Clin. exp. Immunol. (1990) **80**, 376–380

Neonatal myasthenia gravis: antigenic specificities of antibodies in sera from mothers and their infants

S. J. TZARTOS, A. EFTHIMIADIS, E. MOREL*, B. EYMARD† & J.-F. BACH* *Hellenic Pasteur Institute, Athens, Greece, * INSERM U25, CNRS 122, Hopital Necker, Paris, and † Centre Chirurgical Marie Lannelongue, Laboratoire d'Immunologie, Paris, France*

(Accepted for publication 10 January 1990)

SUMMARY

Transient neonatal myasthenia gravis (MG) is a human model of passively transferred MG. In an effort to understand the characteristics of the most pathogenic antibodies in MG, we studied the fine antigenic specificities of anti-AChR antibodies in sera from 21 MG mothers (nine of which had transiently transferred the disease) and 17 of their infants. Although in a few cases significant differences in antibody specificities were observed between mothers and infants, whether myasthenic or not, generally the antigenic specificities of the antibodies in sera from infants were very similar to those of their mothers. Furthermore, no characteristic differences were detected between the antibody repertoires of mothers who transferred the disease and those who did not.

Keywords neonatal myasthenia gravis monoclonal antibodies acetylcholine receptor

INTRODUCTION

The neuromuscular disease myasthenia gravis (MG) is an autoimmune disorder characterized by weakness and fatigability of the skeletal muscles. MG is considered to be caused by the spontaneous development of antibodies against the muscle nicotinic acetylcholine receptor (AChR) (Drachman *et al.*, 1987; Willcox & Vincent 1988; Lindstrom, Shelton & Fugii 1988). These antibodies result in loss of AChRs and also directly block the function of the remaining AChR molecules, thereby causing a defect in neuromuscular transmission. About two-thirds of the anti-AChR antibodies, both from human myasthenic patients and from rats immunized with intact AChR, are directed against an extracellular area of the α-subunit, the main immunogenic region (MIR) (Tzartos & Lindstrom 1980; Tzartos *et al.*, 1982; 1988a; Barkas *et al.*, 1987; Tzartos 1988).

Transient neonatal MG is a human model of passively transferring the disease. Although all newborn babies of myasthenic mothers have anti-AChR antibodies at birth (Keesey *et al.*, 1977; Morel *et al.*, 1988), only a small percentage of them (10–15%) express the myasthenic syndrome (Namba, Brown & Grob 1970). The myasthenic symptoms usually appear a few hours after birth and their average duration is about 3 weeks. The appearance of transient MG does not seem to be related to the severity of the mother's disease (Elias, Butler & Appel 1979; Olanow 1982; Morel *et al.*, 1988). The role of the anti-AChR titre is questionable; while in some reports no correlation between presence of MG and antibody titre in the infants' sera was observed (Lefvert & Osterman 1983; Bartoccioni *et al.*, 1986), in others, MG infants had generally higher titre sera than the healthy infants of MG mothers (Keesey *et al.*, 1977; Donaldson *et al.*, 1981; Morel *et al.*, 1988). It is unclear yet whether differences in fine antigenic specificities play any role in the above discrepancies.

In the present study we are comparing the fine antigenic specificities of the antibodies from MG mothers who do or do not transfer the disease, with those of their infants. We also compare the antigenic specificities of antibodies from MG mothers who transferred with those of mothers who did not transfer the disease. For this purpose an antibody competition technique (Tzartos *et al.*, 1982; 1988b; Whiting *et al.*, 1986) was applied, between the human sera and anti-AChR monoclonal antibodies (MoAbs), of known epitope specificity, for binding on the intact human AChR. The majority, though not all, of both myasthenic and non-myasthenic infants were found to have a repertoire of anti-AChR specificities very similar to their mothers. No significant differences were observed between sera from the two groups of mothers.

