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Experimental autoimmune myasthenia gravis (EAMG) is a model for human myasthenia (MG) that is routinely induced in susceptible rat strains by a single immunization with *Torpedo* acetylcholine receptor (TACHR). TACHR immunization induces anti-AChR Abs that cross-react with self AChR, activate the complement cascade, and promote degradation of the postsynaptic membrane of the neuromuscular junction. In parallel, TACHR-specific T cells are induced, and their specific immunodominant epitope has been mapped to the sequence 97–116 of the AChR α subunit. A proliferative T cell response against the corresponding rat sequence (R97–116) was also found in TACHR-immunized rats. To test whether the rat (self) sequence can be pathogenic, we immunized Lewis rats with R97–116 or T97–116 peptides and evaluated clinical, neurophysiological, and immunological parameters. Clinical signs of the disease were noted only in R97–116-immunized animals and were confirmed by electrophysiological signs of impaired neuromuscular transmission. All animals produced Abs against the immunizing peptide, but anti-rat AChR Abs were observed only in animals immunized with the rat peptide. These findings suggested that EAMG in rats can be induced by a single peptide of the self AChR, that this sequence is recognized by T cells and Abs, and that breakdown of tolerance to a self epitope might be an initiating event in the pathogenesis of rat EAMG and MG. The Journal of Immunology, 2004, 172: 2697–2703.

E xperimentally induced autoimmune myasthenia gravis (EAMG) is an animal model of human myasthenia gravis (MG) (1), a B cell-mediated, T cell-dependent autoimmune disease of the neuromuscular junction in which the nicotinic acetylcholine receptor (AChR) is the autoantigen (2). EAMG is similar to the human disease in its clinical and immunopathologic manifestations and represents a widely used model for the investigation of new therapeutic strategies (1, 3). Etiological factors promoting autoimmunity are still unknown in both EAMG and its human counterpart. AChR-specific T cells play a role in driving autoreactive Ab production and hence the autoimmune response in both EAMG and MG. EAMG is routinely induced in susceptible rat strains (Lewis strain is preferred, but Brown-Norway or Fisher might also be used) by immunization with AChR purified from *Torpedo* electric organ (TACHR) in CFA (3). In Lewis rats, T cells from TACHR-immunized animals recognize the immunodominant sequences a100–116 and a73–90 (4) and a97–112 (5, 6) of *Torpedo* receptor α subunit. Similarly to native AChR, the human AChR a1–210 recombinant protein induced AChR-binding, AChR-modulating, and AChR-blocking autoantibodies in rats when injected once intraocularly as an emulsion (7). In this model, T cells recognized the sequence a125–147 of the human AChR α subunit, and this peptide was then used to induce EAMG (8). To our knowledge, no information is available for the myasthenogenic activity of T cell epitopes derived from rats immunized with self AChR. In this paper we describe the induction of EAMG in Lewis rats by a synthetic peptide corresponding to region a97–116 of the rat AChR α subunit. We found that breaking of tolerance to a single T cell epitope of the self autoantigen induces autoreactive T cells and specific Abs to rat AChR, leading to clinical signs of the disease. A similar mechanism might be of relevance to induction of the corresponding disease in humans.

Materials and Methods

Animals

Female Lewis rats, 6–8 wk of age, were purchased from Charles River (Calco, Italy) and housed at the animal facility of the Neurological Institute Carlo Besta (Milan, Italy). Experiments were approved by the ethical committee of the Institute and were performed in accordance with the Principles of Laboratory Animal Care (European Communities Council Directive 86/609/EEC).

