

## Original Article

# IL-21 does not involve in OVA-induced airway remodeling and chronic airway inflammation

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Received May 18, 2015; Accepted July 10, 2015; Epub July 15, 2015; Published July 30, 2015

**Abstract:** Interleukin (IL-21) is a member of the type I cytokine family with sequence homology to IL-2, IL-4, and IL-15. IL-21 has been reported to improve symptoms of allergic rhinitis in mice. In this study we examined whether IL-21 signaling involved in allergic airway inflammation and remodeling *in vivo* by using ovalbumin (OVA)-induced chronic asthma model. We showed IL-21 level was increased in the serum of asthma models but not bronchoalveolar lavage fluid (BALF). Intranasal administration with recombinant mouse IL-21 or anti-IL-21 receptor (IL-21R) antibody did not affect OVA-induced chronic airway inflammation and airway remodeling *in vivo* and also not affect the expression of IL-13 and TGF- $\beta$  in BALF. Moreover, expression of IL-13 and TGF- $\beta$  was not affected by intranasal administration with recombinant mouse IL-21 or anti-IL-21R antibody. These results indicated that IL-21 signaling might not play an important role in airway inflammation and remodeling.

**Keywords:** IL-21, airway remodeling, airway inflammation

## Introduction

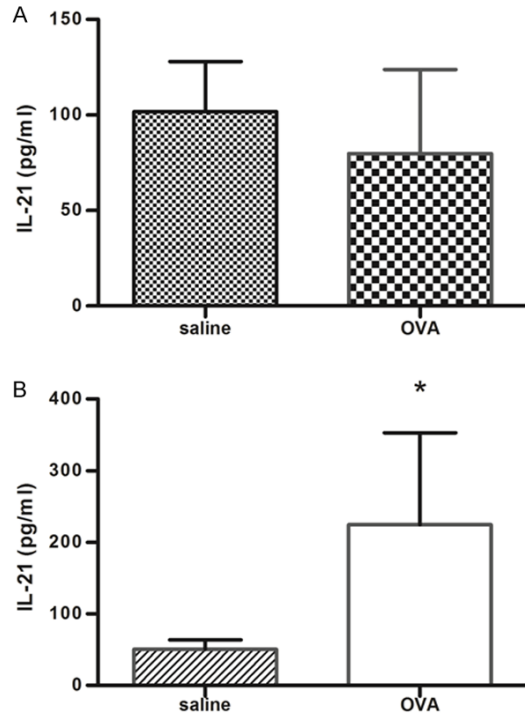
Asthma is a chronic disease characterized by airway inflammation, airway hyperresponsiveness (AHR) and airway remodeling. However, the mechanisms that underlie the development of airway inflammation and airway remodeling are poorly understood. Allergic airway inflammation mainly manifests as airway infiltration of leukocytes such as eosinophils, lymphocytes, and macrophages [1]. Airway remodeling refers to pathologic structural changes such as subepithelial fibrosis, increase of smooth muscle mass in the airway, and increased vascularization [2]. Airway remodeling has been considered to result in more rapid decline of lung function in some asthmatic patients [3]. However, there is no effective therapeutic agent preventing airway remodeling, which urges us to look for new therapeutic targets for asthma.

Interleukin (IL)-21 is a member of the type I cytokine family with sequence homology to IL-2,

IL-4, and IL-15 [4]. The IL-21 receptor complex is composed of two subunits. The common  $\gamma$ -chain subunit is shared with IL-2, IL-4, IL-7, IL-9, IL-13, IL-15 and the private IL-21 receptor (IL-21R) [4, 5]. IL-21 is predominantly produced by activated CD4<sup>+</sup> T cells and natural killer (NK) T cells [6]. IL-21R is expressed by a variety of lymphohematopoietic cells [4]. IL-21 exerts a variety of regulatory effects. Several studies have indicated that IL-21 regulates the differentiation, growth, and activation of Th1 [7-10], Th2 [11], CTL [12] and NK cells [10].

