Increased Susceptibility to Experimental Autoimmune Neuritis after Upregulation of the Autoreactive T Cell Response to Peripheral Myelin Antigen in Apolipoprotein E-Deficient Mice

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Abstract. Experimental autoimmune neuritis (EAN), an acute demyelinating inflammatory disease of the peripheral nervous system (PNS), is a good model for the human counterpart, Guillain-Barré syndrome. Apolipoprotein E (ApoE), a 34-kDa glycosylated protein with multiple biological properties, has been linked both with the innate immune response of mice and with neurological disease. The present study investigated the previously unexplored role of ApoE in autoimmunemediated demyelination. ApoE-deficient (apoE−/−) mice exhibited a greater susceptibility to EAN induced by the PNS myelin P0 protein peptide 180–199, as compared to wild type (apoE+/+) mice. The augmented susceptibility seen in apoE−/− mice was associated with increased inflammatory cell infiltrates in the PNS during the effector phase. Although the 2 groups of mice exhibited no quantitative or proportional differences in splenic lymphocyte populations, the apoE−/− mice showed enhanced antigen-specific proliferation of T cells of spleen, which is related to modified macrophage function, upregulation of Th1 and downregulation of Th2-autoreactive responses to P0 peptide. These effects were shown as increased numbers of IFN-γ expressing cells in the spleen and of IFN-γ, IL-12 and TNF-α expressing cells in the PNS, as well as a decreased IL-10 production by splenic cells in apoE−/− mice. In addition, apoE−/− mice had enhanced antigen-specific antibody responses, which might have contributed to their aggravated EAN. These data provide strong evidence that apoE acts as an inhibitor of this inflammatory and demyelinating disease by upregulating IL-10, as well as by inhibiting Th1 responses and antigen-specific antibody formation. These data may aid the development of new and more effective therapeutic strategies for inflammatory and demyelinating diseases such as Guillain-Barré syndrome.

Key Words: Apolipoprotein E; Cytokines; Experimental autoimmune neuritis (EAN); Guillain-Barré syndrome; Th1/Th2.

INTRODUCTION

Experimental autoimmune neuritis (EAN) is a T cell-mediated, acute, demyelinating inflammatory disease of the peripheral nervous system (PNS) that serves as a model for the human Guillain-Barré syndrome. EAN can be induced in susceptible animal species and strains by active immunization with either PNS tissue, the PNS myelin proteins P2 and P0, or synthetic P2 and P0 peptides emulsified in Freund’s complete adjuvant (1–3). EAN can also be transferred to naïve recipient rats by CD4+ P2- or P0-reactive T cell lines (4–7). Therefore, CD4+ type 1 helper T (Th1) cells play a crucial role in EAN, although the exact sequence of events and molecular mediators of the PNS inflammatory response have not been clearly defined.

Apolipoprotein E (apoE) is a 34-kDa glycosylated protein with multiple biological properties. ApoE is synthesized predominantly in the liver, but also by cells in the spleen, brain, and lung. Hepatic parenchyma cells, glial cells, and macrophages are main cells for the production of apoE in corresponding tissue (8). ApoE has been widely studied in atherosclerosis, a vascular disease characterized by lipoprotein accumulation and immune/inflammatory activation (9–12). ApoE has also been extensively studied in Alzheimer disease, a chronic progressive neurodegenerative disease characterized by pathological neurofibrillary tangles, amyloid plaque deposition, and local expression of inflammatory cytokines (13, 14).

In addition to its physiological role in cholesterol transport, apoE has immunomodulatory properties in vitro and in vivo. ApoE-deficient mice showed abnormal humoral and cellular immune response (15). In vivo, apoE downregulates Th1 immune responses (10), and in vitro, apoE suppressed the lymphocyte proliferation (16, 17). ApoE4 was related to decreased levels of IL-1ra (interleukin 1β receptor antagonist), an anti-inflammatory cytokine, after the initiation of cardiopulmonary bypass (18). The apoE ε4 allele has been found to be associated with faster progression of disability in multiple sclerosis (MS), a T cell-mediated autoimmune disease in the central nervous system (CNS) (19–21). Furthermore, an increased susceptibility to experimental autoimmune encephalomyelitis (EAE), which is an animal model for MS, was seen...
in apoE knockout mice (22). However, the role of apoE in autoimmune disease in the PNS has not been investigated.

