

Significance of Interleukin-17A in patients with nasal polyposis

Yang Shen¹, Chang-Kai Pan¹, Xin-Ye Tang¹, Yu-Cheng Yang¹, Xia Ke¹, Wei Kou¹, Xiao-Qiang Wang¹ and Su-Ling Hong¹

Summary

Background: Interleukin-17A (IL-17A) is a key inflammatory cytokine in many disorders, while the significance of IL-17A in nasal polyposis (NP) is still obscure. This study aimed to investigate the expression of IL-17A in nasal polyps from both atopic and nonatopic patients and its associations with clinical and histological features.

Methods: In all, 30 patients with NP were included, and were grouped into atopic and nonatopic patients according to skin prick test (SPT). Disease severity was evaluated by symptom score, endoscopy score and CT score. Histological characteristics were assessed by eosinophilic infiltration, basement membrane (BM) thickness, epithelial damage, squamous metaplasia, and goblet cell hyperplasia. IL-17A expression in polyps was detected by ELISA and immunohistochemistry.

Results: Endoscopy score and CT score were significantly higher in atopic NP patients than in nonatopic NP patients ($p < 0.05$). IL-17A levels were significantly upregulated in both atopic ($p < 0.01$) and nonatopic ($p < 0.05$) patients versus controls. Furthermore, IL-17A levels were significantly higher in the atopic group versus nonatopic group. Significantly positive correlations were found between IL-17A levels and CT scores, eosinophilic infiltration and BM thicknesses.

Conclusions: These results indicated that expression of IL-17A was significantly upregulated in NP patients and was more severe in atopic NP patients, suggesting that IL-17A

may play an important role in the pathology of NP and atopy may contribute to NP by stimulating the production of IL-17A. (*Asian Pac J Allergy Immunol* 2011;29:169-75)

Key words: nasal polyposis, Interleukin-17A, atopy, nonatopy, eosinophil

Introduction

Nasal polyposis (NP), commonly encountered in clinical otorhinolaryngology, is a chronic inflammatory disease of the nasal cavity and sinus. At present the treatment outcomes of antibiotics, steroids, and surgery for NP are unsatisfactory and the recurrence rate remains high¹. NP has become a more and more important social issue because of its high incidence (2–5% worldwide) and the considerable economic burden associated with the disease².

Over the last 2 decades, the pathogenesis of NP has been studied widely, but it is not clearly understood yet and the role of atopy in the etiology and pathogenesis of NP is still a controversial issue. Nowadays NP is considered to be a multifactorial disease and generally represents a subset of chronic inflammation of the mucous membrane in the paranasal sinus, which is distinct from chronic rhinosinusitis without NP². A variety of allergic^{3,4}, infectious, inflammatory, anatomical⁵, and genetic^{6,7} factors are known to be involved in the origin of NP.

Recently, Interleukin-17 (IL-17) A, a key inflammatory cytokine of Th17 cells, was found significantly upregulated in several disorders: acute hepatic injury⁸, rheumatoid arthritis⁹ and asthma¹⁰. Furthermore, IL-17A levels were reported to be increased in serum from patients with allergic rhinitis^{11, 12} and were related to clinical severity¹². Zhao Y et al.¹³ reported that allergic asthmatic patients showed higher IL-17 concentrations in plasma and culture supernatants than normal controls. Moreover, IL-17A levels were found to be increased in skin affected by allergic contact dermatitis and psoriasis; IL-17A regulates keratinocyte expression of adhesion molecules and

From the ¹ Department of Otorhinolaryngology, The First Affiliated Hospital of Chongqing Medical University, 1#Yixueyuan Road, Chongqing 400016, People's Republic of China

Corresponding author: Su-Ling Hong

E-mail: hsl_prof@163.com

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chemokines¹⁴. Since all these diseases are known to be related to atopy, IL-17A may play an important role in atopic inflammation. Thus, studies examining the correlation between IL-17A and atopy in the pathogenesis of NP is meaningful.

Nowadays, there were some reports regarding the expression of IL-17A in NP. However, the exact function of IL-17A in the pathogenesis of NP still remains obscure. For a better understanding of the role of IL-17A in NP, the present study aimed to evaluate the expression of IL-17A in NP and the different expression between atopic and nonatopic patients, then investigate the association between IL-17A and disease severity. We demonstrated the IL-17A expression in polyp tissue using ELISA and immunohistochemistry in atopic, nonatopic NP patients and controls, then investigated the correlations between IL-17A levels and endoscopy scores, CT scores, eosinophilic infiltration and BM thicknesses. To our knowledge, this is the first study to investigate the expression of IL-17A in atopic and nonatopic patients with NP respectively.

