

Acetylcholine receptor antibody in myasthenia gravis: predominance of IgG subclasses 1 and 3

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SUMMARY

The concentrations of IgG subclass antibodies (Ab) to acetylcholine receptor (AChR) were quantified in 36 patients with myasthenia gravis (MG) treated with pyridostigmine only, and in eight patients who underwent thymectomy, using an IgG subclass-specific immunoprecipitation assay. IgG1, IgG2, IgG3, and IgG4 subclass Ab to AChR were present in 100%, 33%, 64% and 39% of the pyridostigmine-treated patients, respectively. The concentration of IgG1 Ab increased significantly with disease severity as graded by the Osserman-Genkins classification ($r_s = 0.37$, $P < 0.05$). IgG1 and IgG3 subclass protein concentrations were significantly higher ($P < 0.0003$) in the 36 pyridostigmine-treated MG patients than in 44 age- and sex-matched healthy subjects. Thymectomy induced an appreciable reduction in anti-AChR IgG1 concentration in two patients, whereas six patients showed no changes in Ab to AChR. The results support the hypothesis that binding of anti-AChR IgG1 and IgG3 on AChR in the neuromuscular junction followed by complement-mediated cell lysis or phagocytosis, may play a role in the pathogenesis of MG.

Keywords Acetylcholine receptor antibody complement-mediated cell lysis IgG subclasses myasthenia gravis thymectomy

INTRODUCTION

Myasthenia gravis (MG) is an autoimmune disease mediated by circulating antibodies (Ab) of IgG class to acetylcholine receptors (AChR) in the neuromuscular junction of striated muscle. The Ab concentration does not correlate with the severity of disease within a group of MG patients (see review by Vincent, 1980). A clue to this lack of correlation could be variations in the concentrations of the IgG subclasses of Ab to AChR. Determination of anti-AChR IgG subclasses is also relevant to obtain information on the mechanism of cell destruction in postsynaptic folds. Different IgG subclasses are involved in complement activation and in antibody-dependent cellular cytotoxicity (see review by Natvig & Kunkel, 1973), which may play a pathogenic role in MG.

Previous studies of the distribution of anti-AChR IgG subclasses in plasma from MG patients have been divergent. These studies, however, were hampered by the lack of specific methods for

determination of the IgG subclasses (Lefvert & Bergström, 1978; Lefvert, Cuenoad & Fulpius, 1981; Tindall, 1981; Vincent & Bilkhu, 1982). By the availability of monoclonal Ab to IgG subclasses 1–4 it became possible to develop an IgG subclass specific immunoprecipitation assay (Nielsen *et al.*, 1985), allowing a more accurate determination of the anti-AchR IgG subclass concentrations in MG patients.

The aim of the present study has been to quantify the IgG subclass Ab to AchR in a group of patients with MG treated with pyridostigmine only, and to investigate the influence of thymectomy on the IgG subclass concentrations. To elucidate the possible pathogenic role of IgG subclass Ab to AchR we sought for statistical relationships between IgG subclass Ab concentrations and disease severity as graded by the Osserman–Genkins classification (1971), as well as different clinical types of MG as classified by Compston *et al.* (1980). To evaluate whether the observed predominance of IgG1 and IgG3 subclass Ab is part of a more pronounced disturbance of IgG subclass metabolism we also compared IgG subclass protein concentrations in patients with MG and in normal subjects.

MATERIALS AND METHODS

Patients and normal subjects. Two groups of patients with MG have been investigated: The first group contained 36 patients which have been treated with pyridostigmine, but not with prednisone, cytotoxic drugs, plasmapheresis or thymectomy, the clinical features of which are given in Table 1.

The second group contained eight patients who underwent suprasternal thymectomy. Plasma samples were collected before and after the operation over periods of 9–54 months. None of these patients had thymomas nor were treated with immunosuppressive drugs or plasmapheresis. All patients showed clinical improvement in the observation period. Plasma samples were kept at -20°C after addition of 2500 kiu/ml aprotinine to inhibit proteolysis.

