

Apolipoprotein E Deficiency Enhances the Antigen-Presenting Capacity of Schwann Cells

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KEY WORDS

antigen presentation; apolipoprotein E; MHC-II; co-stimulatory molecule; Schwann cells

ABSTRACT

Apolipoprotein E (apoE) has immunomodulatory properties and has been implicated in the pathogenic mechanism of autoimmune diseases. Previously, the authors found that apoE deficiency increased the susceptibility to experimental autoimmune neuritis (EAN), an animal model for human Guillain-Barré syndrome. To further elucidate the mechanism behind apoE deficiency exacerbating EAN, the authors investigated the role of major target and important antigen-presenting cells of the peripheral nerve system, Schwann cells (SCs), in apoE knockout mice. Treatment of apoE deficient SCs with recombinant mouse interferon- γ and lipopolysaccharide resulted in higher MHC-II and CD40 expression as compared with normal SCs derived from wild-type mice. The increased MHC-II and CD40 expression on SCs was accompanied by lower levels of intracellular IL-6 production within SCs of apoE deficiency, which is confirmed by the neutralization with anti IL-6 antibody. The increased antigen-presenting capacity of apoE deficient SCs was further explored by enhancement of T cell proliferation co-cultured with P0 peptide 180–199 specific T cells derived from EAN mice immunized with the P0 peptide. In conclusion, apoE may protect mice from EAN and probably also from chronic inflammatory demyelinating polyneuropathy by affecting the antigen-presenting function of SCs via influence of IL-6 production. © 2007 Wiley-Liss, Inc.

INTRODUCTION

Apolipoprotein E (apoE), a 34-kDa glycosylated protein, is synthesized predominantly in the liver, but also by cells of the spleen, brain, and lung. Hepatic parenchyma cells, glial cells, and macrophages are the major cell populations that produce apoE in the respective tissues (Mahley, 1988). In addition to its physiological role of cholesterol transport, apoE has immunomodulatory properties. For example, apoE can down-regulate Th1 immune responses in mice (Laurat et al., 2001) and inhibit lymphocyte proliferation in human peripheral blood (Avila et al., 1982; Macy et al., 1983). ApoE-deficient mice showed an abnormal humoral and cellular immune response (Laskowitz et al., 2000). Furthermore, the apoE ϵ 4 allele has been found to be associated with the progression of disability in multiple sclerosis (MS), a T cell mediated autoimmune

disease of the central nervous system (CNS) (Chapman et al., 1999, 2001; Sylantiev et al., 1998). Previously, we explored the role of apoE in P0 peptide 180–199 induced experimental autoimmune neuritis (EAN), which is an autoimmune disease of the peripheral nervous system (PNS) and serves as an animal model for human Guillain-Barré syndrome (GBS) (Yu et al., 2004). Our data showed that apoE deficiency increased antigen presentation of macrophages, which may be one of the mechanisms of increased susceptibility of EAN in apoE deficient mice.

In contrast, Schwann cells (SCs) are the myelin forming glial cells in the PNS and have been implicated with an immunoregulatory role in the inflammatory autoimmune neuropathies such as GBS and chronic inflammatory demyelinating polyneuropathy (CIDP) (Koski, 1997). Some studies revealed that SCs can be induced to express MHC-II molecule and thereby act as nonprofessional antigen-presenting cells in the PNS to stimulate T cell proliferation (Argall et al., 1992; Armati et al., 1990; Gold et al., 1995; Lilje and Armati, 1997). For example, MHC-II expression has been found on SCs in GBS and CIDP patients (Pollard et al., 1986, 1987).

However, whether apoE molecule affects SCs' function, especially in autoimmune neuropathy, is still unclear. Here we explored the effect of apoE on the antigen-presenting function of SCs by assessing the expression of MHC-II and the co-stimulatory molecules CD40, CD80, and CD86 on SCs of apoE deficient mice. Since IL-6 is produced by SCs (Koski, 1997) and can inhibit SCs' antigen-presenting function (Kitamura et al., 2005), we also detected the intracellular IL-6 level of apoE deficient SCs.

MATERIALS AND METHODS

Animals

Male apoE knockout (apoE KO) and corresponding wild-type C57BL/6 (H-apoETM1UNC) mice were purchased from

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Bomholtgaard Breeding and Research Centre, Denmark, and bred in the animal facility of Karolinska University Hospital Huddinge, Stockholm, Sweden. ApoE KO mice were generated on a 129 × C57BL/6 (H-apoETM1UNC) genetic background and subsequently backcrossed for 11 generations. The mice used in this study were 10–12 weeks old.

