed that when rat denervated receptor

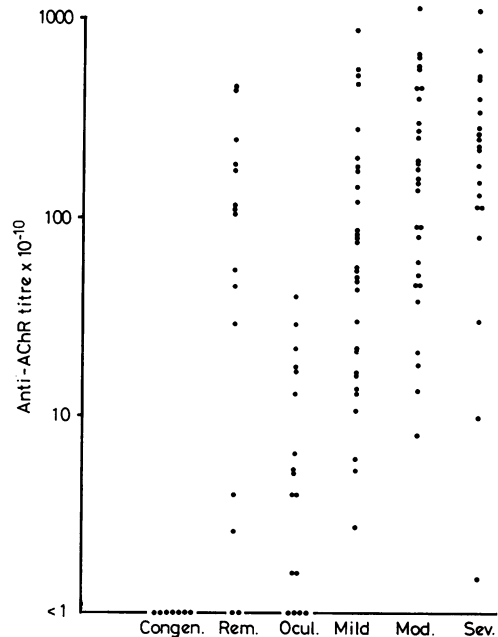


Fig 1 Anti-AChR antibody titres in a group of seven patients with congenital myasthenia, and 111 patients with myasthenia gravis, of whom 15 were in remission, 17 had disease confined to ocular muscles, and the remaining 79 had mild, moderate or severe generalised disease.

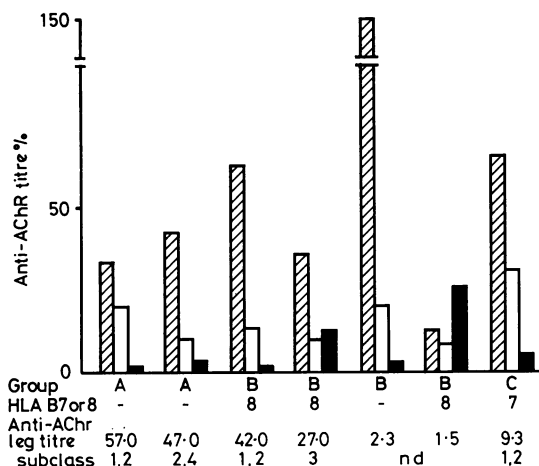


Fig 2 Heterogeneity of anti-AChR antibodies in different individuals. Columns represent reactivity of antibodies against human extraocular muscle AChR (hatched), mouse AChR (open) and the α -BuTx binding site of human leg AChR (black), as a percentage of reactivity with ^{125}I - α -BuTx-AChR from human leg muscle (values). Other findings in each patient are also given. Group A, thymoma; B under 40 years at onset; C over 40 years at onset. Subclass denotes the predominant IgG subclass of the anti-AChR antibody measured with leg muscle.

was used in the conventional immunoprecipitation assay, some patients' antibody titres were substantially underestimated, and in one patient's serum anti-AChR antibody was only measurable when the technique was modified to detect antibodies binding to the α -BuTx site.³⁹

Anti-AChR associated with pencillamine treatment

During the last few years several reports have described patients who developed symptoms of MG during treatment with pencillamine, either for rheumatoid arthritis or for Wilson's disease.⁴⁰ Withdrawal of the drug was associated with clinical improvement in most cases. Anti-AChR antibody has been found in the sera of several such cases⁴¹⁻⁴⁴ and in most studies anti-AChR levels were found to fall rapidly when the pencillamine was withdrawn.

The incidence of MG among pencillamine treated patients appears to be very low but in a study of 54 rheumatoid arthritis patients on pencillamine we have found two with raised anti-AChR titres although neither showed signs of MG.⁴⁵ Many other immunologically based conditions have also been reported during pencillamine treatment,⁴⁶ and most resolve spontaneously when

the treatment is stopped. It seems likely that pencillamine disturbs the maintenance of tolerance in some way but attempts to test this by treatment of experimental animals with long-term pencillamine have not as yet been successful (for example see reference 43).

Anti-AChR in congenital myasthenia

Measurement of anti-AChR antibody has helped to distinguish a group of patients with myasthenia whose symptoms present at or close to birth. We were unable to detect anti-AChR antibody in six patients of this group,⁴⁷ nor did these patients have other auto-antibodies or evidence of an immune disorder. Further studies have indicated that the pathogenesis of their disease is heterogeneous and differs from that of acquired MG.⁴⁸

Heterogeneity of anti-AChR antibodies in MG sera

The AChR as it exists in detergent solution is a fairly large macromolecule, which probably contains four α -BuTx binding sites and four different kinds of subunit. It has been shown that antibodies raised in rats against purified subunits of Torpedo AChR do not cross-react well with the other subunits, suggesting that there is a large degree of antigenic difference between the four subunits (see Lindstrom, page 569). These anti-Torpedo subunit antibodies also react to a limited extent with human Triton-X100 solubilised AChR. It is therefore reasonable to suppose that there are several antigenic domains on the extracted human AChR and that the human anti-AChR in MG sera may contain several antigenic specificities.

