Clin. exp. Immunol. (1982) **49**, 266–272.

Acetylcholine receptor antibody characteristics in myasthenia gravis. II. Patients with penicillamine-induced myasthenia or idiopathic myasthenia of recent onset

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(Accepted for publication 12 March 1982)

SUMMARY

Anti-acetylcholine receptor (anti-AChR) antibody characteristics including light chain, IgG subclass, avidity for denervated human acetylcholine receptor and reaction with various human and mammalian AChR preparations were examined in 11 patients who developed myasthenia during penicillamine treatment of rheumatoid arthritis. Results were compared with those already reported in 35 patients with generalized idiopathic myasthenia gravis (MG). We found significant differences in the avidity and the light chain of the anti-AChR. However, anti-AChR characteristics in 12 patients with recent onset (<4 months' duration) idiopathic MG did not differ significantly from those in patients with penicillamine-induced MG. In the patients with generalized MG a trend was found towards higher percentage of kappa light chain and higher anti-AChR avidity with duration of disease. Anti-acetylcholine receptor antibodies in penicillamine-induced myasthenia gravis therefore appear to be similar to those of idiopathic myasthenia gravis of recent onset.

INTRODUCTION

A form of myasthenia gravis has been described which developed during penicillamine treatment of rheumatoid arthritis and also in two cases of Wilson's disease (Bucknall, Ballint & Dawkins, 1979). Symptoms usually involve the eye muscles first but generalized weakness may occur. More than 80% of cases improve within a few months of stopping penicillamine treatment. Anti-AChR antibodies are present and typically fall when treatment is discontinued (Vincent, Martin & Newsom-Davis, 1978).

Anti-AChR titres in penicillamine-associated MG (pen-MG) tend to be low compared to those found in generalized idiopathic MG and one might expect that the characteristics of the antibody would also prove to be different. We here compare the characteristics of anti-AChR in pen-MG with our previous results in patients with idiopathic disease (Vincent & Newsom-Davis, 1982) and with those in 12 patients with idiopathic MG whose symptoms were of less than 4 months' duration at the time of sampling.

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0009-9104/82/0800-0266$02.00

MATERIALS AND METHODS

Patients. Serum was taken from 11 patients with rheumatoid arthritis who had developed typical MG during penicillamine treatment (duration of symptoms <2 months) which remitted when the drug was withdrawn. Serum samples were also taken at the first available opportunity and a second sample up to 15 months later from 12 patients with idiopathic MG of recent origin (duration <4 months). Details of the patients are given in Table 1.

Table 1. Clinical details of patients

		Recent onset idiopathic MG	
	Penicillamine-associated MG	Thymoma	Non-thymoma
Number studied	11	6	6
M:F	6:5	2:4	3:3
Age at onset (years)	44–70	48–61	14–58
Duration of penicillamine treatment (months)	2–24	Not applicable	
Duration of symptoms at first sampling (months)	0–2	1–4	2–4
Second sample			
Duration of symptoms (months)	n.d.	5–15	6–16
Number on immunosuppression (duration, months)	n.d.	5 (4–12)	2 (1–5)
Number thymectomized (time since operation, months)	n.d.	5 (3–12)	6 (1–9)

Acetylcholine receptor preparations. Several different receptor preparations were used in this study and their origin and the methods of extraction are described in detail elsewhere (Vincent & Newsom-Davis, 1982). Most of the assays were performed using denervated human calf muscle (d-AChR) obtained at amputation from a patient with diabetes mellitus and peripheral neuropathy. Acetylcholine receptor was also extracted from normal human calf muscle (n-AChR) and extraocular muscle (oc-AChR) taken post mortem, from cultured fetal human muscle cells, and from a mouse non-fusing cell line (BC3H1, kindly donated by Dr S. Bevan). The muscle was homogenized, the membranes extracted in Triton X-100 and labelled with 2·5 nM ^{125}I-α-bungarotoxin (^{125}I-α-ButTx) as previously described (Vincent & Newsom-Davis, 1982).