Antigens

AChR was purified from *Torpedo californica* electroplax tissue (Aquatic Research Consultants, San Pedro, CA) by affinity chromatography on Naja siamensis toxin coupled to Sepharose 4B (Pharmacia Biotech, Uppsala, Sweden) (9). The TACHR concentration was determined by [125I]-α-bungarotoxin ([125I]-α-BTX; Amersham Italia, Milan, Italy) binding assay, and the specific activity was 3.7–5.5 pmol [125I]-α-BTX binding sites/mg protein. The purified receptor was analyzed on SDS-PAGE. TACHR preparations were dialyzed extensively against 0.01 M Tris-HCl (pH 7.5), 0.001 M EDTA, 0.1 M NaCl (Tris-HCl buffer), and 0.1% Triton X-100 and were stored at −80°C. TACHR used for T cell proliferations was further dialyzed against Tris-HCl buffer and 0.025% Triton X-100 and filter-sterilized (0.2 µm). The peptides 797–116 (DGDFAIHMTKLLDLYTGGI) and R97–116 (DGDFAIKFKTVKLLDLYTGGI) were synthesized according to Gen-Bank published sequences (J00963 for *Torpedo*, X74832 for rat AChR α

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4 Abbreviations used in this paper: EAMG, experimental autoimmune myasthenia gravis; AChR, acetylcholine receptor; α-BTX, α-bungarotoxin; LNC, lymph node cell; MG, myasthenia gravis; RNS, repetitive nerve stimulation; SI, stimulation index; TACHR, *Torpedo* acetylcholine receptor.
subunit). Peptides were synthesized using F-moc chemistry on an 431A automated peptide synthesizer (PE Applied Biosystems, Foster City, CA). Peptides were purified by reverse phase HPLC; their synthesis was confirmed by mass spectrometry.

Immunization protocols

Female Lewis rats (6–8 wk) were immunized in the hind foot pads with 50 μg of H97–116 peptide in CFA (Sigma-Aldrich, Milan, Italy) supplemented with 1 mg of H37Ra/rat (Difco, Detroit, MI) on day 0 and were boosted on day 30 with the same peptide in IFA (Sigma-Aldrich). EAMG was also induced with 50 μg of purified TACHR in CFA (plus 1 mg of H37Ra/rat).

Clinical evaluation

Each experimental animal was weighed at the beginning of the experiment and twice weekly. The severity of the disease was scored by measuring muscular weakness. Clinical scoring was based on the presence of tremor, hunched posture, muscle strength, and fatigability. Fatigability was assessed after exercise (repetitive paw grips on the cage grid) for 30 s. Disease severity was expressed as follows: grade 0, normal strength and no abnormalities; grade 1, mildly decreased activity and weak grip or cry, more evident at the end of testing; grade 2, clinical signs present before exercise (tremor, head down, hunched posture, weak grip); grade 3, severe clinical signs present before exercise, no grip, moribund; and grade 4, dead. Results are expressed as the mean of the evaluations recorded for each animal at each time point. EAMG in sick animals was confirmed by edrophonium chloride test; repetitive nerve stimulation (RNS) was performed as described previously (10).

AChR content in rat muscle

AChR content in muscles was assayed as described previously (11). Briefly, AChR was solubilized from muscle membranes with Tris-HCl buffer and 2% Triton X-100 overnight at 4°C, and the solutions containing solubilized AChR were clarified by centrifugation at 100,000 × g for 30 min. AChR crude extracts (100 μl, diluted) were incubated with 125I-Aβ-TX for 4 h at room temperature, transferred on DE-81 DEAE disks, and washed with Tris-HCl buffer and 0.5% Triton X-100. Radioactivity was determined by gamma counting. The assay for each sample was performed in duplicate. Values were expressed as picograms per milliliter of culture medium for each cytokine, calculated from a standard curve.

Statistical analysis

The Mann-Whitney U rank test and Student’s t test were used for statistical analysis. The level of significance was p < 0.05.

