

Increased IL-22 level in allergic rhinitis significantly correlates with clinical severity

Jun Tang, Ph.D.,¹ Ping Xiao, Ph.D.,¹ Xi Luo, M.D.,² Jing Bai, M.D.,¹ Wentong Xia, M.D.,² Weixiong Chen, M.D.,¹ Jian Li, M.D.,² Qingqing Yu, M.D.,¹ Sisi Shi, M.D.,¹ Yuxia Xu, M.D.,¹ Zhonglin Mou, M.D.,³ Yuejian Wang, M.D.,¹ and Huabin Li, Ph.D.²

ABSTRACT

Background: IL-22 regulates various processes and has been linked to diverse effects. However, the importance of IL-22 in the pathogenesis of allergic rhinitis (AR) remains poorly understood. This study sought to evaluate the levels of IL-22 and IL-17A in AR patients and their association with clinical severity of AR.

Methods: Thirty-six AR patients and 22 normal controls were enrolled in this study. The frequencies of IL-22⁺, IL-17A⁺, and IL-9⁺ T helper (Th) cells in peripheral blood of AR patients and normal controls were examined by flow cytometry. Serum levels of IL-22 and IL-17A in AR patients and normal controls were determined by ELISA. The clinical relevance of the percentages of IL-22⁺ and IL-17A⁺ Th cells as well as serum IL-22 and IL-17A levels were evaluated.

Results: The frequencies of IL-22⁺ and IL-17A⁺ Th cells, but not IL-9⁺ Th cells, were significantly increased compared with those in normal controls ($p < 0.05$). Frequencies of IL-22⁺ and IL-17A⁺ Th cells in peripheral blood of AR patients significantly correlated with visual analog scale scores of nasal symptoms (nasal congestion and rhinorrhea; $p < 0.05$). Moreover, the serum levels of IL-22 and IL-17A were significantly increased compared with those in normal controls ($p < 0.05$) and significantly correlated with the levels of *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* specific IgE in AR patients.

Conclusion: Our findings suggested that IL-22 as well as IL-17A may play an important role in the regulation of Th2-skewed inflammation in AR patients. (Am J Rhinol Allergy 28, e197–e201, 2014; doi: 10.2500/ajra.2014.28.4088)

Allergic rhinitis (AR) is characterized by T helper type 2 (Th2)-skewed immune response, intense eosinophilic infiltration, goblet cell hyperplasia, and airway hyperreactivity.¹ It is well established that these features are mediated by antigen-specific Th2 cells and their cytokines including IL-4, IL-5, and IL-13.² Recently, the identification of novel Th cell subsets provided new insight into our understanding of the molecular mechanisms involved in the development of allergic inflammation and thus led to revision of the classic Th1/Th2 paradigm.³ For example, there is growing evidence that regulatory T (Treg)-cell insufficiency plays a crucial role in the lack of maintenance of peripheral T-cell tolerance to allergens,⁴ and induction of Treg cells by allergen-specific immunotherapy is considered to represent a promising strategy for the management of AR and asthma.⁵

Additionally, other newly emerged immune/inflammatory cell subsets such as Th17 cells offer further understanding to immune regulation toward distinct T-cell subsets.⁴ Th17 cells are characterized by high expression of the transcription factors retinoic acid-related orphan receptor γ thymus and retinoic acid-related orphan receptor α , which are critical for Th17 differentiation. Th17 cells predominantly produce IL-17A and IL-22, which are two cytokines with broad effects in the lung and other tissues.⁶ Although the exact role of Th17 subset in AR is more complex, several studies revealed that IL-17A is involved in the enhancement of Th2 cell-mediated eosinophil recruitment into the airways in the asthma mouse model,⁷ and serum IL-17A

levels are related to clinical severity in AR patients.^{8,9} On the contrary, IL-22 has been proposed to possess both proinflammatory and anti-inflammatory properties depending on environmental context. For example, Sonnenberg *et al.* found that in the absence of IL-17A, IL-22 serves as a tissue-protective role, and when IL-17A is present IL-22 contributes to the disease pathogenesis in a model of airway inflammation.¹⁰ Other studies reported that the levels of IL-22 mRNA are increased in peripheral blood mononuclear cells in asthma patients¹¹ and that the levels of IL-22 in sera tend to correlate with the severity of asthma.¹² However, the potential involvement of IL-22 in the pathogenesis of AR remains poorly understood.