Methods

Patients

30 patients (18 males, 12 females) between 23 and 70 years of age were included. The diagnosis of NP was made according to the current European EAACI Position Paper on Rhinosinusitis and Nasal Polyps¹⁵ and American guidelines². Clinical data about patients included age, sex, duration of disease, history of asthma, and recurrence of nasal polyps. Symptom scores were assessed according to visual analog scale (VAS)¹⁶. The preoperative computed tomography (CT) scans were graded according to Lund and Mackay¹⁷. The preoperative nasal endoscopy scores were graded according to Lanza and Kennedy¹⁸. Patients with antrochoanal polyps, cystic fibrosis, fungal sinusitis, primary ciliary dyskinesia, or systemic diseases were excluded. 10 patients with a deviated septum were recruited as a control group. These patients had no history of respiratory disease or allergy, and their skin prick test (SPT) results were negative. This study was approved by the ethical committee of Chongqing Medical University. Informed consent was obtained from each patients and control subject before collecting material.

All patients stopped oral and topical application of corticosteroids or antihistamines for at least 4 weeks and received 2-3 days of antibiotics before surgery. The polyp samples were obtained during

surgery. Surgery was performed only when medical management had failed.

Determination of allergy

The test of atopy was based on SPT (Allergopharma, Hamburg, German). The SPT results were assessed in accordance with the recommendations of the Subcommittee on Allergen Standardization and Skin Tests of the European Academy of Allergy and Clinical Immunology³. Patients were considered prick test positive if at least 1 allergen elicited a papule diameter which was as large as or larger than that produced by the positive control (histamine). A total of 18 inhaled allergens were tested, including house dust, grass, tree, mold, food, and weed panel allergens. Most patients had clinical signs consistent with allergic rhinitis.

Tissue preparation

The polyp tissue was obtained from patients during surgery. Control samples were obtained from the mucosa of the inferior nasal concha at the time of operation for nasal septal deviation. The tissue was divided into two parts. One part was fixed immediately in 10% formalin, embedded in paraffin wax, and sectioned at 4-5 μ m thickness to be used for hematoxylin-eosin (HE) staining and immunohistochemistry. The other part was snap-frozen in liquid nitrogen and stored at -80°C for further use in ELISA.

Histological analysis

The tissue sections were stained with HE. Sections were observed under a low-power magnification ($\times 100$) to obtain a general expression. The epithelial remodeling was evaluated by the presence of epithelial damage, squamous metaplasia, and goblet cell hyperplasia. Epithelial damage was defined as epithelial sloughing and loss of integrity of epithelial cells. At a magnification of $\times 400$, maximal basement membrane (BM) thickness was measured in the most severely thickened regions with the use of an image analyzer. The absolute number of eosinophils was counted in 5 fields selected randomly. Then these five counts were averaged as the mean number per high power field. All analysis was done by 2 independent observers who did not know the diagnosis and clinical data.

Table 1. Clinical features of atopic and nonatopic patient with NP

	atopic NP	Nonatopic NP	<i>p</i> value
Sex (male:female)	11:6	7:6	n.s.*
Age (y)	47 (37.5-53.5)	46 (36-55.5)	n.s.
Asthma	2/17	0/13	n.s.*
Duration of NP (y)	5 (2-10)	5 (2-9)	n.s.
Symptom score	11 (8.5-13.5)	13 (11-16)	n.s.
CT score	11 (8.5-17.5)	9 (7-11.5)	0.011
Endoscopy score	8 (5.5-10.5)	5 (3-7)	0.026
Recurrence	5/17	2/13	n.s.*

Data are expressed as medians and interquartile ranges. The level of significance (*p*) was obtained by means of Student's *t* test or the Fisher exact test (marked with *), and the significance level equals a *p* value of 0.05. n.s.= not significant.

Immunohistochemistry

The slides were dewaxed in xylene and dehydrated in alcohol. The endogenous peroxidase was blocked with 3% hydrogen peroxide in phosphate buffered saline for 15 minutes. Then sections were blocked with 10% normal goat serum and incubated with primary polyclonal rabbit antihuman IL-17A Ab (Santa Cruz Biotechnology, Santa Cruz, Calif., USA) overnight at 4 °C. Then samples were incubated in biotinylated secondary antibody (Zhongshan Co., Beijing, China), followed by avidin-peroxidase complex. Color development was achieved with 3', 3'-diaminobenzidine, which results in a brown-stained precipitate in positive cells. Finally, sections were counterstained with hematoxylin and mounted in 5 fields selected randomly.