ApoE-deficient (apoE\(^{-/-}\)) mice generated by targeted gene disruption (23) provide an excellent model for evaluating the immunological importance of apoE. We used this model to characterize the role of apoE in contributing to pathologic autoimmunity in general and to EAN in particular. Our data demonstrate that a significant aggravation of clinical and pathological EAN in apoE\(^{-/-}\) mice was induced by the P0 peptide 180–199. This aggravation was associated with an increased accumulation of inflammatory infiltrates in the PNS, upregulation of Th1, and downregulation of Th2-autoreactive responses to peripheral myelin antigen, as well as enhanced antigen specific antibody responses in apoE\(^{-/-}\) mice.

**MATERIALS AND METHODS**

**Mice**

Male wild-type C57BL/6 (H-2\(^b\), apoE\(^+/+\)) mice and apoE\(^{-/-}\) mice on the C57BL/6 (H-2\(^b\)) background were purchased from Bomholtgard Breeding and Research Centre, Ry, Denmark, and bred in the animal facility of Huddinge University Hospital, Karolinska Institutet, Stockholm, Sweden. The mice used for these experiments were 4- to 5-weeks old and weighed 18 g to 22 g. The EAN model in mice was approved by the South Stockholm Research Animal Ethics Committee, Huddinge County Court, Stockholm, Sweden.

**Peptide Antigen**

The neuritogenic P0 protein peptides, corresponding to amino acids 180–199 of rat PNS myelin P0 protein, were synthesized by solid-phase stepwise elongation using a Tecan/Syro peptide synthesizer (Multisyntech, Bochum, Germany).

**Induction of EAN and Assessment of Clinical Signs**

A total of 47 apoE\(^{-/-}\) and 45 apoE\(^{+/+}\) mice were used in 5 separate experiments. Animals were immunized twice (days 0 and 7) by subcutaneous injection with 120 µg of P0 peptide 180–199 and 0.5 mg of Mycobacterium tuberculosis (strain H37 RA; Difco, Detroit, MI) in 25 µl saline and 25 µl Freund’s incomplete adjuvant. All mice received 400, 200, and 200 ng pertussis toxin (Sigma, St. Louis, MO) by intravenous injection on days −1, 0 and +2 post-immunization (p.i.), respectively. Using a blinded protocol, 2 different examiners assessed clinical signs of EAN immediately before immunization (day 0) and thereafter every day or every second day until day 76 p.i. EAN was scored as follows: 0, normal; 1, flaccid tail, decreased tone in whole tail or mild limb weakness; 2, severe limb weakness or mild hind limb paralysis; 3, moderate hind limb paraparesis; 4, severe hind limb paralysis; and 5, severe tetraparesis.

**Histopathological Assessment**

Six mice from each group were killed on day 24 p.i., at a time when the clinical signs of EAN peaked. Sciatic nerve segments were excised close to the lumbar spinal cord, fixed in 4% formaldehyde, and embedded in paraffin. Multiple longitudinal sections (5- to 6-µm slices) of sciatic nerves were stained with hematoxylin and eosin for evaluation of the extent of mononuclear cell (MNC) infiltration. Tissue areas were measured by image analysis and the results were expressed as cells per mm\(^2\) tissue section.

**Immunohistochemistry**

Segments of sciatic nerves were dissected and snap-frozen in liquid nitrogen. Cryostat sections (10 µm) were exposed to the following monoclonal antibodies (mAbs): rat anti-mouse CD4 (PharMingen, San Diego, CA), rat anti-mouse macrophage, rat anti-mouse CD8 (both from Harlan Sera-Lab, Leicestershire, England), rabbit anti-rat IFN-γ, rabbit anti-rat IL-4 and rabbit anti-rat TNF-α (all from the Central Laboratory Animal Institute (CLAI), Utrecht, The Netherlands) as well as rat anti-mouse IL-12 (Biosource, Camarillo, CA). Sections were stained according to the avidin-biotin technique (Vectastain Elite Kit; Vector Labs, Burlingame, CA). Omission of primary antibodies served as a negative control. Nonspecificity of staining was also controlled on tissue sections. The tissue areas were measured by image analysis and the results expressed as cells per mm\(^2\) tissue section.