MATERIALS AND METHODS

AChR and α-bungarotoxin

Crude muscle extracts derived from amputated human legs were prepared for use as ^{125}I-α-bungarotoxin-labelled antigen (Lindstrom *et al.*, 1981). α-bungarotoxin (from Sigma) was labelled with ^{125}I by the chloramine-T method.

Correspondence: S. Tzartos, Hellenic Pasteur Institute, 127, Vas. Sofias Ave., Athens 11521, Greece.

MoAbs

All MoAbs used were derived from rats immunized with intact AChR from *Electrophorus electricus* electric organs (MoAbs 42, 35 and 25), fetal calf muscles (MoAbs 64, 73 and 66) or human muscles (MoAbs 195 and 203) (Tzartos *et al.*, 1981, 1983, 1986). All but the negative control MoAb 25 bound well to human muscle AChR. MoAb preparations were obtained from hybridoma culture supernatants concentrated by 50% ammonium sulphate. MoAbs 42 (IgG2a), 35 (IgG1), 195 (IgG1) and 202 (IgG1) are directed against the MIR. MoAbs 64 (IgG2a), 73 (IgG1) and 66 (IgG2a) bind to other extracellular sites on the α, β and γ AChR subunits, respectively. However, the binding sites for the latter two MoAbs (73 and 66) are located near the MIR since these MoAbs partially compete with anti-MIR MoAbs (Tzartos *et al.*, 1986; Kordossi & Tzartos, 1989).

Human sera

Clinical and immunological studies of most of the MG patients have been reported elsewhere (Morel *et al.*, 1988). The antibody titres of the sera were determined by radioimmunoassay using human muscle extracts (Lindstrom *et al.*, 1981).

Competition between MoAbs and MG sera for human AChR binding

This was performed in principle as described earlier (Tzartos *et al.*, 1982, 1985, 1988b). In brief, samples of human muscle extracts in PBS plus 0·5% Triton X-100 containing 2 nM AChR labelled with 10 nM ^{125}I-α-bungarotoxin were incubated for 3 h with a large excess of a specific MoAb or control MoAb 25. The mixtures were then dispensed into 96-well plates (20 μl per well, containing 5 fmol AChR) to which were added 20 μl buffer containing about 4 fmol MG serum, supplemented with normal human serum (NHS) to give a total volume of undiluted serum of 0·5 μl (or 2 μl for low titre sera). After 3 h, rabbit anti-human gamma-globulin serum pretreated with normal rat serum (Tzartos *et al.*, 1985) was added, incubated for 1 h, centrifuged and washed. All incubations were performed at 4°C. The radioactivity present in the individual wells was counted. After subtraction of the background counts (radioactivity precipitated with NHS in the presence or absence of specific MoAb; 2–5% of the positive control), the percentage inhibition of binding by the MoAbs was estimated by the equation:

$$\% \text{ inhibition of binding} = \frac{\text{ct/min(MG MoAb25)} - \text{ct/min(MG MoAbX)}}{\text{ct/min (MG MoAb25)}} \times 100$$

where ct/min (MG MoAb25) and ct/min (MG MoAbX) is the radioactivity precipitated by the MG serum in the presence of negative control MoAb 25 or specific MoAbX, respectively. This inhibition (i.e. protection of the AChR against binding of serum antibodies) is assumed to represent the percentage of antibodies present in the test serum capable of recognizing the MoAb-protected region of the AChR. Occasionally, negative inhibition values were obtained. MoAbs are able to cross-link two or more AChR molecules (Tzartos *et al.*, 1986). Thus the negative inhibition values can be attributed to the trapping of these AChR–MoAb complexes by the serum MG antibodies. In the Figs both negative and zero inhibition values are presented as 0% protection.