The number of different anti-AChR antibodies which can simultaneously react with a solubilised AChR molecule has not been determined accurately, but we found that when AChR was exposed to an excess of anti-AChR the size of the resulting complex was quite variable, suggesting that different sera contained different numbers of anti-AChR antibodies.³⁴ We also tried to enumerate the number of different antibodies by isoelectric focusing of α -BuTx-AChR-IgG complexes (formed at limiting anti-AChR concentrations). Only in one patient were we able to show a clearly defined peak suggesting, perhaps, the presence of one "clone" of antibody producing cells. Other patients gave a poorly resolved pattern. Lefvert and Bergström⁴⁹ analysed the elution profiles of several MG IgG preparations after isoelectric focusing and found broad peaks of anti-AChR activity, including bands of IgG which apparently enhanced

α -BuTx binding, and others which inhibited it (see below). Thus isoelectric focusing has not, as yet, satisfactorily demonstrated the number and diversity of anti-AChR antibodies, although it has confirmed that the antibody is polyclonal in most patients. Using subclass specific antisera we have found that most patients have anti-AChR distributed within subclass 1, 2 and 4, but occasionally subclass 3 predominates (Vincent, Lang and Newsom-Davies, unpublished (fig 2)). This contrasts with Lefvert and Bergström's finding since a large proportion of their (Swedish) patients had anti-AChR antibody exclusively or mostly in IgG subclass 3. This was reflected in a high rate of turnover of the antibody studied during thoracic duct drainage.⁵⁰

Mittag and his colleagues⁵¹ raised the possibility that two forms of AChR exist in denervated rat muscle extracts, when they showed that some MG sera prevented a proportion (about 50%) of the AChR from binding to Concanavalin A-sepharose. The ability to inhibit AChR binding to Concanavalin A was not found with all sera. This suggests the presence of at least two different antigenic specificities among anti-AChR antibodies.

The heterogeneity of MG anti-AChR is convincingly demonstrated by the enormous variability in the cross-reactivity of the antibodies with different AChR preparations. This was first shown by Lindstrom and his colleagues who studied the reactivity of 24 MG sera with AChR from different species, including squirrel monkey, rat, a non-fusing mouse cell line, electric eel and Torpedo.⁵² The degree of anti-AChR crossreactivity with each AChR preparation varied considerably between different sera. We have found similar variability in the titre of anti-AChR (fig 2). This variability is not limited to inter-species differences, however, since we found that the titre of anti-AChR when tested against human extraocular muscle was not the same as that found when sera were tested against two different human leg muscle extracts.^{34 35} (fig 2).

One possibility is that leg muscle preparation from amputated limbs may be at least partially denervated by ischaemic changes and coexisting peripheral neuropathy. Indeed, an antigenic difference between rat normal and denervated muscle AChR was first indicated by Almon *et al.*,²⁶ when they found that MG sera did not inhibit α -BuTx binding to normal rat muscle AChR. This observation has recently been confirmed by Weinberg and Hall who found that eight out of ten MG sera reacted preferentially

with denervated rat muscle AChR, but contrasts with the observation that anti-AChR antibodies raised in experimental animals against eel, Torpedo or rat denervated receptors do not distinguish between normal and denervated rat receptor.⁵³

The nature of antigenic domains on the AChR which are responsible for the variable reactivity of anti-AChR antibodies, and the degree to which this is dependent on the varying affinity of the antibodies is not known. However, similar results are found when the reaction of antibody with the α -BuTx binding site on the receptor is investigated. In their original paper Almon *et al* found that about 33% of MG sera inhibited the binding of α -BuTx to rat denervated receptor.²³ Bender *et al*²⁷ found that their assay on frozen sections of human endplates was positive in about 44% of patients. If the ability of MG sera to inhibit α -BuTx binding is quantified, however, it is found that the proportion of antibodies inhibiting α -BuTx binding as a proportion of those which do not interfere with the α -BuTx binding site varies from 0.1—25%³⁴ (fig 2), and there has been at least one report of an MG serum which inhibited α -BuTx binding to rat AChR but did not bind to the α -BuTx-labelled AChR.³⁹

In studying the heterogeneity of anti-AChR antibodies in MG sera we have hoped to correlate our findings with some aspect of the disease, either as regards its clinical severity, the age and sex of the patient, or the thymic pathology. However, to date we have not been able to find any consistent pattern of antibodies either in their subclass or their reaction with various mammalian AChR preparations, which correlate with the clinical status of the patient. Indeed the variability of anti-AChR characteristics among patients within each category (fig 2) greatly exceeds the variability between categories for example (fig 3). The only indication of a group of patients whose antibodies can be distinguished from others is that of those patients whose symptoms are confined to ocular muscles. These patients have the lowest mean antibody titre when measured against human limb muscle and in about 25% of cases values are within the control range.³⁵ However, their sera react equally well with ocular muscle AChR (fig 3), unlike the majority of patients with generalised disease in whom anti-AChR titres against ocular muscle receptor are generally lower than those against limb muscle. This observation not only indicates that antigenic differences between limb and ocular muscle underlie some of the selec-

