Clin. exp. Immunol. (1985) **60,** 637–644.

Anti-acetylcholine receptor idiotypes in myasthenia gravis analysed by rabbit anti-sera

B. LANG, A. J. ROBERTS, A. VINCENT & J. NEWSOM-DAVIS *Department of Neurological Science, Royal Free Hospital School of Medicine, London, UK*

(Accepted for publication 20 December 1984)

SUMMARY

Anti-idiotype sera, raised in rabbits against anti-acetylcholine receptor (AChR) (idiotype) purified from the serum of three myasthenia gravis patients, inhibited binding of homologous idiotype to the AChR by up to 80%. The expression of idiotype in the three individuals changed very little over a period of several years, during which they showed a declining trend in overall anti-AChR antibody. Only one of the four anti-idiotype sera inhibited the binding of anti-AChR from a number of other patients. Our results indicate a consistency of idiotype expression within an individual, and fail to show substantial idiotype sharing between individuals.

Keywords idiotype sharing anti-idiotypes myasthenia gravis anti-acetycholine receptor

INTRODUCTION

Anti-idiotype antibodies may play an important role in the regulation of idiotypic expression. In an autoimmune disease such as myasthenia gravis (MG) in which heterogeneous autoantibodies are directed against the acetycholine receptor (AChR) of the neuromuscular junction (for review see Vincent, 1980), the idiotypes of the anti-AChR in different patients are of great interest, and may be of therapeutic importance if some degree of sharing of idiotypic determinants exists as suggested in previous reports (Lefvert, 1981; Lefvert *et al.*, 1982).

In this study, using rabbit anti-sera raised against anti-AChR purified from a single plasma sample from each of three patients, we have investigated (a) the expression of each patient's idiotypes during several years of treatment, and (b) the degree of idiotypic sharing between different patients with MG. Our results show consistency of idiotypic expression within an individual, but show only very limited sharing of idiotypes between different individuals.

MATERIALS AND METHODS

Preparation of AChR, α-bungarotoxin (α-BuTx) and purified anti-AChR antibodies. Preparation of human AChR from gastrocnemius and soleus muscle of amputated limbs and the iodination of α-BuTx and IgG have been previously described (Newsom-Davis *et al.*, 1978; Lang, Vincent & Newsom-Davis, 1982; Vincent & Newsom-Davis, 1982).

Correspondence: Dr A. Vincent, Department of Neurological Science, Royal Free Hospital School of Medicine, Rowland Hill Street, London NW3 2PF, UK

Plasma was obtained from three female patients who were undergoing therapeutic plasma exchange. Seventeen additional MG patients were also investigated for idiotype sharing. These were selected to be representative of the varied clinical expression of the disease but did not include any patients with purely ocular symptoms. Clinical details of the patients are given in Table 1. The AChR–α-BuTx Sepharose 4B affinity column used to purify anti-AChR antibody (idiotype), and the assays for anti-AChR activity and for human IgG concentrations using competitive radioimmunoassay, were as described previously (Lang *et al.*, 1982).

Immunization of rabbits. Anti-sera to purified anti-AChR antibodies (sp. act. 1–1·57 pmols/μg IgG) were raised in four New Zealand White rabbits by two i.m. injections (2–5 μg purified anti-AChR antibody per injection in Freund's complete adjuvant) followed by four or five s.c. injections in incomplete adjuvant at monthly intervals. The total amount injected into each rabbit was 30–60 μg. Animals were bled 5–10 days after each injection; the serum was separated and stored at −20°C. Pre-immune serum was also stored.

Characterization of anti-idiotypic sera

Anti-human IgG activity. Rabbit sera were assayed for anti-human IgG activity before and after adsorption by human IgG (Miles Chemical Co.) which had been covalently coupled to Sepharose 4B (Pharmacia fine chemicals) at a concentration of 18 mg/ml. Various concentrations (0·2–20 μl) of rabbit immune sera were incubated with ^{125}I-human IgG (1 μg) for 2 h at room temparature. After this time the immune complexes were precipitated by the addition of sheep anti-rabbit IgG (Seward Laboratories Ltd) which had previously been shown to be devoid of anti-human activity. Pre-immune serum was used as control. Results were expressed as μg human IgG precipitated per ml of serum.

