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Mechanisms of Nasal Tolerance Induction in Experimental Autoimmune Myasthenia Gravis: Identification of Regulatory Cells

Fu-Dong Shi,‡ Hulin Li,† Huabing Wang,† Xuefeng Bai,‡ Peter H. van der Meide,‡ Hans Link,† and Hans-Gustaf Ljunggren*

Autoantigen administration via nasal mucosal tissue can induce systemic tolerance more effectively than oral administration in a number of experimental autoimmune diseases, including Ab-mediated experimental autoimmune myasthenia gravis, a murine model of myasthenia gravis. The mechanisms underlying nasal tolerance induction are not clear. In this study, we show that nasal administration of acetylcholine receptor (AChR) in C57BL/6 mice, before immunizations with AChR in adjuvant, results in delayed onset and reduced muscle weakness compared with control mice. The delayed onset and reduced muscle weakness were associated with decreased AChR-specific lymphocyte proliferation and decreased levels of anti-AChR Abs of the IgG2a and IgG2b isotypes in serum. The clinical and immunological changes in the AChR-pretreated C57BL/6 wild-type (wt) mice were comparable with those observed in AChR-pretreated CD8<sup>−/−</sup> mice, indicating that CD8<sup>+</sup> T cells were not required for the generation of nasal tolerance. AChR-pretreated wt and CD8<sup>−/−</sup> mice showed augmented TGF-β and reduced IFN-γ responses, whereas levels of IL-4 were unaltered. Splenocytes from AChR-pretreated wt and CD8<sup>−/−</sup> mice, but not from CD4<sup>−/−</sup> mice, suppressed AChR-specific lymphocyte proliferation. This suppression could be blocked by Abs against TGF-β. Thus, our results demonstrate that the suppression induced in the present model is independent of CD8<sup>+</sup> T cells and suggest the involvement of Ag-specific CD4<sup>+</sup> Th3 cells producing TGF-β.


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Abbreviations used in this paper: EAMG, experimental autoimmune myasthenia gravis; AChR, acetylcholine receptor; B6, C57BL/6; wt, wild type; MBP, myelin basic protein; α-BGT, 125I-α-bungarotoxin; p.i., postimmunization; MNC, mononuclear cell; LN, lymph node; MG, myasthenia gravis.
AChR Abs. Myelin basic protein (MBP) used as control Ag was purified from normal mouse brains (19).

Nasal tolerance induction

A modified schedule described for nasal tolerance induction in rats was used (15). Briefly, each mouse was given a total amount of 150 μg of *Torpedo* AChR IgG PBS into each nostril. Control mice received PBS only. The administrations were performed daily for 10 consecutive days before immunization.

Induction and clinical evaluation of EAMG

Mice were immunized s.c. with 40 μg of AChR in CFA in a total volume of 100 μl and boosted twice on days 25 and 55 after primary immunization with 40 μg of AChR in CFA s.c. The mice were scored every other day after the second immunization for signs of muscle weakness that were characteristic of EAMG. The disease symptoms were graded between 0 and 3 (20): 0, no definite muscle weakness; 1, normal strength at rest but weak with chin on the floor and inability to raise the head after exercise consisting of 20 consecutive paw grips; 2, as grade 1 and weakness at rest; and 3, moribund, dehydrated, and paralyzed. Clinical EAMG was confirmed by an injection of neostigmine bromide and atropine sulfate (20).

RIA for muscle AChR content

Aliquots (2 μl) of 125I-α bungarotoxin (α-BGT) (Amersham, Arlington Heights, IL)-labeled, Triton X-100-solubilized mouse muscle extract were mixed with standard pooled mouse anti-AChR antiserum in triplicate. After incubation, rabbit anti-mouse Ig (Dakopatts, Copenhagen, Denmark) was added. The precipitates were counted in a Packard gamma-counter (Meriden, CT). The percentage of loss of muscle AChR in test mouse muscle was calculated as described previously (21).