Results

Characterization of T cell epitopes in Lewis rats

Different groups mapped T cell epitopes on the α subunit of the AChR in EAMG in Lewis rats, and the identified sequences were closely related to the kind of Ag used to induce EAMG (purified TACHR, Torpedo, or human AChR α subunit recombinant proteins). We focused our T cell studies on the sequence corresponding to residues α97–116 of the AChR α subunit, because of two independent reports indicating that sequences α97–112 (6) and (α100–116) (4) were overlapping immunodominant T cell epitopes in TACHR-induced EAMG in Lewis rats. The region α97–116 differs between Torpedo and rat receptor sequence at positions 104 (His→Lys), 105 (Met→Phe), 108 (Leu→Val), and 115 (Lys→His); 16 of 20 residues are identical (80% homology). Therefore, we wondered whether the sequence R97–116, derived from rat AChR, might be recognized by T cells and represent a self T cell epitope in EAMG. Lewis rats were immunized with TACHR and sacrificed 1) 10 days after immunization, or 2) when EAMG was evident (7–8 wk after immunization). Lymph node cell (LNC) reactivity against TACHR, T97–116, and R97–116 peptides was then evaluated by conventional 3H-thymidine incorporation assay (Fig. 1). As expected, T97–116 peptide was recognized at both time points considered, as it results from processing of the priming Ag (TACHR). On the contrary, LNC recognition of R97–116 of the AChR subunit, because of two independent reports indicating that sequences α97–112 and (α100–116) (4) were overlapping immunodominant T cell epitopes in TACHR-induced EAMG in Lewis rats. The region α97–116 differs between Torpedo and rat receptor sequence at positions 104 (His→Lys), 105 (Met→Phe), 108 (Leu→Val), and 115 (Lys→His); 16 of 20 residues are identical (80% homology). Therefore, we wondered whether the sequence R97–116, derived from rat AChR, might be recognized by T cells and represent a self T cell epitope in EAMG. Lewis rats were immunized with TACHR and sacrificed 1) 10 days after immunization, or 2) when EAMG was evident (7–8 wk after immunization). Lymph node cell (LNC) reactivity against TACHR, T97–116, and R97–116 peptides was then evaluated by conventional 3H-thymidine incorporation assay (Fig. 1). As expected, T97–116 peptide was recognized at both time points considered, as it results from processing of the priming Ag (TACHR). On the contrary, LNC recognition of R97–116 (Fig. 1A) was very low and under the threshold value considered to be a positive response (SI, >3); the specific response against this sequence became evident only 8 wk after immunization (chronic EAMG; Fig. 1B). This result might be explained by the cross-recognition of R97–116 by T97–116-specific cells. To address this issue, Lewis rats were immunized with each peptide, and primary LNC responses (day 10) against immunizing peptides and TACHR were measured. The results are shown in Fig. 2. Both animal groups gave strong responses against each priming peptide at both concentrations used in vitro; partial recognition of the non-immunizing peptide (cross-reactivity) was observed in both groups. No responses were observed against TACHR (not reported in Fig. 2).

EAMG induction

EAMG is routinely induced in the Lewis rat by a single immunization of purified AChR from Torpedo electroplax (20 μg) in CFA. The observed cross-reactivity between R97–116 and T97–
116 peptides at the T cell level suggested testing these sequences as a potential autoantigen able to induce the experimental disease in Lewis rats. Preliminary experiments were performed to define the optimal dose of Ag; a single immunization was not sufficient to induce clinical manifestations of EAMG, although T cell responses as well as anti-peptide Abs were detected in immunized rats (data not shown). The immunization protocol we thereafter adopted was as follows: one immunization with peptide (50 μg) in CFA in the hind footpads on day 0, followed by a booster injection of peptide (50 μg) in IFA 1 mo later. Three independent experiments were performed, and results are reported in Table I. Evaluation of EAMG was assessed by body weight and clinical score; the disease was evident 8 wk after TAChR immunization. EAMG was assessed by body weight and clinical score; the disease was evident 8 wk after TAChR immunization. EAMG was evident in R97–116-immunized and R97–116, and TAChR for 3 days. Proliferative responses were studied at 10 days (A) and 8 wk (B) after immunization, when EAMG was evident. The dotted line indicates an SI of 3. Recognition of R97–116 peptide became evident 8 wk after TAChR immunization.

