Clin. exp. Immunol. (1982) **47**, 119–126.

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

T. BARKAS & J. A. SIMPSON *Glasgow University Department of Neurology, Institute of Neurological Sciences, Southern General Hospital, Glasgow, Scotland*

(Accepted for publication 10 July 1981)

SUMMARY

Anti-idiotype antisera were raised in rabbits by immunization with purified sheep anti-Torpedo receptor antibodies. The antisera were able specifically to block the binding of receptor to the inducing antibodies but not anti-Torpedo antibodies from other animals of the same, or other, species. Rabbits producing the anti-idiotype sera were not protected from experimental autoimmune myasthenia gravis (EAMG). The implications of these observations for the potential use of anti-idiotype antisera in the treatment of myasthenia gravis are discussed.

INTRODUCTION

The human neuromuscular disorder, myasthenia gravis, is now well characterized as an autoimmune disease in which a major self-antigen is the nicotinic acetylcholine receptor. Antibodies to the receptor can be demonstrated in the serum of 90% of myasthenic patients. Experimental models of the disease can be readily induced in a range of species by immunization with nicotinic receptor purified from the electric organs of fish, such as Torpedo (see review by Barkas, 1979). One possible approach to therapy might be the production of anti-idiotype antisera as a means of specific suppression of autoantibody production. Preliminary work along these lines has been reported by Schwarz *et al.*, (1978) who immunized mice with syngeneic lymphocytes previously educated *in vitro* with purified Torpedo receptor. Their anti-idiotype antisera showed considerable cross-reaction of anti-receptor antibodies raised in a number of species, including cross-reactions at the receptor-binding sites. For use as a possible means of therapy, it would obviously be advantageous if one anti-idiotype antiserum could be used for different patients and the cross-reactivity noted above is therefore encouraging. The techniques used to raise the anti-idiotype antisera, however, are not readily applicable to the human situation. A more practicable method is the purification of anti-receptor antibodies which are then used to raise heterologous anti-idiotype antisera. We have adapted this approach using anti-Torpedo receptor antibodies, and have successfully produced anti-idiotype antisera. However, negligible cross-reaction at the receptor-binding site was found between anti-receptor antibodies raised in different animals. The relevance of these findings to possible immunotherapy of myasthenia gravis is discussed.

Correspondence: Dr T. Barkas, Department of Neurology, Institute of Neurological Sciences, Southern General Hospital, 1345 Govan Road, Glasgow G51 4TF, Scotland.

0009-9104/82/0100-0119$02.00 © 1982 Blackwell Scientific Publications

MATERIALS AND METHODS

Preparation of nicotinic acetylcholine receptor and antisera. The nicotinic acetylcholine receptor was purified from the electric organs of *Torpedo marmorata* as described previously (Harvey *et al.*, 1978). Antisera to the purified receptor were raised in rabbits, sheep and mice by two intramuscular injections of 100 μg (10 μg for mice) of receptor firstly in Freund's complete adjuvant then in incomplete adjuvant with an interval of 3 weeks. Animals were bled out when paralysis occurred.

Measurement of receptor levels and antibody titres. Alpha-bungarotoxin (Boehringer) was iodinated by the method of Lindstrom *et al.* (1976) to a specific activity of 100 Ci/mmol. Receptor activity was measured essentially as described by Schmidt & Raftery (1973).

Anti-receptor antibody levels were determined as described by Barkas *et al.* (1978) using a second antibody as precipitant. Sheep anti-rabbit IgG and anti-mouse IgG and donkey anti-sheep IgG were kindly provided by the Scottish Antibody Production Unit, Law Hospital.

Protein was estimated by the method of Dulley & Grieve (1975).

