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Acetylcholine Receptor Antibody in Patients with Myasthenia Gravis

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SUMMARY **The determination of acetylcholine receptor antibody (AChR Ab) titer by an enzyme-linked immunosorbent assay (ELISA) in patients with myasthenia gravis was introduced. The optimal conditions were determined by chequerboard determination. The specificity was confirmed by inhibition tests. The sensitivity is 9 p mole. The comparison of AChR Ab titers among 49 myasthenic patients, 19 non-myasthenic neurological patients and 20 healthy blood donors has shown that it is a highly sensitive, specific, reproducible, rapid, simple and inexpensive method for determining AChR Ab and that it is highly valuable for the diagnosis of myasthenia gravis.**

Myasthenia gravis is an autoimmune disorder.[1,2] Acetylcholine receptor antibody (AChR Ab) has been considered to be mainly responsible for the immunopathogenesis in myasthenia gravis. Detection of AChR Ab is certainly important for either clinical or immunopathological study of myasthenia gravis.

AChR Ab has been determined by radioimmunoassay since 1975.[3,4] The positivity is increasing with the improvement of the determining method.[5] Determination of AChR Ab has been probed by ELISA since 1980.[6] We introduce here an ELISA for determining AChR Ab level, and report its diagnostic value for myasthenia gravis.

MATERIALS AND METHODS

Subjects

Forty-nine patients with myasthenia gravis were included in this study. The diagnosis was confirmed by typical history, positive response to neostigmine and/or a characteristic decrement in the amplitude of the muscle evoked potential with a repetitive supramaximal nerve stimulation at low-frequency. The diagnoses of nineteen non-myasthenic neurological controls included epilepsy (five), vascular headache (five), Parkinsonian syndrome (four), progressive muscular dystrophy (two), amyotrophic lateral sclerosis (two) and trigeminal neuralgia (one). Twenty controls consisted of healthy blood donors.

Materials

The following materials were obtained as indicated : α-bungarotoxin (Sigma Chemical Company, St. Louis, MO); acetylcholine receptor (extracted from electric organ of electric eel, kindly donated by Dr. KK Wan from the Salk Institute for Biological Studies, San Diego, CA); O-phenylenediamine (Sigma); goat anti-human IgG peroxidase conjugate (Institute of Biological Products, Department of Health, Beijing, China, lot. 85-1). The AChR Ab titer of our pooled serum from patients with definite myasthenia gravis was quantitated using standard acetylcholine receptor antiserum donated by Dr. KK Wan. The titer of AChRAb was expressed as moles of ^{125}I-toxin binding sites precipitated per liter of serum. α-bungarotoxin specifically binds acetylcholine receptor, and was used for coating. Polystyrene microtiter plates made in

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Shanghai, China, with 40 wells, were used as the carrying surface for α-bungarotoxin.

Methods

α-bungarotoxin diluted in pH 9.6 coating buffer (0.05 M carbonate-bicarbonate buffer) was first coated (25 p mole/well). The middle layer was AChR (0.2 p mole/well) diluted in PBS-T (0.002 M KH_2PO_4, 0.008 M Na_2HPO_4, 0.137 M NaCl, 0.003 M KCl, 1 % Tween). The sandwich procedures were basically the same as described elsewhere. The optimal conditions were determined by chequerboard titration.

Specific inhibition test

The same volume (56 μl) with different amounts (0, 3.23, and 323 f mole/well) of AChR in PBS-T was added to three series of wells. The same concentration (4.5 p mole- 9.2 n mole/well) of standard AChR Ab (0.5 ml) as competitor was added and incubated for half an hour, then samples to be determined were added as previously described.

Reproducibility

Each of ten MG patients' sera was determined six times. Table 1 shows that the method was

Table 1 Test of reproducibility

Number of sample	Times of test						Mean	S.D.	C.V.%
	1	2	3	4	5	6			
1	1.15	1.13	1.11	1.18	1.20	1.17	1.16	0.03	2.59
2	5.90	6.10	6.20	5.80	6.00	5.70	5.95	0.19	3.19
3	0.60	0.57	0.61	0.59	0.62	0.63	0.60	0.02	3.33
4	0.72	0.70	0.69	0.74	0.75	0.71	0.72	0.02	2.76
5	0.70	0.73	0.71	0.69	0.67	0.68	0.70	0.02	2.86
6	0.44	0.42	0.45	0.46	0.43	0.44	0.44	0.01	2.27
7	1.04	1.00	0.99	1.02	1.03	1.02	1.02	0.02	1.96
8	2.10	2.15	2.12	2.09	2.07	2.05	2.10	0.04	1.90
9	2.50	2.65	2.55	2.47	2.60	2.45	2.54	0.08	3.15
10	4.00	4.10	4.15	4.20	3.90	3.80	4.03	0.15	3.72

$$\text{C.V. \%} = \frac{\text{S.D.}}{\text{Mean}} \times 100$$

Table 2 Determination of optimal conditions with chequerboard titration.