AChR content compared with normal control muscles (16.9 ± 10.6 and 40.5 ± 12.1 pmol/100 g, respectively; p = 0.0007); these values were not different from those in rats immunized with TAChR (12.6 ± 3.9 pmol/100 g; p = 0.46). AChR content in muscles from T97–116-immunized rats (35.1 ± 12.6 pmol/100 g) was not statistically different from the amount of receptor in control muscles (p = 0.47).

Presence of Abs against peptides R97–116, T97–116, and rat (self) AChR in EAMG rats

Blood samples were collected at the beginning of each experiment, before the boost (d = 30), and at the end of experiments to evaluate Ab production. Thirty days after immunization with R97–116 and T97–116 peptides (i.e., blood samples taken before boost), Lewis rats developed anti-peptide Ab; anti-R97–116 Ab were 0.189 ± 0.014, and anti-T97–116 Ab were 0.280 ± 0.019, respectively (ΔOD at 450 nm; mean ± SEM; p = 0.0062; Fig. 5, A and B). These values did not change significantly after boosting with the relevant peptide (ΔOD at 450 nm, 0.177 ± 0.016 and 0.297 ± 0.021, respectively; Fig. 5, A and B). The difference between the two groups was still statistically significant (p = 0.01). We also measured the level of Abs to the nonimmunizing peptides. Anti-T97–116 Ab were found in R97–116-immunized animals, and anti-R97–116 Ab were found in T97–116-immunized rats. Their values did not significantly increase after the boost (Fig. 5, A and B). These results suggest that the presence of anti-peptide Abs was not associated with EAMG onset or progression.

We next measured the level of pathogenic anti-rat AChR Ab by a conventional radioimmunoprecipitation assay (Fig. 5C). Thirty days after immunization (before the boost), the mean anti-rat AChR Ab titers were significantly different among experimental groups: 7.7 ± 1.3 pmol/ml (mean ± SEM) in R97–116-immunized rats vs 3.9 ± 1.8 pmol/ml in T97–116-immunized rats (p = 0.023). At this time, overt signs of EAMG were not evident in any animal, although some rats showed slight fatigability. Anti-rat AChR Ab titers, measured at the end of the experiments, were increased in R97–116-immunized rats (22.5 ± 6.3 pmol/ml) compared with pre-boost levels (7.7 ± 1.3 pmol/ml; p = 0.043) and to levels in the T97–116 group (3.97 ± 0.14; p = 0.014). The low
level of anti-rat AChR Ab in T97–116-immunized rats was not able to impair the neuromuscular transmission, as demonstrated by normal RNS, no clinical signs of EAMG, and normal AChR content. Anti-rat AChR Ab were also measured in the presence of an excess of R97–116 peptide (to saturate anti-peptide Abs present in sera), but we did not observe a statistically significant reduction in anti-rat AChR Ab titers. We did not measure anti-TAChR Ab in peptide-immunized rats because they are not pathogenic; anti-rat and anti-Torpedo AChR Ab titers in TAChR-induced EAMG were 33.5 ± 4.5 and 3630.2 ± 70.5 pmol/ml, respectively.

To determine whether immunization with R97–116 and T97–116 peptides induced different IgG subclasses, we measured IgG subtypes of anti-peptide Abs by ELISA. OD values were similar for IgG1, IgG2a, IgG2b, and IgG2c in the two groups, with a predominance of the IgG2b type (data not shown); this is probably due to the same protocol of immunization and subsequent boost adopted. Anti-rat AChR IgG subclasses were also determined in R97–116-induced EAMG and compared with TAChR-induced EAMG; again, no significant differences were noted (data not shown).