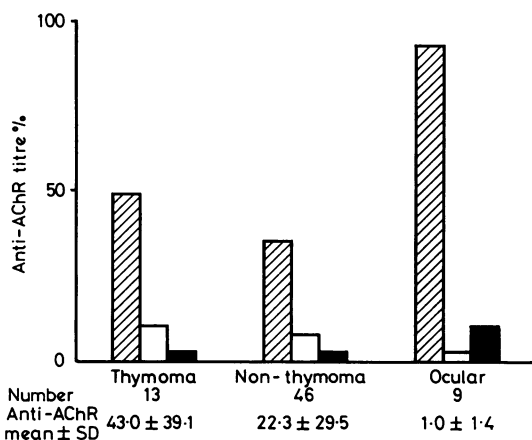


Fig 3 Heterogeneity of anti-AChR in different groups of patients. Symbols as in fig 2. Columns represent mean values for each group.

tivity of myasthenia for the ocular muscles, but also suggest that the nature of the antibodies in ocular patients may be different.

Significance of anti-AChR antibody in pathogenesis of MG

The pathological significance of anti-AChR antibody in MG sera was not immediately acknowledged, partly owing to the poor correlation which was found between antibody levels and the patient's clinical state, and the possibility was considered that the antibody might have a protective function.²⁸ Against this was the observation that thoracic duct drainage results in a short-term clinical remission which can be rapidly reversed by reinjecting the cell-free lymph.¹⁶ This clear demonstration of a role for a humoral factor in MG gained further support when Toyka *et al*⁵⁴ showed that daily injection of MG immunoglobulin into mice resulted in weakness in some animals, reduced miniature endplate potential (mepp) amplitudes and a decreased number of AChRs as shown by reduced α -BuTx binding.

The response of MG patients to plasma exchange,⁵⁵ which reduces serum immunoglobulin, was also consistent with a pathological role for anti-AChR antibody, with a 1 to 5 day time-lag similar to that seen in thoracic duct drainage. Of more importance was the observation that the subsequent rise in anti-AChR antibody after completion of a course of exchanges was associated with clinical deterioration. Muscle strength showed a clear inverse relationship with anti-AChR⁵⁶⁻⁵⁸ but not with other measured constituents such as total IgG and C3, thus supporting

a significant role for anti-AChR in the pathogenesis of the muscle weakness in MG.

The findings in neonatal MG further implicates anti-AChR antibody in myasthenic weakness. In infants born to MG mothers, serum anti-AChR at birth may be similar to that in the mother,⁴ from whom the antibody is derived by placental transfer. Anti-AChR then declines with a $t_{1/2}$ of about ten days^{41, 42} in parallel with clinical improvement. Recent evidence suggests that a factor in amniotic fluid, probably alpha-fetoprotein, can interfere with antibody binding to AChR and this may have a protective function in the infant.⁵⁹

Pathological mechanisms and anti-AChR antibody

There appear to be several mechanisms by which anti-AChR antibody leads to altered neuromuscular transmission and to a reduction in the number of functioning AChRs. Engel and his colleagues (see page 580) have shown that IgG is present on the postsynaptic membrane, and that its distribution follows that of AChR. Components of the complement system (C3 and C9) have also been shown to be present, and this is consistent with the view that a complement-mediated autoimmune destructive process occurs at the postsynaptic membrane, leading to loss of AChR and the characteristic morphological changes at the endplate.

The second mechanism by which anti-AChR antibody causes loss of AChR is through the accelerated degradation that follows cross-linking of the AChRs by antibody (see Drachman *et al*, page 603). However, in the mouse passive transfer model in which accelerated degradation has been observed following MG IgG injection, we have found that the loss of AChR is not a simple function of the amount of antibody bound.⁶⁰ Repeated daily intraperitoneal injections of IgG preparations from some MG individuals, for example, resulted in most of the extractable AChR having antibody bound, and was associated with an increase in the rate of AChR degradation and with a decrease in the total number of AChRs. IgG from other individuals which resulted in a similar amount of antibody binding to AChR had, in contrast, no influence on the rate of degradation or on the total number of AChRs. Moreover, some sera increased the rate of AChR degradation but did not lead to a significant decrease in the number of AChRs, suggesting that binding of antibody to AChR can accelerate resynthesis of AChR as well as increase the rate of degradation. Thus

although antibody binding to receptor may increase the rate of degradation it can also lead to an increased rate of resynthesis, and the balance between these two effects will determine the net number of receptors remaining.

A further way in which anti-AChR antibody could interfere with neuromuscular transmission is by a pharmacological 'block', in which antibody either binds at the ACh site or nearby, and thus interfere with ACh access. Shibuya *et al*¹⁶ demonstrated in the rat neuromuscular preparation that MG sera could induce a variable reduction in miniature endplate potential amplitude. The role of complement in this action was not defined. It seems unlikely that a substantial reversible block occurs *in vivo* since procedures which deplete serum of anti-AChR antibody, such as thoracic duct drainage and plasma exchange, do not normally produce any improvement for at least 24 hours, with a peak response occurring several days after the last exchange. This time lag presumably reflects the time taken for new AChR to be synthesised and deinserted into the postsynaptic membrane, free of anti-AChR antibody.