Table 1. Characteristics of the MG patients and their sera

	Anti-AChR titre (nM)				
	Mother		Child		
Case	Days*	Titre	Days	Titre	MG in infant
1	0	365	0	787	+
2	18	153	12†	11·5	+
			16†	9·1	+
			31†	2·4	+
3	1	127	1	147	+
4	31	123	22	31·8	+
5	5	87	5	88·9	+
6	0	37·5	0	28	+
7	13	99·2	3	69·1	+
8	−15	44	0	21·2	+
9	1	13	1	10·1	+
10	0	515	0	457	−
11	−2	39·1	3	35·9	−
12	24	40·5	10	28·5	−
13	−17	27·3	0	22·3	−
14	0	20·8	0	11	−
15	4	14·9	0	17·5	−
16	6	11·5	3	4·8	−
17	0	11·8	0	8·5	−
17′‡	0	22·8	0†	22·4	−
			12†	7·3	−
18	−7	9	0	3·6	−
19	−7	3·4	0	3·4	−
20	0	11	0	6·7	−
21	0	10·7	0	5·6	−

* Days of collection of the sera. Day 0 refers to the day of birth.

† Sera collected from the same infant, at different days.

‡ Case 17′ represents a new birth of the same mother in case 17.

RESULTS

Some of the characteristics of the mothers and their infants whose sera were used are shown in Table 1. All but one mother with MG infants had anti-AChR titres above 37 nM whereas all but one mother with healthy infants had anti-AChR titres below 41 nM. Antibody titres in infants' sera were generally similar to those of their mothers at day 0. Infant no. 1 had twice the titre of the mother, apparently because IgG concentration in the umbilical cord was higher than in the mother's serum.

Antigenic specificities in the sera were determined by competition experiments between these sera and anti-AChR MoAbs for binding on the ^{125}I-α-bungarotoxin-labelled AChR. The fraction of serum antibodies which were inhibited from binding to the AChR by a protecting MoAb is considered representative of the percentage of human antibodies whose epitopes are occupied by the MoAb used.

Figures 1 and 2 show the antigenic specificities of the antibodies in the sera from MG mothers who transfer (Fig. 1) or do not transfer (Fig. 2) the disease to their infants, and in most cases they are compared with the antigenic specificities of their infants' sera. Antigenic specificities of infants' sera were gener-

