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ORIGINAL ARTICLE

Interleukin-27 Suppresses T Helper-17 Inflammation in Allergic Rhinitis

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ABSTRACT

Background: T helper 17 (Th17) cells and the related cytokines, interleukin (IL)- 17 and IL-23, were proved to play pivotal roles during the development of allergic rhinitis (AR). IL-27, an anti-inflammatory cytokine, has been reported to promote the production of IL-12R and induce Th1 cell responses. However, its effect on Th17 responses was not fully understood. **Objective:** We conducted the present research to explore the role of IL-27 in the regulation of Th17 responses in AR. **Methods:** Thirty confirmed AR patients and 20 controls were recruited for the study. The mRNA expression and protein levels of IL-27 were analyzed employing quantitative PCR (qPCR) and enzyme-linked immunosorbent assay (ELISA), respectively, and their correlations with Th17 cytokines were analyzed. We utilized ELISA and qPCR to analyze the effect of IL-27 on the differentiation of Th17 cells and the production of IL-17 and IL-23 from peripheral blood mononuclear cells (PBMCs). **Results:** We found that the IL-27 levels in AR were downregulated and negatively related to IL-17 and IL-23 levels. The recombinant IL-27 inhibited the mRNA expression of RORγt and the protein expression of IL-17 and IL-23 in PBMCs through MEK, NF-κB, and JNK pathways. **Conclusion:** Our data demonstrated that IL-27 suppressed Th17 responses through MEK, NF-κB, and JNK pathways.

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Keywords: Allergic Rhinitis, IL-17, IL-27, Peripheral Blood Mononuclear Cell, Th17

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INTRODUCTION

Allergic rhinitis (AR) is common in ear-nose-throat clinic with a high prevalence, particularly in children (1,2). AR is characterized by rhinorrhoea, nasal obstruction, epiphora, and nasal itching and affects the quality of life of patients severely. Traditionally, enhanced T helper (Th) 2 inflammation and upregulated Th2 cytokines were thought to be the basis of AR (3,4). However, further studies showed that interleukin-17A (IL-17A)-producing T helper 17 (Th17) cell contributed to the development of AR (5,6). *In vivo* studies using mouse models also suggested that blockade of Th17 inflammation by anti-IL-17 and anti-IL-23 could alleviate the clinical symptoms and Th2 inflammation in ovalbumin induced AR mice, confirming that Th17 and Th2 inflammation interrelated closely (7-9). Interleukin-27 is composed of the subunit of Epstein-Barr virus-induced gene 3 (EBi3) and p28. IL-27 is produced by activated macrophages and dendritic cells (10). IL-27 receptor consists of IL-27Ra (WSX-1/TCCR) and gp130, which could be expressed by various cells such as lymphocytes and antigen-presenting cells (APCs) (11). Previous studies have indicated that IL-27 promoted the production of IL-12 receptor and induced Th1 cell response (12). Nevertheless, IL-27 also exhibits anti-inflammatory role. For example, IL-27Rα-/- mice can develop into lethal immune responses (13). Moreover, previous data have also suggested that IL-27 inhibits Th17 development and increases T-cell production of IL-10 (14,15). In AR, allergen specific immunotherapy has been shown to decrease IL-17 and increase IL-27 in AR patients sensitive to house dust mites (HDMs) (16). Accordingly, we believed that IL-27 may play an important role in Th17 response in AR. In this study, we explored the expression of IL-27 and its regulation in Th17 response in AR patients employing *in vitro* experiments.

MATERIALS AND METHODS

Patients. Thirty patients diagnosed with AR and 20 controls were recruited consecutively from June 2017 to August 2017 at Department of Otolaryngology, RenHe Hospital of Three Gorges University, China. The inclusion criteria comprised clinical history of AR (duration >1 year, persistent symptoms) and allergen test according to Allergic Rhinitis and its Impact on Asthma (ARIA) guideline (2010) (1). The allergen test was performed using skin prick test (wheal diameter >3mm) or specific IgE detection (>0.35 kIU/L, Phadia, Uppsala, Sweden) to common inhalant allergens (pollens, dust mites, pets, molds, and cockroach, etc.). Twenty healthy patients with comparable ages and genders were enrolled as the control. The subjects with diseases such as chronic lung disease or heart disease and those who were using drugs, such as oral corticosteroids, antiepileptics, or immune suppressors, were excluded. Our study was approved by local Ethical Committee, and informed consent was obtained ahead of the study.