Anti-AChR antibody production

The involvement of the thymus in MG, in particular the striking changes of hyperplasia (thymitis) seen predominantly in the younger myasthenic, led us to investigate whether the thymus was a site of significant anti-AChR antibody production. The thymus is already known to be a site of antigen (AChR). Myoid (muscle-like) cells have been identified in the human thymus⁶² and tissue culture of the gland can yield muscle cells which bear AChR.⁶³ Thymic epithelial cells also appear to contain AChR.⁶⁴

Our initial culture studies⁶⁵ showed that thymic lymphocytes from patients with thymic hyperplasia could synthesise antibody in culture. In a larger subsequent study of 35 patients who underwent thymectomy, 17 of 23 patients showing thymitis spontaneously produced anti-AChR in culture, rates of synthesis ranging from 1–42.5 fmoles/10⁶ cells/24 hours. In two patients with thymitis whose thymic cells did not synthesise antibody in culture, the addition of pokeweed mitogen led to production of significant amounts of antibody. In contrast, thymic cells from the 7 patients with thymoma did not synthesise anti-AChR.⁶⁶

A significant positive correlation was observed in the non-thymoma cases between the duration of the illness and the rate of anti-AChR synthesis in culture, and there was a trend for the most

marked changes of thymitis to be found in those with the longest duration of disease, as some⁶⁷ but not all⁶⁸ studies have shown.

The rates of anti-AChR synthesis in culture do not suggest that the thymus is a major site of anti-AChR production even in those showing the highest rates of production. At best, the thymus would be producing about 20 pmoles/24 hours which would represent less than 1% of the synthesis requirements to maintain a serum anti-AChR level of 20 nmoles/litre constant assuming a $t_{1/2}$ of 20 days. Even accepting that antibody synthesis rates *in vivo* are likely to be higher than those *in vitro*, it seems unlikely that the thymus makes more than a small contribution to the total body production of the antibody. Moreover, therapeutic removal of the thymus does not lead to any consistent effect on serum anti-AChR antibody levels in those showing the highest rates of synthesis *in vitro*. Thus the clinical benefit that has often been reported following thymectomy,^{67, 69, 70} particularly in the thymitis cases, does not seem to depend simply on removal of a major site of antibody production. Furthermore, the correlation we have found⁶⁶ between the rate of antibody synthesis by thymic cells and the duration of the disease raises the possibility that involvement of the gland is a secondary event in the disease process.

Peripheral blood lymphocytes (PBL) in culture will also synthesise anti-AChR antibody, but usually require pokeweed mitogen stimulation in contrast to thymic cells.⁷¹ Synthesis can be demonstrated both in non-thymomatous and thymoma cases. Co-culture of PBL with autologous thymus cells that have been subjected to irradiation (1000 rads), which abolishes antibody synthesis and suppressor function,⁷² can enhance antibody production without the need for mitogen stimulation (Newsom-Davis, Willcox, Calder and Vincent, unpublished observations). This is illustrated in fig 4 in which irradiated thymus cells from a patient whose gland showed changes of hyperplasia have been co-cultured at different cell ratios with autologous PBL. This effect might be due to thymus T helper cells or the presence of antigen or both. Irradiated PBL may similarly enhance anti-AChR antibody production when cultured with autologous PBL in the presence of pokeweed mitogen, and in this instance the response would appear to depend on the addition of T helper action.

The nature of the process underlying the breakdown in tolerance to AChR and the production of anti-AChR antibodies is unknown, although viral agents have been suggested.⁷³ A

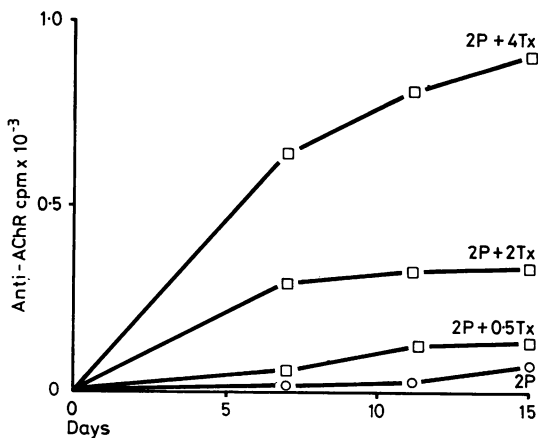


Fig 4 Anti-AChR antibody production in culture by unstimulated peripheral blood lymphocytes from a 35 year old female with myasthenia gravis. Points are the mean of triplicate wells. Each well contains 2×10^6 peripheral blood lymphocytes alone (2P), or with 0.5, 2 and 4×10^6 autologous irradiated (1000 rads) thymic cells (Tx). Irradiated thymic cells cultured alone produced no detectable anti-AChR antibody (not shown).

defect of suppressor mechanisms in childhood MG is suggested by the failure of their lymphocytes to express an antigen dependant suppressor effect, which correlated with a reduced ability of their lymphocytes to express a receptor for sheep red blood cells.⁷⁴ The ability of lymphocytes from normal individuals to exert an antigen dependant suppressor effect *in vitro* could be blocked by IgG from MG patients. Thus suppressor mechanisms may be abnormal in at least some types of MG, but this may be secondary rather than primary, and it is not clear how far these observations are relevant to the specific suppression of anti-AChR antibody production.

