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**Role of apolipoprotein E isoforms and cytokines in  
immune responses and inflammation of the mouse  
peripheral nervous system**

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## ABSTRACT

Experimental autoimmune neuritis (EAN) as an animal model for Guillain-Barré syndrome (GBS) in humans is an immune-mediated disorder affecting the peripheral nervous system (PNS). Apolipoprotein E (apoE) is a glycosylated protein characterized by its wide tissue distribution and multiple biological functions. ApoE can suppress proinflammatory signalings, and vice versa, indicating an intricate apoE-mediated feedback regulation of inflammatory and immune responses. Immune cells together with cytokines produced by various cells contribute to the inflammatory process of EAN by acting as mediators or effectors.

In **Paper I**, the effects of apoE isoforms on the functions of immune cells were investigated. Clinical signs of EAN were most severe in wild type (WT) C57BL/6 mice and apoE4 transgenic (Tg) mice, followed by apoE2 Tg mice and apoE3 Tg mice (WT  $\approx$  E4 > E2 > E3). Proliferation tests of purified T cells from naive mice stimulated with phytohemagglutinin or interleukin (IL)-12 showed isoform-specific differences (WT  $\approx$  E4 > E3  $\approx$  E2). Macrophages from both naive and EAN mice produced nitric oxide (NO) upon inflammatory stimulation in an isoform-dependent manner (WT  $\approx$  E4 > E2 > E3). During the recovery stage of disease, the highest expression of CD178 (FasL) on Schwann cells (SCs) was found in apoE3 Tg mice. In **Paper II**, the effects of different isoforms of apoE on SCs in response to inflammatory stimulation (lipopolysaccharide plus interferon (IFN)- $\gamma$ ) were studied. Upon stimulation, a change in the morphology of cultured SCs was observed. Pronounced production of IL-6 and IL-10 within SCs, and increased levels of IL-6 and NO in culture supernatants were found in an isoform-dependent manner (apoE3 > apoE2  $\approx$  apoE4). Further results indicated that both nuclear factor kappa B (NF $\kappa$ B) and Akt signaling pathways were involved in the process by the same isoform-dependent pattern. In **Paper III**, the role of IFN- $\gamma$ , a signature T helper (Th)1 cytokine, in the pathogenesis of EAN was investigated. The clinical signs of EAN in IFN- $\gamma$  knockout (KO) mice were evidently aggravated. At the peak of EAN, the proportion of IL-17A expressing cells in cauda equina (CE) infiltrating cells, and the serum levels of IL-17A were elevated in IFN- $\gamma$  KO mice. The proportions of MHC II, macrosialin, and IL-12/IL-23p40 expressing cells, relative to total CE infiltrating cells were correspondingly higher in IFN- $\gamma$  KO than WT mice with EAN. In **Paper IV**, the role of tumor necrosis factor alpha (TNF- $\alpha$ ), another Th1 cytokine, in the pathogenesis of EAN was studied. TNF- $\alpha$  deficiency significantly attenuated EAN. Furthermore, TNF- $\alpha$  deficiency induced an antiinflammatory phenotype of macrophages (M2) characterized by reduced production of IL-12 and NO, and enhanced production of IL-10. Moreover, TNF receptor (TNFR)1 monoclonal antibodies markedly suppressed the severity of EAN when they were administered from the beginning of EAN induction.

In summary, our data support an isoform-dependent effect of apoE on EAN. This might be due to the isoform-specific effects of apoE on functions of T cells, macrophages and SCs, which contribute to the distinct clinical courses of EAN. SCs from apoE2 and apoE4 Tg mice bear some dysfunction in producing cytokines (IL-6 and IL-10) and NO as compared with their apoE3 counterparts, probably resulting from their insufficiency to suppress the activation of NF $\kappa$ B and Akt pathways. IFN- $\gamma$  deficiency exacerbates EAN via upregulating Th17 cells despite a mitigated systemic Th1 immune response. TNF- $\alpha$  exacerbates EAN via TNFR1 by inducing the proinflammatory phenotype of macrophage (classically activated macrophage, M1).

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