

Research Article

IL-35 Inhibited Th17 Response in Children with Allergic Rhinitis

Fengmei Xie Quanfu Hu Qinfang Cai Rongwei Yao Shaoji Ouyang

Department of Otolaryngology, Fifth Affiliated Hospital, Southern Medical University, Guangzhou, China

Keywords

IL-35 · Allergic rhinitis · Th17 · IL-17 · IL-23

Abstract

Objective: Previous studies have proved that Th17 (T helper 17) cell subsets, a unique proinflammatory CD4+ T cell lineage, are deeply involved in the pathophysiology of allergic rhinitis (AR). IL-35, secreted mainly by natural Treg (nTreg) and depending on the expression of Foxp3, can effectively alleviate allergen-induced specific airway inflammation. However, the regulation of IL-35 in AR is not clear. **Methods:** Twenty AR children and 20 healthy controls were enrolled. The expression of serum IL-35 protein was detected and the correlation with Th17 cytokines (IL-17, IL-23, IL-27) expression was analyzed by enzyme-linked immunosorbent assay (ELISA). Peripheral blood mononuclear cells were prepared and stimulated by IL-35 to explore its effect on Th17 inflammation. **Results:** Serum IL-35 levels in AR were negatively correlated with serum IL-17 and IL-23 levels in AR. Recombinant IL-35 inhibits the Th17 response of PBMCs, which were mediated by the mitogen-activated protein kinase (MEK) and c-Jun N-terminal kinase (JNK) pathways. **Conclusions:** Our data demonstrate that IL-35 can inhibit Th17 response in AR through MEK and JNK pathways.

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Introduction

Allergic rhinitis (AR) is one of the most common pediatric chronic diseases in otorhinolaryngology. Clinical symptoms such as nasal obstruction, itching, sneezing, and runny nose caused by AR have a serious impact on the quality of life of patients, consuming huge social and medical resources [1]. According to the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines, comprehensive treatment of AR mainly includes allergen avoidance, drug

Shaoji Ouyang
Department of Otolaryngology, Fifth Affiliated Hospital
Southern Medical University
No. 566 Congcheng Avenue, Guangzhou 510900 (China)
E-Mail ouyangshaoji19@sohu.com

treatment, immunotherapy, and patient education [2]. However, up to one fifth of the patients still have symptoms that cannot be effectively controlled after adequate treatment following the guidelines [3]. The main reason is that the pathogenesis of AR is complex, and it is difficult to develop precise treatment methods.

IL-35 is a newly discovered cytokine, which is composed of p35 of the IL-12 alpha chain and the Epstein-Barr virus inducible gene 3 (EBI3) subunit of the IL-27 beta chain. It is secreted mainly by natural Treg (nTreg) and depends on the expression of Foxp3 [4]. IL-35 is distributed in bone marrow, thyroid, blood, and liver. IL-35 receptors (IL-35R) are composed of IL-12R beta 2 and gp130, which can activate the signaling pathways STAT1 and STAT4 [5]. IL-12R beta 2 is mainly expressed by activated T cells and NK cells, while the expression of gp130 is quite common [5].

The main functions of IL-35 include: promoting the functions of regulatory B cells and Treg, inducing iTr35 production, inhibiting the proliferation of effector T cells, and Th17 (T helper 17) cell differentiation in vitro [6–8]. IL-35 can play an inhibitory role in many immune diseases, such as inflammatory bowel disease, autoimmune encephalomyelitis, collagen arthritis, and other diseases [6–8]. In allergic diseases, animal studies have shown that IL-35 can effectively alleviate allergen-induced specific airway inflammation, while intramuscular injection of pVAX-IL-35 can inhibit allergen-specific and total IgE levels in peripheral blood [9]. These studies suggest that IL-35 may be a new target for the treatment of allergic diseases.

The regulation of IL-35 on AR may include the following aspects: IL-35 blockades the development and function of Th2 by inhibiting GATA3 and IL-4 expression and limiting Th2 proliferation, IL-35 mediates the transformation of Th2 cells to Treg [10], and IL-35 reverses IL-17-dependent allergic diseases [11]. However, the regulation of IL-35 on Th17 response in AR is not well established. Therefore, we aimed to explore the expression of IL-35 in AR and its effect on Th17 response.