Recruitment of R97–116 and T97–116 peptides by lymph node T cells

Proliferative responses of LNC were assessed when the clinical condition of the sick animals required ending the experiment. Lymph nodes were aseptically removed and cultured in the presence of R97–116 and T97–116 peptides. A strong proliferative response against R97–116 (Fig. 6A) and T97–116 peptide (Fig. 6B), not statistically different from that obtained with Con A (positive control), was observed. LNCs gave proliferative responses when challenged with purified TAChR. Specific recognition of the relevant (immunizing) peptide as well as partial cross-recognition of the nonimmunizing peptide, observed in preliminary experiments reported above (Fig. 2), were again confirmed. Note that recognition of R97–116 in T97–116-immunized rats (Fig. 6B; mean ± SEM, 63,871 ± 18,059 cpm) was significantly different compared with the T97–116 response (122,457 ± 25,639; p = 0.03) and was similar to that obtained in the presence of TAChR (60,462 ± 19,850). In R97–116-immunized rats (Fig. 6A), the specific response to T97–116 (cross-recognition) was lower than that in R97–116-immunized rats, but not statistically different (120,326 ± 27,004 and 166,192 ± 25,679, respectively), whereas the response to T97–116 was significantly different (p = 0.023) compared with that in the presence of TAChR (47,433 ± 14,494).

The different degree of recognition of the nonimmunizing peptide suggests that T cells recognized specific residues not shared by the two Ags; this is probably due to a different Ag processing of the two peptides.

Lymph node T cell response to peptide R97–116 is associated with a different pattern of cytokine expression compared with T97–116

To investigate the mechanisms associated with EAMG induction by R97–116 peptide, we evaluated Th1- and Th2-type cytokine

Table I. Incidence of EAMG

<table>
<thead>
<tr>
<th>Rats Immunized with</th>
<th>Rats 1</th>
<th>Rats 2</th>
<th>Rats 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAMG incidence (no. (%))</td>
<td>3/4 (75.0)</td>
<td>4/6 (66.7)</td>
<td>6/8 (75.0)</td>
</tr>
<tr>
<td>RNS</td>
<td>2/3</td>
<td>4/6</td>
<td>ND</td>
</tr>
<tr>
<td>EAMG incidence (no. (%))</td>
<td>0/4</td>
<td>0/6</td>
<td>0/4</td>
</tr>
<tr>
<td>Mean clinical score</td>
<td>2.5 ± 0.35</td>
<td>0</td>
<td>3 ± 0.26</td>
</tr>
</tbody>
</table>

a One rat died of EAMG before neurophysiological evaluation.
b ND, Not done.
c RNS was not performed in experiment 3.
release in culture supernatants of lymph node T cells stimulated in vitro with the same peptide used for immunization. We considered IFN-γ and IL-2 as markers for the Th1 subpopulation, and IL-6 and IL-10 as markers for the Th2 subpopulation. Data are reported in Table II. We did not observe any significant modification of Th1-type cytokine levels in supernatant from R97–116-stimulated LNCs derived from R97–116-immunized rats compared with T97–116 stimulated LNCs taken from T97–116-immunized rats. On the contrary, a significantly lower production of IL-6 and IL-10 in the R97–116 group was measured (91% reduction for IL-6, \( p = 0.0017 \); 76% reduction for IL-10, \( p = 0.0013 \)). These data suggest that EAMG induction by R97–116 peptide might be associated with an unbalanced production of cytokines toward the Th1 compartment (i.e., the observed reduction in Th2 compartment implies a functional predominance of the Th1 response in R97–116-immunized rats).

**Discussion**

EAMG is routinely induced using xenogenic AChR purified from *Torpedo* electric tissue (13). TACHR-induced EAMG in susceptible strains (such as Lewis rat) is characterized by high incidence, rapid manifestation of the disease, and quick progression. Clinical signs of the disease are due to anti-TACHR Abs cross-reacting with the self (rat) AChR on the neuromuscular junction. The native receptor contains multiple antigenic determinants, both conformational (B cell epitopes) and linear (T cell epitopes), that are highly effective in activating the host immune system. As occurs in the human disease, Ab production in EAMG is thought to be under the control of AChR-specific T cells. The identification of immunodominant AChR sequences relevant for the T or B cell compartments that are effective in inducing EAMG might be of help in understanding the key immunological processes leading to the disease and hence improve our knowledge of the pathogenesis of MG.