Assay of anti-idiotype activity. The anti-idiotypic activities of the rabbit antisera were investigated by measuring their ability to inhibit the binding of anti-AChR antibodies to ^{125}I-α-BuTx labelled AChR. This included anti-AChR in (a) the original plasma from each patient, (b) the affinity purified idiotypes, (c) the pass through fractions from the affinity column, (d) sera taken during the course of the disease from the three patients and (e) sera from seventeen other representative MG patients. Each anti-AChR preparation was first titrated against 20–30 fmoles ^{125}I-α-BuTx-AChR (see Lang *et al.*, 1982) in order to establish the volume (0·05–5 μl) which precipitated about 50% of the available receptor; this was then used in all subsequent assays. Varying amounts of anti-idiotype sera (previously adsorbed by human IgG), individual pre-immune rabbit sera, pooled ($n=4$) control rabbit sera (adsorbed by human IgG) or PTX buffer (phosphate, pH 7·4, containing 0·1% Triton-X 100) were incubated with this limiting amount of anti-AChR for 2h at room temperature. ^{125}I-α-BuTx-AChR was added and allowed to react for a further 2 h. The human IgG was precipitated by the addition of sheep anti-human IgG devoid of anti-rabbit activity. The precipitation of ^{125}I-α-BuTx-AchR in the presence of anti-idiotype serum was expressed as a percentage of that precipitated in the presence of buffer alone, at each concentration. The 'non-specific' inhibitory effect of pre-immune or pooled control rabbit sera was also expressed as a percentage of buffer control. Assays using plasma or purified idiotype were not necessarily performed at the same time as those on serial serum samples, and in some experiments a different ^{125}I-α-BuTx-AChR preparation and a different control rabbit sera were used. All serial assays, on different samples from an individual patient were, however, performed at the same time.

Specificity of anti-idiotype binding. (1) To eliminate the effect of any residual non-idiotypic anti-human IgG activity, each adsorbed immune rabbit serum was pre-incubated with an excess of control human serum (5–10 μl) before incubation with anti-AChR in the anti-idiotype assay described above.

(2) To test whether anti-idiotype sera contained either anti-AChR activity or anti-α-BuTx activity, the sera were assayed by incubation with ^{125}I-α-BuTx-AChR or ^{125}I-α-BuTx followed by precipitation with sheep anti-rabbit.

(3) To test whether anti-idiotype antibodies were capable of binding to idiotypic determinants outside the antigen combining site of the anti-AChR, ^{125}I-α-BuTx-AChR and anti-AChR antibodies at limiting concentrations were incubated for 2 h. Anti-idiotype sera were added for a further 2 h followed by precipitation with sheep anti-rabbit sera devoid of anti-human activity.