The number of positive cells was analyzed using a magnification of $\times 400$ and scored by 2 independent observers who did not know the diagnosis and clinical data as in the histological study.

ELISA

Polyps was weighed and homogenized in 1 ml 0.9% sodium chloride solution on ice per every 100mg of tissue. Then, the supernatants were centrifuged at 4°C and 3000 rpm for 10 minutes, after which they were collected and stored at -80°C . The levels of IL-17A in the supernatants were assayed using specific ELISA kits (eBioscience, SanDiego, CA, USA) according to the manufacturer's instructions. All assays were performed in duplicate. The results are expressed in pg/ml.

Table 2. Histological features of atopic and nonatopic patients with NP

	atopic NP	Nonatopic NP	<i>p</i> value
Epithelial damage	15/17	10/13	n.s.*
Squamous metaplasia	9/17	5/13	n.s.*
Goblet cell hyperplasia	10/17	4/13	n.s.*
Eosinophil (/HPF)	35.2 (23.6-41.0)	19.8 (10.6-16.5)	< 0.01
BM thickness (μm)	15.3 (8.7-24.0)	7.7 (4.9-11.4)	0.016

Differences between 2 groups were performed using Student's *t* test or the Fisher exact test (marked with *), and the significance level equals a *p* value of 0.05. n.s.= not significant.

Statistical analysis

We used SPSS for Windows 17.0 statistical package program for data analyses. Data are expressed as medians and interquartile ranges. Differences between the values were determined using Student's *t* test. Grouped data were analyzed using a one-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test. When the equal variance test failed, Kruskal Wallis test followed by Mann–Whitney Rank Sum test was used. The categorical presence or absence of structural pathologies was compared by Fisher's exact test. Spearman's test was used to determine correlations between IL-17 and clinical, histological features. $P < 0.05$ was considered significant.

Results

Clinical and histological features

The clinical features of the cases are summarized in Table 1. Among 30 patients with NP included in this study, 17(56.67%) were classified as atopic patients and 13(43.33%) as nonatopic patients. The endoscopy score, and the CT score were significantly higher in the atopic group than in the nonatopic group ($p < 0.05$). However, no statistically significant differences were found between two groups from the standpoint of symptom score, age, sex, duration of disease or recurrence ($p > 0.05$), and asthma was only noted in the atopic group.

Histologically (Figure 1.), the mean number of eosinophils was significantly increased in atopic NP cases, compared with that of the nonatopic group.



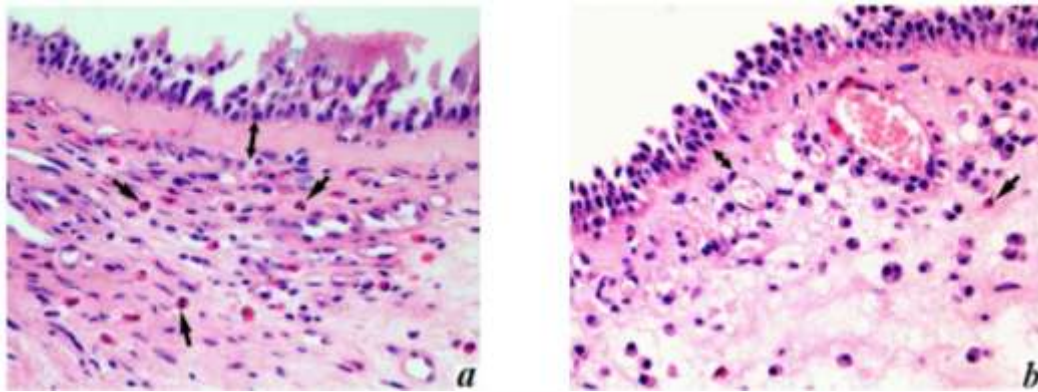


Figure 1. Histological features of NP. (a) Atopic patients' polyps show numerous eosinophils (arrows). The BM (double arrows) is thicker in the polyps from atopic than that from nonatopic patients. (b) Nonatopic patients' polyps show prominent infiltration of inflammatory cells, while eosinophils are scarcer (arrows) and the BM is thinner (double arrows). (HE staining; original magnification: $\times 400$)

The BM was statistically thicker in atopic NP specimens than those from nonatopic NP cases. However, no significant differences were found in terms of epithelial damage, squamous metaplasia and goblet cell hyperplasia ($p > 0.05$). (Table 2.)