Preparation of antibody. Sheep antibodies to the receptor were purified by affinity chromatography. One hundred millilitres of crude Triton extract of Torpedo electric organ (100 g) were mixed with 20 ml of Sepharose-coupled α-cobratoxin (Barkas *et al.*, 1978) for 2 hr at 20°C. After extensive washing with buffer containing 1 M sodium chloride, 50 ml of heat-inactivated sheep antiserum to Torpedo receptor were applied for 1 hr at 20°C. After washing, the bound material was eluted with 25 ml 3 M potassium thiocyanate in phosphate-buffered saline, pH 7·2 (PBS). This treatment was shown to have no effect on antibody activity but irreversibly destroyed receptor antigenicity. After centrifugation, the beads were washed with 10 ml PBS and the combined supernatants dialysed against 2 × 1 l PBS. Negligible toxin-binding activity was detected in the dialysed sample (700 pmol compared with 33,100 bound). However, sheep IgG and antibody to Torpedo receptor could both be demonstrated by immunodiffusion. A precipitate formed on storage at 4°C was removed by centrifugation at 10,000 ***g*** for 20 min. The supernatant was then concentrated to 1 ml and applied to an 86 × 2 cm column of Ultragel ACA34. Elution was carried out in PBS and 1·8-ml fractions were collected. Two peaks of protein were eluted, one at the void volume and one at the position of elution of IgG. Antibody activity and IgG were demonstrated only in the second peak which was pooled and used for further work. Recovery of antibody activity was 137·8 nmol receptor-binding sites from 2,630 nmol applied to the beads of which 444 nmol bound. This represents 11·0 mg antibody. Total protein recovered was 19·2 mg.

Rabbit antibody was purified in a similar fashion.

Preparation of normal sheep IgG and immobilization. A crude sheep IgG preparation was prepared by precipitation of normal sheep IgG with ammonium sulphate (40%) followed by dialysis against PBS. One hundred milligrams of this material were coupled to Sepharose CL-4B by the cyanogen bromide method (Parikh, March & Cuatrecasas, 1974). Absorption of anti-idiotype sera was performed by incubating equal volumes of immunoadsorbent and serum at 20°C for 90 min. Normal sheep IgG was prepared by the method of Ling, Bishop & Jefferis (1977).

Preparation of $F(ab')_2$. Purified sheep anti-receptor antibodies were concentrated to 10 mg/ml and $F(ab')_2$ prepared as described by Stanworth & Turner (1973).

Preparation and radiolabelling of mouse IgG and rabbit anti-mouse IgG. Mouse IgG was isolated by chromatography on protein A–Sepharose. The eluted material was further purified by gel filtration on Ultragel ACA34. IgG was radiolabelled to a specific activity of 500 μCi/mg by the method of McConahey & Dixon (1966). Rabbit anti-mouse IgG antiserum was raised by immunization at 3-weekly intervals with 100-μg amounts of mouse IgG in adjuvant.

Preparation of anti-idiotype antiserum. Anti-idiotype antisera to the purified sheep antibodies were raised in rabbits by intramuscular injection in adjuvant at 3-weekly intervals, firstly in Freund's complete and secondly in Freund's incomplete adjuvant. For the first two injections, 35 μg of sheep antibody were used. This was increased to 100 μg for two further injections. Blood samples were collected at weekly intervals.

Assay of anti-idiotype antisera. (i) Fluid-phase assay. The antisera were tested for their ability to inhibit binding of radiolabelled receptor to anti-Torpedo receptor antibodies. All assays were in quadruplicate. One hundred microlitres (100 ng) of purified sheep antibody in 100-fold-diluted

normal sheep serum or diluted normal serum alone were incubated with 20 μl buffer (10 mM phosphate, 0·1% Triton X100, pH 7·4), normal rabbit serum or test serum for 1 hr at 20°C. One hundred and fifty microlitres of toxin-labelled Torpedo receptor (1·6 pmol) were added and incubated at 4°C for 16 hr. Sufficient donkey anti-sheep IgG to precipitate the sheep IgG was added and incubated at 4°C for 4 hr, followed by centrifugation at 1,500 ***g*** for 10 min and washing with 10 mM phosphate buffer containing 0·1% Triton X100.