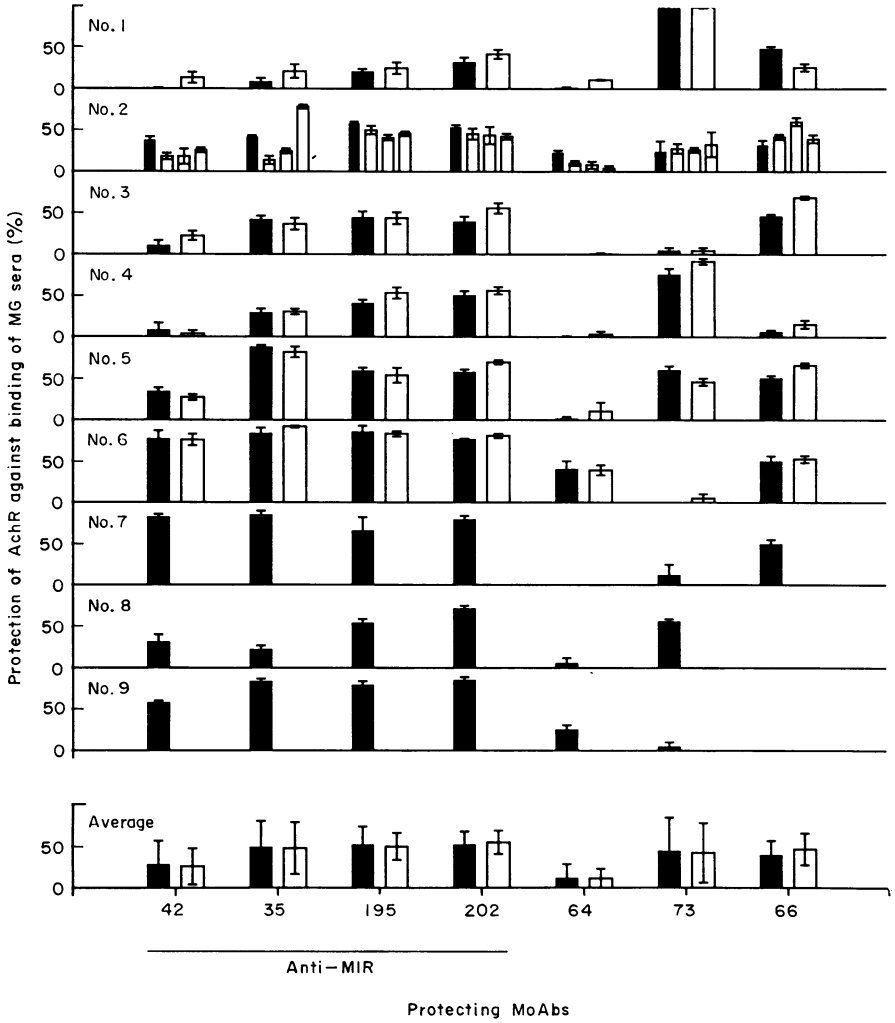

Fig. 1. Anti-AChR specificities in sera from nine MG mothers who transferred the disease to their infants (solid bars) and eight of their infants (open bars) using seven anti-AChR MoAbs. Each bar represents the percentage of inhibition of binding of the serum antibodies on the presence of the corresponding MoAbs. Infant sera in families no. 7, 8 and 9 were not tested. Each serum was usually tested three to four times.

ally very similar to those of their mothers. Differences were in most cases less than 10%, i.e. within the limits of the experimental error of the system. Nevertheless there were a few noted exceptions in which infants' sera differed significantly in some antigenic specificities from those of their mothers (e.g. competition by MoAb 66 in family no. 1). Most of these cases were tested and confirmed several times. No specific trend could be detected; significant differences in antigenic specificities between infants and their mothers were observed in both MG and healthy infants, high and low titres, and they were usually in one or two specificities for each family rather than in all.

The average values for the antigenic specificities of the mothers and infants were also very similar to each other in either group (last line of bars in Figs 1 and 2).

In addition to comparing the sera of newborn babies with those of their mothers, the present data also allowed comparison of sera between mothers who transferred MG and those who did not transfer it. Figure 3 shows that no significant differences existed between the two groups for any of the tested antigenic specificities.

DISCUSSION

This study was performed in an effort to explain why most infants of MG mothers do not exhibit MG symptoms, despite the presence of anti-AChR antibodies in their sera. An explanation might also be found for the discrepancies which are observed between antibody titre and disease severity among the adult MG patients (Lindstrom *et al.*, 1976; Drachman *et al.*, 1982). As far as it concerns the tested antigenic specificities of the anti-AChR antibodies, it was shown that: (1) the sera of the infants, whether myasthenic or not, have generally the same anti-AChR antigenic specificities as those of their mothers. Although the tested antigenic specificities were limited, the overall similarity between the sera of mothers and infants strongly suggests that passive transfer of anti-AChR antibodies in neonatal MG involves the whole or the major part of their mothers' anti-AChR specificities; (2) The antigenic specificities of mothers who transfer the disease do not generally differ from those of mothers who do not transfer it.