**Isolation of MNC from Spleen and Preparation of T cells**

The spleens were removed on day 24 p.i. and single cell suspensions of MNC from individual mice were prepared. These cells were washed 3 times in Iscove’s modified Dulbecco’s medium (Flow Lab, Irvine, UK) supplemented with 1% (v/v) minimum essential medium and 2 mM glutamine (both from Flow Lab), 50 IU/ml penicillin, 60 µg/ml streptomycin (both from Gibco, Paisley, UK). For isolation of T cells, MNC suspensions from Spleen were incubated in 25 cm\(^2\) Falcon culture flask (Becton Dickinson, Franklin Lakes, NJ) with the above serum-free medium for 2 h at 37°C. The nonadherent cells were collected and passed through a nylon wool column (Kisker, Steinfurt, Germany). T cells were obtained by depletion of nylon wool-adherent cells.

**Preparation of Peritoneal Exudate Cells**

Five ml Dulbecco’s medium was injected into the peritoneal cavity of P0 peptide 180–199 immunized mice on the day 24 p.i. After rinsing for 5 min, peritoneal exudate cells (PECs) (macrophages) were eluted from the cavity. These PECs were washed 3 times with medium.

**Flow Cytometry Analysis of Spleen Cells and PECs**

Cell surface phenotypes were identified via immunofluorescence assay. Briefly, \(2 \times 10^5\) MNC from spleen suspended in 50 µl phosphate-buffered saline containing 1% bovine serum albumin and 1% Na\(_2\) (PBS-BSA-Na\(_2\)) were mixed with the following PE- or FITC-conjugated anti-mouse mAbs: anti-CD3, anti-CD4, anti-CD8, anti-CD11b, anti-M3/84 and anti-NK-1.1 (PharMingen), anti-TCR\(\alpha\) and anti-TCR\(\beta\) (Serotec, Oxford, UK) at saturating titers for 30 min at 4°C. Two \(10^5\) PECs suspended in 50 µl PBS were stained with PE- or FITC-conjugated anti-I-A (MHC-II) (Serotec), CD40 (Caltag, Burlingame, CA), CD80 (Serotec), and CD86 (Caltag) antibodies using the same protocol. Cells were subsequently washed twice.
with PBS-BSA-NaN3, re-suspended in PBS-BSA-NaN3 containing 1% paraformaldehyde, and stored at 4°C awaiting flow cytometric analysis with the FACScan (Becton Dickinson, San Jose, CA).

**Cell Proliferation Assays**

PECs (macrophages) from apoE+/+ (PEC+/+) or apoE−/− (PEC−/−) mice were mixed with corresponding T cells (T+/+ or T−/−) at a density of 2 × 10⁶ cells/ml for cultures at a ratio of 1:20 (PEC: T cell). Two hundred µl of cell suspensions were cultured in triplicates in round-bottomed 96-well polystyrene microtiter plates (Nunc, Copenhagen, Denmark). For lymphocyte stimulation, 10 µl of P0 peptide 180–199 was added to cultures at a final concentration of 10 µg/ml for P0 peptide. The concentration had maximum stimulatory effects as assessed in preliminary experiments. After a 60-h incubation, cells were pulsed with ³H-methylthymidine (1 µCi/well, Amer sham, Little Chalfont, UK) and cultured for an additional 12 h. Cells were harvested onto glass fiber filters (Titertek, Skatron, Lierbyen, Norway). ³H-thymidine incorporation was measured in a liquid β-scintillation counter and results expressed as counts per minute (cpm).

