International Immunopharmacology 52 (2017) 51–60

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

# IL-17 contributes to the pathogenesis of obliterative bronchiolitis *via* regulation of M1 macrophages polarization in murine heterotopic trachea transplantation models

Qingshu Meng[a,b,c,1], Jie Liu[c,1], Fang Lin[a,b], Luer Bao[c], Yuyu Jiang[a,b], Liang Zheng[a,b], Jinjun Tie[c], Lin Zhang[a,b], Xiaoting Liang[a], Lu Wei[a,b], Yuan Li[a,b], Huimin Fan[a,b,c], Xiaohui Zhou[a,b,*]

[a] Research Center for Translational Medicine, Shanghai East Hospital, Tongji University School of Medicine, Shanghai, China
[b] Shanghai Heart Failure Research Center, Shanghai, China
[c] Department of Cardiovascular and Thoracic Surgery, Shanghai East Hospital, Tongji University School of Medicine, Shanghai, China

ARTICLE INFO

*Keywords:*
IL-17
Macrophages
Obliterative bronchiolitis
Trachea transplantation

ABSTRACT

Acute allograft rejection is a principal conundrum in lung obliterative bronchiolitis (OB). Monocytes/macrophages infiltration has been proved to be the main reason for acute rejection. IL-17 contributes to the recruitment and function of macrophages. However, the mechanism of IL-17 underlying OB progression remains elusive. In the present study, we showed that the deficiency of IL-17 attenuated the pathology of murine heterotopic trachea allografts. Compared to WT recipients, *IL-17*$^{-/-}$ mice displayed higher frequency of CD206 + cells and lower ratio of CD86 + cells among F4/80 + macrophages in allografts and spleens on day 7 post heterotopic trachea transplantation. Moreover, mRNA levels of pro-inflammatory cytokines including *IL-6*, *TNF-α*, and *IL-1β* decreased in allografts of *IL-17*$^{-/-}$ recipients, but these of *MRC1* and *Arg-1* increased in comparison with WT. IL-17 deficiency can inhibit LPS induced M1 while promote IL-4 induced M2 polarization of bone marrow-derived macrophages. Further data demonstrated that the deficiency of IL-17 suppressed the lipopolysaccharide-induced M1 polarization and function through prevention of phosphorylation of both STAT3 and STAT5. Therefore, IL-17 contributes to OB pathogenesis through regulating macrophages function, thereby it may unravel part of the complexity of IL-17 in OB and enhance future therapeutic development.

## 1. Introduction

Chronic graft dysfunction has always affected long-term allograft survival following lung transplantation. OB and bronchiolitis obliterans syndrome (BOS) contribute most to the risk of this survival. BOS was reported in 49% and 75% of the recipients, at five and ten years after transplantation, respectively [1]. The mechanisms of OB after lung transplantation have not been fully understood. Acute lung allograft rejection has become a main problem in OB progression [2]. During acute rejection (AR), the initial responses are mediated by macrophages. Monocytes/macrophages infiltration is thought to be the marker of acute allograft rejection [3,4]. Depending on different environmental stimuli, macrophages can differentiate into various types with diverse functions, including classically activated macrophages (M1) and alternatively activated macrophages (M2) [5]. Recently, macrophages with different phenotypes and functions have attracted more and more attention. Previous studies demonstrated that macrophages can secrete pro-inflammatory cytokines and display important effects on innate immune responses associated with OB [6–9]. Lack of immunosuppressive macrophages would make lung allografts more susceptible to acute rejection [10].

IL-17, known as IL-17A, was a representative T-helper cell 17 (TH17) cytokine, and was related to OB development in both animal model and human. Some researchers have demonstrated the importance of IL-17 in lung transplantation [11]. In bronchoalveolar lavage during AR, mRNA and protein expressions of IL-17 showed an increase [12]. In addition, from the very early stage post lung transplantation, IL-17 expression can be identified through biopsies [13].