IL-17A in nasal tissue homogenates

The levels of IL-17A were assessed on polyp tissue by means of ELISA tests. IL-17 levels were significantly upregulated in both the atopic ($p < 0.01$) and nonatopic ($p < 0.05$) groups versus controls. Furthermore, when comparing atopic to nonatopic patients, IL-17 levels were significantly higher in the atopic group ($p < 0.05$). (Figure 2.)

Immunohistochemical analysis

Immunohistochemical staining demonstrated that IL-17A expressing cells were detectable in the nasal tissue of both patients with NP and the controls. Consistent with the results of ELISA, the numbers of IL-17A positive cells were significantly greater in nasal polyps of both atopic and nonatopic patients than in the mucosa of the controls ($p < 0.01$ and $p < 0.05$, respectively). Furthermore, the numbers of IL-17A positive cells were statistically higher in the atopic group than the nonatopic group ($p < 0.05$). (Figure 3.)

Correlation analysis

To further determine possible interaction between IL-17A and nasal polyps, we examined the correlations between IL-17A and clinical, histological features of NP patients. As described in Figure 4., There were significantly positive correlations between the levels of IL-17A and CT score, the number of eosinophils and BM thickness

($r = 0.413$, $p = 0.023$; $r = 0.437$, $p = 0.016$; $r = 0.516$, $p < 0.01$). However, we found no correlations between IL-17 and endoscopy scores or symptom scores ($p > 0.05$).

Discussion

In this study, our findings can be summarized as follows: (1) Our data demonstrated increased expression of IL-17A in NP patients and positive correlations between IL-17A and disease severity, suggesting that IL-17A may play an important role in the development of NP; (2) In atopic group, we showed higher levels of IL-17A and more severe clinical and histological features. Thus, we hypothesized that atopy may contribute to NP by stimulating the production of IL-17A. To our knowledge, it was the first time to demonstrate the significantly positive correlations between IL-17A and disease severity in NP patients.

IL-17A, an important proinflammatory cytokine of the IL-17 family, has been found to be elevated in a variety of inflammatory conditions including rheumatoid arthritis¹⁹, gram-negative bacterial pneumonia²⁰, and asthma²¹. The role of IL-17A in regulating lung inflammation has been considered to induce neutrophilic inflammation. In this study, we showed significantly higher IL-17A levels in the polyps of NP patients than in the nasal tissue of controls. The results concur with previous studies evidencing that there was an increased IL-17mRNA and/or proteins expression in NP^{21, 22}. Moreover, the present study demonstrated significant correlations between IL-17A and CT score, the number of eosinophils, BM thickness.

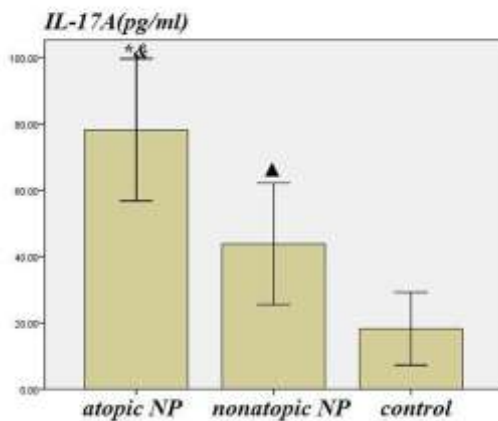


Figure 2. The levels of IL-17 in polyps were detected by ELISA. *: $P < 0.01$, vs. control; ▲: $P < 0.05$, vs. control; &: $P < 0.05$, vs. nonatopic NP.

Based on these results, we propose that IL-17A contribute to the development of NP and have an impact on clinical severity.

Recently, the correlation between IL-17A and eosinophils in the development of airway inflammation has been paid close attention. Eosinophils are one of the most important inflammatory cells in allergic inflammation. They are known to produce and release various proinflammatory cytokines (such as TNF- α , IL-6, IL-8, and IL-1 β) and cytotoxic molecules (such as eosinophil peroxidase and eosinophilic cationic protein), resulting in chronic tissue damage and remodeling in airway²³. About the role of IL-17A in the airway, recent studies reported that IL-17A induced recruitment not only of neutrophils²⁴ but also of eosinophils into the airways in a murine model of asthma²⁵. In addition, Saitoh et al.²⁶

reported that IL-17A was predominantly expressed in both CD4⁺ lymphocytes and eosinophils in NP with asthma. Meanwhile, a previous study demonstrated that human eosinophils were found to express receptors for IL-17A at the protein level and IL-17A could induce the release of chemokines GRO- α /CXCL1, IL-8/CXCL8, and MIP-1 α /CCL4 from eosinophils²³. In our study, we found a significantly positive correlation between IL-17A and eosinophil infiltration and the results of immunohistochemical analysis demonstrated that some of IL-17A positive cells were eosinophils. Based on the results of these researches, we hypothesize that on the one hand eosinophil may be an important source of IL-17A in NP and on the other hand the increased IL-17A levels enhance the recruitment and activation of eosinophils, finally resulting in an eosinophilic self-amplifying process to promote mucosal remodeling in NP.