Methods

Patients

A total of 40 children were enrolled in this study, which included 20 AR children (6–18 years old) and 20 healthy controls. The diagnosis of AR was made according to the Allergic Rhinitis and its Impact on Asthma (ARIA) guideline (2010) [2]. In brief, the information on nasal symptoms, duration of disease, and allergen test were collected for the confirmation of AR. The skin prick test or the detection of serum IgE specific to common inhalant allergens was performed. Patients with other nasal disease or systematic diseases were excluded from our study. Twenty healthy adults were enrolled as controls.

Symptoms such as sneezing, rhinorrhea, itchy nose, and nasal congestion were evaluated on a 0–3 scale (0, none; 1, mild; 2, moderate; and 3, severe), and the total nasal symptom score (TNSS) (0–12) was summed.

Quantitative PCR

Quantitative PCR was performed as previously described [12]. The total RNA was extracted using TRIzol reagent (Life Technologies, Carlsbad, CA, USA). The RNA (1 µg) was reverse-transcribed by cDNA kit (Qiagen). The PCR was performed through an ABI PRISM 7300 Detection System. The relative expression of tested genes was calculated using the comparative ΔC_t method and corrected for the expression of the housekeeping gene. The GAPDH housekeeping gene was used to normalize samples from cells. The primers of RORyt: forward 5'-CCGCTGAGAGGGCTTCAC-3', reverse 5'-TGCAGGAGTAGGCCACATTACA-3'.

ELISA

According to the manufacturer's instructions, the concentrations of IL-35, IL-17, and IL-23 in serum were detected by ELISA kit (Minneapolis R&D System) in duplicate. The detection limits of the assays were as follows: IL-35 (62.5 pg/mL), IL-17 (31.2 pg/mL), and IL-23 (39 pg/mL).

Table 1. Demographic characteristics of the subjects

Groups	AR group	Controls
<i>N</i>	20	20
Sex (male:female)	11:9	10:10
Age, years	10.6 (6–18)	11.2 (6–18)
Total IgE, IU/mL	189.1 (35.6–761.4)*	23.9 (5.5–78.3)
Sensitization		
D1, <i>n</i>	11	–
D2, <i>n</i>	10	–
Cockroach, <i>n</i>	6	–
Cat, <i>n</i>	4	–
Dog, <i>n</i>	2	–
Others, <i>n</i>	0	–
TNSS score	9.1±2.5	–

Values in parentheses are ranges. D1, *Dermatophagoides pteronyssinus*; D2, *Dermatophagoides farinae*; TNSS, total nasal symptom score.

* Compared with the control group, $p < 0.05$.

Preparation of Peripheral Blood Mononuclear Cells and Th17 Cell Differentiation

According to the manufacturer's instructions, peripheral blood mononuclear cells (PBMCs) were isolated from heparinized leukocyte-enriched buffy coats by density gradient centrifugation of lymphoid preparations. In short, the blood was diluted with the same amount of Dulbecco phosphate buffer saline and 2% fetal bovine serum, and carefully stratified on the top of the lymphoid preparations to minimize the mixing of blood and lymphoid preparations. At room temperature, the mixture was centrifuged at 800 *g* for 20 min, and the upper plasma layer was removed and discarded without interfering with the plasma-lymphoid interface. For Th17 differentiation, 20 ng/mL IL-6, 10 ng/mL IL-23, and 0.2 ng/mL TGF- β were added in the medium. In the presence or absence of dust mite extracts (20 μ g/mL hdm; RayBiotech, USA) and/or recombinant IL-35 (R&D, USA), mitogen-activated protein kinase (MEK) inhibitors (20 μ g/mL hdm; RayBiotech), and c-Jun N-terminal kinase (JNK) inhibitors (20 μ g/mL hdm; RayBiotech), the isolated cells were cultured in 1 mL medium (2×10^6 cells/mL) for 3 days in 5% CO₂. The Th17 cytokine expression (IL-23 and IL-17) in supernatant was measured by ELISA.

