Acetylcholine Receptor Antibodies in Myasthenia Gravis: Use of a Qualitative Assay for Diagnostic Purposes

Joel Oger, Robin Kaufman and Kenneth Berry

ABSTRACT: We have modified the techniques of Lindstrom and of Tindall to measure serum acetylcholine receptor antibody using human antigen bound to ^{125}I-alpha Bungarotoxin. By using 10 µl of serum and precipitating antigen-antibody complexes with an excess of staph A, we found that only one out of 43 patients with clinically diagnosed active generalized Myasthenia Gravis had no antibodies. In pooling these results with the results of tests done for diagnostic purposes we found positive results in 54/55 generalized active MG, 8/21 MG in remission, 16/37 ocular MG and 0/55 healthy controls. Two out of 38 non MG were also positive and their clinical diagnosis of botulism and penicillamine treated rheumatoid arthritis have been confirmed by a one year follow-up. Most of these sera were also tested for reactivity with fetal calf AchR. Six out of 49 samples positive with the human receptor were negative with calf receptor. We conclude that our technique is extremely useful for the diagnosis of Myasthenia Gravis and that fetal calf antigen cannot replace human antigen in the assay.

RÉSUMÉ: Les anticorps dirigés contre les récepteurs à l'acétylcholine dans la myasthénie grave. Nous avons modifié le test diagnostique sérologique tel que décrit par Lindstrom et Tindal pour mesurer dans le sérum les anticorps dirigés contre les récepteurs à l'acétylcholine. Le récepteur à l'acétylcholine d'origine humaine marqué avec de l'alpha Bungarotoxine iodée est incubé avec 10 ml de sérum à tester. Après incubation les complexes immuns sont précipités par un excès de staphylocoque A. Parmi 43 cas de myasthénie généralisée dûment diagnostiqués cliniquement nous n'avons retrouvé qu'un sérum négatif. En additionnant les résultats des tests diagnostiques et en tenant compte de l'évolution clinique nous avons trouvé que le test était positif chez 54 des 55 malades testés (98 %) ayant une myasthénie généralisée, 8 des 21 (38 %) myasthénies en rémission, 16 des 37 (43 %) myasthénies oculaires et aucun des 55 contrôles. Deux échantillons sur 38 provenant de malades non myasthéniques se sont également avérés positifs. Dans ces deux cas, les diagnostiques cliniques de polyarthrite rhumatoïde traitée par pénicillamine et de botulisme ont été confirmés par un suivi clinique de un an. La plupart de ces sérums ont aussi été testés en utilisant un antigène foetal d'origine bovine. Six des 49 malades positifs avec l'antigène humain (12 %) se sont avérés négatifs avec l'antigène bovin. Nous concluons que notre technique augmente la sensibilité du test diagnostique de la myasthénie et que l'antigène foetal d'origine bovine ne peut pas remplacer l'antigène d'origine humaine.

Can. J. Neurol. Sci. 1987; 14:297-302

Over the last 10 years, antibodies to the nicotinic acetylcholine receptor (AchRAb) have been found to occur with high frequency in serum of myasthenia gravis (MG) patients.[1-3] They have been localized to the post-synaptic membrane,[4] and can cause disease in mice by passive transfer.[5] Reports that AchRAb against the human receptor are present in 75 to 90% of patients suggest that their detection provides a reliable diagnostic test.

We have modified the techniques of Lindstrom[2] and of Tindall[6] and increased the diagnostic usefulness of this test. A preliminary account of these results has been presented.[7] Since we have made this assay available in July of 1984 as a clinical diagnostic tool across Canada, over 400 samples have been received. In this report we describe our technique and the results obtained in the first year of operation. The limited availability of human receptor prompted us to test the usefulness of fetal calf AchR in diagnosing MG as done by C. Gotti et al.[8]

Techniques

Preparation of Antigen

Acetylcholine receptor (AchR) was solubilized from human or fetal calf muscle for use as an antigen in the assay. Samples