Some previous studies reported that IL-21 suppressed IgE production by B cells. The mice deficient for the IL-21 [13] or the IL-21R [14] gene exhibited enhanced IgE production in response to Antigen (Ag) immunization. Similarly, Hiromura Y et al showed that IL-21 administration into the nostril improved symptoms of allergic rhinitis in mice through preventing of IgE production by B cells and eotaxin production by fibroblasts [15].

## IL-21 does not involve in chronic asthma



**Figure 1.** IL-21 is increased in plasma but not BALF from OVA-challenged mice. IL-21 concentrations in BALF (A) and plasma (B) were assessed 24 hours after the last challenge, as described in Methods. Results were presented as means  $\pm$  SEM. N = 4-6 mice per group; \*P < 0.05 compared with saline-challenged group.

In our previous study, we have demonstrated that administration of IL-21 alone may not alleviate acute allergic lung inflammation [16]. Nevertheless, the effect of IL-21/IL-21R signaling on chronic airway inflammation and airway remodeling has not been explored. Consequently, the goal of the current study is to investigate the effect of IL-21/IL-21R signaling on chronic airway inflammation and airway remodeling. In this study, we used a chronic allergen-induced asthma model and administered them with recombinant mouse IL-21 (rmIL-21) and anti-IL-21R antibody (anti-IL-21R Ab) to examine the *in vivo* effects of IL-21/IL-21R signaling.

### Methods

#### Animals and experimental protocol

Female BALB/c mice were sensitized by intraperitoneal injections of ovalbumin (OVA) (Sigma, St. Louis, MO) on day 0 (0.02 mg/mouse) and day 14 (0.2 mg/mouse). On day 21, mice were

exposed to intranasal OVA challenges (1 mg in 0.2 ml saline) or saline used as control 3 times a week for 4 weeks [17]. Intranasal administration of IL-21 (20 ng/mouse, PeproTech, Rocky Hill, NJ), anti-IL-21R Ab (1  $\mu$ g/mouse, Santa Cruz) or control IgG was performed under light anesthesia 30 minutes before each OVA challenge. All experiment procedures were performed according to the requirements of the Animal Care and Ethics Committee of Tongji Hospital.

#### Bronchoalveolar lavage and lung histology

Bronchoalveolar lavage fluid (BALF) was collected by cannulating the upper part of the trachea, followed by lavage with 1 ml of PBS. Different cell counts were performed on Wright-Giemsa-stained cytopins. The supernatant was stored at  $-80^{\circ}\text{C}$ . To evaluate the extent of inflammation, lung sections were stained with hematoxylin and eosin. Gomori trichrome stain was used to evaluate collagen deposition.

#### Determination of AHR

Airway responsiveness to inhaled methacholine was measured with FlexiVent (SCIREQ Scientific Respiratory Equipment, Inc. Montreal, PQ, Canada) [18]. Mice were anesthetized by intraperitoneal injection of pentobarbital, then tracheostomized and connected to the flexi-vent. Baseline airway resistance was measured in each mouse after nebulization of PBS (vehicle for methacholine) for 10 seconds using an Aeroneb ultrasonic nebulizer (SCIREQ). After baseline measurements, mice were exposed to increasing concentrations of nebulized methacholine.

