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Resistance to Experimental Autoimmune Myasthenia Gravis in IL-6-Deficient Mice Is Associated with Reduced Germinal Center Formation and C3 Production

Caishu Deng, Elzbieta Goluszko, Erdem Tüzün, Huan Yang, and Premkumar Christodoss

To provide direct genetic evidence for a role of IL-6 in experimental autoimmune myasthenia gravis (EAMG), we investigated IL-6 gene KO (IL-6−/−) mice in the C57BL/6 background which were immunized with Torpedo californica acetylcholine receptor (AChR) and evaluated for EAMG. Only 25% of AChR-immunized IL-6−/− mice developed clinical EAMG compared to 83% of C57BL/6 (wild-type) mice. A significant reduction in the secondary anti-AChR Ab of IgG, IgG2b, and IgG2c, but not the primary or secondary IgM response was observed in AChR-immunized IL-6−/− mice, suggesting a possible defect in T cell help and class switching to anti-AChR IgG1, isotype. The AChR-specific lymphocyte proliferative response, IFN-γ, and IL-10 production were suppressed in AChR-immunized IL-6−/− mice. EAMG resistance in IL-6−/− mice was associated with a significant reduction in germinal center formation and decreased serum complement C3 levels. The data provide the first direct genetic evidence for a key role of IL-6 in the autoimmune response to AChR and in EAMG pathogenesis.

We hypothesized that IL-6 also plays a key role in EAMG development because it promotes growth of hematopoietic stem cells, modulates differentiation of activated B cells into plasma cells, facilitates T cell-dependent Ab responses, costimulates T cell differentiation, promotes inflammation, and up-regulates MHC class II expression (12–21). More importantly, cultured MG thymic epithelial cells express high levels of IL-6 mRNA and overproduce IL-6. These cells potentially could be involved in thymic hyperplasia and thymic germinal center (GC) formation in MG patients (22). In addition, AChR-reactive blood mononuclear cells of MG patients express elevated mRNA for IL-6, IL-10, and IL-12 (23). To demonstrate direct genetic evidence for a critical role of IL-6 in EAMG pathogenesis, normally developed IL-6−/− and WT mice were immunized with AChR in CFA. They were then examined for cellular and humoral immune responses to AChR and development of clinical EAMG. The findings reported here provided the first direct genetic evidence for a key role of IL-6 in the autoimmune response to AChR, especially in the production of the anti-AChR IgG1 isotype, GC, and complement C3 and thus contributed to the pathogenesis of Ab-mediated EAMG.

Materials and Methods

AChR and mice

AChR was purified from the electric organ of *Torpedo californica* by α-neurotoxin affinity column (24, 25). *Torpedo AChR* α-chain peptide (α146–162) (7) was synthesized in the protein core laboratory at the University of Texas Medical Branch (Galveston, TX). Seven- to 8-wk-old B6 and IL-6−/− mice in the B6 background were purchased from The Jackson Laboratory (Bar Harbor, ME). All animals were housed in the viral Ab-free barrier facility at the University of Texas Medical Branch and maintained according to the Animal Care and Use Committee Guidelines.

Induction and clinical evaluation of EAMG

For in vivo studies, all mice were anesthetized and immunized with 20 μg AChR emulsified in CFA (Difco, Detroit, MI) s.c. at four sites (two hind footpads and shoulders) on day 0. All of the mice were boosted with 20 μg AChR in CFA s.c. at four sites on the back on days 30 and 60. Mice were screened for clinical EAMG on a daily basis. Clinical muscle weakness was graded (clinical scores) as follows: grade 0, mouse with normal muscle strength; grade 1, normal at rest, with muscle weakness characteristically shown by a hunchback posture, and difficulty to raise the head after exer-
cise, consisting of 20–30 paw grips on cage top grid; grade 2, mouse showed grade 1 symptoms without exercise; grade 3, dehydrated and moribund with grade 2 weakness; and grade 4, dead. Clinical EAMG was also confirmed by i.p. administration of 50 μl neostigmine bromide (0.015 mg/ml), along with atropine sulfate (0.006 mg/ml) in PBS, and observing improvement in muscle strength.