From the Departments of Medicine (Neurology) and the Department of Pathology, University of British Columbia, Vancouver
Received November 14, 1986. Accepted in final form March 12, 1987
Reprint requests to: Dr. J. Oger, Division of Neurology, University Hospital, 2211 Wesbrook Mall, Vancouver, B.C., Canada V6T 2B5

of skeletal muscle were obtained from amputated limbs or autopsy material. Muscle was also obtained from limb muscles of a 4 month old fetal calf. Muscle was stored at −70° until used. Frozen muscles were thawed at 4°, cut into small pieces and homogenized in a blender for 2 to 3 minutes with cold buffer in a 2.5:1 volume ratio. This buffer contained: 0.1 M Na Phosphate buffer pH 7.0, containing 1 μg/ml pepstatin A (Sigma), .01 M sodium azide, 2 x 10^{-4} M phenylmethylsulfonylfluoride and 10^{-4} M Benzethonium chloride to inhibit proteolysis (Buffer A). The homogenate was then centrifuged at 27 x 10^3 g for 30 min. at 4° C. The pellets were recovered and rehomogenized in the same buffer with 2% triton-X (Buffer A-Triton X) to solubilize the AchR from the cell membrane. The mixture was stirred overnight at 4° C. Centrifugation was carried out the next day at 39x10^3 g for one hour. The supernatant was filtered through glass wool. Homogenates were stored in aliquots at −70° C and used within 2 months.

Acetylcholine Receptor Antibody Measurement

A modification of the procedure of Tindall et al[6] was used to assay for precipitating AchRAb. Muscle homogenates were incubated 30 min. at room temperature in the presence of an excess of (1.5 x 10^{-8} M) ^{125}I-alpha bungarotoxin (N.E.N.). Serum was added in triplicate to 4 ml plastic tubes containing 100 μl of labelled muscle homogenate. After incubation overnight at 4° C, a 7% solution of staph A (Sigma P9151) was added. Staph A had been pretreated by heating for 30 minutes (95°) in the presence of 3% SDS and 10% beta-mercaptoethanol. After 20 minutes at room temperature, tubes were washed three times using Buffer A-triton X. Between washes centrifugation was carried out for 5 minutes at 2000 g. Precipitates were counted in a Beckman Gamma 5500. Following preliminary experiments (see results section) optimal amounts of serum to be tested and staph A to precipitate complexes were determined to be 10 μl and 100 μl respectively. For each assay five sera were randomly selected from a group of 50 samples obtained from healthy individuals.

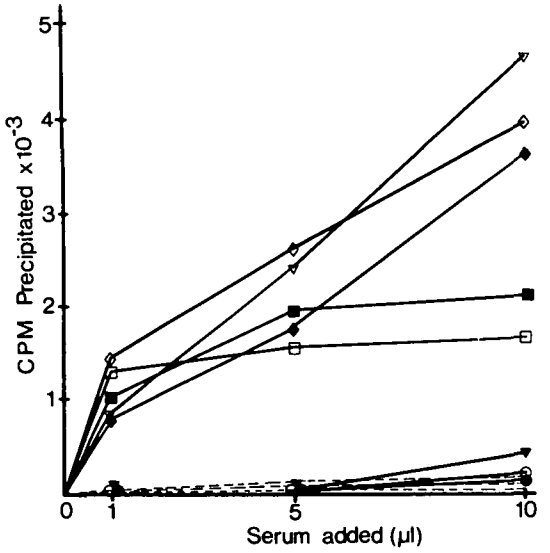

Figure 1 — CPM precipitated in the presence of increasing amounts of human sera: sera with low AchRAb titer are reproduced in Figure 2.

Sample values between 2 and 3 standard deviations over the mean of controls were considered "borderline". Sample values 3 SD over controls were considered positive. Borderline sera were systematically re-assayed using another preparation of human antigen.

Results

A) Optimization of the Assay Using Human Receptor

1. Binding of human AchR by serum Ab

Figure 1 shows the binding of solubilized AchR by serum Ab. When antigen was in excess over serum Ab a direct relationship between serum volume and counts precipitated was observed. We also observed a number of MG sera which differed in their capacity to precipitate AchR. These sera show an early plateau. In our assay system the limit of detection was 3 x 10^{-16} moles of ^{125}-alpha BT binding sites and we obtained false negative results for patients with very low AchR Ab titers unless testing was carried out with at least 5-10 μl of sera (Figure 2). Therefore, 10 μl of serum was considered to be optimum in this assay.