**Enumeration of IFN-γ- and IL-4-Secreting Cells in the Spleen**

A solid-phase enzyme-linked immuno-spot assay (ELISPOT) was used to detect single cells that secreted IFN-γ and IL-4 upon antigen stimulation. Briefly, nitrocellulose-bottomed plates (Microtiter-HAM, Millipore, Bedford, MA) were coated with 100 µl of anti-rat IFN-γ and anti-rat IL-4 mAbs (CLAI), overnight at 4°C. Then 200 µl containing 4 × 10⁶ MNC were added in duplicate with either medium alone (control cultures without antigen) or 10 µl aliquots of P0 peptide 180–199 (final concentration of 10 µg/ml) or PHA (final concentration of 20 µg/ml). After 48-h culture, secreted and bound IFN-γ and IL-4 were visualized by sequential application of rabbit anti-rat IFN-γ polyclonal antibody and rabbit anti-rat IL-4 polyclonal antibody (CLAI), respectively, followed by biotinylated swine anti-rabbit IgG (Sigma) and then avidin-biotin peroxidase complex (ABC Vectastain Elite Kit, Vector). After peroxidase staining, the red-brown immunospots corresponding to the cells that had secreted IFN-γ or IL-4 were counted in a dissection microscope. Results were expressed as numbers of spots per 10⁶ splenic MNC.

**ELISA for Measurement of Cytokine Profile**

Single cell suspensions of P0 peptide 180–199-primed draining MNC were cultured in the presence of P0 peptide 180–199 (10 µg/ml). The supernatants were collected after 48-h culture. Levels of IL-10 and IL-4 in the culture supernatants were measured by optEIA kits (PharMingen).

**ELISA for Measurement of Antigen-Specific IgG Antibodies**

To measure levels of serum IgG and IgG isotypes, we drew blood samples from test mice at day 24 p.i. and pooled sera from each group of 11 animals for ELISA. Microtiter plates (Nunc) were coated with 100 µl/well of purified P0 peptide 180–199 (10 µg/ml) at 4°C overnight. Uncoated sites were blocked with 10% fetal calf serum for 2 h at room temperature. Serum samples (diluted 1:100) were added and incubated for 2 h at room temperature. Plates were then incubated for 1 h with biotinylated anti-mouse IgG (Vector), IgG1 (Serotec), IgG2a (PharMingen), and avidin-horseradish peroxidase conjugate (PharMingen), followed by substrate solution for 30 min. The reactions were stopped with 1M H2SO4 and ODs at 450 nm determined.

**Statistical Analyses**

Differences between groups were evaluated by one-factor analysis of variance (ANOVA). Differences between pairs of groups were tested by Student t test or nonparametric Mann-Whitney U-test. All tests of significance were two-sided. Data are presented as means ± SD and a level of p < 0.05 was considered significant.

**RESULTS**

**Accelerated Initiation and Development of EAN in ApoE−/− Mice**

To explore the role of apoE in the development of EAN, mice were immunized twice with P0 peptide 180–199 in Freund's complete adjuvant. As shown in the Table and Figure 1, all mice acquired EAN. The onset of disease in the apoE+/+ mice ranged between days 10 and 12 after immunization. Of this group, 80% of the animals progressed to mild hind limb paralysis. After the peak response, the mice spontaneously recovered. In contrast, apoE−/− mice immunized with P0 peptide 180–199 experienced an earlier onset of EAN (i.e. at 6 to 8 days p.i.), with a more pervasive and longer lasting course. All apoE−/− mice displayed moderate hind limb paralysis at the peak of their response, which endured significantly longer (61.7 ± 5.8 days vs 51.4 ± 6.6 days, Table).
Enhanced Inflammatory Cell Infiltration into the PNS in ApoE−/− Mice

The entry of inflammatory cells into the PNS is a critical step in the development of EAN. To examine the influence of apoE on recruitment of inflammatory cells to the PNS, we performed histopathological and immunohistochemical studies at the height of the clinical course of EAN (day 24 p.i.). Histopathological evaluation revealed an expanded inflammatory infiltrate composed of more activated macrophages, lymphocytes and granulocytes in sciatic nerve sections from apoE−/− mice (Figs. 2A, 3A), as compared to those from apoE+/+ mice (Figs. 2B, 3A). Immunohistochemical studies comparing the 2 groups showed that the number of CD4+ T cell and macrophage infiltrating sciatic nerves of apoE−/− mice was significantly increased. However, the infiltration of CD8+ T cells was identical in both groups (Fig. 3B). Presumably, apoE inhibited the ability of pro-inflammatory cells to migrate into the PNS, thereby reducing inflammation in the PNS.