Measurements of serum anti-AChR Ab concentration, affinity, and IgG isotype

The serum Ab levels were measured by RIA (18). Briefly, 1 nM of muscle AChR was incubated with 2 nM α-BGT (Amersham). A total of 0.1 μl of serum was added to 1 ml of labeled muscle AChR, followed by rabbit anti-mouse Ig (Dakopatts). The samples were centrifuged, washed, and counted in a gamma-counter. The AChR precipitated minus the background value permitted calculation of the titer in moles of toxin-binding sites bound per liter of serum. After predetermination and adjustment of the anti-AChR IgG Ab levels, the relative affinity of anti-AChR IgG Abs in serum was determined by ELISA using thiocyanate (Sigma) elution (22). Isotypes of anti-AChR IgG Abs were detected using rabbit anti-mouse Ab (Innogenetics, Zwijnaarde, Belgium) at 15 μg/ml. MNCs were cultured as described above. Wells were incubated with or without 4 ng/ml mouse rIL-2 (PharMingen). After 48 h of culture, secreted and bound IFN-γ were visualized by a sequential application of biotinylated detector Ab IFN-γ (Innogenetics) and avidin-biotin complex (Dakopatts). After peroxidase staining, the red-brown immunospots corresponding to the cells that had secreted IFN-γ were enumerated in a dissection microscope.

Statistical analysis

Differences between groups were evaluated by ANOVA. Differences between the groups with respect to disease incidence were analyzed by Fisher’s exact test.

Results

Nasal AChR administration prevents development of clinical EAMG and reduces muscle AChR loss in wt and CD8−/− mice

To establish a protocol for nasal tolerization with AChR in B6 mice, different dosages of AChR were administered nasally to mice that were subsequently immunized s.c. with AChR in CFA and scored for signs of myasthenia gravis (MG). Nasal administration of a total amount of 150, 300, or 600 μg of AChR per mouse divided in 10 consecutive daily administrations was equally effective in preventing the development of EAMG (data not shown). A total of 150 μg of AChR per mouse was adopted as a standard dose and used throughout the present study.

To study the role of CD8+ T cells in the generation of nasal tolerance, wt and CD8−/− mice were administered AChR nasally and subsequently immunized with AChR in CFA three times. The mice were monitored for the muscle weakness characteristic of EAMG. Of the wt mice, 26 of 31 developed muscle weakness, whereas only 6 of 34 wt mice receiving AChR nasally before immunization with AChR developed muscle weakness (p < 0.01). The onset of disease was delayed in the group of wt mice receiving AChR nasally (Table 1). The disease incidence was relatively lower in CD8−/− mice than in wt mice. In total, 18 of 35 AChR-immunized CD8−/− mice developed muscle weakness. In contrast, only 7 of 34 CD8−/− mice receiving AChR nasally before immunization with AChR developed muscle weakness (p < 0.01). The onset of disease was also delayed in the group of CD8−/− mice receiving AChR nasally (Table 1). The mean values of muscle AChR loss in control and AChR-treated wt mice were 68.8 ± 15.5% and 21.8 ± 3.2%, respectively (p < 0.05). The mean values of muscle AChR loss in the control and AChR-treated CD8−/− mice were 42.4 ± 7.2% and 25.5 ± 6.8%, respectively (p < 0.05). Thus, nasal tolerance to AChR can still be effectively induced in the absence of CD8+ T cells.

Nasal AChR administration alters anti-AChR IgG Ab isotype repertoire and affinity

The anti-AChR Abs in MG and EAMG consist mainly of IgG Abs of all subtypes (27, 28). These Abs are responsible for the functional loss of AChR in the neuromuscular junctions (27). Anti-AChR Ab levels were not significantly different in wt and CD8−/−
mice, irrespective of nasal AChR administration before immunization (Fig. 1). However, the affinity of the anti-AChR IgG Abs was lower in both wt and CD8\(^{-/-}\) mice that had received nasal administrations of AChR (Fig. 1). In particular, this was the case for the IgG2a and IgG2b isotypes, whereas affinity levels of IgG1 isotypes were largely unaltered (Fig. 2).

Nasal AChR administration suppresses AChR-reactive lymphocyte proliferation

Proliferative responses to AChR were suppressed in wt as well as in CD8\(^{-/-}\) mice receiving AChR nasally (Fig. 3). The suppression was Ag-specific, because T cells from AChR-treated wt and CD8\(^{-/-}\) mice as well as control wt and CD8\(^{-/-}\) mice proliferated at similar levels in response to the control Ag MBP and to Con A (Fig. 3).