**Immunopathological evaluation of EAMG**

The primary pathology of MG and EAMG in mice is the loss of muscle AChR due to Ab- and complement-mediated attack (26–28). The total concentration of AChR per mouse was determined according to previously published methods and expressed as picomoles of 125I-labeled α-bungarotoxin binding sites (25, 27). The serum anti-mouse AChR Ab was measured by α-bungarotoxin (Amersham, Arlington Heights, IL) RIA (24, 25) and the anti-mouse AChR IgM and IgG isotypes were measured by ELISA (6, 25).

**Lymphocyte proliferative and cytokine assays**

Inguinal and axillary lymph node cells (LNC) were collected at termination of the experiments (day 7 or 90) and 4 × 10^5 cells in 200 μl were exposed in vitro in triplicate wells of 96-well plates to AChR (250 μg/ml) and α146–162 peptide (40 μg/ml) and incubated for 5 days, and lymphocyte proliferative response was measured (7, 25). Culture supernatants were measured for IFN-γ, IL-4, IL-6, and IL-10 by ELISA and IL-2 by cytotoxic T lymphocyte line assay (7, 25).

**Flow cytometry**

Single-cell suspensions of LNC were incubated for 30 min with one of the following Abs: PE-conjugated B7-1, B7-2, and CD40 (BD Pharmingen, San Diego, CA) and FITC-conjugated F(ab′)2, B220, CD4, and CD3 (Caltag Laboratories, Burlingame, CA) anti-mouse mAbs. PE- or FITC-conjugated isotypes were used for controls. Cells were washed twice and then were fixed with 2% paraformaldehyde and analyzed by FACStation flow cytometry (BD Biosciences, San Jose, CA).

**Immunohistochemical staining for GC**

Four-micrometer-thick sections of 10% Formalin-fixed and paraffin-embedded spleens were prepared. Sections were deparaffinized and rehydrated. Endogenous peroxidase activity was quenched with 3% H2O2 in methanol. Sections were blocked for nonspecific binding with normal goat serum diluted 1/20 in DAKO Ab diluent for 15 min. Ag retrieval was done with DAKO Target Retrieval Solution in steam for 20 min. Sections were incubated at room temperature for 30 min. Diluted (1/3 in PBS-0.05% Tween 20) overnight at 4°C. The plates were then blocked with 2% BSA in PBS

**Measurement of serum C3 levels by ELISA**

Ninety-six-well microtiter plates (Dynatech Immulon 2; Dynatech Laboratories, Chantilly, VA) were covered with goat Abs to mouse C3 (ICN Biomedicals/Cappel, Aurora, OH) in 0.1 M sodium carbonate buffer (pH 8.2) overnight at 4°C. The plates were then blocked with 2% BSA in PBS at room temperature for 30 min. Diluted (1/3 in PBS-0.05% Tween 20) serum samples (30 μl) were added and incubated at 37°C for 90 min. After four washes, HRP-conjugated goat anti-mouse C3 complement (ICN Bio- medicals/Cappel), diluted 1/500 in PBS/0.05% Tween 20, was added and incubated at 37°C for 90 min. Subsequently, ABTS substrate solution in 0.1 M citric buffer (pH 4.3) in the presence of H2O2 was added and color was allowed to develop at room temperature in the dark. Plates were read at a wavelength of 405 nm using a Dynatech ELISA reader and the results were expressed as OD values.

**Results**

**AChR-immunized IL-6**−/− mice are resistant to EAMG

IL-6−/− and WT mice were immunized with AChR in CFA on days 0, 30, and 60. In the first experiment, 5 of 12 B6 mice developed typical clinical EAMG after the first boost with AChR and another 5 B6 mice developed clinical EAMG after the second boost with AChR. One of the mice with severe disease died. Overall, 10 (83%) of 12 WT mice developed clinical EAMG in contrast to only 1 of 12 IL-6−/− mice which developed grade 1 and a short course of clinical EAMG after the first boost with AChR. Another 2 of 12 IL-6−/− mice developed grade 1 EAMG after the second boost with AChR. Overall, 3 (25%) of 12 IL-6−/− mice developed clinical EAMG. In the second experiment, the incidence of EAMG in WT and IL-6−/− mice was similar to that in the first experiment, except none of the IL-6 KO mice developed clinical EAMG after the first AChR boost. The kinetics of the incidence and mean severity of clinical EAMG are illustrated in Fig. 1, A and B, for experiment 1 and D and E for experiment 2. In both of the experiments, IL-6−/− mice had a delayed onset, lower total incidence (p = 0.0058), and less severe clinical EAMG (p < 0.05) compared to WT mice. The data demonstrated the first direct genetic evidence for the involvement of IL-6 in the development of clinical EAMG after immunization with AChR.