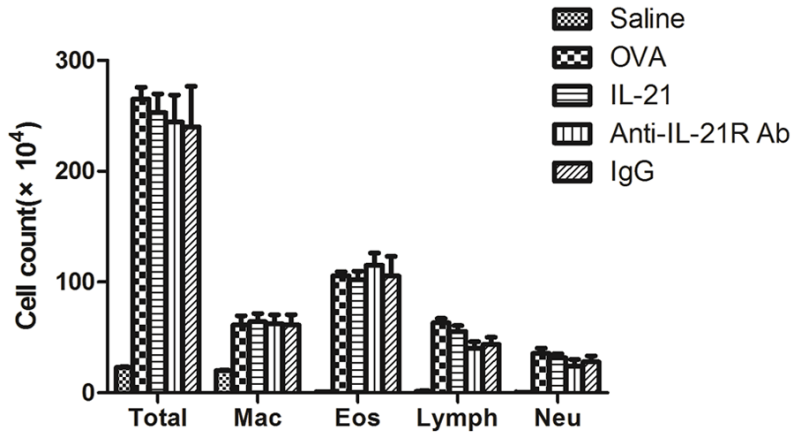
#### Measurement of BALF and serum cytokines

The levels of IL-13 and TGF- $\beta$  in BALF and serum were determined by ELISA (Ebioscience) according to the manufacturer's instructions.

#### Statistical analysis

Data were expressed as means  $\pm$  SEM. Variance analysis was used for statistical comparisons between groups, followed by the Tukey test using GraphPad Prism 5 Software; *p* values of less than 0.05 were considered to indicate statistical significance.

## IL-21 does not involve in chronic asthma



**Figure 2.** IL-21 or Anti-IL-21R Ab treatment does not affect leukocyte accumulation in BALF. The number of inflammatory cells in BALF was determined 24 hours after the last challenge, as described in Methods. Saline; normal control mice treated with saline only; OVA; OVA-sensitized/challenged mice; IL-21; IL-21 treatment and OVA-sensitized/challenged mice; IgG; IgG treatment and OVA-sensitized/challenged mice; Anti-IL-21R Ab; Anti-IL-21R Ab treatment and OVA-sensitized/challenged mice. All data represent means  $\pm$  SEM. N = 4-6 mice per group.

### Results

#### *IL-21 level is increased in plasma from OVA-challenged mice*

To determine whether chronic allergic airway inflammation led to change of IL-21 level, mice were sensitized and challenged with OVA and IL-21 level was determined. As shown in **Figure 1**, compared with those from Saline-challenged mice, levels of IL-21 in serum from OVA-challenged mice were increased significantly while the level of IL-21 from BALFs was not different.

#### *Administration of IL-21 or anti-IL-21R Ab does not reduce lung inflammation and alleviate airway remodeling*

We next determine the effect of IL-21 or anti-IL-21R Ab on lung inflammation and airway remodeling. Compared with control mice challenged with saline, OVA-challenged mice exhibited a significant increase in total leukocyte numbers detected in BALF 24 h after last provocation; differential cell counts revealed that this was due primarily to infiltration of eosinophils, and a small amount of macrophages and lymphocytes (**Figure 2**). IL-21 administration had no effect on total cell counts and eosinophils counts, as well as macrophages, lymphocytes

and neutrophils counts. As compared with IgG, treatment with anti-IL-21R Ab did not affect the counts of total cells, eosinophils, macrophages, lymphocytes and neutrophils.

To evaluate the collagen deposition, lung sections from mice were stained with Gomori trichrome (**Figure 3**). As shown in **Figure 3**, the accumulation of collagen in lung tissues was significantly increased in the OVA-challenged mice compared with saline-challenged mice. Mice treated with IL-21 showed no significant change of collagen deposition compared with saline-challenged mice. In addition, the extent of collagen deposition was not affected by anti-IL-21R Ab treatment, compared with those after treatment with control IgG.

#### *Administration of IL-21 or anti-IL-21R Ab does not reduce the production of IL-13 and TGF- $\beta$*

IL-13 and TGF- $\beta$  concentrations in BALF were measured by ELISA 24 hours after last provocation. The production of IL-13 and TGF- $\beta$  were unchanged by IL-21 or anti-IL-21R Ab treatment (**Figure 4**).