For cross-reaction experiments, suitably diluted anti-Torpedo antisera were substituted for the sheep antibody and precipitation was performed with the appropriate anti-immunoglobulin antiserum.

(ii) Solid-phase assay. Purified sheep antibody or normal sheep IgG was coated onto LP3 tubes (Luckham) by incubating 1-ml aliquots (10 μg/ml in PBS) at 4°C for 4–6 days. Excess protein was washed out with three 1-ml washes of PBS. One millilitre of PBS containing 0·01% gelatin was added and incubated at 20°C for 2 hr. The tubes were then washed or stored at −70°C. For the assay, aliquots of test sera or IgG fractions were added to the tubes followed by sufficient PBS containing 0·05% Tween 20 to make the volume 1 ml total. After 1 hr at 20°C, 15 μl of toxin-labelled receptor (1·6 pmol) were added and incubated at 4°C for 16 hr. All tubes were then washed three times with 1 ml of PBS and counted.

Measurement of anti-sheep IgG and anti-idiotype antibodies in the anti-idiotype antisera. Purified sheep anti-receptor antibodies were iodinated by the method of McConahey & Dixon (1966) to a specific activity of 138 Ci/mmol.

Antibodies to sheep IgG were quantitated by incubating 100 μl PBS–0·5% BSA buffer with 50 μl heat-inactivated rabbit antisera or NRS at 10-fold dilutions for 30 min at 20°C. Fifty microlitres of labelled sheep antibody (0·38 pmol) were then added and incubated for 60 min at 20°C. One hundred microlitres of 10% *Staphylococcus aureus* in PBS—0·5% BSA were added and incubated for 30 min at 20°C. Five hundred microlitres of PBS–BSA were added and the tubes spun at 1,500 ***g*** for 10 min. The supernatants were discarded and the pellets washed with a further 500 μl buffer, then counted.

Anti-idiotype antibodies were measured by the same method except that in the first incubation, heat-inactivated autologous sheep serum from preimmunization bleeds diluted 10-fold in PBS–BSA was used in place of buffer. Experiments using undiluted sheep serum showed no difference compared with 10-fold-diluted serum.

Immobilization of purified sheep anti-receptor antibody and absorption of anti-idiotype serum. The method used was based on that of Avrameas & Ternynck (1969). One millilitre (1 mg) of sheep antibody or PBS was added to 1 ml of heat-inactivated autologous preimmunization serum and dialysed against 0·9% saline, pH 8·6, at 4°C overnight. Potassium phosphate buffer, 1 M pH 7·0 (0·2 ml), was added and followed by 0·3 ml 2·5% aqueous glutaraldehyde. The samples were incubated at room temperature for 5 hr, then at 4°C for 24 hr. The gels formed were gently homogenized and washed extensively with PBS.

One millilitre of heat-inactivated antiserum from rabbit 2 was then added to each gel and incubated at 4°C for 2 hr. After centrifugation at 1,500 ***g*** for 5 min, the sera were tested for anti-idiotype activity in the fluid-phase assay.

RESULTS

Preparation of anti-idiotype antisera

After two injections of purified sheep antibody, antibodies capable of inhibiting the fluid-phase binding of receptor to the purified antibody were elicited (Table 1). As shown in Fig. 1, the effect was clearly detectable at dilutions greater than 100-fold, while normal rabbit serum inhibited the reaction only slightly. Identical results were obtained with heat-inactivated sera. After each boosting dose, the inhibitory activity increased, and remained fairly constant for a period of 3 weeks between injections (Table 1).