Anti-AChR and clinical associations

The high association of this antibody with MG and its specificity with respect to the disease makes it a useful diagnostic test for the disorder. However, about 10% of the patients with generalised MG, according to conventional diagnostic criteria (clinical features, Tensilon responsiveness, EMG changes) do not have detectable antibody, and in ocular cases the proportion is larger (25%). Failure to detect anti-AChR either by the immunoprecipitation method or by inhibition of α -BuTx binding does not necessarily imply that these patients do not have serum anti-AChR activity. The solubilisation of AChR in

the preparation of the antigen used in the assay could lead to loss of antigenic determinants that were critical for those individuals. A patient in whom elevated titres of anti-AChR could not be demonstrated still responded strikingly well to plasma exchange,⁷⁵ indicating that a humoral factor was implicated in her disease as in those in whom anti-AChR titres are elevated.

Anti-AChR levels appear to provide an index of disease severity within the individual, as judged by the effects of plasma exchange.⁵⁶⁻⁵⁸ It is not yet established whether the short-term relationship will prove to be consistent in the longer term. However, patients developing a clinical remission over several months after thymectomy show a decline in serum anti-AChR as do patients showing clinical improvement with immuno-suppressive drug treatment.⁷⁶

Monoclonal gammopathies either of the IgG,⁷⁷⁻⁷⁸ or IgM⁷⁹ type have occasionally occurred in association with MG. Anti-AChR activity was not located in the IgG paraprotein peak in the single instance when this was studied.⁷⁸

Disease heterogeneity and anti-AChR

Analysis of clinical, immunological and HLA-antigen associations in a relatively large group of patients with MG allowed us to define at least three separate categories of the disorder.³⁶ The patients were initially segregated on the basis of thymus pathology (thymoma present or absent) and age of presentation, which was bimodal for the non-thymoma group (fig 5). The three groups

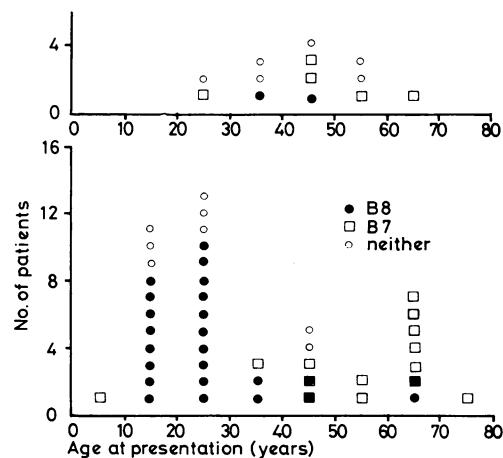


Fig 5 Distribution of patients according to age at presentation and HLA type. Upper panel, 13 cases with thymoma; lower panel, 43 cases without thymoma. Cases with restricted ocular disease are not included.

were: Group A thymoma; Group B non-thymoma, age of onset <40 years; Group C non-thymoma, age of onset >40 years. Group A showed no clear HLA antigen association. Group B, however, was significantly associated with HLA A1, B8 and/or DRw3 and Group C with HLA A3, B7 and/or DRw2 (see also fig 5). These three groups were found to have highly significant differences ($p=0.006$) in their anti-AChR antibody titres, those with thymoma having the highest values and those without thymoma whose illness presented after 40 years of age having the lowest. We have interpreted these data as indicating that more than one mechanism may underlie the breakdown in tolerance which leads to the production of anti-AChR antibody. Since we have found no consistent difference in antibody heterogeneity between these three groups, it seems likely that the basis for the HLA association is in determining to which of these putatively separate mechanisms the individual is susceptible.