No Quantitative or Proportional Uniqueness for Splenic Lymphocytes and PECs of ApoE−/− Mice

To examine the immunomodulatory role of apoE during the development of EAN, we compared the weight, cellularity, and phenotypes of spleen cells of both groups on day 24 p.i. ApoE−/− mice and age-matched apoE+/+ mice showed no significant differences in either weight or splenic cellularity (data not shown). Similarly, FACS analysis showed that the average percentages of CD3+, CD4+, CD8+, αβ-TCR, γδ-TCR, CD3+/NK1.1+, CD3+/NK1.1+ cells, as well as the percentages of M3/84+, CD11b+ cells in the spleens and I-A (MHC-II), CD40, CD80, and CD86 in PECs of apoE−/− mice did not differ from those of apoE+/+ mice (data not shown).

Enhanced T Cell Priming Response and Th1 Cytokine Production in ApoE−/− Mice

To obtain further insight into the mechanisms of apoE regulation and to search for the effects of apoE on antigen-specific T cell activation and on macrophages in T cell proliferation, as well as Th1/Th2 responses in EAN,
we purified spleen T cells and PECs (macrophages) from apoE−/− and apoE+/+ mice at the peak of their disease course (i.e. on day 24 p.i.) for mixed cultures and examined their proliferation in response to P0 peptide 180–199. M+/T+ refer to PEC/T cells from apoE+/+ and M−/T− from apoE−/− mice (5 mice per group). The results are expressed as the mean values ± SD. Differences between groups were evaluated by ANOVA. *p < 0.05, **p < 0.01.

To address the specific role of the apoE in antibody production associated with EAN, we analyzed the antibody response in the target organ, we examined IFN-γ, IL-12, and IL-4 production in sciatic nerve sections from apoE−/− mice at the peak of EAN, the numbers of IFN-γ, IL-12, and TNF-α-positive cells in sciatic nerve sections from apoE−/− mice were significantly higher than those from apoE+/+ mice. In contrast, both groups had an almost identical number of IFN-γ-positive cells in sciatic nerve sections. This pattern of local cytokine secretion suggests a Th1 dominance among effector cell. These results indicate that apoE would inhibit antigen-specific Th1-autoreactive responses and enhance Th2 responses in EAN.

**Elevated Antigen-Specific Antibody Production in ApoE−/− Mice with EAN**

To address the specific role of the apoE in antibody production associated with EAN, we analyzed the antibody responses in apoE−/− and apoE+/+ mice in terms of IgG and IgG subclasses. As shown in Figure 7, the levels of antigen-specific IgG, IgG1, and IgG2a antibodies of apoE−/− mice significantly exceeded those of apoE+/+ mice. The elevated
Fig. 5. The numbers of cytokine-secreting cells and levels of cytokine production by spleen cell supernatants from apoE−/− and apoE+/+ mice immunized with P0 peptide 180–199. Spleen cells were harvested on day 24 p.i. and tested for the numbers of IFN-γ- and IL-4-secreting cells in response to in vitro re-stimulation with P0 peptide 180–199 by ELISPOT assay (A). Levels of IL-10 and IL-4 production were measured in spleen cell supernatants by ELISA after 48 h of culture with P0 peptide 180–199 (B). The results are expressed as the mean values ± SD (6 mice per group). Statistical evaluation compared 2 groups. **p < 0.01.

Fig. 6. The numbers of cytokine-positive cells in the PNS from apoE−/− and apoE+/+ mice immunized with P0 peptide 180–199. Sciatic nerve sections were examined on day 24 p.i. Numbers of IFN-γ-, IL-12-, IL-4- and TNF-α-expressing cells per mm² were determined by immunohistochemistry. The results are expressed as the mean values ± SD (6 mice per group). Statistical evaluation compared 2 groups. **p < 0.01.

