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IL-25 regulates the polarization of macrophages and attenuates obliterative bronchiolitis in murine trachea transplantation models

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ABSTRACT

Obliterative bronchiolitis (OB) remains the major limitations for the long-term survival of allografts after lung transplantation. Th17 cells and IL-17 have been recognized as mediators of the development of OB in both animal models and human beings. IL-25, also called IL-17E, is the only anti-inflammatory cytokine of the IL-17 family, capable of regulating Th17 cells function in autoimmune inflammations. Whether IL-25 affects Th17 cells responses and the development of OB remains poorly understood. Acute rejection (AR) of the lung allograft has been regarded as the main problem for the development of OB, in which infiltrations of monocytes/macrophages play important roles. This study explored the potential role of IL-25 in regulation of macrophages polarization and inhibition of IL-17 production in the progression of OB. Here, we showed that IL-25 directly suppressed the expression of inflammatory cytokines, such as IL-6, IL-23, TNF-α, and IL-1β in LPS-induced pro-inflammatory M1 macrophages in vitro. In vivo data demonstrated that IL-25 deficiency promoted the polarization and function of M1 macrophages and aggravated the progression of OB in murine models of both orthotopic and heterotopic trachea transplantation. In conclusion, these data indicated that IL-25 attenuated OB by suppressing the function of M1 macrophages and IL-17 expression, providing an alternative strategy to intervene the development of OB.

1. Introduction

Lung transplantation remains the only curative strategy for patients with end-stage lung failure. However, the development of obliterative bronchiolitis (OB) or bronchiolitis obliterans syndrome (BOS) following lung transplantation is a key factor which contributes to patients' mortality, affecting up to 50–60% of patients who survive 5 years after lung transplantation [1]. The etiology of OB characterized by an irreversible decline in allograft function due to fibrotic remodeling of small airways is poorly understood.

Th17 cells (interleukin-17 (IL-17)-producing helper T cells) are highly pro-inflammatory cells that are critical for inducing multiple autoimmune diseases [2]. Recent studies revealed the importance of IL-17 and Th17 cells in lung transplantations [3–5]. Elevation of IL-17 mRNA and protein were found in the bronchoalveolar lavage during acute rejections [6]. Data from a large cohort study of 132 lung transplant recipients showed high level expressions of IL-17 and IL-21 in bronchoalveolar lavage biopsies with BOS [4]. Inflammatory cytokine IL-6 plays a vital role in the differentiation of Th17 cells, whereas IL-23 controls Th17 cells expansion or survival, endowing Th17 cells with pathogenic functions [7,8]. In our previous report, we found that blockade of IL-23/IL-17 pathway could attenuate airway obliteration in rat orthotopic trachea transplantation (OTT) [9]. Therefore, modulating the Th17 cells responses and IL-17 production may alleviate the pathology and development of OB.

Acute rejection (AR) of the lung allograft has been proved to be a major risk for the development of BOS [10]. Infiltrations of monocytes/macrophages are recognized as a hallmark of acute allograft rejection [11,12]. It has been revealed that absence of immunosuppressive macrophages may increase the susceptibility of human lung allografts to the rejection process [13]. Based on their responses to environmental stimuli, macrophages can be divided into different populations with distinct functions as classically activated macrophages (M1) and alternatively activated macrophages (M2) [14]. M1, stimulated by LPS or IFN-γ, can secrete high levels of pro-inflammatory cytokines and mediators, such as, TNF-α, IL-1β, IL-6, IL-23, CCL3, iNOS (NOS2) and

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express surface marker CD86. M2 is characterized by producing high levels of anti-inflammatory mediators, such as IL-10, arginase-1 (Arg-1), mannose receptor (MRC1; CD206), TGF-β and a set of chemokines, including CCL17, CCL22 and CCL24. M2 promote tissue remodeling and have immunoregulatory functions. [14].

IL-25, also called IL-17E, is the only anti-inflammatory cytokine of the IL-17 family, was originally identified by James Lee [15], capable of promoting Th2 cell activation and considered to be crucial for both adaptive and innate immune responses [16]. IL-25 exerts its activities by the receptor complex of IL-17-RA and IL-17-RB [17]. Recent study showed that IL-25 could suppress effector macrophages, induce M2 phenotype and reduce renal injury in proteinuric kidney disease [18]. Moreover, IL-25 was demonstrated to limit the expansion of Th17 cells in the intestine by suppressing the expression of macrophage-derived IL-23 [19,20]. Study also revealed that IL-25 inhibited IL-17 production and attenuated experimental encephalitis autoimmune disease (EAE) by regulating the function of dendritic cells (DCs) [21]. These data demonstrated that IL-25 directly regulates the function of macrophages and DCs, and indirectly, suppresses IL-17 production, thus attenuates Th17 cell related diseases.

In this study, we showed that IL-25 directly inhibited the expression of LPS-induced inflammatory cytokines, such as, IL-6, IL-23, TNF-α, and IL-1β in bone marrow derived macrophages (BMDMs) in vitro. In vivo data demonstrated that IL-25 deficiency up-regulated the percentages of M1 macrophages, increased the frequency of Th17 cells and aggravated the progression of OB in murine models of OTT and heterotopic trachea transplantation (HTT). Therefore, IL-25 may regulate the function of macrophages and suppress IL-17 expression in vivo, providing an alternative strategy to attenuate the development of OB.