2. Adding different amounts of staph A (Table 1)

To determine the amount of staph A necessary to precipitate all the antigen-antibody complexes when 10 μl of serum was used we added increasing amounts of staph A. Results of a typical experiment are shown in Table 1. It can be seen that a plateau is reached with 25 μl of staph A. We thus decided to set up the assay with a safe excess, namely 100 μl of staph A.

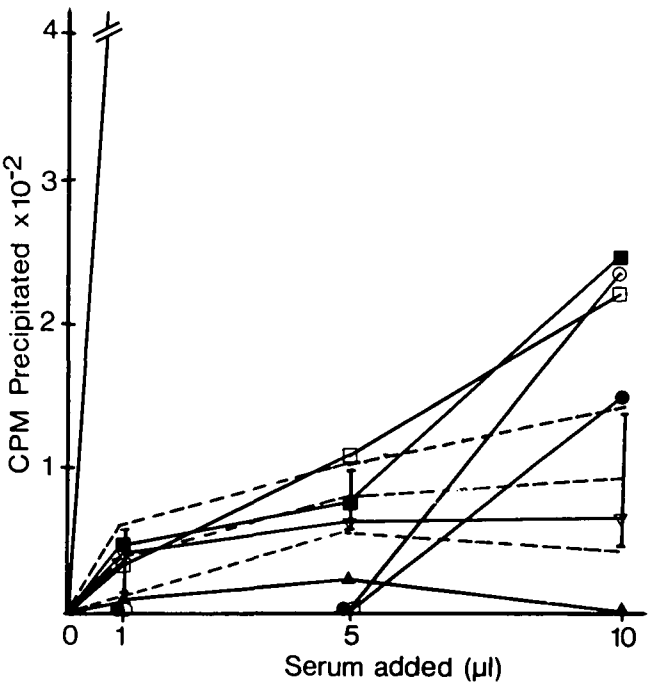

Figure 2 — CPM precipitated in the presence of increasing amounts of human sera having low reactivity. The dotted lines represent the range of controls run in the same experiment. Note the difference in scale between Figure 1 and Figure 2. Out of 6 sera tested and falling in the range of controls when 1 microliter is used, 3 are positive and 1 is borderline when 10 microliters are used.

https://doi.org/10.1017/S0317167100026652 Published online by Cambridge University Press

3. Screening of antigen preparations (Table 2)

Each muscle preparation was tested with a panel of 5 MG (1 of them being negative) and 4 control sera. Only preparations where the positive MG sera, on the average, precipitated twice the CPM precipitated by control sera and which contained a minimum of 3000 precipitable CPM/100 μl of preparation were selected for use in the assay.

B) Results of qualitatative assay using Human Receptor in Patients With Known MG

The following results were generated using the parameters selected as described in the previous section.

1. Active generalized MG (Table 3)

Forty-three patients were known as having active generalized myasthenia gravis. Clinical criteria included extraocular muscle and limb weakness increasing on exercise. All patients had a positive Tensilon test. All improved following Mestinon. Treatment included various combinations of thymectomy, steroids, plasmapheresis and Azathioprine. Forty were positive, two were borderline and only one was found negative. The borderline and negative patients had disease of recent onset (respectively 8 months, 3 months and 4 months). Repeated testing of the same 3 samples confirmed these results. New samples were obtained more than 3 months later from the 2 patients with borderline results: one showed a major increase in its reactivity and had become highly positive; the other remained borderline. The patient with negative results remained negative on repeated sampling. No electrical studies have been performed but this patient fulfilled the criteria of generalized active MG with respiratory distress, reacted to Tensilon® and improved following steroids and thymectomy.