Plasma samples from 44 healthy subjects were used as reference for determination of the IgG subclass protein concentrations in plasma. The reference group matched the patients group regarding age and sex.

Determination of total anti-AchR IgG. The plasma concentrations of anti-AchR IgG was determined as described by Lindstrøm (1977) with the modifications of Vincent & Newsom-Davis (1982). AchR were extracted from skeletal muscle of human amputated legs and labelled with ^{125}I - α -bungarotoxin (α -bgt) followed by incubation with MG patient plasma and precipitation with Ab

Table 1. Clinical features of 36 MG patients treated with pyridostigmine only

	No. of patients	% of total
	25(F)	70(F)
	11(M)	30(M)
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Osserman & Genkins (1971) classification		
I ocular	2	6
IIa mild generalized	8	22
IIb moderate generalized	13	36
III acute severe	8	22
IV chronic severe	5	14
Compston <i>et al.</i> (1980) classification		
A MG and thymoma	0	0
B onset of MG under 40 years	23	64
C onset of MG over 40 years	13	36

(rabbit) to human IgG (Dakopatts, Copenhagen, Denmark). The concentration was expressed as precipitated ^{125}I - α -bgt-AchR in nmol/l plasma. The detection limit of the assay was 0.5 nmol/l.

Determination of anti-AchR IgG subclasses. The assay used for determination of anti-AchR IgG subclass Ab concentrations was performed as previously described in detail (Nielsen *et al.*, 1985). Briefly ^{125}I - α -bgt labelled AchR was incubated with diluted MG patient plasma with AchR excess followed by monoclonal Ab (mouse) to human IgG subclasses (Unipath, London, UK). The amounts of added antibodies were anti-IgG1: 5 μl ; anti-IgG2: 2 μl ; anti-IgG3: 0.25 μl ; or anti-IgG4: 0.25 μl . To tubes containing anti-IgG3 and anti-IgG4 mouse serum was added as IgG carrier. Finally, anti-IgG subclasses were precipitated with Ab (rabbit) to mouse IgG, which has been absorbed with human IgG (Dakopatts, Copenhagen, Denmark). The concentration of anti-AchR IgG of each subclass was expressed as precipitated ^{125}I - α -bgt-AchR in nmol/l plasma after correction for nonspecifically precipitated ^{125}I -activity using normal human plasma as control. The detection limit of the assay was 0.5 nmol/l.

Determination of plasma IgG subclass proteins. The IgG subclass protein concentrations in plasma were determined by radial immunodiffusion (French & Harrison, 1984). Anti-human IgG subclass in 1.4% agarose gel (0.1 M barbitone buffer, pH 8.6) containing 5% PEG 4000 (Merck) was poured on Gelbond film, and 5 μl plasma samples were applied in 2 mm wells. For determination of IgG1 and IgG2 samples were diluted 1 + 6, whereas IgG3 and IgG4 were measured in undiluted plasma. After diffusion for 48 h at 4°C plates were fixed with 1% glutaraldehyde and the immunoprecipitates measured. Pooled normal human serum (Red Cross, Amsterdam, The Netherlands) was used as IgG subclass standards. The content is: IgG1 6.2 mg/ml, IgG2 2.4 mg/ml, IgG3 0.64 mg/ml and IgG4 0.46 mg/ml.

Statistics. The Mann-Whitney *U*-test was used to evaluate whether observed differences in concentrations of IgG subclass Ab to AchR or of IgG subclass proteins between groups of patients or normal subjects were statistically significant. Because only about 1/3 of MG patients had detectable levels of IgG2 and/or IgG4 Ab to AchR, statistical calculation based on ranking on an ordinal scale was not attempted for comparison of groups regarding these IgG subclass Ab. Instead we compared the frequencies of IgG2 and IgG4 positive patients in the groups by the G-test of independence (Sokal & Rohlf, 1981).

