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# The autoimmune response of different mouse strains to T-cell epitopes of the human acetylcholine receptor α subunit

S. BROCKE,* M. DAYAN, J. ROTHBARD,† S. FUCHS & E. MOZES *Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel and †Imperial Cancer Research Fund, Lincoln's Inn Fields, London, U.K.*

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## SUMMARY

The specific recognition of the acetylcholine receptor and its α-subunit by T cells derived from patients with myasthenia gravis or mice with experimental autoimmune myasthenia gravis raises the question of the role of autoreactive T cells in the myasthenic process. Sequences of the acetylcholine receptor α-subunit previously shown to be immunogenic in myasthenic patients were tested for their immunogenicity in various inbred mouse strains. High, intermediate and low T-cell proliferative responses could be observed to peptides representing sequences 195–212 and 259–271 of the human acetylcholine receptor α-subunit. Following immunization with the Torpedo acetylcholine receptor, lymphocytes of SJL mice proliferated efficiently to p195–212 but not to p259–271. On the other hand, lymph node cells of BALB/c mice responded well to p259–271 but not to p195–212. Thus, the influence of the genetic make-up of the examined mice on the immune response to the two peptides could be clearly demonstrated by the existence of strain-dependent immunodominant and cryptic regions on the autoantigen. The differences between the strains were less pronounced when antibody responses were measured to these two T-cell epitopes, although a partial correlation with the proliferative responses could be observed. It can be concluded that epitopes specifically recognized by T lymphocytes of patients with myasthenia gravis also represent specific T-cell epitopes in the autoreactivity to the acetylcholine receptor in mice and that immune responsiveness to these peptides is influenced by the genetic make-up of the responding mouse strains.

## INTRODUCTION

Myasthenia gravis (MG) and its experimental animal model, experimental autoimmune MG (EAMG), are immune disorders characterized by circulating antibodies and lymphocyte autoreactivity to the acetylcholine receptor (AChR). The clinical signs of the disease are caused by an antibody-mediated response, which is carried out and regulated by T and B lymphocytes (Lindstrom, Shelton & Fujii, 1988). Several epitopes on the AChR are specifically recognized by antibodies (Lindstrom *et al.*, 1988; Tzartos & Lindstrom, 1980; Tzartos *et al.*, 1983; Souroujon *et al.*, 1986) and, as shown recently, also by T cells (Yokoi *et al.*, 1987; Brocke *et al.*, 1988; Berrih-Aknin *et al.*, 1987; Harcourt *et al.*, 1988; Fujii & Lindstrom, 1988; Zhang *et al.*, 1988). Immunodominant regions (Tzartos & Lindstrom, 1982; Souroujon *et al.*, 1986; Barkas *et al.*, 1987; Ralston *et al.*, 1987) can be identified as preferred targets for antibody binding, which is directed mainly against the α-subunit of the AChR. The α-subunit is also predominant for T-cell epitopes (Tami, Urso & Krolick, 1987; Hohlfeld *et al.*, 1987), although it has become evident that T- and B-cell epitopes may be different on the AChR. Using peptides representing predicted T-cell epitopes on the human AChR α-subunit, regions could be determined that are preferentially recognized by peripheral blood lymphocytes (PBL) of patients with MG rather than of healthy controls (Brocke *et al.*, 1988; Harcourt *et al.*, 1988).

The formation of a complex between peptide fragments of antigens and products of the major histocompatibility complex (MHC) seems to be an important step for antigen recognition by the T-cell receptor (TcR). In the mouse, the T-cell response to Torpedo AChR is controlled by an H-2 linked Ir gene that maps to the I-A subregion of the MHC (Christadoss, Lennon & David, 1979). Studying MG patients, it has been demonstrated recently that there exists an association between HLA-DR5 and HLA-DR3 and the proliferative response to peptides representing residues 195–212 and 257–269, respectively, of the human AChR α-subunit (Brocke *et al.*, 1988). These results prompted a study of the immunogenicity of synthetic peptides representing sequences of the human AChR and their cross-reactivity with the native antigen on the level of T and B cells, in different inbred

*Present address: Institut of Medical Microbiology, Freie Universität, Berlin, 1000 Berlin 45, FRG.

Abbreviations: AChR, acetylcholine receptor; c.p.m., counts per minute; MG, myasthenia gravis; p, peptide; PBL, peripheral blood lymphocytes; PBS, phosphate-buffered saline; RIA, radioimmunoassay.