Anti-AChR assays. The assays of penicillamine-associated MG sera were performed simultaneously with those of idiopathic generalized MG sera (Vincent & Newsom-Davis, 1982) with which they were compared. All assays were done at the same concentration of AChR (0·1 nM) unless otherwise stated, using ≃4 fmoles of anti-AChR (0·5–2·5 μl serum).

Proportion of light chains and IgG subclasses. The representation of κ and λ light chains was determined by precipitation of antibody–^{125}I-α-ButTx–AChR complexes with 3% polyethylene glycol (PEG)-treated specific anti-sera (Seward Laboratories Ltd.), aided by 3% PEG. The proportion of IgG3 in the anti-AChR was determined similarly using anti-human IgG3 (for details see Vincent & Newsom-Davis, 1982; Vincent & Bilkhu, 1982).

Inhibition of α-ButTx binding. Fifty fmoles of AChR were incubated with approximately 500 fmoles of anti-AChR from each patient. The binding of ^{125}I-a-ButTx at 5 nM was subsequently measured in duplicate by a filter disc assay (Vincent & Newsom-Davis, 1982). Inhibition (number of c.p.m. inhibited compared to control sera incubations) was expressed as % of the number of c.p.m. precipitated based on limiting serum dilutions.

Anti-AChR avidity. A constant amount of serum ($\simeq$ 4 fmoles) was incubated for 4 hr with 100 μl d-AChR at 0·1 and 0·4 nM and the results expressed as a ratio. Seven of the penicillamine-associated sera and all sera from patients with idiopathic MG of recent onset were assayed at varying AChR concentrations (0·03–0·5 nM) and incubated for between 2 and 72 hr, usually 6–8 hr, at room temperature.

Statistics. Statistical differences between the groups were assessed by Student's *t*-test. Where the variances of the groups differed, the significance of the test was tested by the method of Cochrane & Cox (Downie & Heath, 1974).

RESULTS

Penicillamine-associated MG

The anti-AChR titres in pen-MG were significantly lower ($P < 0{\cdot}001$) than the values reported for patients with generalized idiopathic disease (Table 2) and only a little higher than those in patients with purely ocular disease (Vincent & Newsom-Davis, 1982). The mean value for κ light chain (58 ± 19 (s.d.)%) in the anti-AChR was similar to the mean value for the κ light chain in normal human IgG (Eickhoff & Heipertz, 1977), but the values were more widely distributed (Fig. 1). The contribution of IgG 3 subclass was also variable (Fig. 1) and significantly higher than in patients with idiopathic disease (Table 2).

Inhibition of α-BuTx binding

There was relatively little inhibition of α-BuTx binding to d-AChR in the presence of excess antibody, and the values represented between 0 and 4·5% of the total anti-AChR present (see Fig. 1c, Table 2).

Cross-reactivity with other receptor preparations

Pen-MG anti-AChR reacted poorly with n- or oc-AChR compared to d-AChR (Table 2). Four sera showed insignificant reactivity with rat or mouse AChR at the concentration used (0·1 M), and with incubation times of 2–4 hr. However, as shown previously (Vincent & Newsom-Davis, 1982) for ocular MG anti-AChR, when higher concentrations of AChR and longer incubation times were used, cross-reactivity could be demonstrated (not shown). The mean value for cross-reactivity with

Table 2. Results and comparison with previous results in patients with idiopathic MG of long duration

	Generalized idiopathic MG, duration > 1 year§	Penicillamine-associated MG	Idiopathic MG duration < 4 m
Number tested	35	11	12
Anti-AChR titre	71·8 ± 72·4	6·5 ± 3·8‡	55·7 ± 101·9
% κ light chain	79 ± 21	58 ± 18†	66 ± 32
% IgG3	9·8 ± 12·7	18 ± 9·6*	16 ± 10
% anti-α-BuTx site	3·7 ± 3·9	1·9 ± 1·2*	0·7 ± 0·9‡
% anti-normal AChR	90 ± 32	68 ± 13†	n.d.
% anti-ocular AChR	54 ± 21 (21)	55 ± 25 (5)	n.d.
% anti-rat AChR	19 ± 17	9·4 ± 10*	n.d.
% anti-mouse AChR	13 ± 9·4	3·7 ± 5·7‡	n.d.
Anti-AChR avidity (AChR 0·1 nM/AChR 0·4 nM)	0·61 ± 0·1	0·38 ± 0·06*	n.d.
K_D from Scatchard plots ($\times 10^{-12}$ M)	23·5 ± 12·4 (20)	72·7 ± 41·2* (7)	57·0 ± 38·4* (12)

* $P < 0{\cdot}05$, † $P < 0{\cdot}01$, ‡ $P < 0{\cdot}001$, different from generalized group. Results are mean ± s.d.