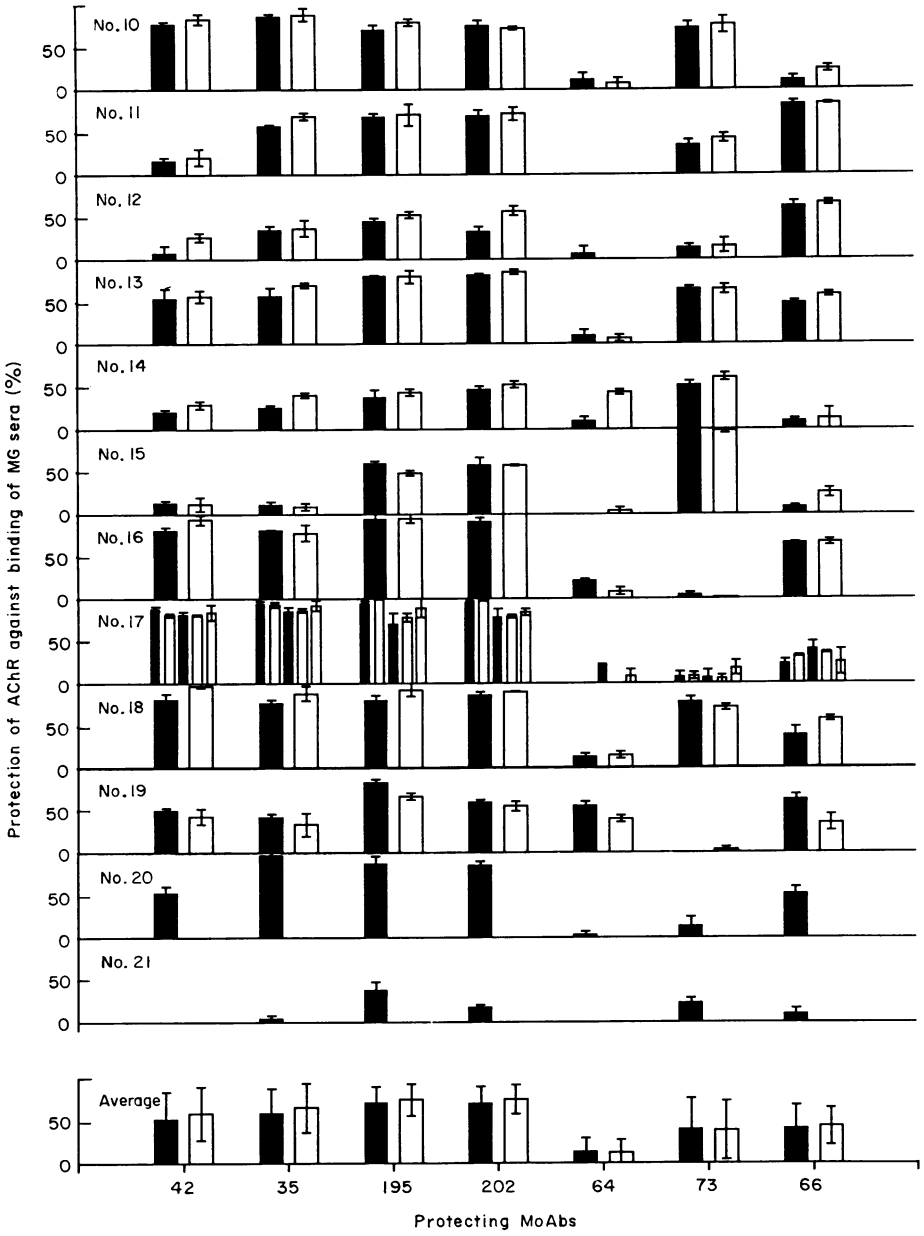

Fig. 2. Anti-AChR specificities in sera from 12 MG mothers with asymptomatic infants (solid bars) and in sera from 11 of their infants (open or other bars). Infant sera in families no. 20 and 21 were not tested.

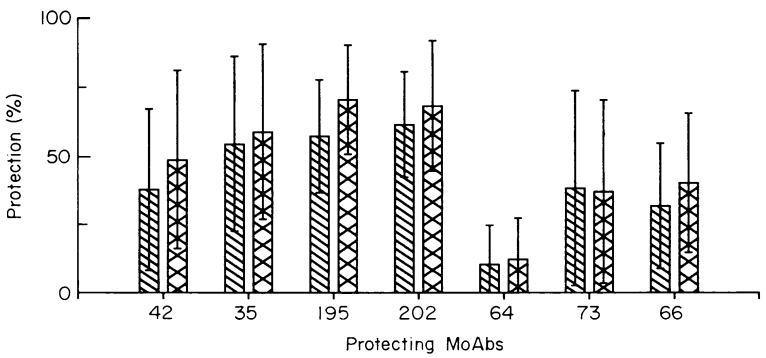

Fig. 3. Comparison of the average inhibition values obtained for the MG mothers who transfer the disease (▨) and those who do not transfer it (⊠).