Correspondence: Professor E. Mozes, Dept. of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.

**Table 1.** Synthetic peptides of the AChR α-subunit

| Peptide | Sequence |
|---|---|
| p195–212 | DTPYLDITYHFVMQRLPL |
| p259–271 | VIVELIPSTSSAV |

Underlined sequences refer to the T-cell motifs of Rothbard & Taylor (1988).

mouse strains. The main objective of the study was the characterization of the immune response to peptides shown to represent major T-cell epitopes in MG. The report shows strain-dependent differences in the immune response potential to the above peptides and demonstrates immunodominant and cryptic epitopes on the AChR α-subunit in various mouse strains.

## MATERIALS AND METHODS

*Mice*

Mice of the different inbred strains C3H.SW, C57BL/6, BALB/c, AKR, C3H/He, SJL and B10.S were obtained from the Jackson Laboratory, Bar Harbor, ME or from Olac, Blackthorn, Bicester, Oxon, U.K. All mice were used at the age of 8–12 weeks.

*Synthetic peptides*

Two peptides corresponding to residues 195–212 and 259–271 of the human AChR α-subunit were synthesized and characterized as previously described for other peptides (Zamvil *et al.*, 1986). The sequences of the peptides are shown in Table 1.

*Torpedo acetylcholine receptor*

AChR was purified from the electric organ of *Torpedo californica* as described previously (Aharonov *et al.*, 1977) with several modifications (Bogen, Mozes & Fuchs, 1984). Elution of AChR from the toxin column and subsequent dialysis were done in 0·025% Triton X-100 instead of 0·1%. The dialysis of the purified receptor was done without EDTA and sodium azide.

*Immunization*

Several groups of five mice were immunized each with 20 μg of the peptide or Torpedo AChR in complete Freund's adjuvant (Difco Laboratories Inc., Detroit, MI) intradermally into the hind footpads. Mice examined for antibody production were boosted 3 weeks later with the same amount of antigen in phosphate-buffered saline (PBS) intradermally.

*Proliferative responses*

Lymph node cells of mice taken 10–14 days after primary immunization were prepared in single cell suspensions and cultured in the presence of different concentrations of either Torpedo AChR or synthetic peptides. The assays were performed in flat-bottomed microtitre plates (Nunc, Roskilde, Denmark) at a cell concentration of $5 \times 10^5$/well in RPMI medium supplemented with 1% syngeneic normal mouse serum (Axelrod & Mozes, 1986), 2 mM glutamine, 1 mM sodium pyruvate, non-essential amino acids, 100 U/ml penicillin, 100 μg/ml streptomycin, 0·25 μg/ml fungizone (Bio Lab, Jerusalem, Israel), $5 \times 10^{-5}$ M 2-mercaptoethanol (Fluka Ab, Buchs, Switzerland) and 10 mM HEPES buffer (Sigma, St Louis, MO). Concanavalin A (Con A; Bio-Makor, Rehovot, Israel) was used as a control for culture conditions. After 4 days of incubation [$^3$H]thymidine (0·5 μCi of 5 Ci/mmol; Nuclear Research Center, Negev, Israel) was added to the cultures for 18 hr. Thereafter, cells were harvested and radioactivity was counted. Results are expressed as mean c.p.m. of triplicate cultures ± SD. SD values did not exceed 10% of mean value.

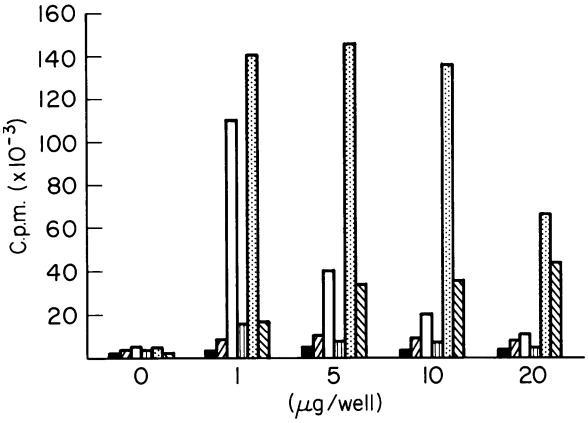


**Figure 1.** Proliferative responses of lymph node cells of p195–212-immunized mice of various inbred mouse strains. Cells ($5 \times 10^5$/well) were cultured in the presence of different concentrations of the peptide. At the end of 4 days incubation period, 0·5 μCi of [$^3$H]thymidine was added. Eighteen hours later cells were harvested, and radioactivity was counted. Results are given as mean c.p.m. of triplicate cultures. SD values did not exceed 10% of the mean. (■) C3H.SW; (▨) C57BL/6; (□) C3H/He; (▩) BALB/c; (▨) SJL; (▧) B10.S.