Symptom Evaluation. Total nasal symptom score (TNSS) was performed as described previously (17,18). The nasal symptoms (sneezing, rhinorrhea, itching, and nasal block) were scored (0, none; 1, mild symptom; 2, moderate symptom; and 3, severe symptom) and summed.

Real-time PCR Analysis. Total RNA was isolated from serum with TRIzol reagent (Life Technologies, Carlsbad, California) according to the manufacturer's instructions.

Subsequently, the RNA was transcribed into cDNA with cDNA Synthesis Kit (Thermo Scientific, CA, USA). PCR reaction conditions were set as follows: 30 cycles at 94°C for 2 min, 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, and 72°C for 5 min. The mRNA expression was measured employing BI7500 real-time PCR system (Applied Biosystems). The primer sequences were listed as follows:
IL-27-5'- CAAGAAGAGGTCCCGTGCTG -3', 5'-TTGAGCCCAGTCCACCACAT-3'; retinoid-related orphan nuclear receptor γt (RORγt) -5'-ATGACATGCAC CTGGAAC ACT-3', 5'-TGTGAGTTCTCCATGCCTAGG-3', β-actin-5'-GGCACC CAGCACAATGAA-3', 5'-GGAAGG TGGACAGCGAGG-3'.

Enzyme-Linked Immunosorbent Assay (ELISA) for Protein Expression. The concentration of IL-27 and Th17 cytokines were determined using ELISA according to the manufacturer's instruction (R&D systems, Minneapolis, Minnesota). All the samples were assayed in duplicate. The detection limits of the test were as follows: IL-27 (156pg/mL), IL-17 (15 pg/mL), IL-23 (16.3 pg/mL).

Peripheral Blood Mononuclear Cells (PBMCs) Preparation. PBMCs were prepared utilizing density-gradient centrifugation. In short, the blood was diluted by the same amount of Dulbecco's PBS and 2% Fetal Bovine Serum. Afterwards, the mixture was layered on top of Lymphoprep carefully to avoid getting mixed. Following centrifugation at 800g for 20 minutes, the upper plasma layer was discarded carefully. The sorted cells (2×10^6 cells/mL) were cultured at 37°C in 5% CO_2 with phytohaemagglutinin (PHA) (50 ng/ml) and ionomycin (500ng/ml) for a 3-days stimulation. For Th17 cell differentiation, PBMCs were stimulated by recombinant 10 ng/ml of IL-1β (Peprotech), 20 ng/ml of IL-6 (eBioscience), 20 ng/ml of IL-23 (eBioscience), 2 ng/ml of TGF-β (eBioscience), 10 μg/ml of anti-IL-4 mAb (eBioscience), and 10 μg/ml anti-IFN-γ mAb (eBioscience). The following antibodies were used in the experiments: IL-27 (50 and 100 ng/ml), mitogen-activated protein kinase kinase (MEK) inhibitors (PD-98059), nuclear factor κB (NF-κB) inhibitor (SN50), c-Jun N-terminal kinase (JNK) inhibitor (SP600125). The mRNA of RORγt was then detected with real-time PCR. The expressions of IL-23 and IL-17 in supernatant were detected employing ELISA.

Statistical Analysis. All the data were represented as mean ± SD. We performed normal distribution and homogeneity of variance analysis. Unpaired t test was performed for the data with normal distribution, and Mann-Whitney U test was done to compare the data with abnormal distribution. Three or more groups were compared using 1-way ANOVA, with Bonferroni post hoc test. The correlations were analyzed with Spearman rank test. P values less than .05 were defined to be statistically significant. The analyses were carried out using GraphPad Prism 6.0 software (GraphPad Software, La Jolla, Calif).

RESULTS

The mRNA and protein levels of IL-27 and Th17 cytokines in AR.