SC Cultures and Stimulation

Sciatic nerves were excised from the apoE KO and wild-type mice and gently stripped of the epineurium. The sciatic nerves were minced and the tissue pieces were cultured in 5 mL RPMI-1640 with 2 mM glutamine and 20 μmol/mL HEPES (Gibco, Paisley, UK) supplemented with 10% (v/v) fetal bovine serum (Gibco), 10 μg/mL insulin (Sigma, St Louis, MO), 0.2 μg/mL sodium selenite (Sigma), 10 μg/mL transferrin bovine (Sigma), 0.01 μmol/mL MEM nonessential amino acid (Sigma), 0.05 mg/mL L-ascorbic acid (Sigma), 6 mg/mL glucose (Sigma), and 50 μg/mL gentamycin (Gibco) on uncoated petri dish (Becton Dickinson, Franklin Lakes, NJ) for 5–6 days. The tissue segments were removed and digested with RPMI-1640 containing 1.25 U/mL dispase, 0.05% (w/v) collagenase, and 0.1% (w/v) hyaluronidase (all from Sigma) at 37°C for 2 h. Thereafter, the digested SC suspension was cultured in the same culture medium plus 2 μM forskolin (Calbiochem, La Jolla, CA) on coated petri dish (for the coating procedure see later).

After culture for 5–6 days, when 80% cells were confluent, the SCs of apoE KO and WT mice, respectively, were stimulated in the same culture medium plus 2 μM forskolin with recombinant mouse interferon-γ (rmIFN-γ, 100 U/mL, Hycult Biotechnology, Uden, Netherlands) and lipopolysaccharide (LPS, 1 μg/mL, Sigma) for 72 h and used thereafter for subsequent experiments. For the antibody-neutralization experiment, the SCs were stimulated as above in the presence of anti IL-6 antibody (1:500, Sigma) for 72 h. The purity of SCs was more than 90% as assessed by flow cytometric analysis using anti-S100 antibody staining (Dako, Glostrup, Denmark), and by microscopic examination, which revealed few contaminating fibroblasts. Although the 90% purity of SCs results from flow cytometry analysis, the remaining 10% were mainly the debris caused by the process of preparation.

Poly-L-lysine and Laminin Coating

Petri dishes were coated following a three-step procedure. First, 0.01% (w/v) poly-L-lysine (Sigma) was applied for 1 h at room temperature (RT). After discarding of the poly-L-lysine solution the surface was washed for three times with PBS. Second, mouse laminin (10 μg/mL, Invitrogen, Carlsbad, CA) was applied for 1 h at RT and then removed. Third, 10% fetal bovine serum in RPMI-1640 was applied for 1 h at RT.

Flow Cytometric Analysis of SCs

The SCs were harvested with 0.25% trypsin-EDTA (Gibco) and washed with 1% bovine serum albumin (BSA)

(Sigma) in PBS. The cells were incubated with PE-conjugated anti-mouse-CD40 and anti-mouse CD86 (both antibodies from Caltag, Burlingame, CA), as well as FITC-conjugated anti-mouse-MHC-II I-AK, and anti-mouse-CD80 (Serotec, Oxford, UK) antibodies for 30 min at 4°C. For staining of intracellular IL-6 production, the SCs were fixed with 2% paraformaldehyde for 20 min at 4°C and permeabilized with 0.5% freshly prepared saponin (Sigma) in PBS containing 1% BSA. The permeabilized cells were incubated with PE-conjugated anti-mouse-IL-6 (PharMingen, San Diego, CA) for 30 min at RT. FITC and PE conjugated isotype antibodies (both from Serotec) were used as negative controls. Cells were subsequently washed twice, resuspended in 1% paraformaldehyde in PBS and stored at 4°C until flow cytometric analysis by a FACScalibur cytometer using CellQuest software (Becton Dickinson, San Jose, CA). Surface molecule expression and intracellular IL-6 production were assessed by determining of the mean fluorescence intensities (MFI) of the SCs labeled with fluorescent antibodies directed against the respective molecules.

Isolation of P0 Peptide 180–199 Specific T Cells

P0 peptide 180–199 specific T cells were collected from the spleens of EAN mice immunized with P0 peptide 180–199. Briefly, wild-typed mice were immunized twice (day 0 and 7) by subcutaneous injection with 120 μg of P0 peptide 180–199 and 0.5 mg of Mycobacterium tuberculosis (strain H 37 RA; Difco, Detroit, MI) in 25 L saline and 25 L Freund's incomplete adjuvant (ICN Biomedicals, Aurora, CO). Additionally, mice received 400, 200, and 200 ng pertussis toxin (Sigma) by intravenous injection on day -1, 0, and +2 postimmunization, respectively. The spleens were removed on the day 30 postimmunization and single cell suspensions of mononuclear cells were prepared. Mononuclear cells suspensions were incubated in 25 cm² Falcon culture flasks (Becton Dickinson) with serum-free RPMI-1640 medium for 1.5 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.