*Radioimmunoassay*

Antibody levels specific to the different peptides in the sera of mice 7 days after the booster injection were determined by solid-phase radioimmunoassay (RIA). Plates were coated with 100 μl of 50 μg/ml peptide or 25 μg/ml Torpedo AChR in PBS containing 0·2% glutaraldehyde and washed with 0·2% gelatin (Merck, Darmstadt, FRG) in PBS. Thereafter, $^{125}$I-labelled goat anti-mouse immunoglobulin was added for 18 hr. After extensive washing of the radioactive reagents, plates were dried and wells were cut out of the plates and counted in a gamma counter. Results are expressed as mean c.p.m. of duplicates ± SD. SD values did not exceed 5% of mean values.

## RESULTS

### Immunogenicity of p195–212 and p259–271 in various inbred mouse strains

Groups of five mice of different mouse strains were immunized with either peptide p195–212 or peptide p259–271, and their lymph nodes were removed 10–14 days after immunization and tested for their ability to proliferate in the presence of the immunizing peptides. Figures 1 and 2 demonstrate representative results of four separate experiments for each mouse strain. As can be seen in Fig. 1, C3H.SW mice were the lowest responders to the peptide and SJL mice responded with the highest specific proliferation. The proliferative responses of the other strains ranged between low and high. The responses to

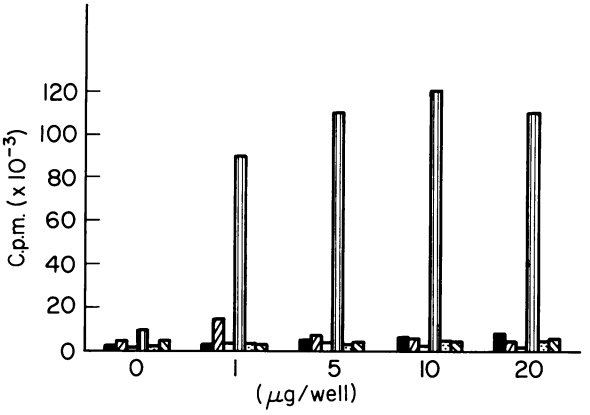


**Figure 2.** Proliferative responses of lymph node cells of p259–271-immunized mice of various inbred mouse strains. Cells ($5 \times 10^5$/well) were cultured in the presence of different concentrations of the peptide. At the end of 4 days incubation period, 0·5 μCi of [$^3$H]thymidine was added. Eighteen hours later cells were harvested, and radioactivity was counted. Results are given as mean c.p.m. of triplicate cultures. SD values did not exceed 10% of the mean. (■) C3H.SW; (■) C57BL/6; (□) C3H/He; (■) BALB/c; (▨) SJL; (▧) B10.S.

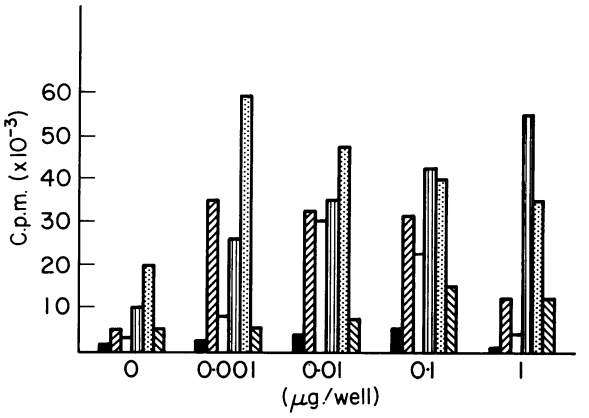


**Figure 3.** Proliferative responses of lymph node cells of Torpedo AChR immunized mice of various inbred mouse strains. Cells ($5 \times 10^5$/wells) were cultured in the presence of different concentrations of the Torpedo AChR. At the end of 4 days incubation period, 0·5 μCi of [$^3$H]thymidine was added. Eighteen hours later cells were harvested and radioactivity was counted. Results are given as mean c.p.m of triplicate cultures. SD values did not exceed 10% of the mean. (■), C3H.SW; (■) C57BL/6; (□) C3H/He; (■) BALB/c; (▨) SJL; (▧) B10.S.

peptide p259–271 were in general lower than to p195–212, as shown in Fig. 2, with the exception of BALB/c mice that responded very efficiently to p259–271.