Conc. of AChR (p mole/well)	Dilution of conjugate	Concentration of coating α-bungarotoxin (p mole/well)											
		25						50					
		Conc. of AChR Ab (nM)						Conc. of AChR Ab (nM)					
		9.22	2.30	0.58	0.14	.036	PBST	9.22	2.30	0.58	0.14	.036	PBST
0.2	1: 5000	1.54	0.82	0.43	0.23	0.08	0.02	1.45	1.02	0.55	0.25	0.14	0.03
	1:10000	1.42	0.72	0.32	0.12	0.07	0.02	0.90	0.45	0.35	0.30	0.12	0.02
0.4	1: 5000	2.30	1.80	1.02	0.60	0.20	0.04	1.50	0.76	0.40	0.20	0.09	0.03
	1:10000	1.20	0.85	0.50	0.18	0.10	0.03	0.68	0.43	0.38	0.25	0.15	0.02

reproducible. The coefficient of variation (C.V.) was lower than 3.72 %; C.V. lower than 10 % was considered to be acceptable.

RESULTS

The determination of optimal conditions and the sensitivity of the method

The four major factors influencing this method were selected by chequerboard titration. These factors included the concentrations of α-bungarotoxin and AChR, the dilution of standard AChR Ab or serum to be determined, and the dilution of goat anti-human IgG conjugated with peroxidase. According to Table 2, we selected the optimal conditions as follows : α-bungarotoxin 25 p mole/well coated at 4°C overnight; AChR 0.2 p mole/well incubated for 1 hour at 37°C; the range of serial concentrations of standard AChR Ab was 9 pM-9.216 nM and incubated for half an hour at 37°C.

The standard curve of AChR Ab in Figure 1 suggested that the sensitivity of this method is 9 p mole.

Specificity of the method and standard curve of AChR Ab

Figure 1 shows that the determined O.D. values correlated inversely with the amount of AChR added when the amount of AChR Ab was fixed.

The range of AChR Ab in which there is a linear relationship between AChR Ab titers and O.D. values in the standard curve is 0.036-4.608 nM.

The comparison of AChR Ab titers in the three groups of subjects

Figure 2 revealed that the AChR Ab titer of patients with

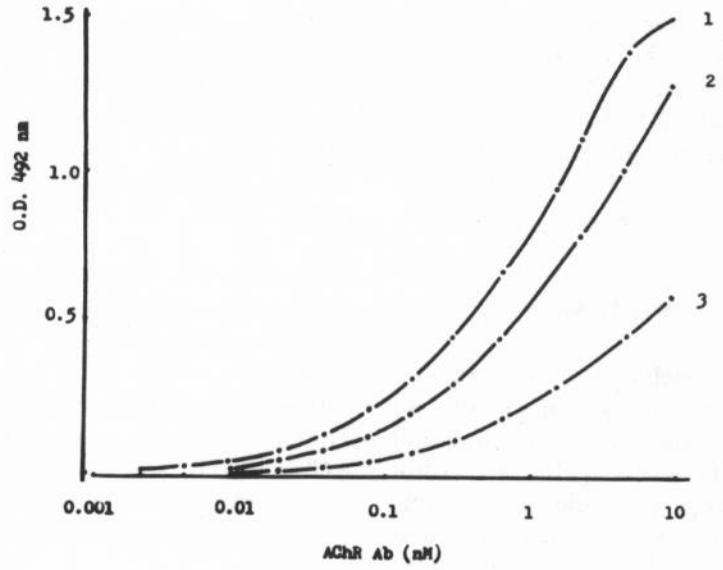

Fig 1. Specificity inhibition test. 1. PBS-T standard curve for AChR Ab (ELISA), 2. AChR 2.23 f mole/well and 3. AChR 223 f mole/well.