Detection of T Cell Proliferation Cultured with SCs

After SCs were stimulated for 72 h with LPS and rmIFN-γ followed by centrifugation, the forskolin-contained medium in SCs culture was removed completely. SCs were harvested with 0.25% trypsin-EDTA (Gibco) and washed with 1% BSA (Sigma) in PBS. Subsequently, the SCs were irradiated with 2500 rad to delete their proliferative capacity and co-cultured with T cells (1.5 × 10⁵ T cells/200 μL/well) at a ratio of SCs:T cells = 1:10 in the presence of P0 peptide 180–199 (final concentration: 10 μg/mL) for 60 h in 96-well round-bottom microtitre plates (Nunc, Copenhagen, Denmark). Thereafter, cells were

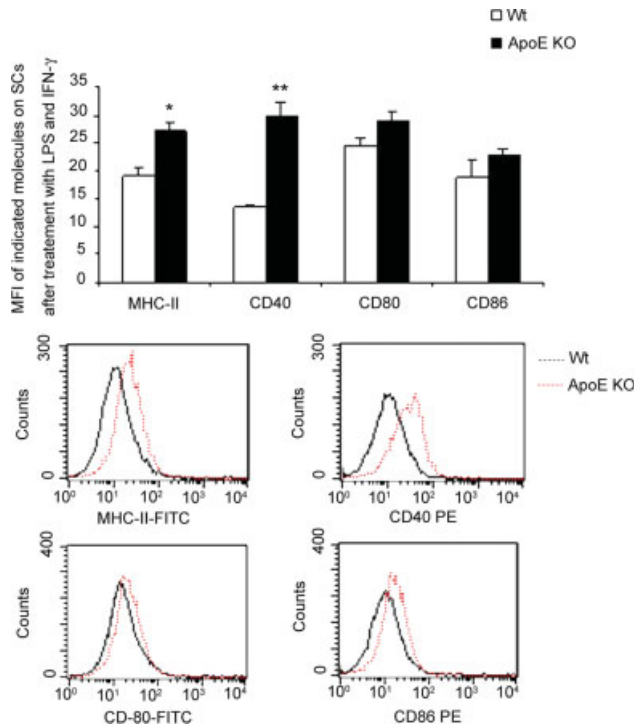


Fig. 1. SCs from 10- to 12-week-old wild-type and apoE KO mice were stimulated with LPS (1 $\mu\text{g}/\text{mL}$) and IFN- γ (100 U/mL) for 72 h. MFI values corresponding to the surface molecule expression of MHC-II, CD40, CD80, and CD86 were determined by flow cytometry. The data are presented as means \pm SD of one representative out of two independent experiments ($n = 4$ in each group). * $P < 0.05$ and ** $P < 0.01$. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

labeled for additional 24 h with 10 μL aliquots containing 1 μCi of [^3H] methylthymidine (Amersham, Little Chalfont, UK) before harvesting. The amount of radioactivity incorporated into DNA was measured in a β -scintillation counter. Cultures were run in duplicates and the results were expressed as cpm.

Statistics

Differences between two groups were tested by Student's t -test. All tests were two-sided. Values are expressed as means \pm SEM, and the level of significance was set at $P < 0.05$.

RESULTS

ApoE Deficiency Increases MHC-II and CD40 Expression on SCs

SCs of wild-type and apoE KO mice were stimulated with 100 U/mL rmIFN- γ and 1 $\mu\text{g}/\text{mL}$ LPS for 72 h and stained with fluorescent antibodies, and the MFI values corresponding to the respective surface molecule expressions were determined by flow cytometry. The MFI values of MHC-II and CD40 on SCs of apoE KO mice were higher compared with those of wild-type mice ($P < 0.05$