### Proliferative responses of Torpedo AChR-primed lymphocytes and cross-reactivity with p195–212 and p259–271

Figure 3 demonstrates the proliferative responses of the different mouse strains to the AChR of *Torpedo californica*. All the mouse strains tested responded to the receptor. C3H.SW mice were the lowest, but still significant responders (Fig. 3).

**Table 2.** Proliferative responses of mice immunized with Torpedo AChR

| Strain | H-2 type | *In vitro* stimulation with: Torpedo AChR | p195–212 | p259–271 |
|---|---|---|---|---|
| C3H.SW | b | 2·4* | 1·5 | 1·2 |
| C57BL/6 | b | 6·4 | 2·4 | 2·7 |
| BALB/c | d | 5·2 | 1·8 | 4 |
| C3H/He | k | 11 | 1·8 | 1·3 |
| SJL | s | 3·0 | 6·8 | 2·1 |
| B10.S | s | 2·9 | 2·4 | 1·4 |

Cells ($5 \times 10^5$/well) were cultured in the presence of different concentrations of either the Torpedo AChR or the peptides. At the end of 4 days incubation period, 0·5 μCi of [$^3$H]thymidine was added. Eighteen hours later cells were harvested, and radioactivity was counted.

*Results are expressed as stimulation indices at the optimal concentration of antigen.

Lymphocytes of various mouse strains immunized with the whole Torpedo AChR were also tested for their cross-reactivity with p195–212 and p259–271 in an *in vitro* proliferation assay. As shown in Table 2, BALB/c lymphocytes responded well to the native Torpedo AChR, and cross-reacted significantly with p259–271, but very weakly with p195–212. On the other hand, lymphocytes of mice of the H-2$^s$ haplotype, SJL and B10.S, cross-reacted with p195–212 after primary immunization with Torpedo AChR. Interestingly, lymph node cells of SJL mice reacted even more strongly to p195–212 than to the immunizing Torpedo AChR (Table 2), but cross-reacted minimally with p259–271 (Table 2). Torpedo AChR-primed lymphocytes of C57BL/6 mice cross-reacted weakly with both peptides, whereas C3H/He cells, which were high responders to AChR, did not respond to either of the peptides by *in vitro* proliferation. C3H.SW lymph node cells responded poorly to the AChR and also did not respond to the synthetic peptides. The above results were reproducible in three to four separate experiments.

### Antibody responses to p195–212, p259–271 and Torpedo AChR in various inbred mouse strains

One week after booster immunization, mice of the various strains were bled, and their pooled sera were tested for reactivity with both peptides and with Torpedo AChR in a solid-phase radioimmunoassay. As demonstrated in Figs 4, 5 and 6, which represent one experiment out of several performed, all strains examined produced antibodies that were specific to the immunizing antigens. The only exceptions were mice of the SJL and B10.S strains that were non-responders at the level of antibody production to p259–271 (Fig. 5) as well as of T-cell responsiveness (Fig. 2). No cross-reactivity with p195–212 and p259–271 on the level of antibody production was detected in sera of Torpedo AChR-immunized mice. The latter mice were bled several times during a long period after immunization and although high antibody titres specific to the Torpedo AChR could be determined, no cross-reactivity with the peptides was observed. This is in contrast with the cross-reactivity detected at the level of T-cell responses (Table 2).

