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Measurement of anti-acetylcholine receptor auto-antibodies in myasthenia gravis

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Two different acetylcholine receptor (AChR) preparations derived from amputated human muscle ($AChR_{AMP}$) and from the human rhabdomyosarcoma cell line TE671 ($AChR_{TE671}$) were compared in radio-immunoprecipitation assays for the detection of AChR auto-antibodies in serum specimens from 20 patients with proven myasthenia gravis. Tests performed with the $AChR_{TE671}$ and $AChR_{AMP}$ antigen preparations were positive in all the patients and in 19/20 respectively. A high degree of correlation (r = 0,94) was evident between the two auto-antigen preparations. Assays based on the use of TE671-derived antigen represent a useful alternative to the conventional assay using $AChR_{AMP}$ for the detection of AChR auto-antibodies.

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The demonstration of acetylcholine receptor (AChR) antibodies is considered to be essential in the diagnosis of suspected myasthenia gravis (MG).[1] These auto-antibodies are routinely assayed in serum specimens by radio-immunoassay (RIA) with AChR preparations from human skeletal muscle radiolabelled by snake toxins, usually α-bungarotoxin.[2] The absence of false-positive results in other auto-immune or neuromuscular diseases demonstrates the high specificity of this assay,[3] while the sensitivity is reported to vary between 70%[1] and 90%.[2]

A modified anti-AChR antibody assay using AChR prepared from the human rhabdomyosarcoma cell line TE671 has recently been described.[4-6] This AChR preparation has the advantages of improved accessibility and homogeneity, while eliminating the requirements for procurement and processing of potentially infectious clinical material.[7] This study compared these two different AChR antigen preparations in the standard radio-immunoprecipitation assay for the detection of AChR auto-antibodies.

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Materials and methods

Patients

Sera from 19 patients with generalised MG and 1 with ocular disease (patient 4) were analysed for AChR antibodies. Of these, 15 satisfied the electrophysiological criteria (electro-decremental response on repeated nerve stimulation and/or increased jitter as measured with single fibre electromyography) for the diagnosis of MG.[8,9] Of the remaining 5 patients, 3 had a positive edrophobium test (patients 5, 6 and 20), while patients 11 and 12 were diagnosed with MG on clinical features (fatigueability and response to treatment) alone. Fifteen were women and 5 men with average ages of 58 and 54 years respectively. The 3 patients younger than 40 years were all female (patients 2, 3 and 7). Patients 1, 2 and 3 were newly diagnosed and untreated, while the others had been diagnosed previously and treated with acetylcholine-esterase inhibitors and various combinations of immunosuppressive agents, thymectomy or plasma exchange.

Control test sera were obtained from 10 healthy individuals (1 man and 9 women with average ages of 24 and 27 years respectively).

Sources of AChR

For the preparation of amputated human muscle AChR ($AChR_{AMP}$) we used human gastrocnemius muscle obtained from the amputated leg of a diabetic patient. The tissue was processed as previously described[1] and the AChR-enriched detergent extract aliquoted and stored at –72°C until used. Immediately prior to use in assays of AChR auto-antibodies, this antigen preparation was labelled for 4 hours at 4°C with ^{125}I-labelled α-bungarotoxin (Amersham International, Amersham, Buckinghamshire, UK, > 200 Ci/mmol) as previously described.[1]

Detergent-solubilised AChR derived from the TE671 cell line ($AChR_{TE671}$) were obtained from a commercial source (RSR Ltd, Pentwyn, Cardiff, UK). These preparations were pre-labelled with ^{125}I-labelled α-bungarotoxin.

Measurement of AChR auto-antibodies

This was accomplished using a standard radio-immunoprecipitation assay.[1] Briefly, when $AChR_{AMP}$ was used as the source of auto-antigen, 50 µl of ^{125}Iα-bungarotoxin-labelled $AChR_{AMP}$ were co-incubated with 5 µl serum overnight at 4°C (the quantities of radiolabelled $AChR_{AMP}$ and serum were pre-determined in standardisation experiments), followed by the addition of 50 µl of goat anti-human IgG (RSR Ltd) and incubation for an additional 4-hour period at 4°C. Thereafter, 1 ml of washing solution (phosphate-buffered saline containing 0,05% Triton-x 100 and 0,01M sodium azide) was added to the tubes, which were centrifuged at 1 500 x g for 30 minutes. The supernatants were then aspirated and the pellets resuspended and the washing procedure repeated. Thereafter the radioactivity in the pellet was measured using a LKB 1261 Multigamma-counter (LKB, Turku, Finland).