MATERIALS AND METHODS

Subjects

This study was approved by the Ethical Committee of the First People's Hospital of Foshan City, and written informed consent was obtained from all of the subjects. Thirty-six adult AR patients that accorded with the standard criterion for AR issued by Allergic Rhinitis and Its Impact on Asthma guidelines and predominantly sensitized to house-dust mites were specifically recruited in the study.¹ Twenty-two nonatopic patients undergoing septoplasty because of anatomic variations were considered as controls. The atopic status of the AR patients and control subjects was evaluated by allergen skin-prick test (SPT) that was performed by using a common panel (house-dust mites, animal danders, mold, birch, and grass). For analysis, blood sampling was collected from all subjects for flow cytometric analysis or other use. The serum levels of *Dermatophagoides pteronyssinus*- and *Dermatophagoides farinae*-specific IgE (sIgE) were determined by using the Unicap system (Phadia, Uppsala, Sweden). None of the subjects used oral or nasal corticosteroids 4 weeks before enrollment. All subjects were nonsmokers and free from upper respiratory tract infection for 4 weeks preceding the study. The demographic data from the patients and normal controls included in this study are listed in Table 1.

Flow Cytometric Analysis

Heparinized peripheral blood was collected and incubated at 37°C in the presence of monensin (1.5 mM), ionomycin (1 mM), and phor-

From the ¹Department of Otolaryngology, The First People's Hospital of Foshan City, Foshan, China, ²Allergy Center, Otorhinolaryngology Hospital, the First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China, and ³Department of Otolaryngology, The Affiliated Hospital of Hainan Medical University, Haikou, China. Funded by National Natural Science Grants of China (81260156, 81371071) and grants from the Ministry of Hygiene (201202005 and 2014BAI07B04).

The authors have no conflicts of interest to declare pertaining to this article. Address correspondence to Yuejian Wang, M.D., Department of Otolaryngology, The First People's Hospital of Foshan City, No. 81, North Lingnan Avenue, Foshan, China 528000, or to Huabin Li, Ph.D., Allergy Center, Otorhinolaryngology Hospital, the First Affiliated Hospital, Sun Yat-sen University, No. 58, Zhongshan 2nd Road, Guangzhou, China, 510080.

E-mail address: wyjian@fsyy.com; allergyli@163.com.
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Table 1 Demographics of AR patients and normal controls

Groups	Controls	AR	p Value
No.	22	36	NS
Sex (M/F)	14:8	26:10	NS
Age (yr)	20–41	23–45	NS
SPT (positive/negative)	0:22	36:0	<0.05
Nasal symptom VAS score (mean ± SD)			
Nasal congestion	—	5.5 ± 3.2	
Nasal rhinorrhea	—	6.1 ± 2.7	
Sneezing	—	5.4 ± 2.9	
Itching	—	4.7 ± 2.5	
Asthma in history and present (yes/no)	0:22	4:32	—
Smoking (yes/no)	0:22	7:29	—
Duration of disease (yr)	—	3–10	—

Age and disease duration are shown as range. Nasal symptom VAS scores are shown as mean ± SD. M = male; F = female; AR = allergic rhinitis; VAS = visual analog scale; NS = not significant.

bol myristate acetate (100 ng/mL; all were purchased from Sigma-Aldrich, St. Louis, MO) in a humidified atmosphere with 5% CO₂ for 6 hours. After incubation, cells were washed in PBS and were resuspended in PBS containing 0.5% bovine serum albumin and 0.01% NaN₃ (staining buffer) at room temperature for 15 minutes. The cells were then washed and incubated with Permeabilization Medium (Invitrogen, Carlsbad, CA) and anti-CD4 and anti-CD3 (BD Pharmingen, San Diego, CA) for 30 minutes at room temperature. After cytokine labeling (by adding anti-IL-22, IL-17A, and IL-9; all were purchased from eBioscience, San Diego, CA), the cells were analyzed on a BD FACS Calibur flow cytometer (BD, Palo Alto, CA) and were analyzed by Cellquest software (BD).

Enzyme-Linked Immunosorbent Assay

The levels of serum IL-22 and IL-17A were measured using ELISA. A commercially available ELISA kit of IL-22 and IL-17A (R&D Systems, Minneapolis, MN) was used according to the manufacturer's protocols. The detection limits for IL-22 and IL-17A were 7.8 and 15.6 pg/mL, respectively. For convenient analysis, all values below the detection limit, were considered to be zero.

