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Blockade of CD47 ameliorates autoimmune inflammation in CNS by suppressing IL-1-triggered infiltration of pathogenic Th17 cells

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ABSTRACT

The migration of Th17 cells into central nervous system (CNS) tissue is the key pathogenic step in experimental autoimmune encephalomyelitis (EAE) model. However, the mechanism underlying the pathogenic Th17 cell migration remains elusive. Here we report that blockade of CD47 with CD47-Fc fusion protein is effective in preventing and curing EAE by impairing infiltration of Th17 cells into CNS. However, CD47 deficiency does not directly impair the migration of Th17 cells. Mechanistic studies showed that CD47 deficiency inhibited degradation of inducible nitric oxide synthase (iNOS) in proteasome of macrophages by Src activation and led to the increased nitric oxide (NO) production. Then NO suppressed inflammasome activation-induced IL-1β production. This lower IL-1β reduces the expression of IL-1R1 and migration-related chemokine receptors on $CD47^{-/-}$ Th17 cells, inhibiting the ability of Th17 cells to infiltrate into the CNS of $CD47^{-/-}$ mice and therefore suppressing EAE development. *In vivo* administration of exogenous IL-1β indeed promoted the infiltration $CD47^{-/-}$ Th17 cells into CNS and antagonized the protective role of CD47 deficiency in EAE pathogenesis. Our results demonstrate a potential preventive and therapeutic application of CD47 blockade in controlling EAE development.

1. Introduction

Experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis (MS), is a myelin-specific autoreactive $CD4^+$ T cell-mediated chronic inflammatory autoimmune disease. Th17 cells have been ascribed as a greater pathogenic impact on the onset and maintenance of EAE than Th1 cells [1–3]. Cytokines and chemokines produced by the innate immune system are known to regulate the differentiation and migration of these Th1 and Th17 cells, however, the detailed mechanisms underlying how these activated T helper cells are then recruited into the CNS remains largely unknown.

Inflammasomes, the innate immune system receptors and sensors, process the proinflammatory cytokines, such as interleukin (IL) -1β and IL-18 in response to pathogens and stresses, link a variety of autoimmune diseases, including MS or EAE [4–6]. IL-1β can induce IL-17 production by synergizing with IL-23 signal [7,8], promote the differentiation of naïve $CD4^+$ T cells into Th17 cells, and directly augment IL-17 production by activated memory $CD4^+$ T cells expressing IL-1R1 [9,10]. $IL\text{-}1R1^{-/-}$ mice exhibit the reduced IL-17 production and are resistant to developing EAE [11], suggesting a connection between IL-1 signaling and Th17 cell function.

Abbreviations: CNS, central nervous system; DCs, dendritic cells; EAE, experimental autoimmune encephalomyelitis; IL, interleukin; iNOS, inducible nitric oxide synthase; *i.p.*,intraperitoneal, *i.v.*,intravenously; LN, lymph node; MS, multiple sclerosis; NO, nitric oxide; Q-PCR, quantitative RT-PCR; SIRPα, signal-regulatory protein alpha; TSP-1, thrombospondin-1.

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Furthermore, mice deficient in NLRP3 are resistant to EAE with decreased immune cell infiltration into the CNS [12]. In contrast to the role of IL-1β in exacerbating EAE pathogenesis, nitric oxide (NO) plays an essential role to negatively regulate the immune responses, as deficiency in inducible nitric oxide synthase (iNOS) leads to the increased EAE susceptibility [13]. We previously reported that NO, produced by regulatory dendritic cells (DCs), inhibited mature DC-initiated T cell proliferation [14], which was further confirmed in a later study showing the pro-apoptotic function of NO on autoreactive T cells [15]. Though many kinds of cellular and molecular components of innate and adaptive immunity have been proposed to be important in the pathogenesis of autoimmune diseases, the mechanism for Th17 cell migration into the CNS, a key step in EAE development, remains to be further illuminated.

CD47 is ubiquitously expressed on hematopoietic and non-hematopoietic cells and physically associated with several