Statistical Analysis

Data were presented as mean $\pm$ SD. Analysis of variance and ad hoc post test were performed for comparison of groups. The Pearson correlation analysis was used to analyze the correlation between biomarker. $p < 0.05$ was considered as significant difference.

Results

The Expression of Serum IL-35 in AR Patients and Its Relationship with Th17 Cytokines and Disease Severity

Table 1 summarizes the characteristics of the participants. Compared with the control group, the protein expression of IL-35 in AR children was significantly lower, while the protein expression of IL-17 and IL-23 was significantly upregulated in AR children ($p < 0.05$) (Fig. 1). The IL-35 protein concentration was also significantly correlated with the total nasal symptom score ($r = -0.56$, $p < 0.05$), indicating that IL-35 levels were correlated with the disease severity. We also found that serum IL-35 levels in AR children were significantly correlated with the serum IL-17 ($r = -0.61$, $p < 0.05$) and IL-23 ($r = -0.64$, $p < 0.05$) levels (Fig. 2).

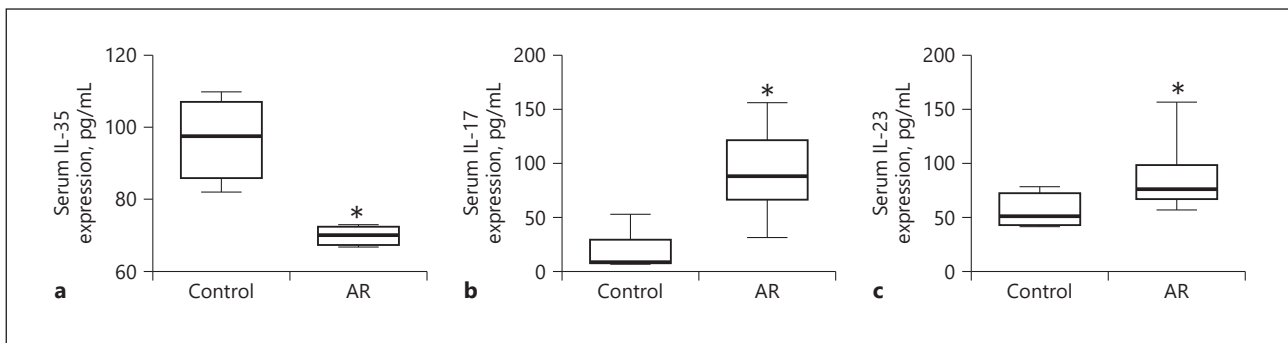


Fig. 1. The expression of serum IL-35 (a), IL-17 (b), and IL-23 (c) in AR children and controls. * $p < 0.05$, compared with controls.