Table 1. Clinical details and results of inhibition studies

					Inhibition by			
					Anti-id/A	Anti−id/B	Anti-id/C	Anti-id/D
					Raised against			
Patient	Sex	Thymic† pathology	Anti-AChR (nM)‡	CRS§	(id-MG/1)	(id-MG/1)	(id-MG/2)	(id-MG/3)
MG/1	F	H	123	17±3·3 (5)	72±5·8 (5)	76±5·5 (5)	28±7·3 (2)	27±2·5 (2)*
MG/2	F	H	44	8·9±2·2 (3)	8·3±8·2 (3)	26±7·4 (3)†	62±2·5 (5)	14±5·6 (3)
MG/3	F	T	32	21±3·1 (3)	15±6·2 (2)	16±9·0 (4)	26±19 (2)	80±3·5 (4)
MG/4	F	H	280	1·5±2·1 (2)	1·0±5·6 (2)	2·5±2·1 (2)	1·0±2·8 (2)	−1 (1)
MG/5	F	—	85	13±2·8 (2)	3 (1)	−11 (1)	28±8·9 (2)	15±6·3 (2)
MG/6	M	T	88	27±2·8 (2)	6·5±6·4 (2)	21±2·1 (2)	17±8·5 (2)	21±3·5 (2)
MG/7	F	H	15	−3±12 (2)	−5 (1)	−3 (1)	−9 (1)	6 (1)
MG/8	F	H	51	5·5±19 (2)	−6 (1)	−1±4·9 (2)	28±1·4 (2)	0±12 (2)
MG/9	M	T	34	20±9·5 (4)	18 (1)	26±4·9 (2)	26 (1)	6·5±9·2 (2)
MG/10	F	M	22	14±1·7 (4)	11 (1)	19 (1)	26±4·2 (2)*	15±3·5 (2)
MG/11	M	T	14	13±4·9 (2)	5·0±11 (2)	7·0±9·9 (2)	14±4·2 (2)	11±5·6 (2)
MG/12	M	—	12	43±11 (4)	31 (1)	42±11 (2)	42±0·7 (2)	26±6·4 (2)
MG/13	F	T	22	14±3·4 (4)	14±0·7 (2)	9·7±6·8 (3)	31±3·5 (2)†	13±4·9 (2)
MG/14	M	—	16	−3·0±12 (2)	−5 (1)	−3 (1)	−9 (1)	6 (1)
MG/15	F	—	2·5	29±8·5 (2)	26±1·4 (2)	32±7·1 (2)	40±1·4 (2)	31±2·1 (2)
MG/16	M	—	2·6	12 (1)	4 (1)	13 (1)	17 (1)	16 (1)
MG/17	M	—	1·6	47±3·5 (2)	13±2·8 (2)	21±3·5 (2)	35±6·4 (2)	41 (1)
MG/18	M	I	3·0	8·0±11 (2)	−10 (1)	6·5±10 (2)	−5·0±2·8 (2)	9 (1)
MG/19	M	H	2·9	37 (1)	5·5±7·0 (2)	18±2·1 (2)	30 (1)	23 (1)
MG/20	M	H	1·2	16 (1)	17 (1)	−1 (1)	10 (1)	6·0±7·1 (2)

* $P<0.05$; † $P<0.01$ Student's *t*-test, significance of inhibition of heterologous anti-AChR.

† H = hyperplasia; T = thymoma; I = involuted; — = thymectomy not performed. In four cases the serum sample tested had been obtained before thymectomy.

‡Anti-AChR titres were derived from titration curves described in the Materials and Methods.

§CRS (control rabbit serum); these are the mean of results with pre-immune sera and pooled control serum in the case of MG/1–3, and pooled control serum only in the case of MG/4–20.

RESULTS

Characterization of anti-idiotype sera

Four anti-idiotype (anti-id) sera were raised. Anti-id/A and /B were raised against anti-AChR from patient MG/1, and anti-id/C and /D against patients MG/2 and MG/3, respectively (Table 1). Both anti-human IgG and anti-AChR idiotype activity were present in the rabbit anti-idiotype sera after the second injection of idiotype and following subsequent boosts. The anti-human IgG activity, which ranged from 200 to 380 μg of IgG precipitated per ml of rabbit serum, could no longer be detected after adsorption of the sera by human IgG–Sepharose. It was not detectable in the pre-immune sera or in the adsorbed pooled control serum.

After adsorption, sera were tested to see if they inhibited the binding of homologous anti-AChR to human AChR using an amount of anti-AChR which precipitated 50% of the available AChR (see Materials and Methods). This limiting concentration was essential for detecting partial inhibition. Twenty microlitres of pre-immune (unadsorbed) rabbit serum inhibited the precipitation of ^{125}I-αBuTx-AChR by a maximum of 22% when compared with buffer controls. Adsorbed, pooled rabbit sera also inhibited by up to 20%. By contrast, the same volume of anti-idiotype serum used against homologous anti-AChR inhibited precipitation by 62–80% (Table 1, top three rows). Inhibition was 53–59% greater than that achieved by the corresponding pre-immune or control serum. Typical titrations of the inhibition by different concentrations of each anti-idiotype serum are shown in Fig. 1.

Anti-id/A and /B (raised against the same anti-AChR from patient MG/1) inhibited precipitation by 72% and 76% respectively at 20 μl; when these sera were used together inhibition was essentially unchanged. For example, in one experiment 10 μl anti-id/A inhibited by 56% 10 μl anti-id/B inhibited by 67%, and 10μl anti-id/A and anti-id/B inhibited by 66%.