In some experimental models, myasthenogenic sequences were identified among B cell epitopes, and these regions were located within the main immunogenic region (\( \alpha_{67–76} \)) (14) or near the \( \alpha \)-BTX binding site-acetylcholine binding site: \( \alpha_{125–147} \) (15) and \( \alpha_{183–200} \) (16). Anti-peptide Abs, able to bind to conformational extracellular domains of the AChR, can directly attack the receptor or interfere with the acetylcholine binding site. Indeed, rats immunized with \( \alpha_{125–147} \) derived from *Torpedo* sequence, either with or without the disulfide link Cys\(^{128}\)-Cys\(^{142}\), produced anti-peptide Abs that bound to the native receptor (17, 18). EAMG in rats has been induced by \( \alpha_{183–200} \) peptide derived from *Torpedo*, but not from human AChR \( \alpha \) subunit sequence (16); these sequences differ in three residues at positions 187, 189, and 191, and the researchers suggested that AChR-blocking Abs were induced. Artificial peptides were synthesized by coupling peptides \( \alpha_{67–76} \) or \( \alpha_{190–195} \) (B cell epitopes) and peptide \( \alpha_{107–116} \) (T cell epitope) with spacing residues (Asn-Pro-Gly-Gly). Lewis rats received multiple injections on days 0, 14, and 28 and were killed on...
day 56. These peptides were highly immunogenic and induced anti-peptide Abs, but rats did not show clinical signs of EAMG (the disease was documented by electrophysiological studies) (19, 20).

Other groups studied the fine antigenic specificity of AChR-reactive T cell lines or clones; different domains were successfully mapped on the α subunit of the receptor, but the identified epitopes were strictly related to the kind of Ag used for EAMG induction. T cells from TACChR-immunized Lewis rats recognized the immunodominant sequences [Tyr100, Gly90, Tyr100(99), Gly90, Tyr100]73–90 (4) and region α97–112 (5, 6) of Torpedo AChR α subunit. T cells primed with these peptides showed significant proliferation when challenged in vitro with either the homologous peptide or intact AChR. When peptides [Tyr100, Gly90, Tyr100(99), Gly90, Tyr100]73–90 were used as immunogen, none of the rats showed clinical signs of EAMG or detectable amounts of Ab to AChR (4).

On the contrary, when Lewis rats were immunized with the recombinant extracellular portion of the human (mammalian) AChR α subunit (α1–210), T lymphocytes preferentially proliferated in response to peptide α125–147 of the human receptor (7). This epitope was further refined and shortened to α129–145 (8), a sequence identical with rat AChR. In this model, Bordetella pertussis was used as supplementary adjuvant to CFA, and rats were repeatedly immunized (two to five times) with this peptide. Clinical signs of EAMG were evident in only three of 23 rats immunized with α129–145, but in none of the rats immunized with α129–145 (8). This immunodominant peptide was also studied by Matsuo et al. (21), but they did not observe clinical signs of the disease in their animals that received only a single injection of peptide in CFA supplemented with B. pertussis.

Our data differ from the studies reported above. A high incidence of the disease was observed when rats were immunized with the self peptide (>70% of rats developed EAMG in the R97–116-immunized groups). EAMG was clearly evident and confirmed by RNS, loss of body weight, and positive EAMG clinical score. The mean EAMG score observed at the end of the experiments was comparable to that usually observed after immunization with TACChR. The model was reproducible, as clinical signs of EAMG were observed in three independent experiments. Moreover, i.p. administration of edrophonium chloride was able to reverse EAMG symptoms in all sick animals. Elevated anti-rat AChR Abs and reduced AChR content in muscles were found only in R97–116-immunized rats. Anti-rat AChR Abs present at low level in T97–116-immunized rats were not able to interfere with the neuromuscular transmission, as demonstrated by normal RNS, no clinical signs of EAMG, and normal muscle AChR content in these animals. It should be considered that immunization of animals started at 8 wk of age compared with 10–14 wk in a previous study (8). The low incidence of clinical EAMG in the model induced by α129–145 can be partially explained by the lower susceptibility to disease induction of older rats (22).