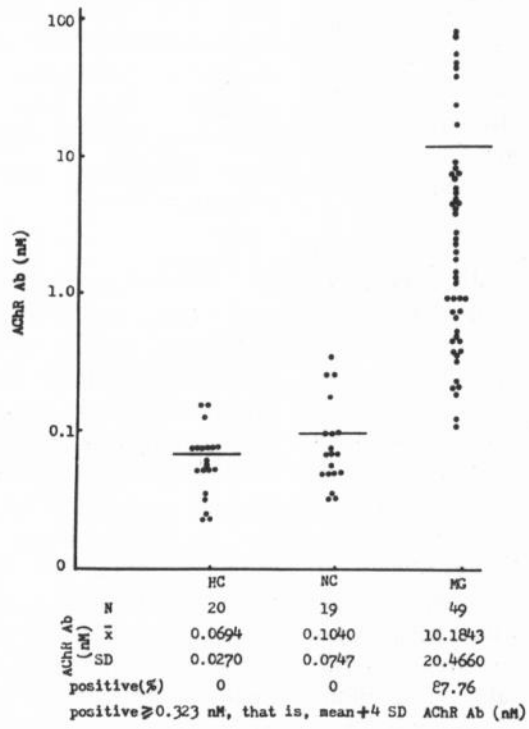

Fig 2. Distribution of AChR Ab titers in healthy controls (HC), non-myasthenic neurological controls (NC), and myasthenia gravis (MG).

myasthenia gravis (10.18 ±20.46) was significantly higher ($p < 0.01$) than that of controls (both healthy and non-myasthenic neurological controls, 0.09 ± 0.06), and that there is no significant difference ($p > 0.1$) of AChR Ab titers between non-myasthenic neurological controls (0.10 ± 0.07) and healthy controls (0.07 ± 0.03).

DISCUSSION

It is well known that myasthenia gravis is an autoimmune disease mainly mediated by humoral immunity involving the acetylcholine receptor in the postsynaptic membrane of the neuromuscular junction.[1,2] It may involve both adults and children.[7,8]

The ultrastructure of the postsynaptic region of the neuromuscular junction is simplified in myasthenia gravis.[7,9,10] The amount of acetylcholine receptor at the motor end plate in myasthenia gravis is reduced,[9,11] and many of the remaining receptors have antibody bound.[11] Immunization of animals with acetylcholine receptor can produce an experimental disease similar in clinical,[12-14] electrophysiological,[15] biochemical and immunochemical[16] aspects to that of MG in man; during its chronic phase, the motor end plates exhibit an ultrastructure similar to that found in myasthenia gravis,[17] the receptor content of muscle is reduced,[10] and many of the remaining receptors also have antibody bound.[18] Myasthenic symptoms can be produced in animals by passive transfer of IgG from patients with myasthenia gravis,[19] or of antibody from rats with experimental autoimmune myasthenia gravis to normal rats.[20] Circulating AChR Abs are found in 80-95 % of patients with myasthenia gravis but not in patients with other neuromuscular or autoimmune diseases or in normal controls.[1,3,5,21] The AChR Ab crosses the placenta and its presence in the circulation of the newborn is associated with the appearance of transient neonatal myasthenia gravis.[22] Removal of AChR Ab by thoracic duct drainage or plasmapheresis induces clinical relief. Reinfusion of cell free lymph or IgG provokes the reappearance or aggravation of the clinical manifestation.[2,23-25] In short, AChR Ab is mainly responsible for the immunopathogenesis in myasthenia gravis. Therefore, determination of serum AChR Ab is very important for either clinical or immunopathological study of myasthenia gravis.

AChR Ab titer has been determined by radioimmunoassay since 1975[3,4] and the titer essentially correlates well with patient severity.[1] The main drawbacks of radioimmunoassay are the short half-life of ^{125}I, the potential danger for technicians, the requirement for expensive equipment (e.g. γ-counter, *etc.*) and protecting apparatus, and the complexity and time consuming nature of the procedure.

ELISA is a serological technique that has been used to determine AChR Ab since 1980.[6] AChR is bound into the well by the specific binding between AChR and α-bungarotoxin coated in the well. If there are AChR Abs in the patient serum, the AChR Ab will be fixed into wells by specific binding between antigen and antibody. Our data revealed that AChR Ab titer in patients with myasthenia gravis is significantly higher than both healthy and non myasthenic neurological controls ($P < 0.01$, Fig.2). This suggests that this method of determining AChR Ab is highly valuable for the diagnosis of myasthenia gravis.

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