Table 1 depicts the demographic data of the study population. Regarding the cases, sex ratio and age were comparable between AR and the control groups. The mRNA (2.944e-005 ± 7.428e-006 vs 0.000359 ± 9.863e-005) and protein (199.1 ± 9.2 vs 419.2 ± 58.7 pg/mL) level of IL-27 in the AR group were significantly lower than those in the controls ($p<0.001$, Figure 1).

Table 1. Demographic characteristic of the study population.

Groups	AR group	Control
Number	30	20
Sex (Male:Female)	16:14	12:8
Age (months)	25.6 (18-45)	28.4 (18-42)
TNSS	7.9 (6-10)	0
ECPa (ng/ml)	69 (19.0-164.0) *	12.5 (4.8-78.0)
IgEa (IU/ml)	158.7 (56-463.0) *	23.0 (12.3-64.0)

a Data presented as median values (minimum-maximum). * compared with control group, p<0.05.

The protein level of IL-17 (91.6 ± 7.7 vs 50.6 ± 5.06 pg/mL) and IL-23 (74.4 ± 9.8 vs 46.1 ± 4.79 pg/mL) in the AR group were more upregulated than those in the controls (p<0.001, Figure 1). Our results also revealed that the decreased IL-27 protein level was negatively related to IL-17 (r=-0.56, p=0.002) and IL-23 (r=-0.51, p=0.006) protein expression in the AR (Figure 2). The decreased IL-27 protein level was also negatively related to total nasal symptom score in the AR, suggesting that IL-27 may be contributed to disease severity (r=-0.58, p=0.001) (Figure 2).

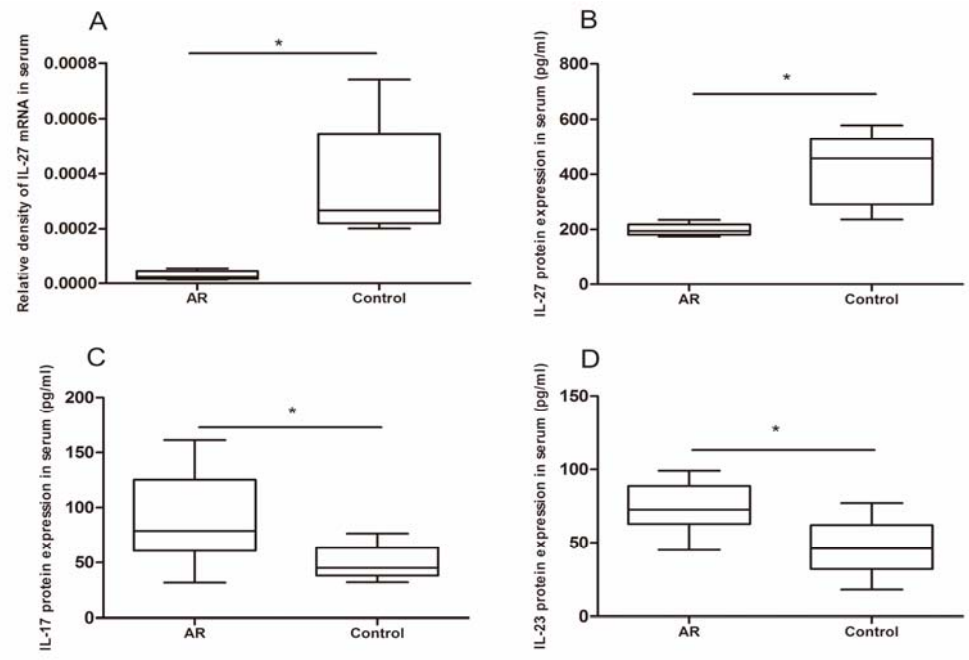

Figure 1. The expression of IL-27 and Th17 cytokines between AR and the controls. A,B. Serum IL-27 mRNA and protein expression between AR patients and the normal controls. C,D. Serum IL-17 and IL-23 protein expression between AR patients and the normal controls. (C,D). AR, allergic rhinitis. * Compared with the control, p<0.05.

IL-27 inhibit Th17 inflammation of PBMCs.