Nasal AChR administration alters cytokine responses

Upon activation, Th cells differentiate into Th1, Th2, and Th3 functional subgroups that are characterized by their ability to produce IFN-\(\gamma\), IL-4, and TGF-\(\beta\), respectively (29). The production of anti-AChR Abs in EAMG and MG is regulated by these cytokines (27). We have shown that the suppression of EAMG in Lewis rats by nasal administration of AChR correlates with decreased numbers of IFN-\(\gamma\) and increased numbers of TGF-\(\beta\) mRNA-expressing cells (30, 31). Thus, the altered anti-AChR IgG Ab repertoire and affinity in the tolerized mice should theoretically be determined by the altered cytokine profile in these mice. To demonstrate this, we determined IFN-\(\gamma\), IL-4, and TGF-\(\beta\) production in the culture

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**Table I. Effects of nasal administration of AChR on the development of EAMG in wt and CD8\(^{-/-}\) mice**

<table>
<thead>
<tr>
<th>Mice Tolerization Regimen*</th>
<th>No. of Mice per Group</th>
<th>Disease Incidence</th>
<th>Median Day of Onset</th>
<th>Mean Maximal Severity of EAMG (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt PBS</td>
<td>31</td>
<td>26/31 (84%)</td>
<td>37</td>
<td>1.67 ± 0.82</td>
</tr>
<tr>
<td>wt AChR</td>
<td>34</td>
<td>6/34 (18%)</td>
<td>54</td>
<td>0.35 ± 0.32</td>
</tr>
<tr>
<td>wt AChR, anti-TGF-(\beta)</td>
<td>6</td>
<td>4/6 (66%)</td>
<td>32</td>
<td>1.88 ± 0.75</td>
</tr>
<tr>
<td>wt AChR, control Ab</td>
<td>4</td>
<td>1/4 (25%)</td>
<td>50</td>
<td>0.28 ± 0.11</td>
</tr>
<tr>
<td>CD8(^{-/-}) PBS</td>
<td>35</td>
<td>18/35 (51%)</td>
<td>40</td>
<td>0.98 ± 0.63</td>
</tr>
<tr>
<td>CD8(^{-/-}) AChR</td>
<td>34</td>
<td>7/34 (21%)</td>
<td>57</td>
<td>0.27 ± 0.20</td>
</tr>
<tr>
<td>CD8(^{-/-}) AChR, anti-TGF-(\beta)</td>
<td>6</td>
<td>3/6 (50%)</td>
<td>34</td>
<td>1.55 ± 0.58</td>
</tr>
<tr>
<td>CD8(^{-/-}) AChR, control Ab</td>
<td>4</td>
<td>1/4 (25)</td>
<td>51</td>
<td>1.77 ± 0.80</td>
</tr>
</tbody>
</table>

* Mice were treated with AChR or PBS nasally and subsequently immunized with AChR in CFA. In some experiments, the mice receiving AChR nasally were given 1 mg of anti-TGF-\(\beta\) or isotype control Ab at the time of immunization followed by 500 \(\mu\)g of mAbs weekly until the termination of experiments.

**FIGURE 1.** Anti-AChR IgG Ab responses on day 90 p.i. Serum anti-AChR Ab concentrations were measured by RIA and expressed as moles of \(\alpha\)-BGT binding sites per liter of serum (upper panel). The anti-AChR IgG Ab affinity index was measured by potassium thiocyanate ELISA (lower panel). Results are expressed as mean values ± SD. The samples tested in this figure are from the mice indicated in Table I. For mice receiving PBS or AChR, \(n = 10\). For mice receiving AChR in conjunction with treatment with anti-TGF-\(\beta\) or control mAb, \(n = 4\). * \(p < 0.05\); # \(p < 0.01\). Comparisons were between tolerized and nontolerized PBS-treated mice, respectively.

**FIGURE 2.** Anti-AChR IgG Ab isotypes measured by ELISA. The serum samples used were the same as those used in Fig. 1. No differences in levels of anti-AChR IgG Ab isotypes in tolerized mice and in tolerized mice treated with isotype control Ab were observed (data not shown). Results are expressed as mean values ± SD. * \(p < 0.05\); # \(p < 0.01\). Comparisons were made between tolerized and nontolerized mice, respectively.
were unaltered in the tolerized mice. In contrast, wt and CD8−/− mice treated with anti-TGF-β but not isotype control Ab had similar anti-AChR IgG Ab affinity (Fig. 1) as well as similar levels anti-AChR IgG2a and IgG2b isotypes (Fig. 2). Similarly, the suppression of AChR-specific proliferation and IFN-γ production were nearly completely reversed by anti-TGF-β Ab treatment (Table II, Fig. 3).