Evaluation of Nasal Symptom Severity

The visual analog scale (VAS) was used to score the individual nasal symptoms including nasal congestion, rhinorrhea, sneezing, and itching. The scale ranges from 0 (no symptoms) to 10 (very troublesome symptoms). Patients were asked to position a cross on a line corresponding to their own perception of nasal symptom severity.

Statistical Analysis

Data were expressed as the median and interquartile range except for additional note, and were analyzed via nonparametric Mann–Whitney U test. Correlations between different quantitative variables were established using Spearman's correlation coefficient. A value of p < 0.05 was considered statistically significant.

RESULTS

Patient Characteristics

Thirty-six AR patients between 23 and 47 years of age were enrolled in the present study (Table 1). There AR patients were predom-

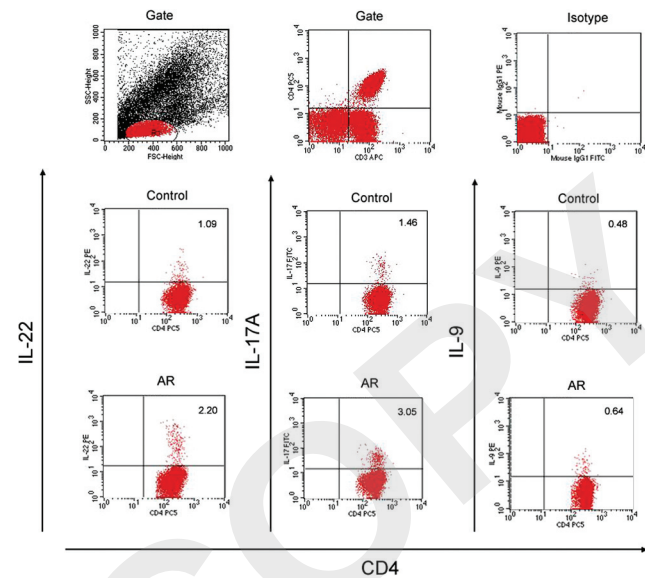


Figure 1. Representative results of flow cytometric analysis of IL-22⁺, IL-17A⁺, and IL-9⁺ T helper (Th) cells in peripheral blood of allergic rhinitis (AR) patients and normal controls. Samples were live gated on lymphocytes by forward and side scatter gates, and limits for fluorescence-positive cells were set using nonspecific IgG antibodies.

inantly sensitized to house-dust mites (*D. pteronyssinus* and *D. farinae*) as suggested by SPT. Other sensitized allergens included cat dander, birch, grasses, *Parietaria*, etc. The mean disease duration was 3.2 ± 1.4 years (mean ± SD); four of them had a history of asthma. The individual nasal symptom scores (VAS) were as follows: nasal congestion, 5.5 ± 3.2; nasal rhinorrhea, 6.1 ± 2.7; sneezing, 5.4 ± 2.9; and nasal itching, 4.7 ± 2.5. Of 36 AR patients, 18 patients were examined for serum concentrations of *D. pteronyssinus* and *D. farinae* sIgE using the Unicap system. The mean levels of *D. pteronyssinus* and *D. farinae* sIgE were 6.7 ± 8.9 kU/L and 10.7 ± 13.3 kU/L (mean ± SD), respectively.

Frequencies of IL-22⁺, IL-17A⁺, and IL-9⁺ Th Cells in Peripheral Blood of AR Patients

To investigate the importance of IL-22 in AR patients, we first examined the frequencies of IL-22⁺, IL-17A⁺, and IL-9⁺ Th cells in peripheral blood of AR patients and normal controls by flow cytometry. For the convenience of analysis, the representative results of flow cytometric analysis of IL-22⁺CD4⁺ cells, IL-17A⁺CD4⁺ cells, and IL-9⁺CD4⁺ cells in peripheral blood of AR patients and normal controls are shown in Fig. 1. IL-22⁺CD4⁺ cells, IL-17A⁺CD4⁺ cells, and IL-9⁺CD4⁺ cells were detectable in almost all subjects. As shown in Fig. 2, we found the frequencies of IL-22⁺CD4⁺ cells and IL-17A⁺CD4⁺ cells were significantly increased compared with those in normal controls (p < 0.05). Conversely, no significant difference was observed in frequency of IL-9⁺CD4⁺ cells between AR patients and normal controls (p > 0.05).