References

- 1 Simpson JA. Myasthenia gravis. A new hypothesis. *Scot Med J* 1960; **5**:419-36.
- 2 Kibrick S. Myasthenia gravis in the newborn. *Paediatrics* (Boston) 1954; **14**:365-86.
- 3 Namba T, Brown SB, Grob D. Neonatal myasthenia gravis. Report of two cases and review of the literature. *Paediatrics* (Boston) 1970; **43**:488-507.
- 4 Keeseey J, Lindstrom J, Cokely H, Herrman C. Anti-acetylcholine receptor antibody in neonatal myasthenia gravis. *N Engl J Med* 1977; **296**:55.
- 5 Campbell H, Bramwell E. Myasthenia gravis. *Brain* 1900; **23**:277-336.
- 6 Buzzard EF. The clinical history and post-mortem examination of five cases of myasthenia gravis. *Brain* 1905; **28**:438-83.
- 7 Walker MB. Treatment of myasthenia gravis with physostigmine. *Lancet* 1934; **1**:1200-1.
- 8 Dale HH, Feldberg W. Chemical transmission at motor nerve endings in voluntary muscle. *J Physiol (Lond)* 1934; **81**:39-40.
- 9 Lindsley DB. Myographic and electromyographic studies of myasthenia gravis. *Brain* 1935; **58**:470-82.
- 10 Wilson A, Stoner HB. Myasthenia gravis: a consideration of its causation in a study of fourteen cases. *Quart J Med* 1944; **13**:1-18.
- 11 Lammers W, Most Van Spijk Dvd. Unsuccessful attempt to demonstrate a paralytic factor in serum of myasthenia gravis patients. *Nature* 1954; **173**:1192-3.
- 12 Nastuk WL, Strauss AJL, Osserman KE. Search for a neuromuscular blocking agent in the blood of patients with myasthenia gravis. *Am J Med* 1959; **26**:394-409.
- 13 Parkes JD, McKinna JA. Effects of thymic extract on the neuromuscular junction. *Nature* 1967; **214**:1116-7.
- 14 Namba T, Grob D. Effect of serum and serum globulin of patients with myasthenia gravis on neuromuscular transmission. *Neurology* 1969; **19**:173-84.
- 15 Stricker E, Tholen H, Massini MA, Staub H. The effect of haemodialysis in myasthenia gravis. *J Neurol Neurosurg Psychiatry* 1960; **23**:291-4.
- 16 Bergström K, Franksson C, Matell G, von Reis G. The effects of thoracic duct lymph drainage in myasthenia gravis. *Europ Neurol* 1973; **9**:157-67.
- 17 Strauss AJK, Seefal BC, Hsu KC, Burkholder PM, Nastuk WL, Osserman KE. Immunofluorescence demonstration of a muscle binding complement fixing serum globulin fraction in myasthenia gravis. *Proc Soc Exp Biol* 1960; **105**:184-91.
- 18 Feltkamp TEW, van den Berg-Loonen PM, Nijemhuis LE, Engelfriet CP, van Rossum AL, van Loghem JJ. Myasthenia gravis, autoantibodies and HLA antigens. *Brit Med J* 1974; **1**:131-3.
- 19 Oosterhuis HJGH, Feltkamp TEW, van Rossum AL, van den Berg-Loonen, Nijemhuis LE. HLA antigens, autoantibody production, and associated disease in thymoma patients, with and without myasthenia gravis. *Ann NY Acad Sci* 1976; **274**:468-74.
- 20 Vettors JM. Myasthenia gravis. *Lancet* 1966; **1**:314-5.
- 21 Peers J, McDonald BL, Dawkins RL. The reactivity of the antistriational antibodies associated with thymoma and myasthenia gravis. *Clin exp Immunol* 1977; **27**:66-73.
- 22 Van der Geld HWR, Strauss AJL. Myasthenia gravis: Immunological relationship between striated muscle and thymus. *Lancet* 1966; **1**:57-60.
- 23 Almon RR, Andrews CG, Appel SH. Serum globulin in myasthenia gravis: inhibition of a bungarotoxin binding to acetylcholine receptors. *Science* 1974; **186**:55-7.
- 24 Chang CE, Lee CY. Isolation of neurotoxins from the venom of bungarus multicinctus and their modes of neuromuscular blocking action. *Arch Int Pharmacodyn Ther* 1962; **114**:241-57.
- 25 Miledi R, Potter LT. Acetylcholine receptors in muscle fibres. *Nature* 1971; **233**:599-603.
- 26 Almon RR, Appel SH. Interaction of myasthenic serum globulin with the acetylcholine receptor. *Biochem Biophys Acta* 1975; **393**:66-77.
- 27 Bender AN, Ringel SP, Engel WK, Daniels MP, Vogel Z. Myasthenia gravis: a serum factor blocking acetylcholine receptors of the human neuromuscular junction. *Lancet* 1975; **1**:607-9.
- 28 Aharanov A, Abramsky O, Tarrab-Hazdai R, Fuchs S. Humoral antibodies to acetylcholine receptor in patients with myasthenia gravis. *Lancet* 1975; **2**:340-2.