*Abbreviations:* OB, obliterative bronchiolitis; H & E, hematoxylin-eosin; HTT, heterotopic trachea transplantation; KO, knock-out; LPS, lipopolysaccharide; WT, wild-type; BMDMs, bone marrow-derived macrophages

* Correspondence to: X. Zhou, Research Center for Translational Medicine, Shanghai East Hospital, Tongji University School of Medicine, Shanghai Heart Failure Research Center, No. 150, Jimo Rd., Pudong, Shanghai 200120, China.
*E-mail address:* zxh100@tongji.edu.cn (X. Zhou).
[1] Q.M. and J. L. contribute equally to this work.

http://dx.doi.org/10.1016/j.intimp.2017.08.022
Received 14 March 2017; Received in revised form 2 August 2017; Accepted 25 August 2017
Available online 31 August 2017
1567-5769/ © 2017 Elsevier B.V. All rights reserved.

Our previous results found that block of IL-17 by anti-IL-23 antibody (Ab) can attenuate OB in rat orthotopic trachea transplantation (OTT) models [14]. Following researches demonstrated that block of IL-17 using gene therapy of lentivirus IL-10 or IL-17A: Fc fusion protein neutralization could abate AR in murine heterotopic trachea transplantation (HTT) models [15,16]. Furthermore, inhibition of IL-17 by iTregs transfer can prolong allografts survival time and attenuate AR progression [17] and block of IL-17 by IL-17A antagonistic antibody can reduce the incidence of OB in lung allografts [18]. However, the mechanism of IL-17 underlying OB remains poorly understood.

IL-17 plays a role through binding to IL-17RA and IL-17-RC (receptor complex) [19,20]. Previous report found that IL-17 induced inflammation was mainly mediated by neutrophils [21]. Recent results indicated that IL-17 is of high importance in macrophage recruitment and production of cytokines, thus it can combine innate and acquired immunity [22–25]. Another report indicated that through IL-17 and IL-17RA/IL-17RC, the lung tumor tissues can induce primary peritoneal macrophages as well as RAW264.7 macrophages migration [25]. In addition, IL-17 also participates in proinflammatory cytokine production in macrophages induced by BCG [24]. These results suggested that macrophages were involved in inflammatory responses induced by IL-17.

In this current research, possible functions and polarizations of macrophage induced by IL-17 were studied, and potential mechanism of M1 macrophages in phenotype and function alterations induced by IL-17 was evaluated. From above all, these results indicate that IL-17 may contribute to the pathogenesis of OB by regulating the function of macrophages.

## 2. Materials and methods

### 2.1. Mice

Nine to ten week old specific pathogen-free (C57BL/6; Balb/c) male mice were obtained from Shanghai Laboratory Animal Company (Shanghai, China). *IL-17*$^{-/-}$ (*IL-17A*$^{-/-}$) mice (C57BL/6 background) were obtained from Prof. Chen Dong in University of Texas MD Anderson Cancer Center. Study protocols were reviewed and approved by the Institutional Animal Care and Use Committee of Tongji University.

### 2.2. Heterotopic trachea transplantation

HTT was performed as described before [26]. *IL-17A*$^{-/-}$ or WT mice were dealt to have vertical incisions on bilateral back. Each subcutaneous pocket was implanted with a four-ring donor trachea through incision. WT C57BL/6 mice were set as controls without grafts. Grafts were removed following animals sacrifice on day 7 after transplantation. Both control and isograft groups consist of 3–5 mice. All allograft groups consist of 5–7 mice. Each experiment was performed for at least 3 times. TRIzol (Ambion life technologies, USA) was used to harvest grafts for qRT-PCR. Tissues used for histological analysis were fixed in paraformaldehyde, followed by hematoxylin-eosin (H & E) staining.

### 2.3. Histological analysis

Trachea allografts were harvested on day 7 after surgery and were processed with fixation and paraffin embedding. Following that, H & E was used for 5-μm-thick sections staining.