In very few cases, significant differences in the antigenic specificities were observed between infants' and their mothers' sera. We do not know the reason for these differences. It has been suggested that some of the antibodies in neonatal MG are produced in the infants rather than in their mothers (Lefvert & Osterman 1983). Alternatively, the few differences may be due to selected elimination of some antibody specificities possibly enriched in certain Ig subclasses (Vernet-der Garabedian *et al.*, 1989).

Whatever the explanation may be for the few observed differences, the overwhelming similarities in antigenic specificities between mothers and their infants, and the absence of characteristic differences between the two groups of mothers (transferring or not transferring the disease) suggest that antibody specificity is not the determining factor for inducing MG symptoms in infants of MG mothers. The rather weak correlation between antibody titre and newborn MG (Keesey *et al.*, 1977; Donaldson *et al.*, 1981; Morel *et al.*, 1988) may have the same yet uncertain explanation as that for the lack of correlation between severity of the disease and antibody titre in adult MG patients. This may be due to different overall sensitivity of the different patients to the anti-AChR antibodies because of variations of several intrinsic factors.

ACKNOWLEDGMENTS

We thank Dr T. Dosios and his colleagues for providing valuable material, A. Kokla for technical assistance and Dr R. Matsas for useful discussions.

Supported by grants from the Greek General Secretariat of Research and Technology (S.J.T.), the Association des Myopathes de France (S.J.T. and E.M.) and the Muscular Dystrophy Association of America (S.J.T.).

REFERENCES

Barkas, T., Mauron, A., Roth, B., Alliod, C., Tzartos, S.J. & Ballivet, M. (1987) Mapping the main immunogenic region and toxin binding site of the nicotinic acetylcholine receptor. *Science* **235,** 77.

Bartoccioni, E., Evoli, A., Casali, C., Scoppetta, C., Tonali, P. & Provenzano, C. (1986) Neonatal myasthenia gravis: clinical and immunological study of seven mothers and their newborn infants. *J. Neuroimmunol.* **12,** 155.

Donaldson, J.O., Penn, A.S., Lisak, R.P., Abramsky, O., Brenner, T. & Schotland, D.L. (1981) Antiacetylcholine receptor antibody in neonatal myastaenia gravis. *Am. J. Dis. Child.* **135,** 222.

Drachman, D., de Silva, S., Ramsay, D. & Pestronk, A. (1987) Humoral pathogenesis of myasthenia gravis. *Ann. NY Acad. Sci.* **505,** 90.

Drachman, D.B., Adams, R.N., Josifek, L.F. & Self, S.G. (1982) Functional activities of autoantibodies to acetylcholine receptors and the clinical severity of myasthenia gravis. *N. Engl. J. Med.* **307,** 769.

Elias, S.B., Butler, I. & Appel, S.H. (1979) Neonatal myasthenia gravis in the infant of a myasthenic mother in remission. *Ann. Neurol.* **6,** 72.

Keesey, J., Lindstrom, J., Cokey, H. & Hermann, C. (1977) Anti-acetylcholine receptor antibody in neonatal myasthenia gravis. *N. Engl. J. Med.* **296,** 55.

Kordossi, A.A. & Tzartos, S.J. (1989) Monoclonal antibodies against the main immunogenic region of the acetylcholine receptor. Mapping on the intact molecule. *J. Neuroimmunol.* **23,** 35.

Lefvert, A.K. & Osterman, P.O. (1983) Newborn infants to myasthenic mothers: a clinical study and an investigation of acetylcholine receptor antibodies in 17 children. *Neurology* **33,** 133.

Lindstrom, J., Einarson, B. & Tzartos, S.J. (1981) Production and assay of antibodies to acetylcholine receptor. *Meth. Enzymol.* **74,** 432.

Lindstrom, J., Shelton, D. & Fugii, Y. (1988) Myasthenia gravis. *Advances in Immunology* **42,** 233.