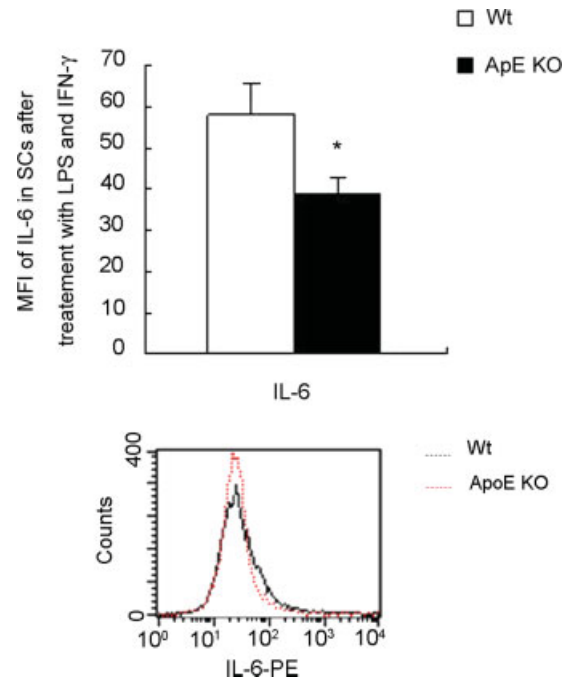


Fig. 2. SCs from 10- to 12-week-old wild-type and apoE KO mice were stimulated with LPS (1 $\mu\text{g}/\text{mL}$) and IFN- γ (100 U/mL) for 72 h. MFI values corresponding to the intracellular IL-6 expression of SCs were determined by flow cytometry. The data are presented as means \pm SD of one representative out of two independent experiments ($n = 4$ in each group). * $P < 0.05$. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

for both). However, only a nonsignificant tendency of increasing levels of MFI of CD80 and CD86 expression was seen in SCs of apoE KO mice compared with wild-type mice (Fig. 1).

ApoE Deficiency Decreases the Intracellular IL-6 Production of SCs

After stimulation with 100 U/mL rmIFN- γ and 1 $\mu\text{g}/\text{mL}$ LPS for 72 h, intracellular IL-6 production by SCs was detected by flow cytometry. The MFI for IL-6 was significantly lower in SCs of apoE KO mice compared with wild-type mice ($P < 0.05$) (Fig. 2).

Neutralization of Intracellular IL-6 Could Up-Regulate the Surface Molecular Expression of MHC-II and CD40 in SCs

Coincidentally with the observation described a neutralization experiment with anti IL-6 antibodies was performed. After exposure to anti IL-6 antibody for 72 h, the SCs were analyzed for CD40 and MHC-II expression by flow cytometry. There was a significant increase of MFI values of CD40 in the SCs of apoE KO compared with wild-type mice ($P < 0.05$) (Fig. 3). However, a significant

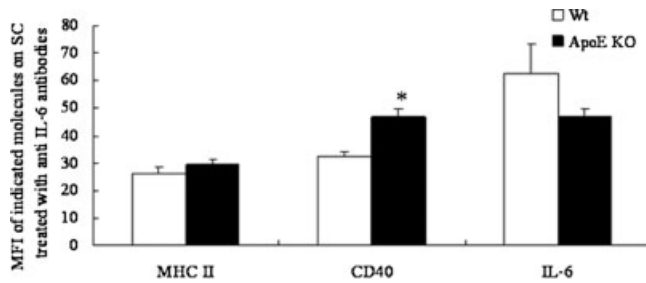


Fig. 3. SCs from 10- 12-week-old wild-type and apoE KO mice were stimulated with LPS (1 $\mu\text{g}/\text{mL}$) and IFN- γ (100 U/mL) and neutralized with the anti IL-6 antibody (1:500 dilution) for 72 h. MFI values corresponding to the expression of the surface molecules MHC-II and CD40 and of the intracellular molecule IL-6 were determined by flow cytometry. The data are presented as means \pm SD of one representative out of two independent experiments ($n = 4$ in each group). * $P < 0.05$.

up-regulation of MHC-II was not detected in the ApoE KO group compared with wild-type mice. We exposed the SCs to three different concentrations of anti IL-6 antibodies equivalent to dilutions of 1:250, 1:500, and 1:1000. The results were similar at each concentration in three independent experiments (data not shown).

ApoE Deficiency Enhances Antigen Presenting Capacity of SCs

To investigate the effect of apoE deficiency on the antigen-presenting capacity of SCs, SCs from apoE KO and wild-type mice were irradiated and co-cultured with P0 peptide 180–199 specific T cells from wild-type EAN mice in the presence of P0 peptide 180–199. T cells co-cultured with SCs of apoE KO mice showed a higher level of proliferation compared with T cells with SCs of wild-type mice ($P < 0.05$) (Fig. 4). This indicates that apoE deficiency enhances the antigen-presenting capacity of the SCs.