Relationship between Frequencies of IL-22⁺ and IL-17A⁺ Th Cells and Symptom Severity Assessed by VAS in AR Patients

To determine the association of IL-22⁺CD4⁺ cells and IL-17A⁺CD4⁺ cells with clinical severity, we recorded the individual nasal symptom (nasal congestion, rhinorrhea, sneezing, and itch) score using VAS scale and evaluated the relationship between frequencies of IL-22⁺CD4⁺ cells and IL-17A⁺CD4⁺ cells and nasal symptom score in AR patients. As shown in Fig. 3, we found the

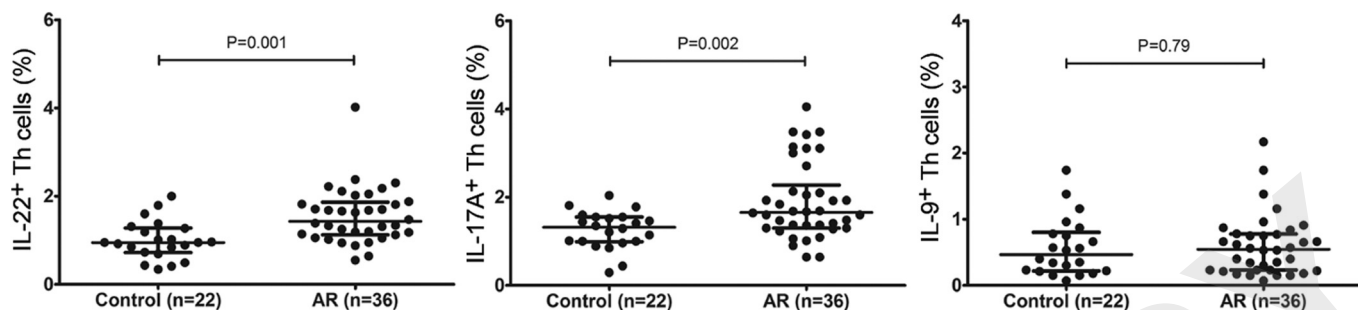


Figure 2. Frequencies of IL-22⁺, IL-17A⁺, and IL-9⁺ T helper (Th) cells in peripheral blood of allergic rhinitis (AR) patients. The frequencies of IL-22⁺ and IL-17A⁺ Th cells but not IL-9⁺ Th cells were significantly increased compared with those in normal controls.

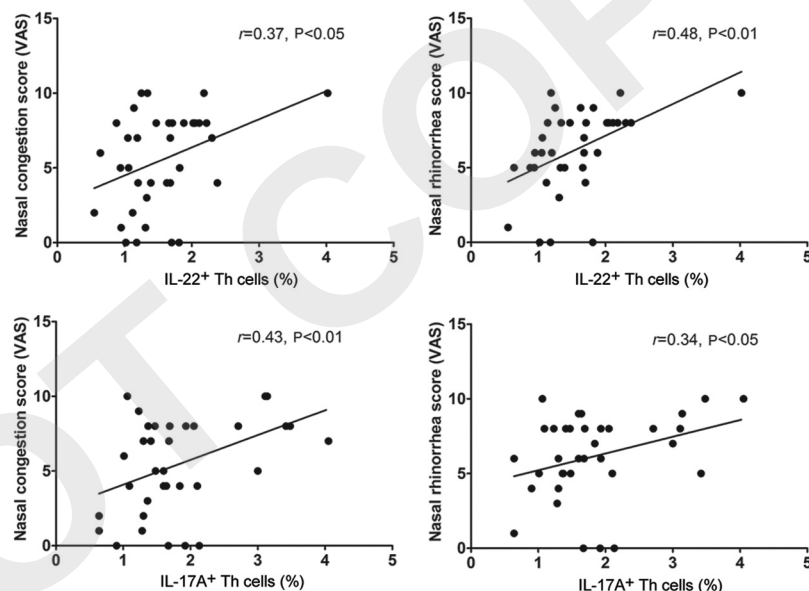


Figure 3. Relationship between frequencies of IL-22⁺ and IL-17A⁺ T helper (Th) cells and symptom severity assessed by visual analog scale (VAS) in allergic rhinitis (AR) patients. Frequencies of IL-22⁺ and IL-17A⁺ Th cells in peripheral blood of AR patients significantly correlated with VAS scores of nasal symptoms (nasal congestion and rhinorrhea).