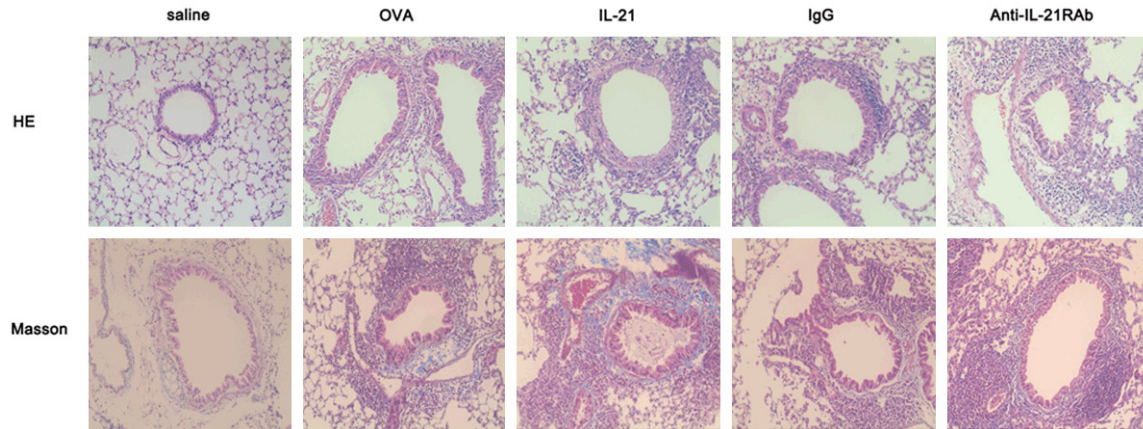
#### *Treatment with IL-21 or anti-IL-21R Ab does not reduced AHR in chronic allergic asthma*

To determine the effects of IL-21 or anti-IL-21R Ab on lung function, airway resistance was measured 24 h after the last provocation. In OVA-challenged mice, airway resistance was increased in a dose-dependent manner after exposure to methacholine (**Figure 5**). IL-21 treatment did not inhibit the increased airway reactivity to methacholine. Anti-IL-21R Ab treatment did not alter the methacholine-induced AHR, compared with control IgG treatment.

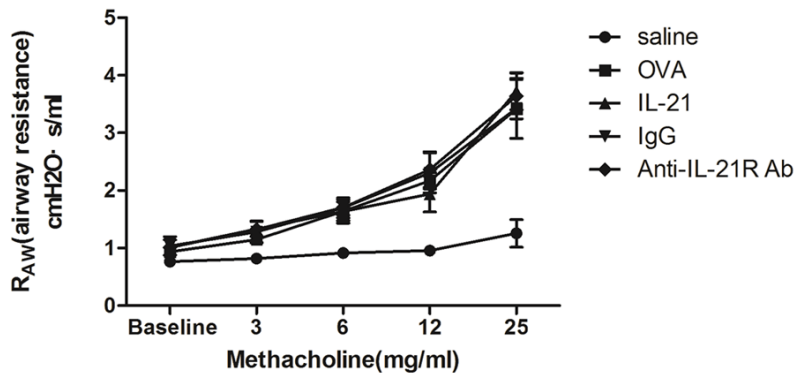
### Discussion

In the present study, we investigated the impact of IL-21 signaling on the regulation of chronic allergic airway inflammation and remodeling

## IL-21 does not involve in chronic asthma



**Figure 3.** IL-21 or Anti-IL-21R Ab treatment does not affect OVA-induced airway remodeling. Sections were stained with haematoxylin and eosin (top panel) or Gomori trichrome (bottom panel). Representative results of six different experiments are shown (original magnification:  $\times 200$ ).



**Figure 4.** IL-21 or Anti-IL-21R Ab treatment does not affect OVA-induced airway hyperresponsiveness (AHR). Mice were first exposed to nebulized saline, followed by increasing doses (3 to 25) mg/ml of nebulized methacholine for 3 minutes each. Breathing indices were read for 3 minutes after each nebulization, and enhanced pause values were determined. All data represent means  $\pm$  SEM. N = 4-6 mice per group.

induced by OVA exposure. We showed that either IL-21 or anti-IL21R Ab: (1) did not affect OVA-induced airway inflammation and airway remodeling; (2) did not effect on OVA-induced AHR; (3) did not modulate the expression of inflammation and remodeling related cytokines. Taken together, these data suggested that IL-21 might not play a critical role during the development of allergic airway remodeling.