Suppression of IFN-γ-secreting cells in wt as well as in CD8−/− mice receiving AChR cannot be reversed by addition of IL-2

Nasal administration of AChR suppressed Th1 cytokine IFN-γ production. To further determine whether this T cell subset was selectively anergized, we enumerated the IFN-γ-secreting cells among MNCs in the presence and absence of IL-2 in the cultures. Consistent with cytokine ELISA data, the numbers of AChR-reactive IFN-γ-secreting cells were lower in wt and CD8−/− mice that had received AChR nasally (Fig. 4). IL-2 preincubation increased the numbers of IFN-γ-secreting cells in control mice; however, it did not increase the numbers of IFN-γ-secreting cells in the tolerized mice. Thus, the present findings do not support induction of anergy as a possible explanation for the observed results, although this possibility cannot be formally excluded in our system.

Table II. Altered AChR-specific cytokine profile in AChR-pretreated wt and CD8−/− mice, and effects of neutralizing TGF-β

<table>
<thead>
<tr>
<th>Mice</th>
<th>IFN-γ</th>
<th>IL-4</th>
<th>TGF-β1</th>
<th>IFN-γ</th>
<th>IL-4</th>
<th>TGF-β1</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt, PBS</td>
<td>2200 ± 439</td>
<td>208 ± 47</td>
<td>870 ± 225</td>
<td>1872 ± 422</td>
<td>187 ± 33</td>
<td>852 ± 188</td>
</tr>
<tr>
<td>wt, AChR</td>
<td>870 ± 225</td>
<td>193 ± 24</td>
<td>2011 ± 421</td>
<td>973 ± 255</td>
<td>177 ± 55</td>
<td>1540 ± 328</td>
</tr>
<tr>
<td>wt, AChR + anti-TGF-β</td>
<td>2330 ± 425</td>
<td>184 ± 67</td>
<td>45 ± 22</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD8−/−, PBS</td>
<td>1689 ± 326</td>
<td>200 ± 38</td>
<td>800 ± 189</td>
<td>1438 ± 400</td>
<td>168 ± 22</td>
<td>851 ± 212</td>
</tr>
<tr>
<td>CD8−/−, AChR</td>
<td>700 ± 208</td>
<td>202 ± 25</td>
<td>1988 ± 442</td>
<td>633 ± 212</td>
<td>170 ± 42</td>
<td>1424 ± 266</td>
</tr>
<tr>
<td>CD8−/−, AChR + anti-TGF-β</td>
<td>1427 ± 334</td>
<td>211 ± 47</td>
<td>0.00 ± 0.00</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Mice were first treated with AChR or PBS nasally and subsequently immunized with AChR in CFA. In some experiments, the mice receiving AChR nasally were given 1 mg of anti-TGF-β or isotype control Ab at the time of immunization followed by 500 μg of mAbs weekly until the termination of experiments. Mice were killed at 14 days p.i., and draining LN cells were isolated. The LN cells were cultured with or without AChR (10 μg/ml). The culture supernatants were collected after 48 h. Cytokine production in tolerized mice was not affected by isotype control Ab (data not shown). Spontaneous cytokine release: IFN-γ, 326 ± 77; IL-4, undetectable; and TGF-β, 274 ± 89. Results represent one of two independent experiments and are expressed as mean values ± SD. There were four mice in each group.

**ND** not done.
Table III.  

<table>
<thead>
<tr>
<th>Mice</th>
<th>Protocol*</th>
<th>Anti-TGF-β in vivo</th>
<th>Control mAb in vivo</th>
<th>Anti-TGF-β in vitro</th>
<th>Control mAb in vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>Control</td>
<td>8780 ± 693</td>
<td>8500 ± 343</td>
<td>8800 ± 443</td>
<td>8933 ± 222</td>
</tr>
<tr>
<td>wt</td>
<td>PBS</td>
<td>9800 ± 777</td>
<td>10845 ± 444</td>
<td>7900 ± 354</td>
<td>9932 ± 870</td>
</tr>
<tr>
<td>wt</td>
<td>AChR</td>
<td>3360 ± 534</td>
<td>9932 ± 683</td>
<td>3932 ± 423</td>
<td>7560 ± 455</td>
</tr>
<tr>
<td>CD4(^{-/-})</td>
<td>AChR</td>
<td>8500 ± 432</td>
<td>9325 ± 630</td>
<td>8325 ± 730</td>
<td>7902 ± 258</td>
</tr>
<tr>
<td>CD8(^{-/-})</td>
<td>AChR</td>
<td>4508 ± 674</td>
<td>8024 ± 538</td>
<td>3424 ± 638</td>
<td>8056 ± 408</td>
</tr>
</tbody>
</table>