§ Results from Vincent & Newsom-Davis, 1982.

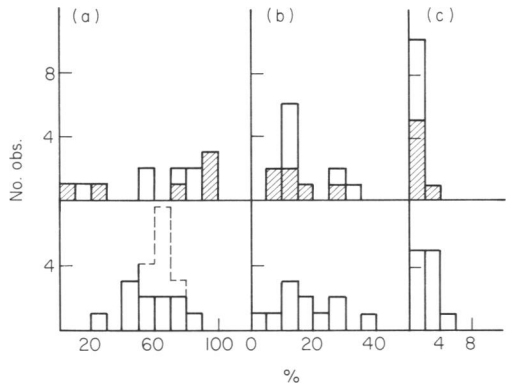

Fig. 1. Antibody characteristics in patients with penicillamine-associated MG and in those with idiopathic MG of recent onset. The histograms show (a) % κ, (b) % IgG3 and (c) % of antibodies inhibiting α-BuTx binding, in the pen-MG cases (below), and idiopathic MG patients of recent onset (above); the thymoma cases are shown as hatched columns. The dashed line represents the distribution of values in normal IgG (Eickoff & Heipertz, 1977).

mouse AChR, using short incubation times (Table 2), was significantly lower than that found for generalized idiopathic MG.

Anti-AChR avidity

In patients with pen-MG the ratio of c.p.m. precipitated at two concentrations of d-AChR differed significantly from that in patients with generalized idiopathic disease (Table 2). Seven sera were also examined at varying concentrations of AChR and incubation times. Typical results from one pen-MG patient and one patient with idiopathic MG are shown in Fig. 2a, and redrawn as Scatchard plots in Fig. 2b. Serum from the pen-MG patient reacted more slowly and showed only

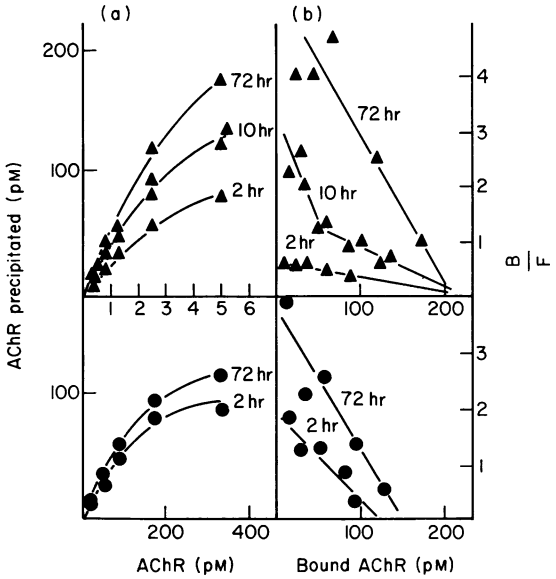

Fig. 2. Avidity of anti-AChR. (a) the binding of sera from a pen-MG case (upper panel) and a typical idiopathic case (lower panel) are illustrated at various AChR concentrations and at different times of incubation. In (b) Scatchard plots of the data show an increase in 'avidity' with time of incubation particularly in pen-MG but no change in the total AChR precipitated (intercepts on the abscissa).