The primary pathology in MG and EAMG is a significant reduction of muscle AChR due to Ab- and complement-mediated attack of the NMJ (26–28). The number of α-bungarotoxin binding sites in the muscles, which reflect the amount of functionally available muscle AChR, were measured in AChR-immunized WT and IL-6−/− mice. The functional AChR in AChR-immunized IL-6−/− mice was significantly higher (p < 0.05) than that of WT mice (Fig. 1, C, Expt. 1, and F, Expt. 2). Therefore, the lower incidence of EAMG in IL-6−/− mice correlated with higher available functional muscle AChR. Although mice with disease have significantly more AChR loss compared to mice without disease (27), the muscle AChR levels do not reveal a significant correlation with severity of disease. This could be due to the differential neuromuscular transmission safety margin in each mouse.

**Predominant suppression of secondary anti-AChR IgG2 in AChR-immunized IL-6−/− mice**

Sera from individual mice were collected at various time points after the first immunization with AChR. The anti-AChR IgG Ab response was measured first by RIA. In both of the experiments, compared to AChR-immunized WT mice, AChR-immunized IL-6−/− mice had reduced anti-mouse AChR IgG at most of the time points tested (Fig. 2). ELISA was adopted to measure serum anti-AChR IgM and IgG isotypes. After the third AChR immunization (day 74), when a significant number of WT mice developed clinical EAMG, the anti-AChR Abs belonging to the IgG, IgG2α, and IgG2a isotypes, but not IgM isotype, were significantly reduced in AChR-immunized IL-6−/− mice compared to AChR-immunized WT mice in both experiments (Fig. 3). After the primary AChR immunization or after boosting with AChR, no significant suppression of anti-AChR IgM Abs was observed in IL-6−/− mice compared to WT mice (data not shown). Therefore, IL-6 deficiency could have led to defective T cell help and/or defective class switching of anti-AChR IgM to IgG2 isotype, thus contributing to the resistance of clinical EAMG. Our data imply that IL-6 is critical for producing the secondary anti-AChR Ab response.
AChR-immunized IL-6−/− mice have reduced cellular immune responses to AChR and its dominant peptide α146–162

To test the lymphocyte response to AChR and its peptides, LNC pressed in IL-6−/− mice were stimulated in vitro with AChR and α146–162 peptide. Proliferation of AChR and α146–162 peptide-specific lymphocyte and production of IFN-γ, IL-10, and IL-4 were measured. At termination of the short-term (day 7) and long-term experiments (day 90), IL-6−/− mice demonstrated a reduced proliferative response to AChR and α146–162 peptide compared to the proliferative response of cells from WT mice (Fig. 4, A and D). AChR and α146–162 peptide-specific IFN-γ and IL-10 production were also significantly suppressed in IL-6−/− mice compared to WT mice in the day 7 assay. In all cultures, IL-4 was undetectable in ELISA. These data suggest that the primary and established lymphocyte responses to AChR and its peptide were suppressed in IL-6−/− mice. Furthermore, the data implicate IL-6 as having an in vivo regulatory role in AChR and α146–162 peptide-specific IFN-γ and IL-10 production, especially in the established (long-term) immune response, since these cytokines were suppressed in AChR-immunized IL-6−/− mice.

We also measured IL-6 in the supernatant of AChR and α146–162 peptide-challenged AChR immune lymphocytes (long term). AChR and α146–162 peptide-specific WT lymphocytes produced response to AChR and its dominant peptide α146–162 peptide-specimen binding sites in AChR-immunized B6 and IL-6 KO mice (C and F). EAMG clinical incidence was significantly lower (p < 0.05) in IL-6 KO mice when compared to that in B6 mice using Fisher’s exact test from days 21 to 64 (first experiment) and days 10 to 54 (second experiment) after the second AChR immunization. EAMG mean clinical severity (score) in IL-6−/− mice was significantly lower (p < 0.05) in IL-6 KO mice. The error bars are SE. In both experiments 1 and 2, AChR-immunized B6 mice had significantly reduced levels of functional muscle AChR compared to IL-6 KO mice (p < 0.05 by Student’s t test).