The following case illustrates a borderline result as well as the absence of a close correlation between clinical signs and AchRAb activity. In July of 1985 a 20 year old woman presented with drooping of the eyelids, fatiguable upward gaze and proximal limb weakness on exercise. Tensilon test was positive. After one week of symptoms AchR antibody levels against human receptor were found to be borderline (CPM precipitated 2300, 5 controls 2175±65, mean + 2SD = 2295). She responded dramatically to Mestinon. Mestinon was discontinued in November of 1985 and she was found to be in complete remission in December of 1985 when a second sample of serum was obtained. This showed 6267 CPM precipitated (controls precipitated 2845±47 CPM, mean +3SD = 2986). Follow up studies one year later showed that she remained in complete remission but has persistent high levels of AchRAb circulating in her serum.

2. Healthy Controls

Fifty-five healthy controls were tested. All were negative.

3. Generalized MG in remission

Patients were assessed as having generalized MG in remission if they fulfilled the following criteria: past history of generalized MG clinically substantiated by a neurologist, absence of clinical signs of MG in the absence of treatment. Twenty-one patients fulfilling these criteria were tested; 5 (24%) were positive, 5 (24%) were borderline and 11 (52%) were negative. On re-assay of the 5 borderline samples 2 were negative, 2 borderline and 1 positive.

4. Ocular myasthenia

Ocular MG was defined as weakness clinically localized to eye muscles without bulbar or limb involvement. It manifested itself by diplopia or ptosis only, increasing with exertion and as the day passed. The diagnosis rested clinically on the improvement following injection of Tensilon. Nineteen out of 37 (51.8%) were found to be positive; 3 were found to be borderline and 15 to be negative. On repeated assay of the 3 borderline samples one was found negative and 2 were again found to exhibit borderline reactivity.

C) Diagnostic Test in Possible Generalized MG

Fifty-one patients presenting with possible generalized MG not diagnosed at the time of sampling were tested, 13 were found to be positive, 33 were negative and 5 borderline.

Table 1: CPM precipitated in the presence of increasing amounts of staph A

	10 μl human sera added		
Volume of staph A added (μl)	CPM Precipitated by NHS (a)	CPM Precipitated by one MG Serum (b)	Net Counts (b-a)
10	470	2860	2390
25	820	5080	4260
50	1200	5470	4270
100	1840	5900	4060
200	2310	6530	4220
300	2640	7060	4420

Table 2: Reactivity of 5 MG sera and 5 control sera with different human AchR preparations (CPM precipitated)

		20	1	12	16
MG positive	1	7175	3568	1846	1837
	2	9792	2800	1739	1802
	3	8503	3519	1518	1766
	4	2599	3377	1604	2046
Mean		7017	3316	1676	1862
MG negative	5	1807	ND	1374	1712
Control	1	1803	1645	1449	1811
	2	1774	1495	1396	1771
	3	1745	1689	1415	1812
	4	1706	1669	1392	1797
	5	2071	1534	1443	1777
Mean		1819	1605	1419	1793
SD		144	86	26	19

Table 3: Validation of the diagnostic assay (human AchR)

	+	+/−	−
Active generalized MG* (43)	40	2	1
MG generalized remission (21)	5	5	11
Ocular MG (37)	19	3	15
Healthy controls (55)	0	0	55

* diagnosis made before AchRAb assay

+/− means borderline (CPM precipitated between 2 and 3 standard deviations above the mean of the 5 healthy controls assayed on the same day)

https://doi.org/10.1017/S0317167100026652 Published online by Cambridge University Press

Response to Mestinon as well as clinical follow-up of 3-12 months confirmed that 12 of the 13 positive indeed had generalized myasthenia gravis. One had a diagnosis of botulism on clinical and epidemiological grounds; no block was found on repetitive stimulation of the ulnar nerve. However, its serum was not positive for Botulinum Toxin by injection to mice using the technique described in Kautter and Lynt.[9] One year after the acute episode this patient is clinically well. Serum is still low positive but single fiber EMG is normal.

Thirty-three sera were found to be negative. Diagnoses after follow up included: botulism (n = 6), psychasthenia (n = 7), Eaton Lambert syndrome (n = 5), 9 cases of muscle disease (2 dystrophia myotonica, 1 muscular dystrophy, 1 steroid myopathy, 1 limb girdle dystrophy, 4 polymyositis) Guillain Barre Strohl disease (n = 2), Amyotrophic Lateral Sclerosis (ALS) (n = 3) and paraneoplastic sensory neuropathy (n = 1).