\* Unfractionated 10\(^6\) splenic MNCs (putative suppressor cells) from mice that had been receiving AChR or PBS nasally prior to immunization with AChR in CFA were prepared at 14 days p.i. Splenic MNCs from naive mice were included and are indicated as “control.” A fraction of the mice were injected i.p. with 1 mg of anti-TGF-β or isotype control Ab at the time of immunization followed by 500 μg of mAbs weekly until the termination of experiments. Responder cells (4 × 10\(^5\)) were prepared from the LNs of CD4\(^{+}\) and CD8\(^{+}\) mice at 14 days p.i. The ratio of suppressor to responder cells in cocultures was 2.5:1. The cultures were stimulated with AChR at a concentration of 10 μg/ml. After 3 days of incubation, the cells were pulsed for 18 h with 10-μl aliquots containing 1 μCi[\(^{3}H\)]thymidine (Amersham). Proliferation response was determined by thymidine incorporation. For in vitro blockade, 4 ng/ml anti-mouse TGF-β or isotype control Abs were added to the coculture. The data were expressed as mean (four mice in each group) ± SD. The range of background proliferation or proliferation in response to control Ag MBP was 3200–4111 cpm.

Discussion

In the present study, we show that nasal administration of AChR prevents the development of EAMG in B6 mice. The suppression of disease, AChR-specific T cell responses, and alteration of the anti-AChR Ab isotypes in the AChR-treated B6 wt mice were comparable with those observed in AChR-treated CD8\(^{-/-}\) mice, indicating that CD8\(^{+}\) T cells are not required for the generation of nasal tolerance. AChR-treated wt and CD8\(^{-/-}\) mice showed augmented TGF-β and reduced IFN-γ responses, whereas levels of IL-4 were unaltered. Splenocytes from wt as well as from CD8\(^{-/-}\) mice, but not from CD4\(^{-/-}\) mice, suppressed AChR-primed lymphocyte proliferation. This suppression could be blocked by Abs against TGF-β. Thus, the present results extend our previous observations in rats (15–17) and suggest that active suppression by the sensitization of CD4\(^{+}\) Th3 cells producing TGF-β plays a major role in the generation of nasal tolerance.

Nasal tolerance induction is sometimes associated with immune deviation from a Th1 to a Th2 phenotype in T cell-mediated autoimmune diseases (12) and is associated with AChR peptides in at least one study of nasal tolerance induction against AChR (32). However, in the present study, Th2 cytokine IL-4 responses were neither enhanced nor suppressed by the nasal administration of AChR, suggesting that the suppression of Th1 cytokines is a result of up-regulation of Th3 cytokines rather than Th2 cytokines in this system. Previous studies on the tolerance induction by nasal administration of AChR in the rat model of EAMG have indicated that the suppression of disease development was likely due to TGF-β-secreting cells (30, 31). The present study provides evidence in support of active suppression by the sensitization of Th3 cells producing TGF-β in nasal tolerance induction. TGF-β production was augmented, and the effects of in vitro suppression of AChR-primed lymphocyte proliferation could be blocked by anti-TGF-β Abs.

The role of CD8\(^{+}\) T cells in the pathogenesis of EAMG has recently been investigated. Shenoy et al. (33) reported that β₂-microglobulin\(^{-/-}\) mice with deficient MHC class I expression and a reduced number of CD8\(^{+}\) cells showed a more severe EAMG than corresponding wt mice. In contrast, Zhang et al. (26, 34) reported that the depletion of CD8\(^{+}\) T cells by either Abs or gene targeting reduces the severity of EAMG in Lewis rats and in B6 mice. Differences in in vivo systems and in the antigenic properties of AChR preparations may account for the discrepancies observed in these studies. The present results indicate that disease development was relatively mild in CD8\(^{-/-}\) mice compared with wt mice. In part, this could be explained by the ability of CD8\(^{+}\) T cells to help autoreactive B cells by secreting an array of cytokines and by expressing the CD40 ligand (35). However, it is unlikely that CD8\(^{+}\) T cells function as effector cells in EAMG pathogenesis. B cell-deficient mice have normal CD8\(^{+}\) T cell cytotoxic functions but remain completely free from EAMG because no anti-AChR Ab is produced (36, 37). The numbers of infiltrating CD8\(^{+}\) T cells in the neuromuscular junctions are very sparse in patients with MG as well as in animals with EAMG (37, 38).