DISCUSSION

SCs have pleiotropic functions in the inflammatory demyelinating diseases of the PNS, such as GBS and CIDP. In addition to being the target of the autoimmune destruction, they can secrete trophic factors, cytokines like IL-1, IL-6, and IL-13, complement, and myelin proteins (Koski, 1997), which contribute to the axonal outgrowth and sprouting, neuronal survival, and immunoregulation within the PNS. In the recovery phases of the autoimmune demyelinating diseases, SCs express FasL and thereby contribute to the elimination of invading autoreactive T cells (Wohlleben et al., 2000). In contrast, previous studies revealed that SCs are able to express MHC-I and MHC-II after stimulation with inflammatory cytokines and to stimulate specific T cell proliferation (Armati et al., 1990; Gold et al., 1995; Lilje and Armati, 1997), which can be blocked by anti-MHC-II antibody (Gold et al., 1995). Here, we demonstrate that apoE deficiency increased MHC-II and CD40 expression on SCs, down-regulated the intracellular IL-6 production of SCs,

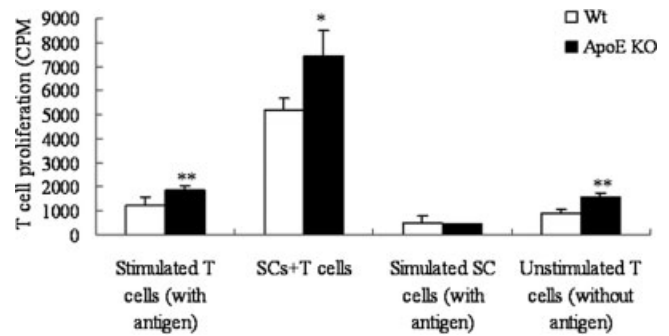


Fig. 4. SCs from 10- to 12-week-old wild-type and apoE KO mice, stimulated with LPS (1 $\mu\text{g}/\text{mL}$) and IFN- γ (100 U/mL) for 72 h and irradiated with 2500 rad, were co-cultured with P0 peptide 180–199 specific T cells from wild-type EAN mice for 60 h at ratio of 1:10 in the presence of P0 180–199 peptide. ^3H -thymidine incorporation was measured after a 24 h pulse with ^3H -methylthymidine. The data are presented as means \pm SD of one representative out of two independent experiments ($n = 4$ in each group). * $P < 0.05$ and ** $P < 0.01$.

and enhanced the antigen-presenting capacity of SCs, indicating that apoE can modulate the immune response within the PNS by acting on SCs, the nonprofessional antigen-presenting cells within the PNS.

It has been evidenced that apoE is involved in the autoimmune diseases. For example, the apoE $\epsilon 4$ allele is associated with a progression of disability in MS (Chapman et al., 2001). ApoE deficiency exacerbated experimental allergic encephalomyelitis (EAE), an animal model for MS, and EAN, as well as increased antigen specific T cell proliferation in both EAE and EAN (Karussis et al., 2003; Yu et al., 2004). Furthermore, increased antigen-presenting function of macrophages of apoE KO mice was found in EAN (Yu et al., 2004) and up-regulated expression of MHC-II, CD40 and CD80 on macrophages of apoE deficient mice resulted in enhancement of their antigen-presenting function (Tenger and Zhou 2003).

IL-6 is a pleiotropic cytokine with effects on cell growth, differentiation, and migration during the immune responses and inflammation (Hirano, 1998). It can orchestrate both pro- and anti-inflammatory processes because of its promoting effects on both IL-2 and IL-4 production (Jones, 2005). Recently, Park et al. (2004) demonstrated that IL-6 inhibits the dendritic cell maturation through STAT3 activation and they found an enhanced expression of MHC-II and CD86 on the dendritic cells of IL-6 KO mice. Additionally, Kitamura et al. (2005) discovered that IL-6-STAT3-mediated activation of the lysosomal enzyme cathepsin S decreased the intracellular H2-DM and MHC-II $\alpha\beta$ dimer levels in dendritic cells, which attenuated the subsequent CD4 $^+$ T cell activation. These findings show that IL-6 can inhibit MHC-II and CD 86 expression on the professional antigen presenting cells, but the mechanisms behind this phenomenon have not been clarified finally. In the current study, SCs as a source of IL-6 in the PNS (Koski, 1997) were observed to decrease the expression of intracellular IL-6 accompanied with higher levels of MHC-II and CD 40 expression on apoE deficient SCs. Moreover, an enhanced antigen-presenting function of SCs was found in apoE KO mice for

P0 peptide 180–199 specific T cell. These results were confirmed by exposure to anti IL-6 antibodies, by which treatment CD40 was up-regulated in the SCs of apoE KO mice compared with SCs of wild-type mice.

In conclusion, apoE deficiency increases CD40 and MHC-II expression on SCs. We hypothesize that this occurs via down-regulation of intracellular IL-6 production with the consequence of an enhancement of the antigen-presenting function of the SCs.

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