Fig. 7. Antigen-specific antibody responses in apoE−/− and apoE+/+ mice immunized with P0 peptide 180–199. The concentrations of anti-P0 peptide 180–199 IgG, IgG1 and IgG2a were measured by ELISA on day 24 p.i. (11 mice per group). The results are expressed as the mean values ± SD. Statistical evaluation compared 2 groups. **p < 0.01. One representative experiment out of three is shown.

amounts of antibodies indicate that apoE can inhibit antigen-specific antibody responses during the course of EAN.

DISCUSSION

ApoE, as well as apoE-containing lipoproteins and multimers of synthetic apoE peptides can inhibit mitogen- and antigen-induced proliferation of cultured lymphocytes (24–26). Furthermore, apoE may play an immunomodulatory role and engage in neurotrophic or neuroprotective functions in the CNS (27). ApoE-deficient mice are highly susceptible to infection due to having impaired immune responses to bacteria and viruses (28–30).

Using the model of EAN established in apoE−/− mice, we investigated the role of apoE in autoimmune-mediated demyelination. Our results show that the apoE molecule is involved in the initiation and development of EAN and that an apoE deficiency effectively worsens clinical and pathological EAN. In addition, we delineated several potential mechanisms by which apoE could participate in the pathogenesis of EAN.

Firstly, apoE−/− mice showed an apparent T cell enhancement in response to priming with peripheral nerve myelin antigen. The proliferation of T cells from spleen was markedly increased in apoE−/− mice as compared to apoE+/+ mice, although no quantitative or proportional differences were found in their splenic lymphocyte populations. Therefore, apoE may be required for inhibiting activation of CD4+ T cells and induction of differential CD4+ T cell polarization. Furthermore, the effect of enhancement of T cell response in apoE−/− mice is associated with macrophage functions. ApoE deficiency may modify or heighten macrophage functions, which resulted in a strong T cell response.

Macrophages play a crucial part in autoimmune neuropathies and are putatively involved in all steps of the
pathogenic process (31, 32). Because apoE is synthesized predominantly by macrophages in the blood cells, it seems likely that the enhanced immune response in the P0-peptide immunized apoE−/− mice resulted from a modified function of macrophages rather than T cells. Although the autoreactive Th1 cells of apoE−/− mice were clearly upregulated in response to P0 peptide, the macrophages of apoE−/− mice can also increase the T cell proliferation of apoE+/+ mice. The cause might be an enhanced antigen-presenting cell function or other functions of macrophages at different stages during induction of the immune response. However, molecules such as CD40, CD80, CD86, and MHC-II, which are related to the antigen presentation, are not clearly involved in the pathogenic process, and the mechanisms need to be studied further.

Secondly, shifting the Th1/Th2 balance towards Th2 cells may be one mechanism underlying increased susceptibility to EAN in apoE−/− mice. Cells from apoE−/− mice produced higher levels of IFN-γ, IL-12, and TNF-α and lower levels of IL-10 than their counterparts. Th cells can be divided into Th1 and Th2 subpopulations with contrasting and cross-regulating cytokine profiles that may play a decisive role in the initiation and limitation of an autoimmune process (33). Dysregulation of the Th1/Th2 balance can result in cancer, allergy, and autoimmunity (34). In EAN, Th1 cytokines predominate and mediate inflammatory damage, whereas Th2 cytokines have been associated with remissions and recovery from disease (3, 35–37). The Th1 cytokines, IFN-γ, IL-12, and TNF-α, have all been linked to disease expression in EAN (3, 38, 39). The presence of CD4+ T cells and T cell-activation by antigen-presenting cells are necessary conditions for the initiation and development of EAN. This function is at least partly linked to their secretion of IFN-γ. Convincing evidence asserts that a sufficiently high level of IFN-γ-producing cells in blood, lymph nodes, and PNS tissue roughly parallels the presence of clinical EAN, consistent with an inflammatory role of Th1-promoting cytokines in the pathogenesis of EAN (3, 39). Mice deficient in the IFN-γ receptor are resistant to EAN induction (40) and raised IFN-γ correlates positively with increasingly severe EAN. The higher numbers of IFN-γ-secreting Th1 cells we detected in apoE−/− mice indicate that IFN-γ production accelerates EAN in these animals. However, an apparent contrary effect of IFN-γ was seen in EAE, a CNS disease that closely resembles EAN, in which IFN-γ protected mice from EAE (41, 42). The reasons for the opposite result of IFN-γ in EAE are not completely understood. One reason could be that IFN-γ plays a double role in autoimmune diseases, that is, IFN-γ can enhance inflammatory response by several pathways, but the production of IFN-γ benefits the host (e.g. promoting infiltrating lymphocyte undergoing apoptosis). Finally, the effect of IFN-γ on autoimmune disorders may depend on an adequate balance between pro-inflammatory and anti-inflammatory cytokines.