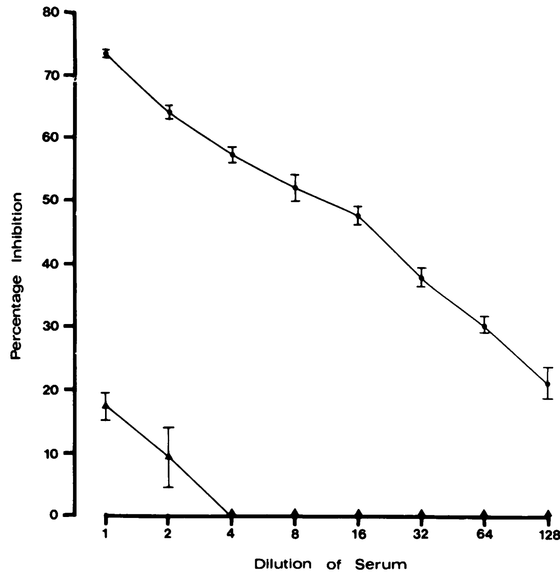

Fig. 1. Inhibition of the binding of radiolabelled receptor to purified sheep antibodies by anti-idiotype serum A. The serum used was from rabbit A 1 week after the third injection. Results are the mean and standard deviation of three experiments. (●) Serum from rabbit A, (▲) serum from normal rabbit.

Definition as anti-idiotype

Inhibition of the precipitation assay could be explained by factors other than anti-idiotype antibodies. The anti-idiotype antisera were tested for anti-receptor antibody activity but none was found. The results were shown not to be due to non-specific effects on the subsequent precipitation by anti-sheep IgG antibodies. Absorption of the anti-idiotype antiserum with immobilized normal sheep IgG had no effect on the result. Moreover, no decrease in radiolabelled receptor precipitated was found if the anti-idiotype antiserum was added after the labelled receptor rather than before it. This was confirmed by the observation that the anti-idiotype antiserum did not affect the binding of

Table 1. Inhibition of binding of radiolabelled receptor to purified sheep antibody by anti-idiotype antisera

No. of injections of antibody	Time after injection (weeks)	Percentage inhibition of binding of receptor	
		Rabbit A*	Rabbit B*
2	1	65·6	47·4
2	2	62·0	46·0
3	1	78·1	67·3
3	2	76·3	66·0
3	3	74·5	70·7
4	1	83·0	82·1

Inhibition was calculated using the equation:

$$\frac{(Ab+B)-(Ab+T)}{(Ab+B)-(N+B)}$$

where (Ab + B) is the c.p.m. with sheep antibody plus buffer, (Ab + T) is the c.p.m. with sheep antibody plus test serum, (N + B) is the c.p.m. with normal sheep serum plus buffer. All other details are given in the Materials and Methods section.

* Source of serum.

labelled mouse IgG to sheep anti-mouse IgG as assessed by immune precipitation with anti-sheep IgG (Table 2). The antiserum was shown to be effective using $F(ab')_2$ fragments of pure sheep IgG (Table 2). Direct confirmation that the inhibition observed was not due to altered precipitation came from experiments using solid-phase purified sheep antibodies (Table 3) where a similar blocking effect was observed.

However, the results might still be explained by anti-allotype antibodies if the allotype site was present in the $F(ab')_2$ region in such a position that bound antibody could cause steric hindrance of the binding of antigen. That this was not so was demonstrated by the fact that exactly the same inhibition of receptor binding was found in the presence of a 100-fold excess of normal IgG obtained from preimmunization bleeds from the same sheep (sheep 1) used to prepare the purified antibody ($77{\cdot}0 \pm 3{\cdot}7\%$, mean and standard deviation of two experiments) and of IgG from a serum pool from other sheep ($75{\cdot}2 \pm 8{\cdot}0\%$). Identical results were obtained with a 100-fold or 200-fold excess of normal autologous IgG ($74{\cdot}9 \pm 1{\cdot}84\%$ with 100-fold excess, $78{\cdot}5 \pm 1{\cdot}41\%$ with 200-fold excess). This last experiment clearly defines the antisera as anti-idiotype. Moreover, the inhibitory activity of the antisera was absorbed by purified sheep anti-Torpedo antibody immobilized in the presence of autologous preimmunization serum, but not by the serum alone.

The final titres of anti-sheep IgG and anti-idiotype antibodies were respectively 682 and 85 nM for rabbit A and 866 and 108 nM for rabbit B.