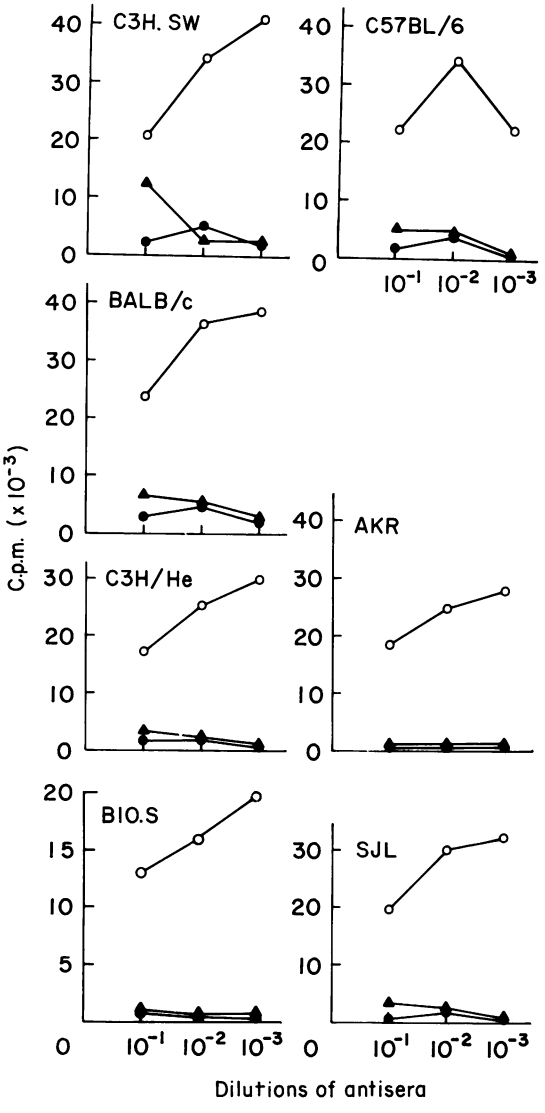


**Figure 4.** Antibody activity in the sera of p195–212-immunized inbred mouse strains. Microtitre plates were coated with p195–212 (○), p259–271 (●) and Torpedo AChR (▲). Specific binding of the sera was detected by $^{125}$I-labelled goat anti-mouse Ig antibody in a RIA. Pooled sera of five mice in a group were diluted as indicated. Results are given as mean of duplicates. SD values did not exceed 5% of the mean.

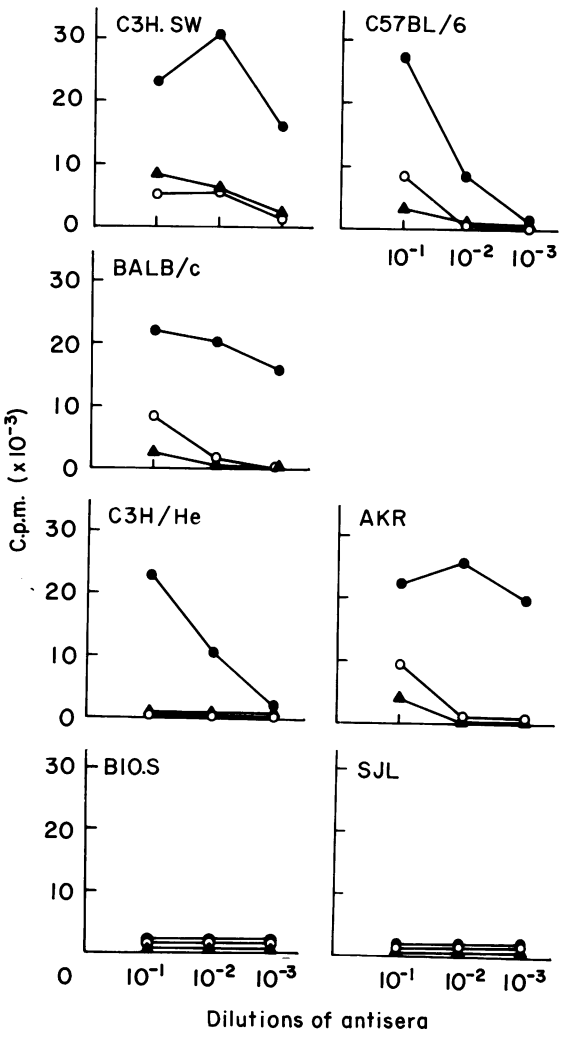


**Figure 5.** Antibody activity in the sera of p259–271-immunized inbred mouse strains. Microtitre plates were coated with p195–212 (○), p259–271 (●) and Torpedo AChR (▲). Specific binding of the sera was detected by $^{125}$I-labelled goat anti-mouse Ig antibody in a RIA. Pooled sera of five mice in a group were diluted as indicated. Results are given as mean of duplicates. SD values did not exceed 5% of the mean.