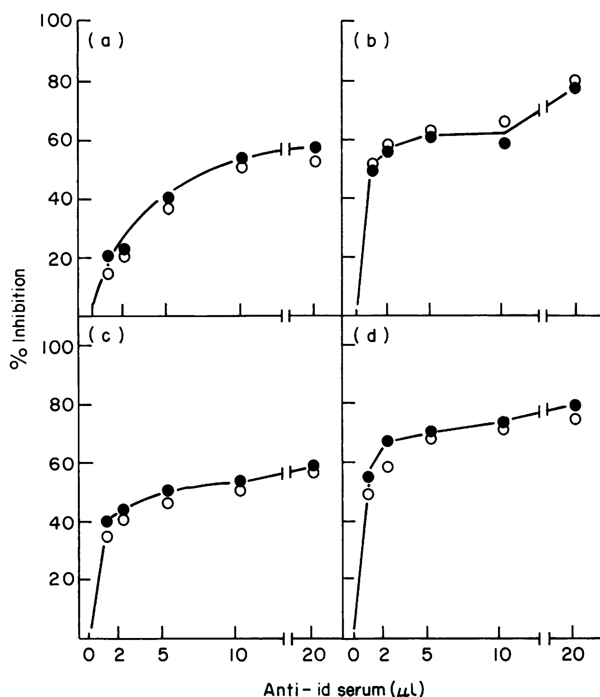

Fig. 1. Inhibition of autologous anti-AChR by anti-id sera /A, /B, /C and /D (a,b,c,d). Results shown are of a single representative experiment. Filled circles are in the absence and open circles in the presence of 10 μl control human serum to absorb any anti-human IgG activity remaining in the anti-id preparations. Normal rabbit serum values have been subtracted.

If anti-idiotype sera were pre-incubated with control human serum before incubation with anti-AChR, similar results were obtained (Fig. 1a–d); this confirmed that the anti-idiotypes were not recognising determinants on the anti-AChR IgG which were common to human IgG. Anti-idiotype sera showed no reactivity with AChR or ^{125}I-α-BuTx itself.

Anti-id/D was used to investigate the idiotypic profile of the autologous anti-AChR during purification on the AChR affinity column. The first few pass through fractions contained very little anti-AChR but the percentage inhibition by 20 μl anti-id/D (68–78% maximum) did not differ significantly from the starting material (72%). The purified idiotype subsequently eluted from the column was inhibited to a slighly greater extent (85%).

Longitudinal study of idiotype expression

Anti-id/B, /C and /D were used to investigate the expression of homologous idiotype in sera taken from each patient at intervals over a period of several years during which they underwent repeated plasma exchange, immunosuppressive drug treatment and, in MG/2, thymectomy. During this time serum anti-AChR levels showed considerable variation superimposed on a strongly declining trend; the degree of inhibition, however, of anti-AChR activity by anti-id sera raised against the individual's purified idiotype varied very little. The only marked change in idiotype expression occurred transiently in patient MG/3 after a period of cyclophosphamide treatment (Fig. 2). During and immediately after this time total anti-AChR antibody levels showed an upward trend. Patient MG/1 also received cyclophosphamide early in the study but showed no clear change in idiotype expression (not shown). The maximum inhibition by 20 μl anti-id serum of anti-AChR in serum samples taken at various times during the period of observation and assayed using a single AChR preparation was 59·1 ± 8·0% (mean ± s.d., n = 19) and 38·5 ± 3·8% (mean ± s.d., n = 10) for patients /1 and /2 respectively. Each of the idiotype preparations from patients MG/1 and MG/2 used for raising the anti-sera was prepared from plasma taken within the first few months of observation whereas that from patient MG/3 was taken later (Fig. 2).