Table 2. Effect of anti-idiotype antiserum on precipitation of labelled mouse IgG by sheep anti-mouse IgG and on the precipitation of receptor by $F(ab')_2$ fragments of pure sheep anti-Torpedo receptor antibodies

Serum added	Precipitated mouse IgG (c.p.m.)	Precipitated receptor (c.p.m.)
Normal rabbit	19,420	8,910
Anti-idiotype A	18,355	2,456
Anti-idiotype A after absorption with normal sheep IgG	19,198	3,191

Total mouse IgG added was 53,800 c.p.m. Total labelled receptor added was 49,940 c.p.m. The antiserum used was from rabbit A 1 week after the fourth injection. Results of blanks using normal sheep IgG have been subtracted.

Table 3. Inhibition of binding of radiolabelled receptor to immobilized purified sheep antibodies

		Radioactivity bound (c.p.m.)	
Sample added	Volume (μl)	Experiment 1	Experiment 2
Buffer alone	—	12,669	14,039
IgG from anti-idiotype A	50	9,889	10,045
IgG from anti-idiotype A	100	—	4,890
Serum from anti-idiotype A	50	—	157
Normal rabbit serum	50	—	12,041

Total receptor added was 48,000 c.p.m. The concentration of the IgG from rabbit A was 1 mg/ml. Background counts have been subtracted. The antiserum used was from rabbit A 1 week after the fourth injection.

Table 4. Binding of radiolabelled receptor to anti-Torpedo receptor antibodies from a range of animals in the presence of anti-idiotype antisera raised to antibody from sheep 1

	Radioactivity precipitated (c.p.m.)		
Source of antibodies	Normal rabbit serum	Anti-idiotype A	Anti-idiotype B
Purified antibody from sheep 1	6,378 ± 255	—	1,633 ± 178
in normal sheep serum	7,465 ± 590	2,573 ± 129	—
Serum from sheep 1	5,646 ± 91	—	2,772 ± 209
	6,332	4,017 ± 83	—
Serum from sheep 2	12,003 ± 267	—	11,370 ± 9
	14,827 ± 2,584	15,073 ± 2,415	—
Serum from sheep 3	10,047 ± 9	—	9,380 ± 2
	12,746 ± 1,312	12,630 ± 3,174	—
Serum from rabbit 1	10,635 ± 381	8,439 ± 622	10,582 ± 211
Serum from rabbit 2	3,706 ± 310	4,677 ± 490	4,250 ± 389
Serum from rabbit 3	5,733 ± 370	5,887 ± 180	6,033 ± 415
Serum from rabbit 4	11,091 ± 705	11,866 ± 630	11,673 ± 152
Purified rabbit antibody	14,875	15,240 ± 601	15,064 ± 139
Serum from mouse	14,896 ± 1,122	16,298 ± 757	15,159 ± 1,228

Total receptor added was 40,000 c.p.m. Anti-Torpedo antisera were diluted to give approximately equivalent amounts of antibody. The results are the mean and standard deviation of two experiments. The antisera were those taken 1 week after the fourth injection. Results of blanks using normal sheep serum with the appropriate rabbit serum have been subtracted.

Effect of anti-idiotype sera on antibodies from other animals

The antisera were tested for their ability to prevent the binding of receptor to antisera from three sheep (Tables 4 & 5). Inhibition was only observed with serum from the sheep (sheep 1) from which the antibodies were prepared. Inhibition was less than with purified antibody.

Similarly, with a number of antisera from rabbits and mice, little inhibition was observed (Tables 4 & 5).

Effect of anti-idiotype antisera on experimental myasthenia

Experimental autoimmune myasthenia gravis (EAMG) is readily and reproducibly induced in rabbits by two intramuscular injections of 100 μg purified Torpedo receptor in adjuvant. Animals producing the anti-idiotype antisera were tested for susceptibility to EAMG. Onset and severity of disease were identical to normal EAMG, showing no protection.