AChR-immunized IL-6−/− mice are resistant to clinical EAMG. Mice were immunized with AChR in CFA on days 0, 30, and 60. Kinetics of the accumulated clinical incidence of EAMG (A and D), severity (B and E), and muscle AChR content expressed as bungarotoxin binding sites in AChR-immunized B6 and IL-6 KO mice (C and F). EAMG clinical incidence was significantly lower (p < 0.05) in IL-6 KO mice when compared to that in B6 mice using Fisher’s exact test from days 21 to 64 (first experiment) and days 10 to 54 (second experiment) after the second AChR immunization. EAMG mean clinical severity (score) in the first experiment was significantly lower (p < 0.05) in experiment 1 using Student’s t test from days 21 to 64, after the second AChR immunization in IL-6 KO mice when compared to B6 mice. In the second experiment, the mean clinical severity between days 10 and 54 was significantly lower (p < 0.05) in IL-6 KO mice. The error bars are SE. In both experiments 1 and 2, AChR-immunized B6 mice had significantly reduced levels of functional muscle AChR compared to IL-6 KO mice (p < 0.05 by Student’s t test).

FIGURE 1. AChR-immunized IL-6 KO mice are resistant to clinical EAMG. Mice were immunized with AChR in CFA on days 0, 30, and 60. Kinetics of the accumulated clinical incidence of EAMG (A and D), severity (B and E), and muscle AChR content expressed as bungarotoxin binding sites in AChR-immunized B6 and IL-6 KO mice (C and F). EAMG clinical incidence was significantly lower (p < 0.05) in IL-6 KO mice when compared to that in B6 mice using Fisher’s exact test from days 21 to 64 (first experiment) and days 10 to 54 (second experiment) after the second AChR immunization. EAMG mean clinical severity (score) in the first experiment was significantly lower (p < 0.05) in experiment 1 using Student’s t test from days 21 to 64, after the second AChR immunization in IL-6 KO mice when compared to B6 mice. In the second experiment, the mean clinical severity between days 10 and 54 was significantly lower (p < 0.05) in IL-6 KO mice. The error bars are SE. In both experiments 1 and 2, AChR-immunized B6 mice had significantly reduced levels of functional muscle AChR compared to IL-6 KO mice (p < 0.05 by Student’s t test).

FIGURE 2. Anti-AChR IgG responses were suppressed in AChR-immunized IL-6 KO mice. Anti-AChR Abs in sera derived from experiments 1 (A) and 2 (B) were measured by 125I-labeled α-bungarotoxin RIA. *, p < 0.05 by Student’s t test.

FIGURE 3. Predominant suppression of anti-AChR IgG2 Abs in AChR-immunized IL-6 KO mice. Mice described in Fig. 1A were bled 74 days after the first immunization. Anti-mouse AChR isotype titers were determined using ELISA on mouse affinity-purified, AChR-coated plates. *, p < 0.05 using Student’s t test. One representation of two independent experiments.
detectable levels of IL-6 and logically very little IL-6 was produced by AChR and α146–162 peptide-specific lymphocyte-proliferative responses and IFN-γ and IL-10 production. LNC were collected on day 7 or 90 from mice that were evaluated for clinical EAMG. T cell proliferation (short term; A; long term, D) and supernatant levels of IFN-γ and IL-10 (short term, B and C; long term, E and F). The mean background (medium) cpm of B6 mice was 1154 and for IL-6KO mice was 2254 for the short-term lymphoproliferative assay (A). The mean background (medium) cpm of B6 mice was 2848 and for IL-6 KO mice was 2866 for the long-term lymphoproliferative assay (D). The error bars are SE. *p < 0.05 using Student’s t test.