## DISCUSSION

In the present study, mouse strain differences in the immune response potential to two peptides, representing sequences of the human AChR α-subunit, have been demonstrated. In addition, cross-reactivity was observed between Torpedo AChR and these two epitopes in various inbred mouse strains. It is noteworthy that the two peptides analysed in this study, p195–212 and p259–271, were chosen since they were shown to stimulate PBL of myasthenic patients specifically in association with certain HLA-DR determinants (Brocke *et al.*, 1988). These peptides are now shown to include epitopes for murine as well as human T cells.

The two peptides p195–212 and p259–271 are good immunogens and elicited significant T-cell as well as antibody responses upon immunization without any carrier protein. However, it should be noted that on a molar basis the optimal doses of peptides required for immunization of mice or for triggering *in vitro* T-cell responses were higher to three or two orders of magnitude, respectively, than the doses of Torpedo AChR used to elicit the above immune responses.

Although p195–212 induced antigen-specific lymphocyte responses in all mouse strains examined, the proliferative responses ranged between low to high in the different mouse strains (Fig. 1). Only BALB/c mice were high responders to peptide p259–271 (Fig. 2), and they also responded to it after immunization with Torpedo AChR (Table 2). In striking contrast, SJL mice were the best T-cell responders to p195–212 but the worst responders to p259–271; they also failed to produce antibodies to this peptide (Fig. 5). These results suggest a general low or non-responsiveness of H-2$^s$ mice to p259–271, as the B10.S strain behaved similarly (Figs 2 and 5).

Dominant and cryptic epitopes could be determined by *in vitro* stimulation of Torpedo AChR-primed lymph node cells

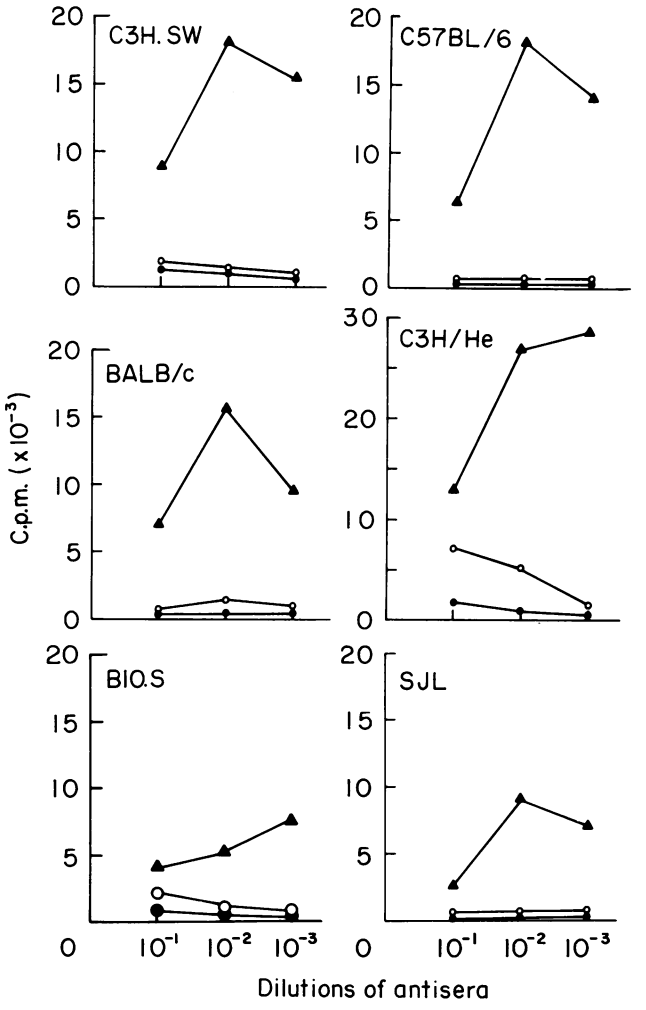


**Figure 6.** Antibody activity in the sera of Torpedo AChR-immunized inbred mouse strains. Microtitre plates were coated with p195–212 (○), p259–271 (●) and Torpedo AChR (▲). Specific binding of the sera was detected by $^{125}$I-labelled goat anti-mouse Ig antibody in a RIA. Pooled sera of five mice in a group were diluted as indicated. Results are given as mean of duplicates. SD values did not exceed 5% of the mean.

with p195–212 and p259–271 (Table 2). The rationale of testing cross-reactivity between the Torpedo AChR α-subunit and human receptor-derived peptides stems from the fact that peptide p259–271 is identical to the corresponding Torpedo region and that p195–212 of the human α-subunit sequence differs from that of the Torpedo sequence by only two amino acids, namely at position 206 Val vs Ile and at position 210 Leu vs Ile (Noda *et al.*, 1983). After immunization with Torpedo AChR, lymphocytes of BALB/c mice proliferated efficiently to p259–271, whereas Torpedo AChR-primed lymph node cells of SJL mice were high responders to p195–212 (Table 2). These results correlate well with the proliferative responses of lymphocytes of these strains after immunization with the peptides (Figs 1 and 2).