2. Materials and methods

2.1. Mice

Specific pathogen-free, female and male mice (C57BL/6; BALB/c) were purchased from Shanghai Laboratory Animal Company (Shanghai, China). IL-25$^{-/-}$ mice (C57BL/6 background) were kindly provided by Prof. Chen Dong (The University of Texas MD Anderson Cancer Center, Houston, TX). 8–12-week-old mice were used and cared for in this experiment. All experiments were performed in accordance with protocols approved by the institutional animal care and use committee of Tongji University.

2.2. Orthotopic trachea transplantation

OTT was performed as previously described [22,23]. Briefly, after the donors (BALB/c mice) were euthanized by intraperitoneally injecting pentobarbital, the trachea of donor was separated from the esophagus by blunt dissection, excised surgically from the second trachea ring to the ninth and placed in DPBS (Corning, USA) until transplantation via a midline cervical incision. This eight-ring donor trachea was implanted end-to-end into the recipient wild-type (WT) C57BL/6 mice or IL-25$^{-/-}$ C57BL/6 mice. WT C57BL/6 mice without transplantation were used as normal control. Donor C57BL/6 mice to recipient C57BL/6 mice were served as the isograft group. Donor BALB/c mice to recipient C57BL/6 mice were served as the allograft group. Mice were sacrificed on day 7, 14 and 30 after transplantations and grafts were removed by blunt dissection. Three mice were included in each group and each separate experiment was repeated for three times. The grafts were harvested half

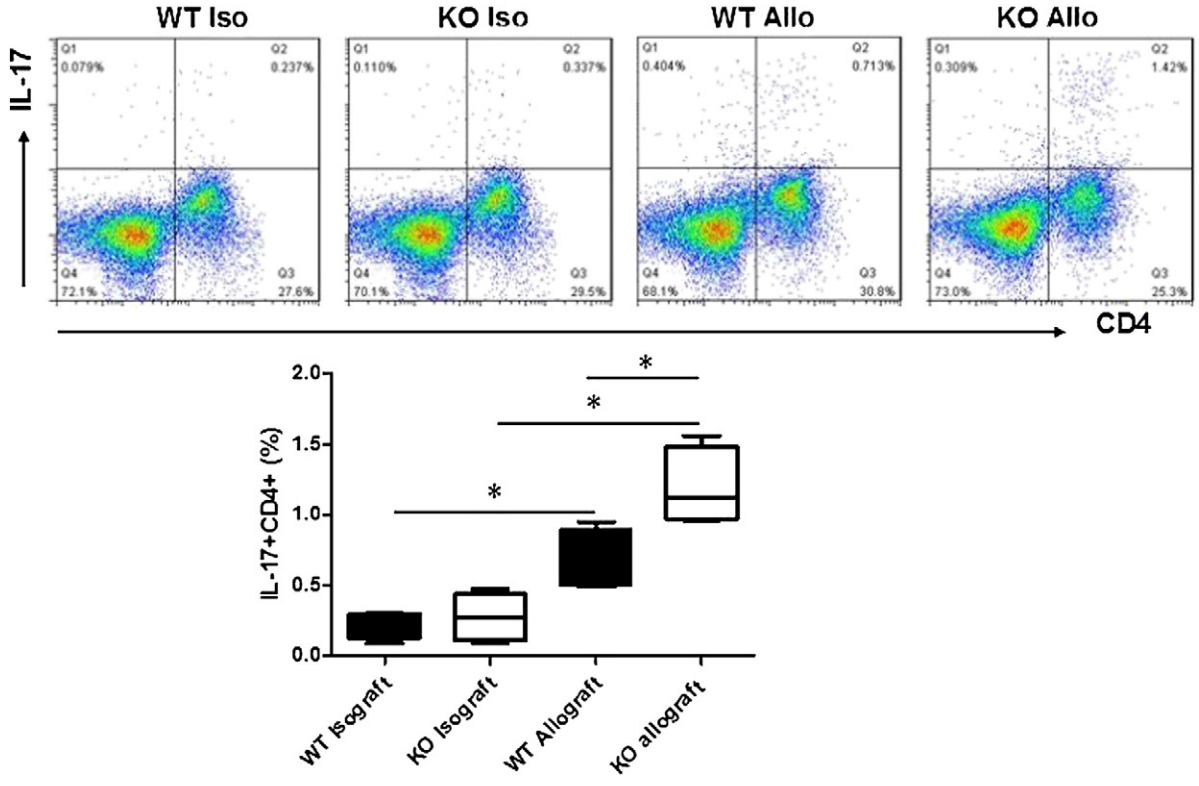

Fig. 1. IL-25 deficiency increased the percentages of Th17 cells in spleens of OTT mice. Mice with isografts and allografts after OTT were killed on Day 7. WT Iso = C57BL/6 WT mice with syngeneic graft; KO Iso = IL-25$^{-/-}$C57BL/6 mice with syngeneic graft. WT Allo = C57BL/6 WT mice with allogeneic graft; KO Allo = IL-25$^{-/-}$C57BL/6 mice with allogeneic graft. The percentages of CD4 + IL-17 + in splenocytes were analyzed by flow cytometry. For intracellular staining, cells were counted and seeded 3×10^6 for each well in 48-well plate. Cells were stimulated with ionomycin and PMA for 1 h, and with monensin for another 3 h. Values indicated mean ± SEM of three separate experiments and representative data. $^*P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$.