When the 5 borderline samples were repeated, 3 were found to be negative. Final clinical diagnoses after follow up were: seizure disorder (1), polymyositis (1), Eaton Lambert syndrome (1). Two were again found to be borderline and are, thus, considered positive in final analysis. These included one patient with generalized MG in remission and one with penicillamine treated rheumatoid arthritis. At the time of writing, this patient had not yet developed any clinical or electrical sign of MG.

D) Disease Associations

One patient with Multiple Sclerosis and clinical ocular MG was found positive. None of 14 other MS cases with fatigue were found positive. One patient with systemic lupus erythematosus and generalized MG had antibodies to AchR. Two other lupus patients without clinical signs of MG were negative.

E) Final Results

If one considers as positive those sera which tested positive on initial assay as well as those who tested positive or borderline on reassay after an initial borderline result, the results of our experience to date are as presented in Table 4. From this table one can calculate the specificity of the assay (number of negative samples divided by number tested). This reaches 100% in healthy individuals and 94.7% in other neurological diseases entering into the differential diagnoses of generalized MG. This is a very conservative figure as the two patients with other neurological disorders and a positive test include a case of presumed botulism and a case of penicillamine treated rheumatoid arthritis. In any event none of these patients has electrical signs of MG. The sensitivity of the assay (number of samples positive divided by number of individuals) when done and interpreted as described was 98.2% in generalized active MG, 38% during remission and 56.7% in ocular MG.

F) Comparison between results of AchR Ab using human and fetal calf antigens

One hundred and twenty-nine sera were assayed using both antigens. Results are presented in Table 5: 121 sera were concordant (43+ with both antigens and 78− with both antigens). Obviously this is an outstanding correlation. There were 2 types of discrepant results: 1) 6 of 49 patients (12%) positive with human antigen were negative when the fetal calf Ag was used; they included 5 patients who definitely had MG (four generalized active and 1 ocular); the remaining patient is the one who had been clinically diagnosed as having botulism. 2) Two patients were found to test positive with fetal calf antigen and negative with human antigen. One had MG but was in remission, the second had Parkinson's disease.

Discussion

We have used an excess of staph A to precipitate the antigen-antibody complexes in the assay for AchRAb. Staphylococcus A does not precipitate IgM and IgA in human serum. It is known[10] that AchRAb are rarely of the IgM and IgA class. Staphylococcus A precipitates readily the IgG 1, IgG 2 and IgG 4 subclasses but reacts poorly with IgG 3. Lefvert[11] has suggested that some patients had antibodies to the acetylcholine receptor which were exclusively of the IgG 3 subtype. Tindall[6] et al on the other hand in a series of 92 patients found 100% correlation between the results obtained by precipitation using a double antibody system and using staphylococcus A. Our results indicating that only 1 out of 55 myasthenic patients with generalized active disease was negative using staph A precipitation would indicate that IgG subclass restriction is not a determinant factor; it is also possible to hypothesize that treatment of staphylococcus A with SDS and Mercaptoethanol increases the affinity of staph A for IgG 3.

Most authors quantify the amount of AchRAb present in serum and express their results in terms of nanomoles of alpha BT precipitated per liter of serum. Due to the limited amount of AchR present in the human muscle preparations (1-2 x 10^{-10} M) they have to use small amounts of serum in the assay.[2,3,6] We have shown here that some MG sera did not contain enough antibodies to be differentiated from controls if less than 10 µl of undiluted serum was used. Thus, in order to maximize the sensitivity of the assay as a diagnostic test, we use 10 µl of

Table 4: Acetylcholine receptor antibodies (qualitative assay in a series of 206 consecutive samples)

	+	−	% +
Active generalized MG (55)	54	1	98.2
MG generalized remission (21)	8	13	38.0
Ocular MG (37)	21	16	56.7
Healthy (55)	0	55	0
Non-MG (38)	2*	36	5.2