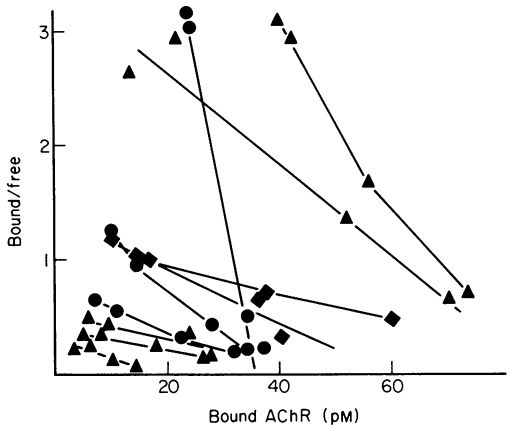

Fig. 3. Avidity of anti-AChR. Scatchard plots from patients with pen-MG (▲), thymomatous MG of recent onset (●) and non-thymomatous MG of recent onset (♦). Note that there is a wide range of binding avidities, as indicated by the slopes of the lines. Incubation time = 10 hr.

low avidity binding with short incubations. With longer incubation the apparent avidity increased and showed an intermediate phase in which more than one binding constant could be seen. Serum from the patient with typical generalized idiopathic MG showed faster equilibration and relatively little increase in binding after 2 hr incubation (Fig. 2). Anti-AChR from patients with typical generalized idiopathic MG seldom showed a low avidity component except at the very highest AChR concentrations that we used (1 nM) (Vincent & Newsom-Davis, 1982). When an incubation period of 6–8 hr was used some of the pen-MG sera showed more than one binding avidity but two had high avidity anti-AChR indistinguishable from that found in idiopathic MG (Fig. 3). The mean results of binding avidity in pen-MG are different from those of idiopathic MG (Table 2).

Patients with recent onset myasthenia gravis

Anti-AChR from 12 patients, in whom symptoms had presented less than 4 months before the serum samples, was examined for the percentage of κ light chain, IgG3 contribution, inhibition of α-BuTx binding and anti-AChR avidity (Fig. 1 and Table 2). Compared to the values in pen-MG, the mean anti-AChR titre was higher but the difference was not significant. The mean percentage of κ light chain in the anti-AChR was very similar though the distribution was less symmetrical. The percentage of IgG3 was also similar but the percentage of antibodies inhibiting α-BuTx binding to AChR was lower. Interestingly, this characteristic of anti-AChR in patients with recent onset MG was also significantly lower than that in patients with idiopathic disease of longer duration.

The antibody avidity in patients of recent onset idiopathic MG showed similar variability to that found in patients with pen-MG and the mean value was significantly different from that in patients of longer duration. Several of the patients had relatively low antibody avidity (Fig. 3) but four patients gave values similar to those found in patients with longer duration of disease.

Antibody characteristics and duration of disease

We looked at one further sample from each of the 12 recent onset idiopathic MG patients (results not shown). There was a striking change in antibody titre in many of the patients between the first and second sample, probably attributable to the fact that most had undergone thymectomy and several had started prednisone treatment in the interval. Antibody characteristics also showed some changes but the mean results were not significantly different between the two samples (not shown). However, when the results from pen-MG and recent onset idiopathic MG patients were compared with results from all patients with generalized disease of longer duration (Fig. 4) there was a trend towards higher avidity and higher percentage of κ light chain with time, although this was not significant (Pearson correlation coefficient).

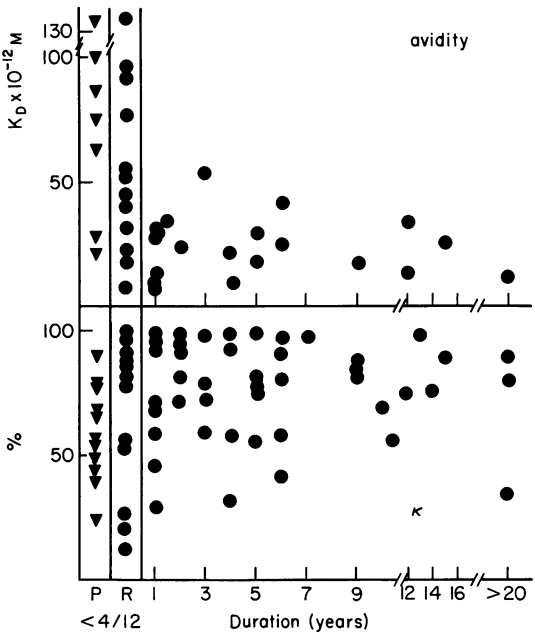

Fig. 4. Antibody characteristics and duration of symptoms. (a) results of antibody avidity determinations on patients with pen-MG (P), idiopathic MG of recent onset (R), and patients with generalized idiopathic disease of variable duration (Vincent & Newsom-Davis, 1982 and unpublished data). (b) percentage of κ light chain.