DISCUSSION

In the present work, we have demonstrated the feasibility of preparing heterologous anti-idiotype antisera to anti-acetylcholine receptor antibodies by immunization with relatively small quantities of purified antibody. The antisera produced are capable of blocking the binding of radiolabelled receptor to the inducing antibodies by a factor of up to 75% as compared with normal rabbit serum. The antisera have been clearly defined as being directed against the idiotypes of the antibodies. Firstly, inhibition of binding of antigen is often itself taken as evidence that antisera are

Table 5. Binding of radiolabelled receptor to anti-Torpedo receptor antibodies expressed as a percentage of that in the absence of anti-idiotype antisera

Source of antibodies	Percentage binding of receptor	
	Anti-idiotype A	Anti-idiotype B
Purified antibody from sheep 1 in normal sheep serum	34·5	25·6
Serum from sheep 1	63·4	49·1
Serum from sheep 2	102	94·7
Serum from sheep 3	99·1	93·4
Serum from rabbit 1	79·4	102
Serum from rabbit 2	118	115
Serum from rabbit 3	103	105
Serum from rabbit 4	107	105
Purified rabbit antibody	99·6	102
Serum from mouse	109	102

The results are taken from Table 4. Binding is calculated from the equation:

$$\frac{(\mathrm{Ab+Id})-(\mathrm{N+Id})}{(\mathrm{Ab}+\mathrm{N_r})-(\mathrm{N}+\mathrm{N_r})}$$

where (Ab + Id) is c.p.m. with the appropriate antibody and anti-idiotype serum, (N + Id) is c.p.m. with the appropriate normal serum and anti-idiotype serum, ($Ab+N_R$) is c.p.m. with the appropriate antibody and normal rabbit serum and ($N+N_R$) is c.p.m. with the appropriate normal serum and normal rabbit serum.

anti-idiotypic. We have also shown that the effect is observed using $F(ab')_2$ fragments of antibody and is specific in that it does not affect the antigen binding of sheep antibodies of different specificity. However, none of these results excluded the possibility that the antisera are anti-allotypic and not anti-idiotypic. The former possibility was shown not to be plausible as a large excess of normal IgG from the sheep used for the preparation of the idiotypes did not influence the result. The antiserum can therefore be defined as anti-idiotypic.

The blocking effect of the antiserum was shown to be restricted almost totally to the original inducing antibodies (Tables 4 & 5) and had no effect on anti-Torpedo antibodies from other animals. This is identical to the situation with anti-tetanus antibodies in man (Geha & Weinberg, 1978) but contrasts with the work of Schwarz *et al.* (1978) who used antisera raised in mice by immunization with syngeneic lymphocytes educated *in vitro* with purified Torpedo receptor. These authors tested their antisera by two methods; firstly, by direct binding to immobilized anti-receptor antibody and secondly by inhibition of the binding of radiolabelled receptor to fluid-phase anti-receptor antibody. Possibly the difference might be explained by the different methodology for production or assay of the antisera. For example, it is not clear from the previous work whether pooled lymphocytes or antisera were used. However, if therapeutic use of antisera in humans was envisaged, the approach used would have to be that taken in the present work. If similar lack of cross-reaction was found with human antibodies, this would imply that individual antisera would have to be prepared for each patient to be treated. In those cases with low levels of antibody, this would be extremely impracticable. However, the outlook might not be so bleak as this. Firstly, the present study relates only to those idiotypic sites where bound antibody can interfere with subsequent binding of receptor. Idiotypic sites outside this area have not yet been studied but it is possible that they might show more cross-reaction of the type observed by Schwarz *et al.* (1978). Secondly, the antibody response in humans might be much more restricted than in experimental

animals as shown for antibody production to thyroglobulin (Nye, Pontes de Carvalho & Roitt, 1980).