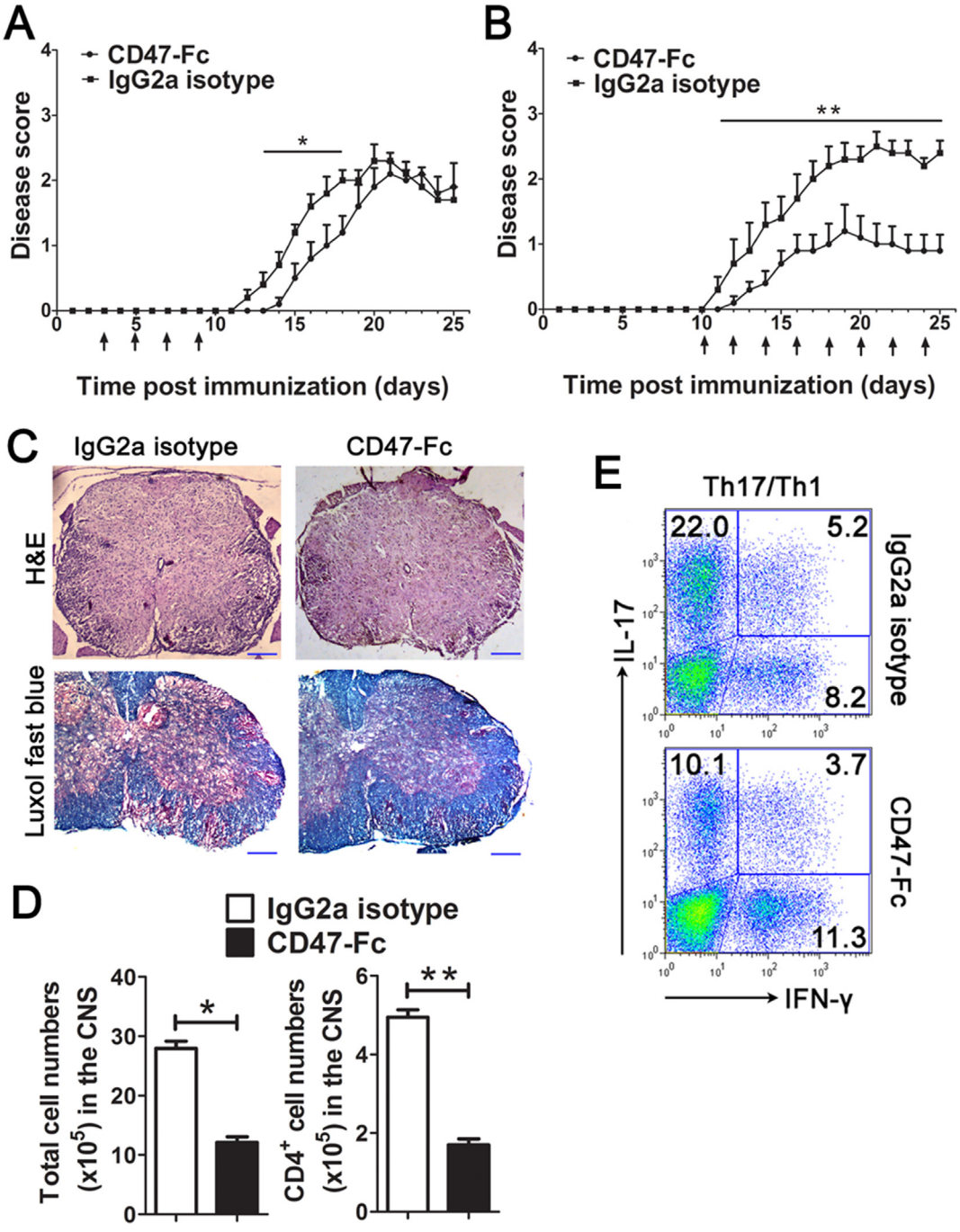

Fig. 1. Blockade of CD47 attenuates pathogenesis of EAE. (**A**, **B**) Clinical EAE scores (mean ± S.D) of WT mice at various times after immunization with MOG_{35-55} in CFA. Mice were *i.p.* injected with 200 μg of either CD47-Fc fusion protein or IgG2a isotype control every other day from day 3 to day 9 (arrows) for the prevention protocol (**A**) and day 10 to day 24 (arrows) for therapeutic protocol (**B**). For prevention protocol $^{*}P < 0.05$ from day 14 to 18 by Mann-Whitney test; for therapeutic protocol, $^{**}P < 0.01$ from day 11 to 25 by Mann-Whitney test. (**C**) Histopathology of CNS sections at day 18 after immunization of mice that received IgG2a isotype control or CD47-Fc fusion protein on the therapeutic protocol; sections were stained with H&E for inflammation (upper) (scale bars: 200 μm) and Luxol fast blue (lower) to evaluate the degree of demyelination (scale bars: 100 μm). (**D**, **E**) Total cell numbers and $CD4^{+}$ cell numbers (**D**) as well as Th17 and Th1 cell percentage (**E**) on day 18 in the CNS of WT mice treated with IgG2a isotype control or CD47-Fc fusion protein on the therapeutic protocol. $^{*}P < 0.05$; $^{**}P < 0.01$ (two-tailed Student's t-test).