Cell surface marker expression in AChR-immunized IL-6−/− mice

The total axillary and inguinal LNC numbers (Fig. 6, B and D) and the expression of cell surface markers, MHC class II, B220, B7-2, B7-1, CD40, and CD4 were measured in IL-6−/− and WT mice 7 days after a single immunization (Fig. 6A) with AChR in CFA and at termination of the long-term experiment (Fig. 6C; 90 days after the first immunization with AChR in CFA). On day 90, we also detected D3 expression. These data suggested that expansion of lymphoid cells after AChR/CFA immunization was not impaired in IL-6−/− mice. The total number of draining LNC was comparable to those in AChR-immunized WT and IL-6−/− mice both on days 7 and 90 (Fig. 6, B and D). There were no obvious differences in the cell surface expression of MHC class II, B220, B7-1, B7-2, and CD40 molecules between AChR-immunized IL-6−/− and WT mice (Fig. 6C). The cell surface molecule expression on normal B6 and IL-6−/− mice (for day 90 age matched) is given in the legend to Fig. 6. MHC class II, CD40, and B220 molecule expression were augmented and CD3 and CD4 molecule expression were reduced in both B6 and IL-6−/− mice after AChR immunization. There was no change in the expression of B7-2 and B7-1 molecules on day 90 after AChR immunizations. A similar result was obtained in the second long-term experiment (data not shown). In both B6 and IL-6−/− mice, B cells have expanded and T cells are reduced after AChR immunization.

Reduced number and size of GC in the AChR-immunized IL-6−/− mice

Spleens from IL-6−/− and WT mice were isolated at day 90 after primary immunization with AChR/CFA and prepared for immunohistochemistry using PNA as the marker for GC. A significant reduction in the number and size of GC in AChR-immunized IL-6−/− mice was observed compared to WT mice (Table I and Fig. 7).

Decreased serum C3 levels in AChR-immunized IL-6−/− mice

Serum C3 levels of AChR-immunized WT and IL-6−/− mice were measured by ELISA in serum samples obtained 2 wk after the first, second, and third AChR immunizations. This complement component was chosen for its key role in the complement activation cascade. C3 levels were found to be elevated in WT mice after the third immunization. By contrast, serum C3 levels of IL-6−/− mice revealed a slight downward, steady-state deviation from initial levels (Fig. 8). The failure of up-regulation of C3 could have contributed to the resistance to EAMG induction in the IL-6−/− mice.

Discussion

IL-6−/− mice developed normally and acquired a normal immune system. The number of thymocytes and peripheral T cells in IL-6−/− mice was slightly reduced (29, 30), but these cells exhibited normal expression patterns of the TCR α-, β-, γ-, and δ-chains, CD4, and CD8 molecules (29). B cells in the bone marrow and spleens of IL-6−/− mice expressed B220, IgM, IgD, and CD23 within normal ranges (29). The T cell-dependent IgM response to vesicular stomatitis virus was normal, but the IgG response was 5–10 times reduced in IL-6−/− mice compared to B6 mice (29).
The polyclonal T cell responses were comparable between IL-6/−/− and WT mice (30). IL-6/−/− mice had a reduced Ag-specific, T cell-proliferative response, but a normal response to mitogen (30). IL-6/−/− mice were resistant to experimental autoimmune encephalomyelitis (EAE); however, in vivo IL-6 reconstitution into IL-6/−/− mice could recover their susceptibility to EAE (31). These data suggest that IL-6/−/− mice did not have embryonic or postnatal developmental defects, but rather that IL-6 promotes both the cellular and humoral immune responses. IL-6 has been shown to be involved in the development of myelin oligodendrocyte glycoprotein-induced EAE (30), Ag-induced arthritis (32), lupus in NZB/W F1 mice (33), and cyclophosphamide-induced autoimmune diabetes in nonobese diabetic/WEHI mice (34).

Approximately 15% of MG patients have thymomas (35, 36).

Table I. Reduced number of GC in the AChR immunized IL-6 KO mice

<table>
<thead>
<tr>
<th>Expt. No.</th>
<th>Strain</th>
<th>No. of mice</th>
<th>Number of GC</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>B6</td>
<td>4</td>
<td>14.3 ± 1.12</td>
<td>0.005</td>
</tr>
<tr>
<td>2</td>
<td>IL-6/−/−</td>
<td>4</td>
<td>2.78 ± 2.4</td>
<td>0.00015</td>
</tr>
<tr>
<td>2</td>
<td>B6</td>
<td>7</td>
<td>7.6 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>IL-6/−/−</td>
<td>8</td>
<td>0.88 ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>

* Spleens from IL-6/−/− and control WT were isolated at day 90 after primary immunization with AChR/CFA and prepared for immunohistochemistry using PNA as the marker for GC.