Idiotype sharing between patients

Sharing of anti-AChR idiotypes between the three patients and 17 others who were selected to

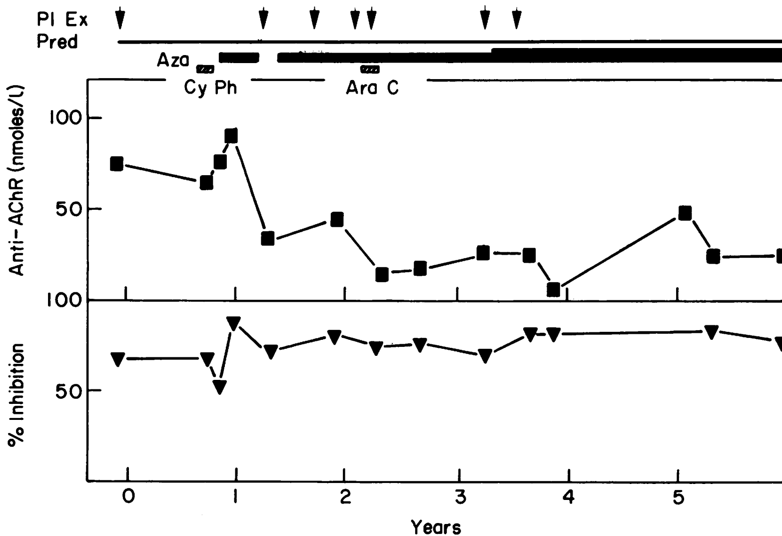

Fig. 2. Inhibition of anti-AChR (patient MG/3) by anti-id serum (D) over a period of 6 years observation during which she was treated with plasma exchange (Pl. Ex), prednisolone (Pred), azathioprine (Aza) and short courses of cyclophosphamide (Cy Ph) and arabinoside C (Ara C). Upper plot shows anti-AChR titre (nmoles/l) and lower plot shows percentage inhibition by 20 μl of anti-id serum. Normal rabbit serum controls have been subtracted. The plasma used for preparing id-MG/3 was obtained from the first bag of the last plasma exchange.

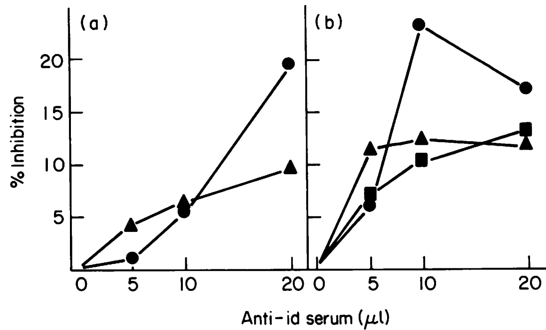

Fig. 3. Inhibition of heterologous anti-AChR by anti-id sera. (a) inhibition of patient MG/2 anti-AChR by anti-id B (●) and of MG/l by anti-id D (▲). (b) Inhibition of anti-AChR from patients MG/5 (■), MG/10 (▲), and MG/13 (●) by anti-id C. Normal rabbit serum controls have been subtracted in all cases.

represent different clinical forms of the disease was investigated by comparing anti-id inhibition with inhibition by pre-immune or pooled control rabbit sera. Inhibition by the latter was highly variable, ranging from 0–47·5% (Table 1), and in some cases exceeded that by anti-id raised against heterologous anti-AChR. This was in spite of the fact that the control serum was also adsorbed against human IgG and contained no detectable anti-human IgG activity. Compared with this, presumably, non-specific inhibition, anti-id/A did not cross-react significantly with any MG anti-AChR (Table 1). Twenty microlitres of anti-id/B inhibited MG/2 by 17% but the inhibition had clearly not reached a plateau (Fig. 3a); anti-id/D only crossreacted with one sera (MG/l) significantly, inhibition not exceeding 10% at 20 μl. Anti-id/C was the only serum of the four anti-id preparations which showed cross-reactivity with more than one MG anti-AChR. Inhibition was greater than 10% above control values in six cases (Table 1 & Fig. 3b), but only reached significance in two (MG/10 and MG/13) of the 19 heterologous MG sera investigated.

DISCUSSION

Antisera raised in rabbits against anti-AChR purified from the plasma of three myasthenic patients have shown, in each case, high levels of anti-id antibody. After adsorption against normal human IgG to remove anti-human IgG activity, a low concentration of anti-id sera inhibited 62–80% of the binding of autologous anti-AChR to the receptor. The specificity of this inhibition was demonstrated by its failure to be adsorbed out by large values of normal human IgG, and by the inability of the antisera to precipitate either AChR or α-BuTx directly or AChR–anti-AChR complexes which had been pre-formed. Thus the inhibition observed appeared to be specific for the antigen combining site of the anti-AChR antibodies and is therefore by definition idiotypic.