### 2.4. Murine spleen and allograft mono-nuclear cells isolation

The spleen was minced and single cells were harvested. The trachea was minced and incubated with 200 U/mL collagenase VI, 50 U/mL DNase I, and 0.1 M EDTA for half an hour at 37 °C. The obtained suspension was applied to the Mouse Lymphocyte Separation Medium (DAKEWE, China) and was centrifuged for half an hour at 400*g*. Mononuclear cells were then isolated from the interface of the top and middle layer and washed for subsequent stimulation or staining with fluorescence-conjugated antibodies.

### 2.5. Isolation and culture of murine bone marrow-derived macrophages (BMDMs)

The murine BMDMs were isolated as previously described [7]. WT or *IL-17A*$^{-/-}$ mice aged 6 to 8 weeks were used to obtain femurs. BMDMs were co-cultured with 10% FBS, 30% L929 and 60% DMEM in suspension. Then IL-4 (20 ng/mL; PEPROTECH, USA), lipopolysaccharide (LPS, 100 ng/mL; Sigma, USA) or IL-17 (100 ng/mL; PEPROTECH, USA) was used to treat the cells. qPCR was performed with production of 4 h treatment and flow cytometry was performed using production of 16 h treatment.

### 2.6. Cell staining and flow cytometry

BMDMs, allograft and spleen single cell suspension following HTT was prepared. Antibodies of fluorescence conjugation against F4/80, CD86, and CD206 (BioLegend, USA) were used. For intracellular staining, PMA of 200 ng/mL and ionomycin of 250 ng/mL were applied to stimulate splenocytes for 1 h, after that monensin (1:1000) (Sigma, USA) was used for another 3 h and then splenocytes were harvested. IL-10 and TNF-*α* antibodies of fluorescence conjugation (BioLegend, USA) were used for splenocytes. Data were collected using a FACScan flow cytometer (BD Biosciences, USA). Gates were set on the population of mono-nuclear macrophages and FlowJo Software (TreeStar, USA) was used for analysis.

### 2.7. Macrophages depletion

As previously described, macrophages functional depletion was performed [27]. Briefly, 48 h and 24 h before the trachea transplantation intravenous injection was performed using empty liposomes or 250 μL clodronate liposomes (see http://clodronateliposomes.com).

### 2.8. Quantitative RT-PCR (qRT-PCR)

PrimeScript RT reagent Kits (TaKaRa, Japan) were applied to reversely transcribe RNAs (1 μg) isolated from BMDMs and trachea to cDNA. Fast Real-Time PCR System (7900HT; Applied Biosystems, Singapore) and SYBR Green MasterMix (Applied Biosystems, UK) were applied for qRT-PRC. Analysis was performed [28], and primers were listed below:

*mGAPDH*,5′-AACTTTGGCATTGTGGAAGG-3′, 5′-ACACATTGGGGGTAGGAACA-3′;

*mIL-1β*,5′-CGAGGCTAATAGGCTCATCT-3′, 5′-GTTTGGAAGCAGCCCTTCAT-3′;

*mTNF-α*,5′-AGCCGATGGGTTGTACCTTGTCTA-3′, 5′-TGAGATAGCAAATCGGCTGACGGT-3′;

*mIL-6*,5′-TGATGCACTTGCAGAAAACA-3′, 5′-ACCAGAGGAAATTTTCAATAGGC-3′;

*mArg-1*,5′-CAGAAGAATGGAAGAGTCAG-3′, 5′-CAGATATGCAGGGAGTCACC-3′;

*mMRC1*,5′-AACAAGAATGGTGGGCAGTC-3′, 5′-AACTCCTCGTCCGTCTGTC-3′.

### 2.9. Nitrite assay

A colorimetric method was used to measure Nitrite. A standard curve based on complete-medium cultured sodium nitrite was prepared to evaluate nitrite concentration through comparison with samples [29].

Download English Version:

https://daneshyari.com/en/article/5555241

Download Persian Version:

https://daneshyari.com/article/5555241

Daneshyari.com