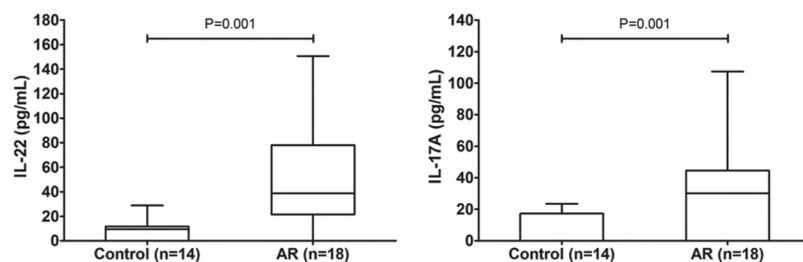


Figure 4. Serum levels of IL-22 and IL-17A in allergic rhinitis (AR) patients and normal controls. The levels of IL-22 and IL-17A were significantly increased compared with those in normal controls.

frequencies of IL-22⁺CD4⁺ cells were significantly associated with nasal congestion and rhinorrhea in AR patients ($p < 0.05$). No significant relationship was observed between the frequencies of IL-22⁺CD4⁺ cells and sneezing and itching. Similarly, the frequencies of IL-17A⁺CD4⁺ cells were significantly associated with nasal congestion and rhinorrhea in AR patients ($p < 0.05$). No significant relationship was observed between the frequencies of IL-17A⁺CD4⁺ cells and sneezing and itching as well.

Relationship between Serum Levels of IL-22 and IL-17A and Level of sIgE in AR Patients

To further characterize the role of IL-22- and IL-17A-related cytokines in the pathogenesis of AR, we next examined the serum levels of IL-22 and IL-17A in 18 AR patients and 14 normal controls. As shown in Fig. 4, we found the levels of IL-22 and IL-17A were significantly increased compared with those in normal controls ($p <$

0.05). Moreover, when correlated serum levels of IL-22 and IL-17A with levels of *D. pteronyssinus* and *D. farinae* sIgE in AR patients, we found serum levels of IL-22 and IL-17A were significantly associated with the levels of *D. pteronyssinus* and *D. farinae* sIgE in AR patients (Fig. 5). Because these AR patients were predominantly sensitized to house-dust mites (*D. pteronyssinus* and *D. farinae*) as suggested by SPT, sIgE to birch and other aeroallergens have not been routinely examined and no correlation to sIgE to birch and other aeroallergens was evaluated.

DISCUSSION

In the present study, we showed IL-22⁺ and IL-17A⁺ Th cells, as well as serum levels of IL-22 and IL-17A, were significantly increased in AR patients. Moreover, we provided the first evidence that IL-22⁺ Th cells, in addition to IL-17A⁺ Th cells, were associated with the clinical severity of AR patients. These findings therefore expand our

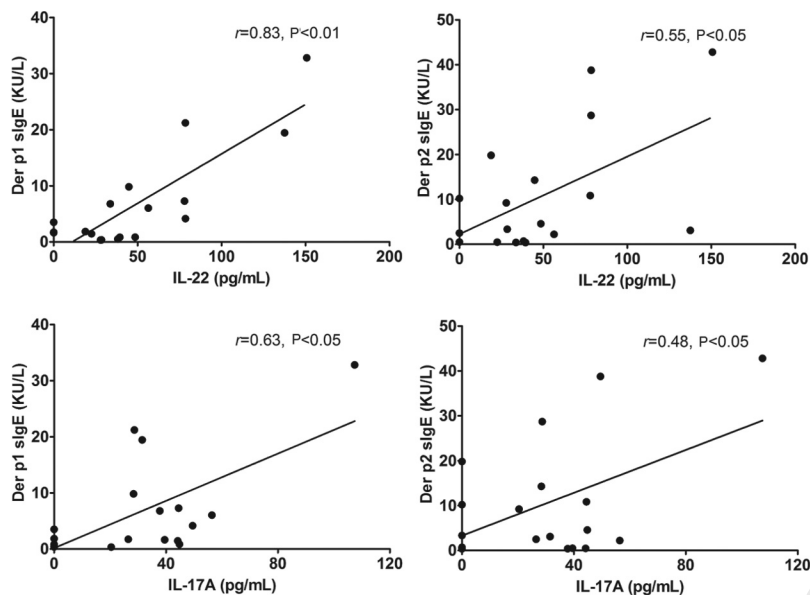


Figure 5. Relationship between serum levels of IL-22 and IL-17A and level of specific IgE (sIgE) in allergic rhinitis (AR) patients. Serum levels of IL-22 and IL-17A significantly correlated with the levels of Der p1 and Der p2 sIgE in AR patients.

understanding on the pathogenesis of AR and contribute to designing optimal therapeutic strategy.