* 1 case of (?) botulism
* 1 case of R.A. treated with penicillamine (see text)

Table 5: Correlations between AchRAb results using human and fetal calf antigens

Fetal Antigen	Human Antigen	No. of Sera	
+	+	43	
−	−	78	
−	+	6	4MG gen. active 1 ?Botulism 1 Ocular MG
+	−	2	1 Non-MG 1 MG remission

https://doi.org/10.1017/S0317167100026652 Published online by Cambridge University Press

serum to be tested. Taking the option of using a large amount of human serum in the assay has prevented us from quantifying the AchRAb but by the same token may have been the essential factor in improving the sensitivity of this test as a diagnostic tool. As we have established that 100 µl of a 7% staph A suspension has enough IgG binding capacity to precipitate all the complexes generated by 10 µl of serum, we know that we are working in excess of staph A. We have also shown that different sera showed different maximum precipitating ability in the presence of the same antigen preparation. A finding confirming the results of Vincent and Newsom-Davis[12] and which has been attributed to the polyclonality of human antibodies.

That blocking antibodies exist, which compete with the alpha BT binding site, has been recognized.[14] Vincent and Newsom-Davis[12] suggested that, at antibody excess, blocking antibodies could displace the alpha BT and decrease the precipitated CPM. This is of concern to us as we use a larger amount of serum in the assay than other authors do. In our dilution curves (Figure 1) we have not found blocking antibodies that could render the assay negative. It is understandable that Vincent and Newsom-Davis, using different precipitation techniques for the different point of their precipitation curve, could obtain an apparent decrease of the precipitated counts when 10 µl is used.

Indeed in the group of 43 patients with duly diagnosed active generalized MG we found only 1 negative serum. Forty-two sera precipitated more AchR than the sum of the 2 standard deviations over the average of 5 controls tested in the same run. Two of these sera were found to be "borderline", i.e. to precipitate between 2 and 3 standard deviation above the mean of the controls run in parallel. When they were re-assayed the same results were again obtained. We interpret this as indicating a low level of antibodies. This interpretation is confirmed by our finding of 5 such borderline sera in 21 MG in remission and 3 out of 37 ocular MG. It is known that ocular MG and generalized MG in remission have lower levels of AchRAb.

We report here that 54 of 55 patients with active generalized MG (98%) were found positive. This compares favorably with results obtained by other authors using the human antigens who reported 84% of 61,[8] 90% of 50,[13] 85% of 153,[12] 90% of 104.[10] We attribute our higher sensitivity to the larger amount of serum used in the assay and to reassaying samples showing borderline results. We also found 57% of cases of ocular MG and 38% of patients in remission to be positive. These findings agree with those above mentioned references.

We found healthy individuals to be negative, but in a series of 38 non-MG patients two were found to be positive. This reduces the specificity of the test from an optimum of 100% to 94.7%. It is of note, however, that one of these patients was diagnosed on clinical and epidemiological grounds as having botulism and that no block was present on repetitive stimulation of the ulnar nerve. Nevertheless, no toxin was found in his serum by the Bio-assay for botulism toxin.[9] This patient is clinically and electrically healthy one year after the acute episode. The second non-MG to have AchR Ab was a patient with rheumatoid arthritis treated with penicillamine. Penicillamine treatment can induce MG but this patient had normal electrical studies. These "false positive" results may, in fact, be interpreted as suggesting subclinical MG.

Cross reactivity of human antibodies with non-mammalian receptors is limited[15] but fetal mammalian muscle does contain more extra-junctional receptors and the AchR yield is greater. C. Gotti et al[8] have found good reactivity with the AchR isolated from fetal calf. Use of fetal calf antigen would increase the availability of antigen and render the assay less cumbersome. In a series of 61 patients they found only one sample negative with human receptor which reacted with fetal calf receptor but three sera positive with human went undetected using fetal antigen. We have confirmed the good correlation reported by these authors and found 112 sera to be concordant. However, five sera from MG patients were positive with human antigen but negative with calf antigen, thus reducing the sensitivity of the assay. We conclude that, despite easier access to fetal calf antigen, it is difficult to accept a reduction in the sensitivity of one of the most specific assays ever found in the clinical neurologist's armamentarium.