Lindstrom, J.M., Seybold, M.E., Lennon, V.A., Whittingham, S. & Duane, D. (1976) Antibody to acetylcholine receptor in myasthenia gravis: prevalence, clinical correlates and diagnostic value. *Neurology* **26,** 1054.

Morel, E., Eymard, B., Vernet der-Garabedian, B., Pannier, C., Dulac, O. & Bach, J.F. (1988) Neonatal myasthenia gravis-A new clinical and immunologic appraisal on 30 cases. *Neurology* **38,** 138.

Namba, T., Brown, S.B. & Grob, D. (1970) Neonatal myasthenia gravis: report of two cases and review of literature. *Pediatrics* **45,** 488.

Olanow, C.W. (1982) Neonatal myasthenia gravis in the infant of an asymptomatic thymectomized mother. *Can. J. Neurol. Sci.* **9,** 85.

Tzartos, J. (1988) Myasthenia gravis studied by monoclonal antibodies to the acetylcholine receptor. *In Vivo* **3,** 105.

Tzartos, S.J. & Lindstrom, J.M. (1980) Monoclonal antibodies to probe acetylcholine receptor structure: localization of the main immunogenic region and detection of similarities between subunits. *Proc. Natl. Acad. Sci. USA* **77,** 755.

Tzartos, S.J., Rand, D.E., Einarson, B.E. & Lindstrom, J.M. (1981) Mapping of surface structures of *Electrophorus* acetylcholine receptor using monoclonal antibodies *J. Biol. Chem.* **256,** 8635.

Tzartos, S.J., Seybold, M. & Lindstrom, J. (1982) Specificities of antibodies to AChR in sera from myasthenia gravis patients measured by monoclonal antibodies. *Proc. Natl. Acad. Sci. USA* **79,** 188.

Tzartos, S.J., Langeberg, L., Hochschwender, S. & Lindstrom, J. (1983) Demonstration of a main immunogenic region on acetylcholine receptors from human muscle using monoclonal antibodies to human receptor. *FEBS Lett.* **158,** 116.

Tzartos, S.J., Sophianos, D. & Efthimiadis, A. (1985) Role of the main immunogenic region of AChR in myastenia gravis. An Fab mAb protects against antigenic modulation by human sera. *J. Immunol.* **134,** 2343.

Tzartos, S., Langeberg, L., Hochschwender, S., Swanson, L. & Lindstrom, J. (1986) Characteristics of monoclonal antibodies to denatured Torpedo and native calf acetylcholine receptors: species, subunit and region specificity. *J. Neuroimmunol.* **10,** 235.

Tzartos, S.J., Kokla, A., Walgrave, S. & Conti-Tronconi, B. (1988a) Localization of the main immunogenic region of human muscle acetylcholine receptor to residues 67–76 of the α-subunit. *Proc. Natl. Acad. Sci. USA.* **85,** 2899.

Tzartos, S.J., Morel, E., Efthimiadis, A., Bustarret, A.F., D'Anglejan, J., Drosos, A. & Moutsopoulos, H.M. (1988b) Fine antigenic specificities of the antibodies in sera from patients with D-Penicillamine-induced myasthenia gravis. *Clin. Exp. Immunol.* **74,** 80.

Vernet-der Garabedian, B., Eymard, B., Bach, J.F. & Morel, E. (1989) Alpha-bungarotoxin blocking antibodies in neonatal myasthenia gravis: frequency and selectivity. *J. Neurobiol.* **21,** 41.

Whiting, P.J., Vincent, A. & Newsom-Davis, J. (1986) Myasthenia gravis: monoclonal anti-human acetylcholine receptor antibodies used to analyze antibody specificities and responses to treatment. *Neurology* **36,** 612.

Willcox, N. & Vincent, A. (1988) Myasthenia gravis as an example of organ-specific autoimmune disease. In *B Lymphocytes in Human Disease* (ed. by A. G. Bird & J. Calvert), p. 469. Blackwell, Oxford.