- 29 Mittag T, Kornfeld P, Tormay A, Woo C. Detection of anti-acetylcholine receptor factors in serum and thymus from patients with myasthenia gravis. *N Engl J Med* 1976; **294**:691-4.
- 30 Lindstrom J. An assay for antibodies to human acetylcholine receptor in serum from patients with myasthenia gravis. *Clin Immunol Immunopathol* 1977; **7**:36-43.
- 31 Lindstrom JM, Seybold ME, Lennon A, Whittingham S, Duane DD. Antibody to acetylcholine receptor in myasthenia gravis. Prevalence, clinical correlates and diagnostic value. *Neurology* 1976; **26**:1054-9.
- 32 Monnier VM, Fulpius BW. A radioimmunoassay for the quantitative evaluation of anti-human acetylcholine receptor antibodies in myasthenia gravis. *Clin Exp Immunol* 1977; **29**:16-22.
- 33 Lefvert AK, Bergström K, Matell G, Osterman PO, Pirskanen R. Determination of acetylcholine receptor antibody in myasthenia gravis: clinical usefulness and pathogenetic implications. *J Neurol Neurosurg Psychiatry* 1978; **41**:394-403.
- 34 Vincent A, Newsom-Davis J. Studies on α -Bungarotoxin and anti-acetylcholine receptor antibody binding to the human acetylcholine receptor in "Neurotoxins: Tools in Neurobiology". *Adv Cytopharmacol* 1978; **3**:269-78.
- 35 Compston DAS, Vincent A, Newsom-Davis J, Batchelor JR. Clinical, pathological, HLA antigen and immunological evidence for disease heterogeneity in myasthenia gravis. *Brain* 1980; (in press).
- 36 Bradley RJ, Dwyer D, Morley BJ, Robinson G, Kemp GE, Oh SJ. Humoral immunity in myasthenia gravis: relationship to disease severity and steroid treatment. *Lancet* 1978; **2**:96.
- 37 Brenner T, Zweiman B, Abramsky O, Tarrab-Hazdai R, Lisak RP, Fuchs S. Radioimmunoassay of antibodies to acetylcholine receptor in serum of myasthenia gravis patients. *Isr J Med Sci* 1978; **14**:986-9.
- 38 Sondag-Tschroots IRJM, Schulz-Raateland RCM, van Walbeck HK, Feltkamp TEW. Antibodies to motor endplates demonstrated with the immunofluorescent technique. *Clin Exp Immunol* 1979; **37**:323-7.
- 39 Dwyer DA, Bradley RJ, Oh SJ, Kemp GE. A modified assay for antibody against the nicotinic acetylcholine receptor in myasthenia gravis. *Clin Exp Immunol* 1979; **37**:448-51.
- 40 Bucknall DC, Dixon A St J, Glick EN, Woodland J, Zutshi DW. Myasthenia gravis associated with penicillamine treatment for rheumatoid arthritis. *Brit Med J* 1965; **1**:600-2.
- 41 Masters CL, Dawkins RL, Zilko PJ, Simpson JA, Leedman RJ, Lindström J. Penicillamine-associated myasthenia gravis, anti-acetylcholine receptor and antistriational antibodies. *Am J Med* 1977; **63**:689-94.
- 42 Vincent A, Newsom-Davis J, Martin V. Anti-acetylcholine receptor antibodies in D-penicillamine-associated myasthenia gravis. *Lancet* 1978; **1**:1254.
- 43 Russell AS, Lindström JM. Penicillamine induced myasthenia gravis associated with antibodies to acetylcholine receptor. *Neurology* 1978; **28**:847-9.
- 44 Argov Z, Nicholson L, Fawcett PRW, Mastaglia FL, Hall M. Neuromuscular transmission and acetylcholine receptor antibodies in rheumatoid arthritis patients on penicillamine. *Lancet* 1980; **1**:203.
- 45 Martin V, Vincent A, Clarke C. Anti-acetylcholine receptor antibodies in rheumatoid arthritis. Submitted for publication.
- 46 Lyle WH. Penicillamine. In "Clinics in rheumatic diseases." Huskisson EC, Ed, 1979; **5**: No. 2. 569-601.
- 47 Vincent A, Newsom-Davis J. Absence of anti-acetylcholine receptor antibodies in congenital myasthenia gravis. *Lancet* 1979; **i**:441-2.
- 48 Vincent A, Cull-Candy S, Newsom-Davis J, Trautmann A, Molenaar P. Congenital myasthenia: a study of five cases. (In preparation).
- 49 Lefvert AK, Bergström K. Acetylcholine receptor antibody in myasthenia gravis: purification and characterisation. *Scand J Immunol* 1978; **8**: 525-34.
- 50 Lefvert AK. Immunoglobulins in myasthenia gravis. Kinetic properties of the acetylcholine-receptor antibody studied during lymph drainage. *Clin Exp Immunol* 1978; **34**:111-7.
- 51 Mittag TW, Tormay A, Massa T. Heterogeneity of acetylcholine receptors in denervated muscle: interactions of receptors with immunoglobulins from patients with myasthenia gravis. *Mol Pharmacol* 1978; **14**:60-8.
- 52 Lindström J, Campbell M, Nave B. Specificities of antibodies to acetylcholine receptors. *Muscle and Nerve* 1978; **1**:140-5.
- 53 Weinberg CB, Hall ZW. Antibodies from patients with myasthenia gravis recognise determinants unique to extra-junctional acetylcholine receptors. *Proc Natl Acad Sci* 1979; **76**:504-8.
- 54 Toyka KV, Drachman DB, Griffin DE, Pestronk A, Winkelstein JA, Fishbeck KH, Kao I. Myasthenia gravis. Study of humoral immune mechanism by passive transfer to mice. *N Engl J Med* 1977; **296**:125-31.
- 55 Pinching AJ, Peters DK, Newsom-Davis J. Remission of myasthenia gravis following plasma-exchange. *Lancet* 1976; **ii**:1373-6.
- 56 Vincent A, Pinching AJ, Newsom-Davis J. Circulating anti-acetylcholine receptor antibody in myasthenia gravis treated by plasma exchange. *Neurology* 1977; **27**:364.
- 57 Dau PC, Lindström JM, Cassel JK, Denys EH, Shev EE, Spitter LE. Plasmapheresis and immunosuppressive drug therapy in myasthenia gravis. *New Engl J Med* 1977; **297**:1134-40.
- 58 Newsom-Davis J, Pinching AJ, Vincent A, Wilson SG. Function of circulating antibody to acetylcholine receptor in myasthenia gravis investigated by plasma exchange. *Neurology* 1978; **28**:266-72