Association between disease severity, as graded by the Osserman–Genkins classification, and anti-AchR IgG and IgG subclass levels was sought for by calculation of Spearman's coefficient of correlation (r_s) with correction for ties. Due to the high number of tied observations in which IgG2 and/or IgG4 Ab to AchR were absent, it was not possible to calculate valid estimates of r_s for correlation of disease severity with levels of these IgG subclass Ab. Differences with a two-sided probability equal to or less than 0.05 were considered statistically significant.

RESULTS

The concentrations of IgG and IgG subclass Ab to AchR in 36 patients with MG treated with pyridostigmine only, are shown in Fig. 1. All patients had IgG1 Ab, whereas 12 (33%), 23 (64%), and 14 (39%) had IgG2, IgG3, and IgG4 Ab, respectively. Only six patients demonstrated Ab to AchR of all four subclasses, and three among these had severe disease activity (Osserman–Genkins, grade 4). The concentration of Ab to AchR in patients with different disease severity as graded by Osserman–Genkins classification are shown in Table 2. Although the median concentration of IgG1 Ab (Table 2) tended to increase between groups with consecutive grades of disease severity, the changes were not significant. The relationship between disease severity and total IgG, IgG1, and IgG3 Ab concentrations was further investigated by calculation of Spearman's coefficient of correlation. In doing this, we assumed that an Osserman–Genkins grade reflected increased disease severity. We found that both total IgG Ab and IgG3 Ab concentrations tended to be associated with severity of disease ($r_s = 0.30$ and 0.29 ; $0.05 < P < 0.10$); but only the IgG1 Ab concentration shows significant correlation ($r_s = 0.37$; $P < 0.05$).

The anti-AchR IgG and IgG subclass concentrations in 23 patients belonging to Compston's class B and 13 patients belonging to class C were compared. The concentrations of IgG, IgG1, and

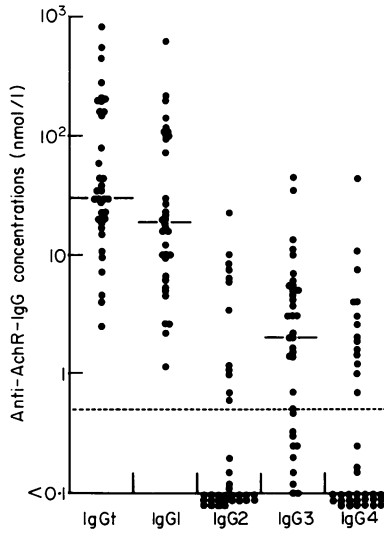


Fig. 1. Anti-AchR IgG subclass concentrations (nmol/l) in 36 MG patients treated with pyridostigmine only. The median values of IgG, IgG1 and IgG3 Ab concentrations are shown as horizontal bars. The detection limit of the assay (0.5 nmol/l) is shown as a broken line.

IgG3 Ab were not significantly different in the two groups. Neither was the frequency of IgG2 Ab positive or IgG4 Ab positive patients different (data not shown).

Concentrations of IgG and IgG subclass Ab to AchR were also determined in eight patients who underwent thymectomy. Due to different periods of follow-up, and different sampling intervals the data were not considered suitable for statistical analysis. As estimated from Fig. 2, six of eight patients showed no changes in anti-AchR IgG or IgG subclass concentrations during the observation period. An appreciable reduction of anti-AchR IgG to 27% and 72% of preoperative values was seen in two patients (Nos 2 and 4 in Fig.2). Patient no.2 had Ab to AchR of all four

Table 2. Anti-AchR and IgG subclass concentration (nmol/l) in MG patients with different disease severity graded according to Osserman & Genkins (1971); data are presented as the median value (25 to 75 percentiles)