FIGURE 7. GC number and size are reduced in IL-6-deficient mice. Spleens from IL-6-deficient mice (A) and control C57BL/6 mice (B) were isolated at day 90 after primary immunization with AChR/CFA and prepared for immunohistochemistry using PNA as the marker for GC. Original magnification, ×250. One representation of 12 PNA-stained spleens from IL-6-deficient (A) and 11 spleens from control B6 mice (B) from two independent experiments.
the development of thymic hypertrophy and GC formation because of its effect on B cell maturation and differentiation. The reduced size of the GC in IL-6−/− mice (37) further supports the above possibility. Therefore, our evidence on the critical role of IL-6 in EAMG development has important implications in MG pathogenesis, including the development of thymic hypertrophy and thymomas. We have not observed any histological changes in the thymi of AChR-immunized B6 mice (data not shown). Also, we do not know whether IL-6 is produced by thymic epithelial cells of AChR-immunized B6 mice. In EAMG the site of autosensitization is AChR draining lymph node and spleen in B6 mice. In MG the site of autosensitization could be the thymus and/or the peripheral lymphoid organs. Therefore, the thymic pathology seen in most MG patients may not be evident in B6 mice with EAMG. Whether thymic changes could be observed after a long time (4–6 mo) following AChR immunizations is yet to be studied in B6 mice.

There is strong evidence for a facilitative role for IFN-γ (8, 9) and IL-10 (38) in EAMG pathogenesis. IL-12 and IL-18 also participate in the development of EAMG (10, 39). It appears that IL-6 in vivo could regulate production of AChR and α146–162-specific IL-2, IFN-γ, and IL-10 production. It is possible that down-regulation of IL-6 could suppress the production of IL-2, IFN-γ, IL-10, and other cytokines which facilitate the development and maintenance of the autoimmune response to AChR. TNFR p55 and p75 gene KO mice were also resistant to EAMG and blocking endogenous TNF function by soluble human recombinant TNFRFc suppressed ongoing clinical EAMG, thus implicating TNF as an important cytokine in EAMG pathogenesis (40, 41). We have also demonstrated evidence for a role for lymphotoxin in the development of EAMG (42). None of the lymphotoxin α (TNF-β)-deficient mice immunized with AChR developed clinical EAMG and failed to switch anti-AChR IgM isotype to IgG (42). From our published and unpublished studies on various cytokines or their receptor KO mice, we suggest that a cytokine hierarchy exists in the development of EAMG, with IL-6, TNF, and IL-18 playing the hierarchical role in its development. Dissecting out the role of these cytokines in the afferent and effector phase of autoimmune response to AChR would pave the way for effectively treating the disease by therapeutic down-regulation of the cytokines involved in the effector phase. Further studies will suggest whether IL-6 and TNF act in concert or one of them regulates the production of the other.

The serum C3 levels of AChR-immunized IL-6−/− mice were reduced gradually, whereas those of B6 mice increased considerably after a brief period of decline. Serum C3 levels of AChR-immunized mice seldom drop off to very low levels after the second immunization, probably due to the fact that repetitive immunizations increase the rate of serum C3 consumption and thus contributed to the suppression of EAMG in IL-6−/− mice. The importance of anti-AChR IgG3 Abs in EAMG pathogenesis was first demonstrated in our previous study (7), in which tolerance to AChR α146–162 peptide significantly suppressed anti-AChR IgG3 Abs. Furthermore, one should note that IgG3, is a complement-fixing Ab. However, the primary IgM anti-AChR Ab response was comparable between AChR-immunized IL-6−/− and B6 mice. These data are consistent with the observation in NZB/W F1 mice (44) and suggest that AChR-immunized IL-6−/− mice have an impaired ability to switch to the anti-AChR IgG isotype, especially IgG3. The reduction in serum C3 in IL-6−/− mice could have contributed to the lower muscle AChR loss. Because anti-AChR IgG Abs and C3 activation contribute to the autoimmune destruction of AChR, therapeutic down-regulation of IL-6 could control the onslaught of an autoimmune attack at the NMJ in EAMG and probably in MG. After induction of EAMG/MG remission via IL-6 down-regulation by IL-6 antagonists, Ag-specific tolerance (e.g., high-dose AChR T cell epitope tolerance (7)) could be used as a maintenance therapy. A similar therapeutic approach could be attempted in other Ab-mediated autoimmune diseases.

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References