There are several possible explanations for the failure to achieve 100% inhibition. First, a proportion of the antibodies might be so heterogeneous that no particular idiotype was present in sufficient concentration to evoke an anti-id response; second, a proportion of the idiotypes might not be recognised by the rabbit as foreign; third, some of the idiotypes might be lost during purification. This latter possibility seems less likely, however, since the maximum inhibition of anti-AChR in the original plasma from MG/3 and in the purified idiotype presentation, by anti-id/D, were very similar.

The maximum inhibition achieved by anti-id sera varied with the different AChR preparations used in the assay. Preliminary observations suggest that higher inhibition was achieved when the muscle extract contained highly denervated AChR, and somewhat lower inhibitions were found when muscle extracts contained normal junctional AChR. This would not be surprising since the anti-AChR idiotypes were purified on affinity columns which mostly employed denervated AChR, and suggests that some of the idiotypes of antibodies binding preferentially to normal AChR may differ from those which bind to denervated AChR (cf. Vincent & Newsom-Davis, 1982).

The inhibition found with pre-immune sera or absorbed pooled control serum was highly variable but related better to the particular anti-AChR being tested than to the control rabbit serum being used (Table 1). Moreover, inhibition by the individual pre-immune sera was similar to that by the pooled control serum, and none of these contained detectable anti-human IgG activity (i.e. < 1 μg of human IgG precipitated/ml of serum, unpublished observations). We can provide no explanation for this variable and non-specific inhibition but it is possible that the interaction of some patients' anti-AChR antibodies with the receptor is particularly vulnerable to the addition of large amounts of serum proteins.

The degree to which anti-id sera inhibited anti-AChR from the individual patients remained remarkably constant over a period of several years despite considerable variations in the anti-AChR titre brought about by plasma exchange, immunosuppressive treatment or thymectomy. In only one case was there clear evidence of a change in idiotypic expression; this appeared to be associated with a 6 week course of cyclophosphamide therapy and, interestingly a temporary rise in total anti-AChR level.

The rabbit anti-id sera detected little evidence of idiotype sharing between these three and 17 other MG patients. Only anti-id/C, raised against anti-AChR from MG/2, appeared to interact with anti-AChR from more than one of the 19 other MG patients investigated; in each case, with this anti-id serum, a plateau of inhibition was found in excess anti-id (see Fig. 3b) suggesting that the anti-id only bound a subpopulation of the anti-AChR. Anti-id/B and /C each cross-reacted with one MG serum, and anti-id /A showed no cross-reactivity. These observations appear to differ from those of Lefvert (1981) who reported evidence of idiotype sharing both by inhibition studies and precipitation of ^{125}I-labelled anti-id IgG by MG sera. As in our studies, however, inhibition of anti-AChR activity was only achieved at relatively high concentrations of the anti-id IgG, indicating limited cross-reaction. Moreover, only a small proportion (< 1%) of the unpurified ^{125}I-anti-id IgG was precipitated (Lefvert, 1981) making evaluation of the results very difficult. The different conclusion reached, therefore, depends on the interpretation rather than on the results themselves.

Human anti-thyroglobulin antibodies also show a paucity of idiotypic sharing. In one study (Matsuyama, Fukumori & Tanaka, 1982) only one of three antisera showed cross-reactivity of varying degree with a number of other patients' antibody, and in another using a solid phase assay, one of four cross reacted with four of nine heterologous anti-thyroglobulin antibodies (Delves & Roitt, 1984). The latter study suggests the existence of both private and public idiotypes in an individual's serum which can also be inferred from the partial inhibition by cross-reacting anti-id that we observed.

In experimental autoimmune MG (EAMG) in which animals are immunized against AChR, idiotypic sharing can occur. Schwartz *et al.* (1978) raised anti-idiotype sera in mice against lymphocytes sensitized *in vitro* and found considerable sharing of anti-AChR idiotypes among different species of animal immunized *in vivo* with Torpedo AChR; they did not investigate human MG sera. Lennon & Lambert (1981) also found sharing of idiotypes between anti-AChR from different inbred mice, whereas Barkas & Simpson (1982) found little evidence of sharing between rabbits immunized against Torpedo AChR. These results tend to confirm the suggestion that the cross-reactivity of idiotypes in inbred mice is greater than that of human antibodies (Geha & Weinberg, 1978).