Anti-AChR Abs are characterized by high affinity and specificity for the intact conformation of the Ag; Abs that are directed against extracellular AChR domains are directly pathogenic, whereas those that are specific for unaccessible domains (intracellular or intrachain) are probably not directly involved in degradation of the neuromuscular junction. We believe that anti-peptide Abs induced in our model belong to the second type, because region 97–116 encompasses the β5 and β5’ domains of the AChR α subunit, that seem to be (at least partially) hidden and in close contact with the ε subunit (23).

In mouse models of EAMG, pathogenic anti-AChR Abs belong to the IgG2a subclass and are associated with Th1-type responses. Although the anti-TACChR Ab response is IgG2b dominant in Lewis rats and IgG1 dominant in Brown-Norway rats, both strains are susceptible to EAMG (24) as all IgG subclasses are capable of binding complement and promote degradation of the neuromuscular junction. We wondered whether the IgG subclass composition of anti-peptide Abs might be taken into account for EAMG induction in R97–116-immunized rats, but we did not observe any difference.

Yeh et al. (25) studied the immune reactivity to peptide α100–116 from TACChR α subunit, and their data proved that T cells reactive with this peptide can provide help for the anti-AChR Ab.

Table II. In vitro Th1 and Th2 cytokines production

<table>
<thead>
<tr>
<th></th>
<th>IFN-γ (pg/ml)</th>
<th>IL-2 (pg/ml)</th>
<th>IL-6 (pg/ml)</th>
<th>IL-10 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LNCs from R97-116 immunized animals</td>
<td>2949.1 ± 278.3a</td>
<td>228.5 ± 45.4a</td>
<td>3.1 ± 2.3b</td>
<td>36.4 ± 9.7c</td>
</tr>
<tr>
<td>LNCs from T97-116 immunized animals</td>
<td>3466.3 ± 153.9</td>
<td>147.7 ± 8.4</td>
<td>34.2 ± 6.5</td>
<td>150.8 ± 21.8</td>
</tr>
</tbody>
</table>

a p = NS.
b p = 0.0017.
c p = 0.0013.
response; moreover, they suggested that the level of T cell help (to Ab production) can be separated from T cell proliferation in response to α100–116 variants with proline or threonine at position 106. It must be noted that the sequence they are studied from is TACr, but with Tyr at position 100, whereas published sequences are characterized by Phe\(^1\)\(^{100}\), as were the rat and Torpedo 97–116 peptides we used. We have not performed specific experiments to evaluate whether Tyr\(^1\) or Phe\(^1\) substitution might be relevant for immune sensitization to AChR. Moreover, amino acid residues that differ between rat and Torpedo α97–116 sequences (His\(\leadsto\)Lys, position 104; Met\(\leadsto\)Phe, position 105; Leu\(\leadsto\)Val, position 108; and Lys\(\leadsto\)His, position 115) need to be studied at the single amino acid level, looking for those residues that might be crucial in terms of peptide presentation or recognition by T cells and subsequently for help to B cells. Indeed, peptide α97–116 contains the proposed binding residues for the rat MHC class II molecule RT1.B (26). We cannot exclude that our rat and torpedo peptides (that are four residues longer than the described peptide 101–116) could bind to rat MHC with different anchor residues or frames. Alternatively, processing of our 20-mer peptides might generate shortened sequences with different affinities to class II molecules. Hence, AChR-reactive T cell clones with different patterns of cytokine production or help in Ab production might be generated.

It has been demonstrated that muscle cells are able to present epitopes derived from the AChR to specific CD4\(^+\) T cells. These T cells could, in turn, initiate or maintain the anti-AChR response that is responsible for AChR loss in MG. More interestingly, T cell activation results in IFN-γ secretion and killing of the myoblast (18). Indeed, a reduced Th2 response with a relative unbalance of T helper cells could, in turn, initiate or maintain the anti-AChR response. It has been demonstrated that muscle cells are able to present antigenic epitopes using recombinant proteins; studies on experimental autoimmune myasthenia gravis. Immunol. 71:538.