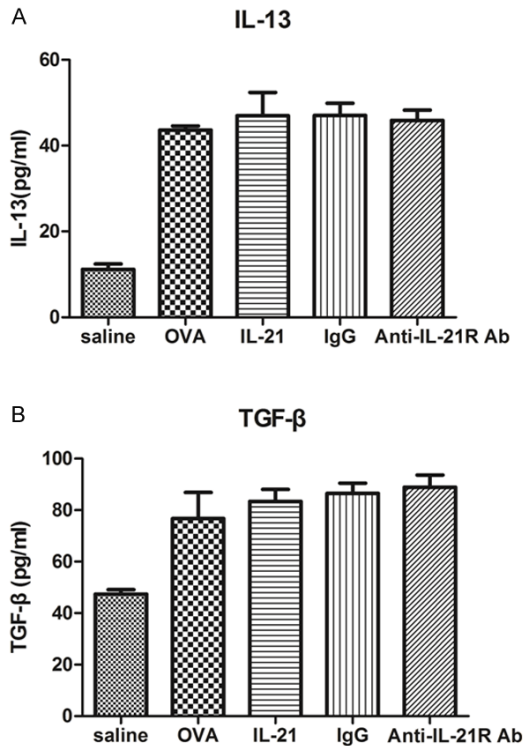
Our previous study showed that the serum IL-21 production was increased in asthma patients, herein; we found that IL-21 was up-regulated in the serum of OVA-challenged mice but not BALF. Therefore, we hypothesized that IL-21 may involve in the pathogenesis of asthma. To

test our hypothesis, we treated mice with IL-21 or anti-IL-21R Ab before every OVA provocation. Hironura and coworkers documented that IL-21 suppressed eosinophils infiltration in nasal-associated lymphoid tissue through preventing eotaxins production by nasal fibroblasts [15]. To validate the effect of IL-21 as a positive control in our study, we repeated their study by using an allergic rhinitis model treated with IL-21 before challenge, and we found decreased eosinophilic infiltration of the nasal mucosa. In previous

study we found exogenous administration of IL-21 may not alleviate allergic lung inflammation [16]. In this study, we demonstrated that intranasal administration of IL-21 had no effect on allergen-induced chronic airway inflammation and remodeling. Similarly, expression of IL-13 and TGF- $\beta$  in the BALF were not modified by IL-21 or anti-IL-21R Ab. One should note that, asthma and allergic rhinitis may have much in common, however, there might be some other factors that would contribute to this discrepancy and more data were needed to interpret this discrepancy.

In order to further test our hypothesis, we examined whether IL-21 or anti-IL-21R Ab could

## IL-21 does not involve in chronic asthma



**Figure 5.** IL-21 or Anti-IL-21R Ab treatment does not affect cytokine production in BALF. IL-13 and TGF- $\beta$  concentrations in BALF were assessed 24 hours after last challenge, as described in Methods. Results were presented as means  $\pm$  SEM. N = 4-6 mice per group.

affect OVA-induced AHR. However, as seen in the chronic asthma model, neither IL-21 nor anti-IL-21R Ab exerted effect on airway resistance. Based on the evidence we concluded that IL-21 signaling did not involve in the OVA-induced AHR.

Taken together, our findings suggest that the chronic airway inflammation and remodeling response triggered by OVA exposure occurred independently of IL-21 activity. Our study is the first study to report that IL-21 has little effect on development of chronic airway inflammation and airway remodeling. However, more research is needed to prove our results further.

### Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 81170021 and No. 30900647).

### Disclosure of conflict of interest

None.

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