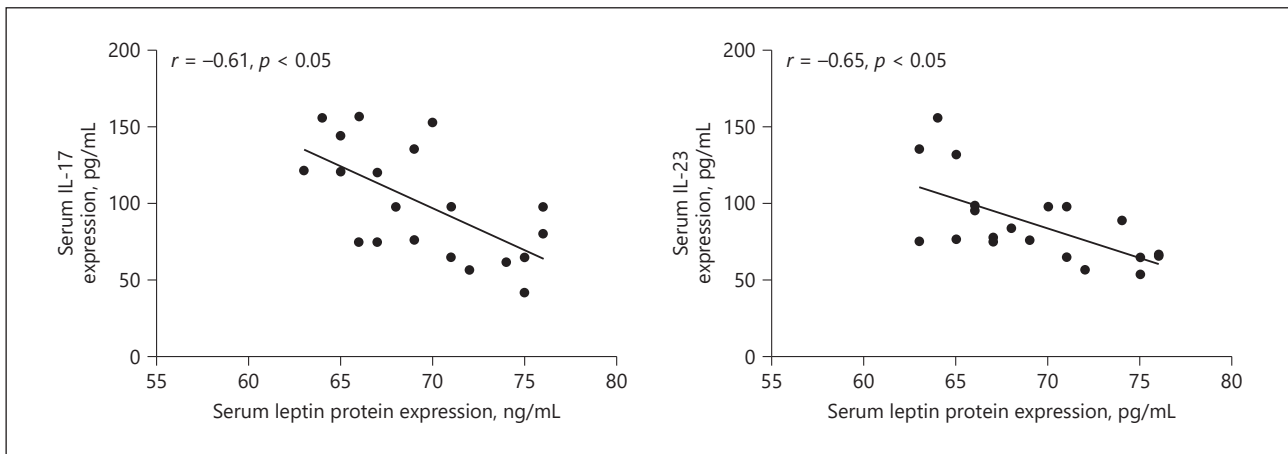


Fig. 2. Correlation between serum IL-35 and Th17 cytokines (IL-17, IL-23) in AR children.

Th17 Cell Differentiation Inhibited by IL-35 and the Related Signal Pathway

The results showed that IL-35 inhibited the relative expression of ROR γ t. In addition, downregulation of IL-17 and IL-23 proteins after IL-35 stimulation was found from PBMCs in AR patients. In addition, IL-35-mediated IL-17 inhibition may be inhibited by MEK and JNK inhibitors (Fig. 3). We also examined the effect of Th17 cytokines (IL-17, IL-23) on the expression of IL-35. Our results showed that IL-17 and IL-23 can inhibit the expression of IL-35 (Fig. 3).

Discussion

In the present study, our results showed that the downregulation of IL-35 is associated with enhanced Th17 response in AR children. We provided evidence that IL-35 inhibits Th17 response by downregulation of IL-23 and IL-17. The above regulation may be through the pathways of MEK and JNK. These results suggest that IL-35 may be a promising target for the treatment of AR. IL-35, composed of EB13 and IL-12 p35, belongs to the IL-12 cytokine family. It is well known that IL-35 induces Treg proliferation and expansion, while inhibiting Th17 cell differentiation [4]. Consistent with our findings, IL-35 plays an important role in the