It is noteworthy that although lymph nodes of C3H/He mice proliferated well to the Torpedo AChR (Table 2), they did not cross-react with either p195–212 or p259–271. Thus, it is likely that although C3H/He mice did respond upon immunization with the peptides (Figs 1 and 2) the latter (in contrast to the case in SJL and BALB/c mice) are not the dominant T-cell epitopes recognized by T cells of this strain when immunized with the whole AChR molecules.

The differences in immune responses of the various mouse strains and the cross-reactions observed between AChR and the above peptides were mainly at the level of T cells rather than of antibody responses. However, these results are not unexpected since p195–212 and p259–271 were selected as potential T-cell epitopes (Rothbard & Taylor, 1988). Indeed, they have been shown previously to stimulate T cells of MG patients (Brocke *et al.*, 1988) and of different mouse strains (Figs 1 and 2). The two peptides utilized in the present study are likely to be relevant in the autoimmune response to the AChR since they specifically stimulated T cells of MG patients and of AChR-immunized mice. The latter immune responses appear to be genetically regulated.

These results suggest that I-A and/or I-E molecules of different H-2 haplotypes are able to present p195–212 and p259–271 to T cells of the examined mice. It has been demonstrated that immunogenic peptides bind directly to class II MHC products (Babbitt *et al.*, 1985; Buus *et al.*, 1986; Brown *et al.*, 1987) and that the peptide/MHC complex is recognized by the T-cell antigen receptor (Davis & Bjorkman, 1988). Indeed, recent results in our laboratory demonstrated direct binding of biotinylated p195–212 to antigen-presenting cells of various mouse strains, which correlates with their proliferative responses (Mozes *et al.*, 1989).

## ACKNOWLEDGMENTS

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## REFERENCES

Aharonov A., Tarrab-Hazdai R., Silman I. & Fuchs S. (1977) Immunochemical studies on acetylcholine receptor from Torpedo Californica. *Immunochemistry*, **14**, 129.

Axelrod O. & Mozes E. (1986) Analysis of the biological functions and fine specificity of (T,G)-A--L specific T cell clones. *Immunobiol.* **172**, 99.

Babbitt B., Allen P., Matsueda G., Haber E. & Unanue E. (1985) Binding of immunogenic peptides to Ia histocompatibility molecules. *Nature (Lond.)*, **317**, 359.

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.

Berrih-Aknin S., Cohen-Kaminsky S., Neumann D., Bach J.F. & Fuchs S. (1987) Proliferative responses to acetylcholine receptor peptides in myasthenia gravis. *J. Neuroimmunol.* **16**, 17.

Bogen S., Mozes E. & Fuchs, S. (1984) Induction of acetylcholine receptor-specific suppression. *J. exp. Med.* **159**, 292.

Brocke S., Brautbar C., Steinman L., Abramsky O., Rothbard J., Neumann D., Fuchs S. & Mozes E. (1988) *In vitro* proliferative responses and antibody titers specific to human acetylcholine receptor synthetic peptides in patients with myasthenia gravis and relation to HLA class II genes. *J. clin. Invest.* **82**, 1894.

Brown J.H., Jardetzky T., Saper M.A., Samraoui B., Bjorkman P.J. & Wiley D.C. (1987) A hypothetical model of the foreign antigen binding site of Class II histocompatibility molecules. *Nature (Lond.)*, **332**, 845.

BUUS S., COLON S., SMITH C., FREED J.H., MILES C. & GREY H.M. (1986) Interaction between a 'processed' ovalbumin peptide and Ia. *Proc. natl. Acad. Sci. U.S.A.* **83,** 3968.

CHRISTADOSS P., LENNON V.A. & DAVID C. (1979) Genetic control of experimental autoimmune myasthenia gravis in mice. I. Lymphocyte proliferative response to acetylcholine receptor is under H-2 linked Ir gene control. *J. Immunol.* **123,** 2540.