T cells producing Th2 cytokines confer protection against EAN. For example, the administration of IL-4 or IL-10 in vivo markedly suppresses EAN (1, 43). IL-10 may play a more critical role than IL-4 in the regulation of EAE (44). Additionally, macrophages are also a main source of IL-10 (45), but basically in the recovery phase of EAN. Therefore, it is less probable that lower level of IL-10 detected in apoE−/− mice at the height of their disease was produced from macrophages that altered function. In the current study, a deficiency of apoE was found to block the production of IL-10 upon autoantigen stimulation, suggesting the importance of apoE in Th2 polarization.

A third possible mechanism of apoE deficiency is an impaired blood-nerve barrier (46), which caused an enhanced homing of inflammatory cells to the PNS and a heightening of their function during the effector phase. The effect might contribute to the aggravation of EAN. Accordingly, our results revealed increased CD4+ T cell and macrophage infiltration into the PNS in apoE−/− mice. This observation suggests that apoE may inhibit the migration of blood-derived inflammatory cells across the blood-nerve barrier or the transduction of chemotactic signals for migration. This is a critical step in the migratory process of inflammatory cells across tight endothelial junctions (47, 48). Although macrophage numbers were increased in the PNS of apoE knockout mice, the lack of apoE secretion by these cells was likely to have enhanced PNS injury. It is possible that after nerve injury, macrophages within the sciatic nerve start to produce apoE that is important for removal of debris from damaged cells as well as stimulation of PNS regeneration (49). Alternatively, apoE may govern P0 peptide-induced cytokine production in the PNS. The IFN-γ increase seen in the PNS of apoE−/− mice may result in an upregulated production of Th1-dependent chemokines that could control tissue migration of effector T cells, leading in turn to more severe disease (50, 51). Furthermore, increases of the pro-inflammatory cytokines IL-12 and TNF-α also contributed to perpetuating inflammatory demyelination in apoE−/− mice with EAN. Therefore, the pattern of local cytokine secretion documented here suggests a Th1-cell dominance among effector cells in the EAN of apoE−/− mice and at least partial suppression of local action of these Th2 cells in the PNS.

Finally, the enhanced antigen-specific antibody responses of apoE−/− mice may act to worsen their EAN since apoE deficiency affected the primary T-dependent antibody responses. Optimal activation of B cells and differentiation into Ig-secreting cells depend on the helper
effects of CD4+ Th cells and are mediated via both direct Th-B cell interaction and the elaboration of antigen-specific cytokines (52). Although EAN is predominantly a T cell-mediated autoimmune inflammation of the CNS, antibodies also participate in this disease (53). In the apoE+/− mice we used, the levels of antigen-specific IgG, IgG1, and IgG2a in sera increased significantly after immunization with P0-peptide. This increased antibody responsiveness may have derived from enhanced T cell priming, which can help B cells, or may have been due to the direct involvement of apoE in antibody production or B cell differentiation. However, a shift towards Th1 antibody production did not appear in apoE−/− mice. The reason may be that apoE deficiency could not fully affect B cell differentiation and IgG isotypes.

In summary, we provide strong evidence that apoE acts as an inhibitor of inflammation and demyelination during EAN by decreasing the T-cell proliferation via modified macrophage function, upregulating IL-10, and damping Th1 and antigen-specific antibody responses. Further studies of the regulatory interaction between apoE and T cells, macrophages, and B cells may lead to identification of key molecules involved in the regulation of autoimmune disease, in which the CD4+ T cell subset has been suggested to be central for pathogenesis.

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