It is our conclusion that we have increased the sensitivity of the AchRAb measurement by increasing the amount of serum used in the assay. We have also introduced the notion of a borderline result. That is, where counts precipitated by test serum are between 2-3 SD over the mean of 5 controls run in parallel. In our hands individuals who repeatedly show borderline results do have myasthenia gravis. This test is now commercially available in Canada and we report here that its sensitivity is 98% in generalized active MG and that it is specific for MG in 95% of the cases.

Acknowledgements

We want to acknowledge the invaluable technical assistance of T. Aziz and the excellent secretarial skills of Catherine Schikowski. We are also indebted to all the neurologists of British Columbia for sending us follow-up notes concerning these patients and particularly Drs. A. Anzarut, J. Beckman, R. Davison, A. Eisen, G. Gibson, S. Hashimoto, B. Jones, M. Jones, D. Paty, P. Rees, C. Simpson, V. Sweeney, B. Tessler and M.J. Wong.

Part of the work reported here was funded in part by a grant from the Woodward's Foundation.

References

1. Appel SH, Almon RR, Levy N. Acetylcholine receptor antibodies in myasthenia gravis. N Engl J Med 1975; 293: 760-761.
2. Lindstrom JM, Seybold MF, Lennon VA, et al. Antibody to acetylcholine receptor in myasthenia gravis: prevalence, clinical correlates and diagnostic value. Neurology 1976; 26: 1054-1059.
3. Lefvert AK, Bergstrom K, Matell G, et al. Determination of acetylcholine receptor antibodies in myasthenia gravis: clinical correlates and pathogenic implications. J Neurol Neurosurg Psych 1978; 41: 394-403.
4. Engel AG, Lambert EA, Howard FM. Immune complexes at the motor end plate in myasthenia gravis. Mayo Clin Proc 1977; 52: 267-280.
5. Toyka KV, Drachman DB, Petonk A, et al. Myasthenia gravis: passive transfer from man to mouse. Science 1985; 190: 397-399.
6. Tindall RSA, Kent M, Wells L. A rapid immunosorbant radioimmunoassay for anti-acetylcholine receptor antibody. J Imm Meth 1981; 45: 1-14.
7. Kaufman R, Oger J. Antibodies to the acetylcholine receptor in myasthenia: comparison between human and fetal calf antigens. Ann NY Acad Sc 1986; 475: 413-414.
8. Gotti C, Mantegazza R, Clementi F. New antigen for antibody detection in myasthenia gravis. Neurology 1984; 34: 374-377.
9. Kautter DA, Lynt RK. Clostridium Botulinum *In*: Compendium of Methods for microbiological examination of food, 2nd edition, Speck ML, ed. American Public Health Inc., Washington 1984; 468-482.
10. Tindall RSA. Humoral immunity in MG: clinical correlations of anti-receptor antibody avidity and titer. NYAS 1981; 377: 316-329.

11. Lefvert AK. Acetylcholine receptor antibody in myasthenia gravis: purification and characterization. Scand J Imm 1978; 8: 525-533.
12. Vincent A, Newsom-Davis J. Acetylcholine receptor antibody as a diagnostic test for myasthenia gravis: results in 153 validated cases and 2967 diagnostic assays. J Neurol Neurosurg and Psych 1985; 48: 1246-1252.
13. Garlepp MJ, Kay RH, Dawkins RL. The diagnostic significance of autoantibodies to the acetylcholine receptor. J Neuroimm 1982; 3: 337-350.
14. Falpius BW, Miskin R, Reich F. Antibodies from myasthenic patients that prevent cholinergic agents from binding to nicotinic receptors. Proc Nat Acad Sc (USA) 1980; 77: 4326-4330.
15. Lindstrom J, Campbell M, Nate B. Specificities of antibodies to acetylcholine receptor. Muscle and Nerve 1978; 1: 140-145.

https://doi.org/10.1017/S0317167100026652 Published online by Cambridge University Press