DISCUSSION

A small proportion of patients undergoing treatment with d-penicillamine develop MG (Bucknall *et al.*, 1979). This form of MG is of potential interest experimentally because it may represent a disease which could be induced in laboratory animals, although attempts to do this have not yet been convincing (Aldrich, Kim & Sanders, 1979; Burres *et al.*, 1979). The validity of this model would depend to some extent on whether the anti-AChR antibodies in pen-MG are similar to those found in idiopathic MG. We have shown here that as a group pen-MG anti-AChR antibodies can be distinguished from those in patients with idiopathic MG by the proportion of κ light chains, the proportion of IgG3, and the anti-AChR avidity. However, when these patients' antibodies were compared to those in patients with recent onset idiopathic MG the differences were not significant. In both groups of patients recent onset of disease was associated with variability in the percentage of κ light chain and in antibody avidity.

None of the pen-MG anti-AChR was 100% κ or λ so clearly penicillamine-induced anti-AChR is not usually monoclonal. Pen-MG anti-AChR also reacted with mouse or rat AChR suggesting that these antibodies are not as restricted in their heterogeneity as has been reported elsewhere (Dawkins, Christiansen & Garlepp, 1981). This discrepancy could relate to the AChR concentration and incubation times used. Cross-reacting antibody might not be evident if the AChR concentration was low and the incubation period short.

Pen-MG anti-AChR measured using short incubation times appeared to be of lower avidity than that found in patients with generalized MG. This difference in avidity however was not so evident when longer incubations were used, and at intermediate time intervals there was sometimes evidence for two different binding constants. The reason for these findings is not clear but they could reflect a time-dependent formation of divalent binding. If this is the case, the two binding sites would presumably be on the same AChR molecule since there was no apparent increase in the intercept value at differing times of incubation (Fig. 2).

Our results suggest that anti-AChR characteristics change during the course of the disease and that antibodies present early on are polyclonal and of relatively low avidity. The trend towards higher proportion of κ light chain and higher avidity with time could be explained not only by the natural course of the disease, but also by the influence of treatment. Twenty-eight out of the 47 patients with idiopathic MG of more than 1 year's duration were on prednisone treatment, and 25 patients had had thymectomy.

Serum anti-AChR levels in pen-MG usually fall too rapidly after stopping treatment for analysis of the influence of duration. However, we have recently found anti-AChR in two patients with rheumatoid arthritis receiving penicillamine treatment who did not have any sign of MG (Martin, Vincent & Clarke, 1980). It is interesting that anti-AChR in these patients, 6 months after anti-AChR was first detected, was more than 85% κ light chain with k_{Ds} of $< 30 \times 10^{-12}$ M (Vincent & Martin, unpublished results). These findings suggest that characteristics of penicillamine-induced anti-AChR could also be influenced by duration.

The percentage of IgG3 was not significantly higher in patients with recent onset idiopathic MG than in those with longer duration of the disease. Moreover antibody inhibiting α-BuTx binding was particularly low. This does not support the suggestion of Fulpius *et al.*, (1981) that antibody early on in the disease is IgG3 and specifically directed at the a-BuTx or acetylcholine binding site.

Our results show that anti-AChR in pen-MG, although different from that in the majority of patients with idiopathic disease, was not distinguishable from antibody in patients with disease of recent onset. This suggests that the nature of the loss of tolerance may be similar in the two conditions. Thus if pen-MG can be reliably induced in experimental animals, it could provide a useful model of the human disease, particularly for testing experimental approaches to specific forms of therapy.

We thank the many doctors who have sent serum from patients with penicillamine-induced myasthenia. The work was supported by the Medical Research Council.

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