Osserman- Genkins grade (no. of patients)	IgG	IgG1	IgG2	IgG3	IgG4
1 (2)	3.6 (2.5-4.7)	2.45 (2.2-2.7)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)
2A (8)	37.2 (14.5-135.5)	12.1 (6.6-61.5)	0.0 (0.0-3.3)	1.8 (0.0-3.4)	0.0 (0.0-1.0)
2B (13)	30.0 (20.5-152.0)	16.3 (5.0-103.0)	0.0 (0.0-0.0)	2.2 (0.0-4.5)	1.0 (0.0-2.0)
3 (8)	36.5 (29.5-375.5)	22.4 (18.5-160.0)	0.0 (0.0-0.5)	1.9 (0.0-5.6)	0.0 (0.0-0.4)
4 (5)	80.0 (23.5-200.0)	30.0 (12.0-72.0)	3.5 (1.2-8.4)	5.6 (1.4-7.0)	1.4 (0.0-2.6)
All patients	32.5 (19.9-163.5)	17.4 (8.2-97.5)	0.0 (0.0-1.1)	2.0 (0.0-5.1)	0.0 (0.0-1.8)

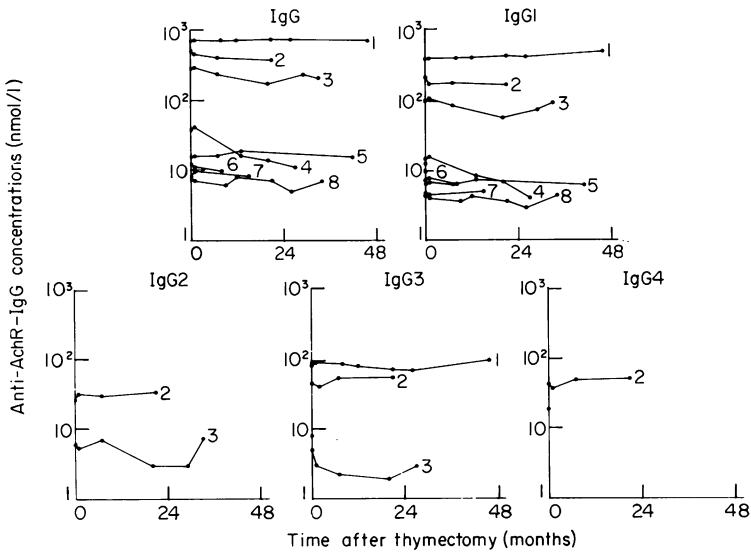


Fig. 2. Anti-AchR IgG and IgG subclass concentrations (nmol/l) in eight patients with MG after suprasternal thymectomy. Preoperative values are marked on the ordinate. On the abscissa is indicated the time after thymectomy. The patients are numbered from 1–8.

Table 3. IgG subclass protein concentrations (g/l) in MG patients and healthy subjects; data are presented as the median value (25 to 75 percentiles)

Group (no. of subjects)	IgG1	IgG2	IgG3	IgG4
MG patients (36)	7.2 (6.2–8.7)	3.2 (2.1–4.8)	0.69 (0.56–0.99)	0.63 (0.24–1.3)
Healthy subjects (44)	6.0 (4.5–6.2)	2.7 (1.7–3.7)	0.40 (0.29–0.60)	0.46 (0.23–0.90)

The concentrations of IgG1 and IgG3 proteins were significantly higher ($P < 0.0003$) in MG patients than in healthy subjects.

subclasses, but the reduction in total anti-AchR IgG was only reflected in IgG1. Patient no.4 had only IgG1 Ab.

The concentrations of IgG1 and IgG3 proteins were significantly higher in the 36 pyridostigmine-treated patients than in healthy subjects ($P < 0.0003$) (Table 3). In patients belonging to Compston's group B the IgG1 subclass protein concentration was significantly higher than in group C (7.3 g/l (6.2–8.7) versus 6.2 g/l (5.5–8.2); $P < 0.05$), whereas the concentrations of IgG2, IgG3 and IgG4 subclass proteins were not significantly different.