There is much controversy regarding whether CD8\(^{+}\) T cells actively participate in the induction of oral tolerance. CD8\(^{+}\) T cells were identified as “the suppressor cells” in early studies (39, 40). Recent studies have shown that CD8\(^{+}\) T cells alone are not sufficient to mediate the active suppression in oral tolerance induced in the T cell-mediated experimental autoimmune encephalomyelitis and experimental autoimmune uveitis (9, 41). The mechanisms of nasal tolerance induction have been suggested to be analogous to those of oral tolerance (42). However, there are a number of

FIGURE 4. Numbers of IFN-γ-secreting cells and effects of preincubation with IL-2. Mice were first treated with AChR or PBS nasally and subsequently immunized with AChR in CFA. Mice were killed at 14 days p.i., and LN MNCs were harvested and cultured in the presence or absence of AChR (10 μg/ml). rIL-2 (4 ng/ml) was applied to every second well in parallel. * p < 0.05. Comparisons were made between tol­erized and non-tolerized mice, respectively. Results represent one of two independent experiments and are expressed as mean values ± SD. There were four mice in each group. The numbers of IFN-γ-secreting cells in response to control Ag MBP or no Ag at all were <3.
structural differences between the upper respiratory tract and gastrointestinal mucosa. For example, the ratio of CD4+ vs CD8+ cells, cytokine milieu, and Ag presentation and costimulation requirements differ (43, 44). These differences suggest that the mechanisms operating in the generation of peripheral tolerance at these two different mucosal surfaces might differ.

Our study has shown that nasal tolerance can be readily established in CD8−/− mice. Because both CD4+ and CD8+ T cells contribute to the production of TGF-β (24, 29), the establishment of tolerance against EAMG in CD8−/− mice suggested that nasal administration of AChR before immunization regulates the CD4+ Th3 subset, to compensate for the absence of CD8+ T cells, and mediates nasal tolerance to AChR. Therefore, the cellular requirements for CD4+ and CD8+ T cells in the generation of EAMG and the generation of nasal tolerance are distinctly different.

Under certain circumstances, when the suppression of autoggressive T cells does not always parallel the suppression of autoreactive B cells, sensitization of Th2 cells and augmented Ab production can occur (45, 46), which may be detrimental. This could be one of the confronting problems in applying mucosal tolerance induction in the treatment of MG. In the present study, in accordance with the differential regulation of Th subsets, IgG2a and IgG2b Abs and affinity were selectively reduced, whereas the total anti-AChR Ab levels were similarly high in the tolerized mice compared with the control mice. Thus, nasal tolerance does not appear to significantly alter the production of anti-AChR Abs, but rather changes their isotype repertoire. At present, the mechanisms underlying this consistent observation are not clear (15, 23). Nasal tolerance induction in EAMG may be associated with the suppression of certain pathogenic Ab subtypes of high affinity to AChR. IFN-γ-dependent anti-AChR IgG2a and IgG2b subtypes were suggested to be pathogenic in B6 mice in several recent studies (47–50). In contrast, IL-4 is not required for the development of EAMG in B6 mice (51). Thus, the suppression of IFN-γ responses and of IgG2a and IgG2b and other productions of pathogenic Ab subtypes may, in part, be responsible for the tolerance induction achieved by nasal administration of AChR in the EAMG model.

Although the current therapy of MG with immunosuppressive drugs is reasonably effective, such treatment must be continued indefinitely and may result in global suppression of the immune system, with increased risks of infection and neoplasia (25). A clinical trial of Ag-specific therapy for MG has not been initiated, but is currently the subject of intensive investigations. The present study provides insight into the mechanisms of nasal tolerance induction and should facilitate the design of an ideal treatment of MG.

Acknowledgments

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