DAVIS M.M. & BJORKMAN P.J. (1988) T-cell antigen receptor genes and T-cell recognition. *Nature (Lond.)*, **334,** 395.

FUJII Y. & LINDSTROM J. (1988) Specificity of the T cell immune response to acetylcholine receptor in experimental autoimmune myasthenia gravis. *J. Immunol.* **140,** 1830.

HARCOURT G.C., SOMMER N., ROTHBARD J., WILLCOX, H.N.A., & NEWSOM-DAVIS J. (1988) A juxta-membrane epitope on the human acetylcholine receptor recognized by T cells in myasthenia gravis. *J. clin. Invest.* **82,** 1295.

HOHLFELD R., TOYKA K.V., TZARTOS S.J., CARSON W. & CONTI-TRONCONI, B.M. (1987) Human T-helper lymphocytes in myasthenia gravis recognize the nicotinic receptor α-subunit. *Proc. natl. Acad. Sci. U.S.A.* **84,** 5379.

LINDSTROM J., SHELTON D. & FUJII Y. (1988) Myasthenia gravis. *Adv. Immunol.* **42,** 233.

MOZES E., DAYAN M., ZISMAN E., BROCKE S., LICHT A. & PECHT I. (1989) Direct binding of myasthenia gravis related epitope to MHC clone II molecules on living murine antigen presenting cells. *EMBO J.* **8,** 4049.

NODA M., FURUTANI Y., TAKAHASHI H., TOYOSATO M., TANABE T., SHIMIZU S., KIKYOTANI S., KAYANO T., HIROSE T., INAYAMA S. & NUMA S. (1983) Cloning and sequence analysis of calf cDNA and human genomic DNA encoding α-subunit precursor of muscle acetylcholine receptor. *Nature (Lond.)*, **305,** 818.

RALSTON S., SARIN V., THANB N.L., RIVIERN J., FOX J.L. & LINDSTROM J. (1987) Synthetic peptides used to locate the α-bungaro-toxin binding site and immunogenic regions on α subunits of the nicotinic acetylcholine receptor. *Biochemistry*, **26,** 3266.

ROTHBARD J.B. & TAYLOR W.R. (1988) A sequence pattern common to T cell epitopes. *EMBO J.* **7,** 93.

SOUROUJON M.C., NEUMANN D., PIZZIGHELLA S., SAFRAN A. & FUCHS S. (1986) Localization of a highly immunogenic region on the acetylcholine receptor α-subunit. *Biochem. Biophys. Res. Commun.* **135,** 82.

TAMI J.A., URSO O.F. & KROLICK K.A. (1987) T cell hybridomas reactive with the acetylcholine receptor and its subunits. *J. Immunol.* **138,** 732.

TZARTOS S. & LINDSTROM J.M. (1980) Monoclonal antibodies used to probe acetylcholine receptor structure: localization of the main immunogenic region and detection of similarities between subunits. *Proc. natl. Acad. Sci. U.S.A.* **77,** 755.

TZARTOS S. & LINDSTROM J.M. (1982) Specificities of antibodies to acetylcholine receptors in sera from myasthenia gravis patients measured by monoclonal antibodies. *Proc. natl. Acad. Sci. U.S.A.* **79,** 188.

TZARTOS S., LONGEBERG L., HOCHSCHWENDNER 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.

YOKOI T., MULAC-JERICEVIC B., KURISAKI J. & ATASSI M. (1987) T lymphocyte recognition of acetylcholine receptor: localization of the full T cell recognition profile on the extracellular part of the α chain of Torpedo Californica acetylcholine receptor. *Eur. J. Immunol.* **17,** 1697.

ZAMVIL S., MITCHELL D., MOORE A., KITAMURA K., STEINMANN L. & ROTHBARD J. (1986) T-cell epitope of the autoantigen myelin basic protein that induces encephalomyelitis. *Nature (Lond.)*, **324,** 258.

ZHANG Y., BARKAS T., JUILLERAT M., SCHWENDIMANN B. & WEKERLE H. (1988) T cell epitopes in experimental autoimmune myasthenia gravis of the rat: strain-specific epitopes and cross-reaction between two distinct segments of the α-chain of the nicotinic acetylcholine receptor (Torpedo Californica). *Eur. J. Immunol.* **18,** 551.