DISCUSSION

The present study shows that IgG1 and IgG3 subclass Ab to AchR are found in 100% and 64% of 36 pyridostigmine-treated MG patients, whereas IgG2 and IgG4 are present in only 33% and 39% of the patients. This is in accordance with previous reports by Lefvert & Bergström (1978) and Lefvert

et al. (1981). Thus, use of highly specific monoclonal Ab to IgG subclasses 1–4 (Djurup *et al.*, 1984) in an immunoprecipitation assay (Nielsen *et al.*, 1985) gave similar results to the preparative methods of Lefvert and colleagues (1978; 1981). In contrast, immunoprecipitation of anti-AchR IgG subclasses with polyclonal antibodies showed a distribution similar to the IgG subclass protein distribution in normal human plasma (Vincent & Bilkhu, 1982). The reason for this discrepancy is not known, but MG patients seem to be heterogenous because anti-AchR IgG2 and IgG4 were present in only 1/3 of our patients (cf. Fig.1). Other possibilities may be the cross-reactivity of polyclonal Ab with other IgG subclasses (Djurup *et al.*, 1984), and a higher susceptibility of IgG3 to proteolysis, which was prevented by addition of aprotinin in our study.

The finding of a significant positive correlation between anti-AchR IgG1 concentration and severity of disease as graded by the Osserman–Genkins classification indicates that IgG1 Ab to AchR are involved in the immune attack on end-plates in MG. The IgG3 Ab concentration tended also to be positively correlated with disease severity, but the correlation was not statistically significant ($0.05 < P < 0.10$). However, taking into consideration the lower frequency of IgG3 Ab positive patients (64% versus 100% IgG1 Ab positive), it is likely that both complement-binding IgG subclasses are involved in the Ab-dependent destruction of AchR. Interestingly, the concentrations of anti-AchR IgG2 and IgG4 showed no negative correlation with disease severity suggesting that non-complement fixing IgG subclasses are not a determining factor in low grade disease by blocking complement-mediated cell destruction.

The amelioration of symptoms in eight MG patients after thymectomy was followed by reduction of total anti-AchR-IgG concentration in only two patients, accounted for by a decrease in IgG1, whereas in six patients both total anti-AchR-IgG and IgG subclasses remained unaltered. Two previous studies have reported decreased levels of anti-AchR IgG following thymectomy (Scadding, Thomas & Havard, 1977; Lefvert *et al.* 1978), whereas Roses *et al.* (1981) observed no change. The reason for these controversies are unclear, but MG patients may be heterogenous with respect to the pathogenic role of circulating Ab to AchR.

In MG patients treated with pyridostigmine only, IgG1 and IgG3 protein concentrations were significantly higher than in normal subjects. This observation suggests that the occurrence of high IgG1 and IgG3 Ab to AchR is part of a more profound disturbance of the immune system. The increased levels of IgG1 and IgG3 proteins may be due to either increased synthesis, e.g. polyclonal B-cell activation, or decreased catabolism, e.g. overloading of the phagocytic capacity of the reticuloendothelial system caused by circulating complexes of AchR, IgG and complement. Studies of IgG1 and IgG3 metabolism in MG patients are needed to investigate these possibilities.

In conclusion, the predominance of IgG1 and IgG3 Ab to AchR, and the correlation between IgG1 Ab concentration and disease severity lends support to the hypothesis that Ab-dependent complement-mediated cell lysis or phagocytosis may play roles in the damage of neuromuscular end-plates. This is supported by the experience that plasma exchange with about 60% reduction in circulating IgG levels is followed by clinical improvement (Vincent, 1980). Recently, selective removal of plasma IgG by specific absorption to protein A from *Staphylococcus aureus* has been proposed as a therapeutic procedure in MG (Szpirt *et al.*, 1985). Protein A binds IgG1, IgG2, IgG4, but only a few quantitatively insignificant allotypes of IgG3 (Van Loghem *et al.*, 1982), and this treatment is therefore restricted to MG patients with low or absent anti-AchR IgG3.

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