We found that IL-27 decreased the relative expression of RORγt in PBMCs induced by PHA in a dose-dependent manner, indicating that IL-27 inhibited the differentiation of Th17 cells directly ($p<0.05$) (Figure 3A). Furthermore, the production of IL-17 and IL-23 by PBMCs in the AR patients was significantly downregulated following IL-27 stimulation in a dose-dependent manner, providing evidence that IL-27 directly inhibited the function of Th17 cells ($p<0.05$) (Figure 3B,3C).

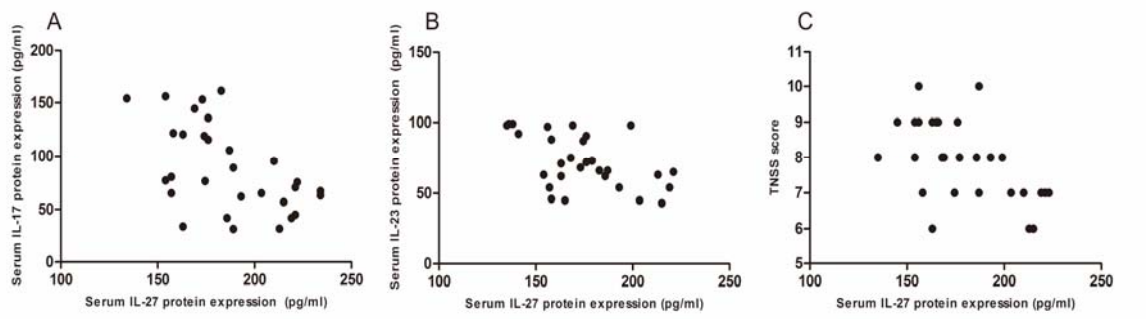

Figure 2. The correlation between serum IL-27 protein expression, serum IL-17 protein expression, IL-23 protein expression, and total nasal symptom score (TNSS) (A-C).

Our data demonstrated that the protein expression of IL-10 and TGF-beta did not change after IL-27 stimulation (data not shown), which implies that the inhibition of Th17 cell by IL-27 was not through IL-10 or TGF-beta. We also found that the addition of SN50 (NF-κB inhibitor), SP (JNK inhibitor), and PD (MEK inhibitors) significantly antagonizes the effect of IL-27 on IL-17 and IL-23 production by Th17 cells, suggesting that these signaling pathways were involved in IL-27 mediated Th17 cells function regulation ($p<0.05$) (Figure 3B,3C).

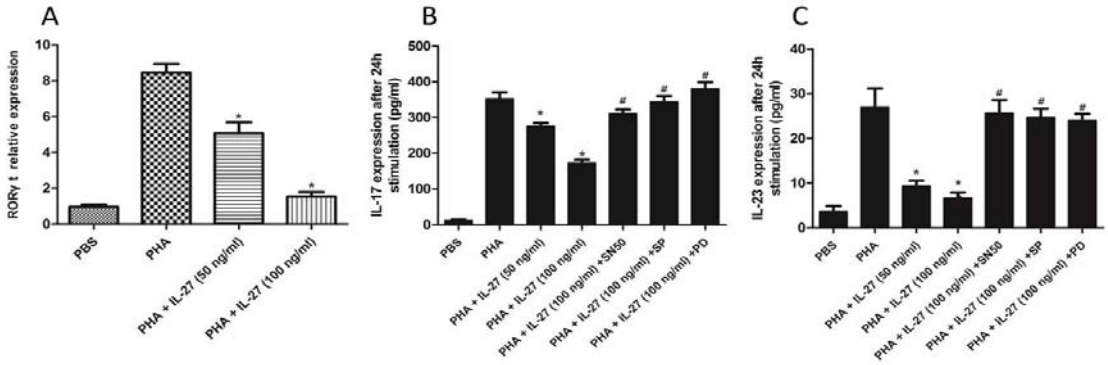

Figure 3. IL-27 inhibited Th17 cell differentiation and function. A. RORγt relative expression was inhibited with IL-27. B,C. The protein expression of IL-17 and IL-23 by PBMCs were inhibited with IL-27 and these effects were inhibited with NF-κB, MEK, and JNK inhibitors (A-C). PHA, phytohaemagglutinin, PD, mitogen-activated protein kinase inhibitors, SN50, nuclear factor κB (NF-κB) inhibitor), SP, c-Jun N-terminal kinase (JNK) inhibitor. * Compared with the PHA group, $p<0.05$. # Compared with PHA+ IL-27 (100 ng/mL) group, $p<0.05$.