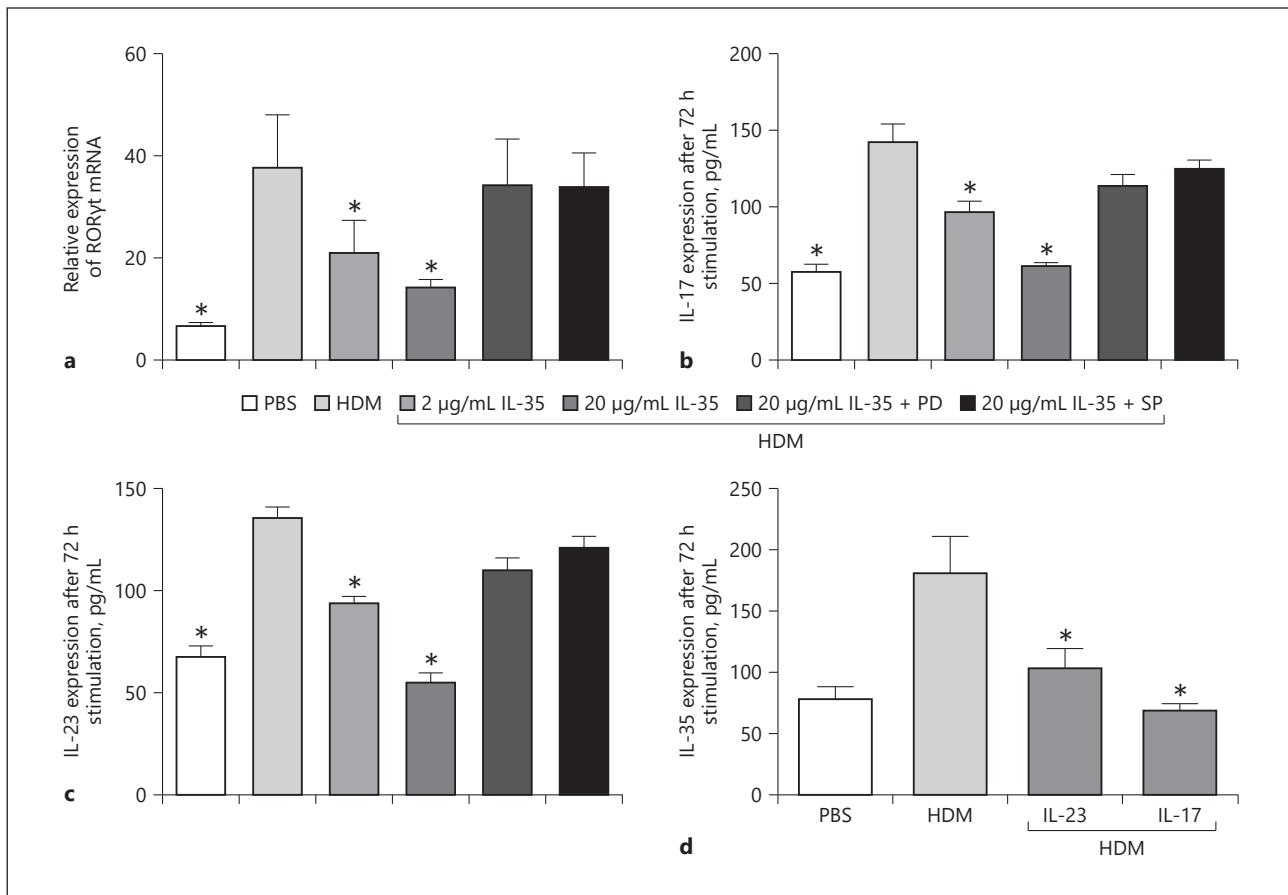


Fig. 3. The expression of RORγt (a), IL-17 (b), and IL-23 (c) by Th17 cells from AR patients after IL-35 stimulation. The effect of IL-17 and IL-23 on the expression of IL-35 was also shown (d). HDM, house dust mite; PD, MEK inhibitors; SP, JNK inhibitor. * $p < 0.05$, compared with the HDM group.

suppression of the development and the differentiation of Th17 in autoimmune diseases [13] by inducing cell cycle arrest in G1 without inducing apoptosis [14]. Whitehead et al. [15] also showed that IL-35 production by Treg cells suppressed the levels of IL-17 and Th17-mediated allergic airway hyperresponsiveness.

The role of IL-35 in AR was not fully understood yet. In their study, Yokota et al. [16] showed that IL-35 inhibited allergic T-cell response and had the ability to modulate the production of IL-2, IL-4, IL-5, IL-10, IL-13, IL-17, IL-23, IL-27, and TNF-α in mice with AR. In another study, Shamji et al. [17] found that IL-35 inhibited IL-5 and IL-13 production by ILC2s in the presence of IL-25 or IL-33 and allergen-driven TH2 cytokines by effector T cells. However, the effect of IL-35 on Th17 response in AR was not reported so far.

In this study, we first measured the protein expression of IL-35 and Th17 cytokines in AR children. Our results showed that downregulated IL-35 was correlated with serum IL-17 and IL-23 level, suggesting that IL-35 may be involved in Th17 response in AR. To prove the direct effect of IL-35 on Th17 response, we stimulated Th17 cells in vitro. IL-35 inhibited the differentiation of Th17 cells by decreasing the relative expression of RORγt. Besides, the function of Th17 cells was also inhibited, manifesting as downregulation of IL-17 and IL-23 protein after IL-35 stimulation. We also found that the above process was mediated through MEK and JNK pathways.

To explore the interaction between IL-35 and Th17 cytokines, we also studied the effect of Th17 cytokines (IL-17, IL-23) on the expression of IL-35 and found that IL-17 and IL-23 can inhibit the expression of IL-35, suggesting the positive feedback between IL-35 and Th17 cytokines. In sum, our data demonstrate that IL-35 can inhibit differentiation and function of Th17 cells in AR through MEK and JNK pathways, which provide potential treatment target for AR.

Statement of Ethics

This study was approved by the local ethics committee, and the parents gave written informed consent.

Disclosure Statement

The authors have no conflicts of interest to declare.

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