Th17 cells are newly emerging Th cell subsets that link the immune response to tissue inflammation. IL-17A and IL-22 are the predominant prototype cytokines.¹³ Although the main function of Th17 cells is supposed to be induction of protection against potentially harmful fungi and extracellular bacteria, a large battery of experiments with IL-17 neutralizing antibodies,¹⁴ as well as relatively high levels of IL-17A,¹⁵ showed the potential role of Th17 cells in the pathogenesis of nasal diseases. For example, Wang *et al.* showed IL-17 antagonism significantly attenuated Th2 responses and infiltration of neutrophil and eosinophil in AR mouse model.¹⁶ Quan *et al.* also reported that IL-17A-deficient mice showed a significant decrease in allergic symptoms, serum IgE levels, and eosinophil infiltration; decreased histamine and cysteinyl leukotriene release; and significantly lowered degranulation and secretion of TNF- α in marrow-derived mast cells.¹⁷ These findings together showed the potential role of anti-Th17 cells in the management of AR. We previously reported that increased MUC5AC expression was associated with nasal IL-17A levels in AR, and IL-17A was found to affect the inhibition of MUC5AC by promoting GR- β mRNA expression in nasal epithelial cells.⁹ In this study, we further confirmed that circulating Th17 cells is associated with disease severity of AR, and serum IL-17A is significantly associated with house-dust mite sIgE levels.

In contrast to IL-17A, IL-22 is a more recently identified Th17-derived cytokine that plays a complicated and important role in inflammatory diseases. The data of IL-22 in the pathogenesis of asthma is somewhat contradictory by exhibiting both proinflammatory and anti-inflammatory properties. The proinflammatory properties of IL-22 are shown as IL-22 is detected at the sites of allergic airway inflammation,¹⁸ and serum levels of IL-22 are higher in patients with severe asthma than those seen in patients with mild asthma and healthy control subjects. In addition,¹⁹ blocking IL-22 during Th2 sensitization significantly decreased eosinophilic inflammation, Th2 cytokine production, airway hyperresponsiveness, and mucus hyperplasia in a mouse model of asthma.^{20,21} On the other hand, several studies showed that IL-22 neutralization during allergen challenge increased airway inflammation and Th2 cytokine production and that treatment with recombinant IL-22 during allergen challenge attenuated it.^{22,23} These findings raise the possibility that the functions of IL-22 are influenced by the inflammatory milieu present in the lung. However, the importance of IL-22 cells in AR has still been largely unknown.

In the present study, we found the frequencies of IL-22⁺ Th cells, as well as IL-17A⁺ Th cells, were significantly increased in AR patients. The increased IL-22⁺ and IL-17A⁺ Th cells were significantly associated with two major nasal symptom scores. Therefore, we provided the evidence that circulating IL-22⁺ Th cells may be considered as a helpful indicator of disease severity. Moreover, we examined the association of IL-22 with house-dust mite sIgE levels in AR patients. Consequently, we found serum IL-22 levels were associated with sIgE levels in AR patients. Based on our results that the percentages of IL-22⁺ and IL-17A⁺ Th cells are associated with clinical severity, as well as other findings that IL-22 contributes to the airway inflammation in the presence of IL-17A, we postulated IL-22⁺ Th cells may exert its proallergic effect under the instruction of secreted IL-17A in AR patients. Additional study concerning the underlying mechanism of IL-22 is required to help us to better understand the pathogenesis of AR.

CONCLUSION

Our findings suggested that IL-22⁺ Th cells, in addition to IL-17A⁺ Th cells, were increased in AR patients. In addition, the levels of IL-22 and IL-17A were found to be associated with disease severity and sIgE level. Therefore, IL-22 as well as IL-17A may play an important role in the regulation of Th2-skewed inflammation in AR patients.

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