Another point of possible relevance is the observation that less inhibition was observed using whole serum from sheep 1 compared with purified antibodies from sheep 1 added back to normal sheep serum, even though the amount of antibody used present in the whole serum sample was less (Table 4). This suggests the possibility that only a subfraction of antibody has been purified by the present method. This could result from lack of binding of low-affinity antibodies to the affinity resin or lack of release of high-affinity antibodies from the resin. If this were the case, the method would require altering so as to obtain a full spectrum of idiotypes.

No protection against EAMG in rabbits was affected by prior immunization with purified sheep antibodies. Similar results were obtained using purified rabbit antibodies (results not shown). This would not be surprising if there were no cross-reaction between the idiotypes. However, rabbits immunized with receptor–rabbit antibody complexes were protected from EAMG (Barkas & Simpson, submitted). The difference between these two systems is being investigated.

This work was supported by the Muscular Dystrophy Group of Great Britain. The technical assistance of Mrs J. Gairns and Mr I. MacDonald are gratefully acknowledged, as is the secretarial assistance of Mrs M. McColl.

REFERENCES

AVRAMEAS, S. & TERNYNCK, T. (1969) The cross-linking of proteins with glutaraldehyde and its use for the preparation of immunoadsorbents. *Immunochemistry*, **6**, 53.

BARKAS, T. (1979) Myasthenia gravis, the acetylcholine receptor and the immune response. *Int. J. Immunopharmacol.* **1**, 263.

BARKAS, T., HARRISON, R., LUNT, G.G. & WATSON, C.M.J. (1978) Immunochemistry of the acetylcholine receptor. *Biochem. Soc. Trans.* **6**, 636.

DULLEY, J.R. & GRIEVE, P.A. (1975) A simple technique for eliminating interference by detergents in the Lowry method of protein determination. *Anal. Biochem.* **64**, 136.

GEHA, R.S. & WEINBERG, R.P. (1978) Anti-idiotype antisera in man. I. Production and immunochemical characterization of anti-idiotype antisera to human antitetanus antibodies. *J. Immunol.* **121**, 1518.

HARVEY, A.L., BARKAS, T., HARRISON, R. & LUNT, G.G. (1978) Inhibition of receptor function in cultured chick myotubes by antiserum to purified Torpedo acetylcholine receptor and myasthenic sera. In *The Biochemistry of Myasthenia Gravis and Muscular Dystrophy* (ed. by G. G. Lunt and R. M. Marchbanks), p. 167. Academic Press, London.

LINDSTROM, J., LENNON, V., SEYBOLD, M. & WHITTINGHAM, S. (1976) Experimental autoimmune myasthenia gravis and myasthenia gravis: biochemical and immunochemical aspects. *Ann. N.Y. Acad. Sci.* **274**, 254.

LING, N.R., BISHOP, S. & JEFFERIS, R. (1977) Use of antibody-coated red cells for the sensitive detection of antigen and in rosette tests for cells bearing surface immunoglobulins. *J. Immunol. Methods*, **15**, 279.

MCCONAHEY, P. & DIXON, F.J. (1966) A method of trace iodination of proteins for immunological studies. *Int. Arch. Allergy appl. Immunol.* **29**, 185.

NYE, L., PONTES DE CARVALHO, L.C. & ROITT, I.M. (1980) Restrictions in the response to autologous thyroglobulin in the human. *Clin. exp. Immunol.* **41**, 252.

PARIKH, I., MARCH, S.C. & CUATRECASAS, S. (1974) Topics in the methodology of substitution reactions with agarose. In *Methods in Enzymology* (ed. by W. B. Jakoby and M. Wilchek), Vol. XXXIV, pp. 77–102. Academic Press, New York.

SCHMIDT, J. & RAFTERY, M.A. (1973) A simple assay for the study of solubilised acetylcholine receptor. *Anal. Biochem.* **52**, 349.

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

STANWORTH, D.R. & TURNER, M.W. (1973) Immunochemical analysis of immunoglobulins and their subunits. In *Handbook of Experimental Immunology* 2nd edn (ed. by D. M. Weir), pp. 10–16. Blackwell, Oxford.