- 59 Abramsky O, Brenner T, Lisak RP, Zeidman A, Beyth Y. Significance in neonatal myasthenia gravis of inhibitory effect of amniotic fluid on binding of antibodies to acetylcholine receptor. *Lancet* 1979; **ii**:1333-5.
- 60 Wilson SG, Vincent A, Newsom-Davis J. Effect of human myasthenic immunoglobulin G on mouse acetylcholine receptor. *Clin Sci* 1980; **57**: 20.
- 61 Shibuya N, Katzutake M, Nakazawa Y. Serum factor blocks neuromuscular transmission in myasthenia gravis: Electro physiologic study with intracellular micro-electrodes. *Neurology* 1978; **28**:804-11.
- 62 Henry K. An unusual thymic tumour with a striated muscle (myoid) component (with a brief review of the literature on myoid cells). *Brit J Dis Chest* 1972; **66**:291-9.
- 63 Kao I, Drachman DB. Thymic muscle cells bear acetylcholine receptors: possible relation to myasthenia gravis. *Science* 1977; **195**:74-5.
- 64 Engel WK, Trotter JL, McFarlin DE, McIntosh CL. Thymic epithelial cell contains acetylcholine receptor. *Lancet* 1977; **i**:1310-1.
- 65 Vincent A, Scadding GK, Thomas HC, Newsom-Davis J. In-vitro synthesis of anti-acetylcholine-receptor antibody by thymic lymphocytes in myasthenia gravis. *Lancet* 1978; **i**:305-7.
- 66 Scadding GK, Newsom-Davis J, Vincent A, Henry K. In preparation.
- 67 Papatestas AE, Alpert LS, Osserman KE, Osserman RS, Kark AE. Studies in myasthenia gravis: Effects of thymectomy. *Am J Med* 1971; **50**: 465-74.
- 68 Veters JM, Simpson JA. Comparison of thymic histology with response to thymectomy in myasthenia gravis. *J Neurol Neurosurg Psychiat* 1974; **37**:1139-45.
- 69 Simpson JA. An evaluation of thymectomy in myasthenia gravis. *Brain* 1958; **81**:112-44.
- 70 Henson RA, Stern GM, Thompson VC. Thymectomy for myasthenia gravis. *Brain* 1965; **88**:11-28.
- 71 Clarke C, Vincent A, Newsom-Davis J. Studies on anti-acetylcholine receptor antibody synthesis by peripheral blood lymphocytes in myasthenia gravis. *Clin Sci* 1979; **56**:1.
- 72 Siegal FP, Siegal M. Enhancement by irradiated T cells of human plasma cell production: dissection of helper and suppressor functions in vitro. *J Immunol* 1977; **118**:642-7.
- 73 Datta SK, Schwartz RS. Infectious (?) myasthenia. *New Engl J Med* 1974; 1304-5.
- 74 Shore A, Limatibul S, Dosch HM, Gelfand EW. Identification of two serum components regulating the expression of T-lymphocyte function in childhood myasthenia gravis. *New Engl J Med* 1979; **301**:625-9.
- 75 Newsom-Davis J. Plasma exchange in myasthenia gravis. *Plasma Therapy* 1979; **1**:17-31.
- 76 Newsom-Davis J, Wilson SG, Vincent A, Ward CD. Long term effects of repeated plasma exchange for myasthenia gravis. *Lancet* 1979; **i**: 464-8.
- 77 Rowland LP, Osserman EF, Scharfman WB. Myasthenia gravis with a myeloma-type, gamma G (IgG) Immunoglobulin abnormality. *Am J Med* 1969; **46**:599-605.
- 78 Somer H, Pirskanen R, Lefvert AK, Wasastjerna C, Korittinen A. Myasthenia gravis and monoclonal IgG gammopathy. *Neurology* 1979; **29**: 842-7.
- 79 Modigliani E, Charbord P, Landman C. Myasthenie avec gammopathie monoclonale IgG Kappa et presence d'anticorps antimuscles stries. *Ann Med Interene (Paris)* 1975; **124**:214-45.