DISCUSSION

In the present study we provided evidence that IL-27 might contribute to the inhibition of Th17 inflammation. IL-27 could downregulate RORγt and Th17 cytokines (IL-17, IL-23) expression directly in PBMCs in vitro. Herein, we provided a new molecular mechanism in Th17 regulation in the AR patients. IL-27 is a newly identified heterodimeric cytokine. Previous studies have suggested that IL-27 regulated the proliferation of naive $CD4^{+}$T cells and B cells (11). Our previous study suggested that IL-27 significantly inhibited Th2 cytokines expression from PBMCs in the AR (19). Moreover, intranasal administration of IL-27 inhibited nasal allergic responses and symptoms in a mouse model of ovalbumin (OVA) antigen sensitized and challenged asthma (20). IL-27 also exerts an anti-inflammatory effect by inhibiting the development of Th17 cells and promoting the development of regulatory T cells in various diseases (21-23). Concerning allergic diseases, in a study, Chen found that experimental allergic conjunctivitis of mice model presented with more severe allergic symptoms, both TSLP-driven Th2 inflammation and Th17 responses, characterized by elevated levels of IL-17A, IL-17F, and RORγt (24). However, the effect of IL-27 on Th17 response in AR was not explored. According to our results, the decreased IL-27 protein level was negatively associated with IL-17 and IL-23 protein, providing preliminary evidence that IL-27 regulates Th17 inflammation in AR. RORγt is a transcription factor essential for the Th17 development. Our data implied that IL-27 inhibited the relative expression of RORγt as well as IL-17 and IL-23 protein expression in PHA-induced PBMCs PHA, providing direct evidence that IL-27 negatively affects Th17 differentiation and function of Th17 cells in AR. Consistently, a study by Mohamed suggested that IL-27 had a strong inhibitory effect on the function of Th17 cells during differentiation, yet IL-27 has little effect on committed Th17 cells in this regard (25). In this study, we also added IL-27 during the Th17 cells differentiation, to see whether IL-27 affected the committed Th17 cells in the AR needed further exploration. Moreover, Denise demonstrated the inhibitive role of IL-27 on activated T cells, specifically Th17 cells in experimental autoimmune encephalomyelitis (26). In animal models, previous studies have indicated that IL-27Rα-/- mice were sensitive to Th17-mediated neuro-disease (27). The author believed that IL-27 inhibited Th17 inflammation by inducing IL-10 production by T cells and modulating regulatory T-cell (Treg) functions. Similarly, a study by Su *et al.* reported that IL-27 has the potential to ameliorate the overwhelming inflammation in patients with rheumatoid arthritis through a reciprocal regulation of Th17 and Treg cells (28). Nonetheless, our data suggested that IL-27 did not alter IL-10 production from PBMCs in AR patients. These differences depicted that the Treg was of great necessity for the regulation of IL-10 expression. Since IL-27 inhibits IL-17 and IL-23 expression stimulated by PHA, the addition of SN50 (NF-κB inhibitor), SP (JNK inhibitor), and PD (MEK inhibitors) antagonizes the effect of IL-27 on IL-17 and IL-23 production by Th17 cells. This depicts that these signaling pathways play important roles in IL-27 mediated Th17 cells regulation. Interestingly, Peter suggested that Th17 differentiation was inhibited once the ratio of p-STAT3/p-STAT1 was less than one. Their results also showed that IL-27 could upregulate the expression of p-STAT3 so as to induce Th17 differentiation in the absence of STAT1 (29). In conclusion, our data shed light to the fact that IL-27 suppresses Th17 inflammation through NF-κB, MEK, and JNK pathway. IL-27 might be utilized as a potential target for the prevention and treatment of AR.

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