Recently, Dwyer *et al.* (1983) have presented evidence that MG sera contain naturally occurring anti-id antibodies; these were detected by their reaction with a single monoclonal anti-AChR antibody raised against narcine AChR. This monoclonal anti-AChR antibody may therefore present some widely shared idiotypic determinant. Our results, in showing that only a few MG patients have cross-reactive anti-AChR idiotypes, do not rule out the possibility of other shared idiotypes to which these four rabbits were unresponsive. To exclude this a larger study would be required. However, the very limited idiotypic sharing that we have demonstrated reflects the heterogeneity of anti-AChR reported in a number of studies (Lindstrom, Campbell & Nave, 1978; Vincent & Newsom-Davis, 1982) and suggests that the most immunogenic idiotypes are restricted to the individual.

This work was supported by the Medical Research Council.

REFERENCES

BARKAS, T. & SIMPSON, J.A. (1982) Lack of inter-animal cross-reaction of anti-acetylcholine receptor antibodies at the receptor binding site as demonstrated by heterologous anti-idiotype antisera: implications for immunotherapy of myasthenia gravis. *Clin. exp. Immunol.* **47,** 119.

DELVES, P.J. & ROITT, I.M. (1984) Idiotypic determinants on human thyroglobulin autoantibodies derived from the serum of Hasimoto patients and EB virus transformed cell lines. *Clin. exp. Immunol.* **57,** 33.

DWYER, D.S., BRADLEY, R.J., URQUHART, C.K. & KEARNEY, J.F. (1983) Naturally occurring anti-idiotypic antibodies in myasthenia gravis patients. *Nature,* **301,** 611.

GEHA, R.S. & WEINBERG, R.P. (1978) Anti-idiotypic antisera in man. *J. Immunol.* **121,** 1518.

LANG, B., VINCENT, A. & NEWSOM-DAVIS, J. (1982) Purification of anti-acetylcholine receptor antibody from patients with myasthenia gravis. *J. Immunol. Meth.* **51,** 371.

LEFVERT, A.K. (1981) Anti-idiotype antibodies against the receptor antibodies in myasthenia gravis. *Scand. J. Immunol.* **13,** 493.

LEFVERT, A.K., JAMES, R.W., ALLOID, C. & FULPIUS, B.W. (1982) A monoclonal anti-idiotypic antibody against anti-receptor antibodies from myasthenic patients. *Eur. J. Immunol.* **12,** 790.

LENNON, V.A. & LAMBERT, E.H. (1981) Monoclonal antibodies to acetylcholine receptors: evidence for a dominant idiotype and requirement of complement for pathogenicity. *Ann. N.Y. Acad. Sci.* **377,** 77.

LINDSTROM, J., CAMBELL, M. & NAVE, B. (1978) Specificities of antibodies to acetylcholine receptors. *Muscle Nerve,* **1,** 140.

MATSUYAMA, T., FUKUMORI, J. & TANAKA, H. (1983) Evidence of unique idiotypic determinants on human anti-thyroglobulin antibodies. *Clin. exp. Immunol.* **51,** 381.

NEWSOM-DAVIS, J., PINCHING, A.J., VINCENT, A. & WILSON, S.G. (1978) Function of circulating antibody to acetylcholine receptor in myasthenia gravis investigated by plasma exchange. *Neurology,* **28,** 266.

SCHWARTZ, M., NOVICK, D., GIVOL, D. & FUCHS, S. (1978) Induction of anti-idiotype antibodies by immunisation with syngeneic spleen cells educated with acetylcholine receptors. *Nature,* **273,** 543.

VINCENT, A. (1980) Immunology of acetylcholine receptor in relation to myasthenia gravis. *Physiol. Rev.* **60,** 756.

VINCENT, A. & NEWSOM-DAVIS, J. (1982) Acetylcholine receptor antibody characteristics in myasthenia gravis. I. Patients with generalized myasthenia or disease restricted to ocular muscles. *Clin. exp. Immunol.* **49,** 257.