The method was almost identical when the $AChR_{TE671}$ was used in the RIA procedure, except that shorter incubation

periods were used (2 hours at room temperature during incubation of $AChR_{TE671}$ (50 µl) with serum (5 µl) and a further 2 hours at 4°C following addition of goat anti-human IgG).

With both types of AChR preparation the auto-antibody concentrations in serum specimens are expressed as nanomoles/litre (nmoles/l) toxin bound.[1]

Results

Comparison of $AChR_{AMP}$ and $AChR_{TE671}$

The antibody concentrations in the sera of the patients with MG are shown in Table I. Although antibody titres were generally higher in assays using the $AChR_{AMP}$ preparation, a high degree of correlation (r = 0,94) between the $AChR_{AMP}$ and $AChR_{TE671}$ antibody titres was observed. Elevated levels of anti-AChR antibodies were found in the sera of 20/20 and 19/20 MG patients with the $AChR_{TE671}$ and $AChR_{AMP}$ auto-antigen preparations respectively; those of the 3 untreated patients were somewhat higher than those of the 17 treated patients (15,7 nmoles/l v. 8,3 nmoles/l for untreated and treated patients respectively using $AChR_{AMP}$; the corresponding values with $AChR_{TE671}$ were 12,4 nmoles/l and 6,3 nmoles/l).

Table I. Serum AChR auto-antibody titres in patients with myasthenia gravis

Patient	Sex	Age (yrs)	$AChR_{TE671}$	$AChR_{AMP}$
1	F	43	7,159	5,773
2	F	39	13,025	18,096
3	F	13	16,925	23,247
4	F	63	11,105	12,447
5	M	73	2,950	3,565
6	F	52	11,040	34,630
7	F	39	14,380	25,310
8	F	63	4,978	4,217
9	F	79	14,990	9,100
10	F	53	8,540	8,760
11	F	61	1,611	0,891
12	F	53	2,011	1,636
13	M	60	3,971	4,091
14	M	55	0,939	0,504
15	M	50	0,791	0,492
16	M	51	2,700	4,670
17	F	49	0,390	0,280
18	F	77	12,653	16,793
19	F	59	4,564	4,093
20	F	51	8,927	9,000

Results are expressed as nmoles/l toxin bound with cut-off points of 0,28 and 0,32 for the $AChR_{TE671}$ and $AChR_{AMP}$ preparations respectively.

The mean values ± the standard deviation of the 10 control sera were 0,16 ± 0,16 nmoles/l and 0,10 ± 0,17 nmoles/l with the $AChR_{AMP}$ and $AChR_{TE671}$ preparations respectively. Cut-off points were taken as the mean value for each AChR preparation ± 1 standard deviation.

Discussion

In this study we have demonstrated a close correlation (r = 0,94) between the $AChR_{AMP}$ and $AChR_{TE671}$ antibody concentrations in serum specimens from patients with MG. These observations are in agreement with several previous studies.[4,5,7] Moreover, we have also demonstrated the usefulness of the recently introduced, commercially available $AChR_{TE671}$-based AChR-antibody RIA kit. This method is clearly of comparable sensitivity and specificity to $AChR_{AMP}$-based methods but, in addition, has several distinct advantages, including elimination of procurement of tissue, cumbersome antigen processing procedures and antigen radiolabelling steps. With the removal of these potential deterrents, it is probable that an increasing number of laboratories will introduce this important serodiagnostic test.

Although a high degree of correlation was observed when the two different AChR preparations were used, titres observed with $AChR_{AMP}$ were generally higher (by about 25% on average). This difference has been reported by others [7] and may be related to TE671's expressing only the extrajunctional isoform of AChR, whereas $AChR_{AMP}$ contains both junctional and extrajunctional AChR.[10]

In conclusion, the commercially available $AChR_{TE671}$-based auto-antibody detection kit compares remarkably well with conventional $AChR_{AMP}$-based methods and is a more-than-useful alternative.

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