The role of atopy in the pathophysiology of NP has been a controversial topic for many years. Some previous studies suggested that atopy did not significantly affect inflammatory mediators and eosinophilic infiltration in NP^{27, 28, 29}. However, a recent study demonstrated that patients with NP were sensitive to most common allergens in our environment and exhibited a clear-cut correlation with other allergic factors³. Moreover, Alatas et al.³⁰ showed that NP in allergic and nonallergic patients might differ in their histology and their histologic responses to steroid therapy. Cheng W et al.³¹ suggested that nasal polyps from atopic patients had more TH2 cells and eosinophils than nonatopic patients' polyps did and there might be some different immune responses between two groups. These histological and immunological differences between atopic and nonatopic patients imply that

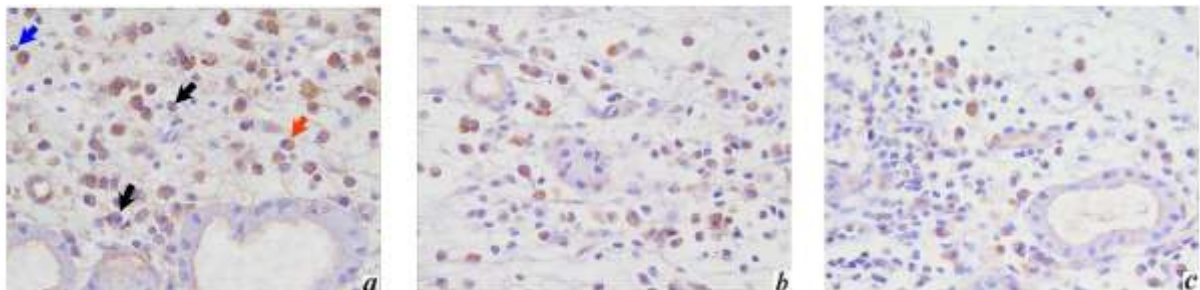


Figure 3. Immunohistochemical staining of IL-17A in tissue from atopic NP (a), nonatopic NP (b) and controls (c). IL-17A-positive cells are prominent eosinophils (black arrows), plasma cells (orange arrow), and lymphocytes (blue arrow). (Original magnification: $\times 400$)

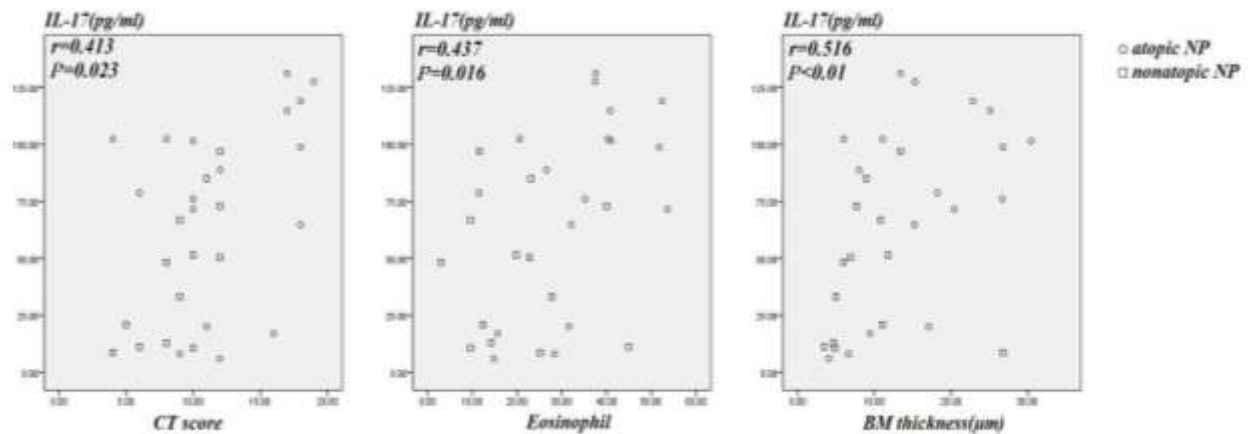


Figure 4. Spearman correlations between IL-17A concentrations and CT score, tissue eosinophil and BM thickness.

deeper research between the two types of NP should be performed. In our study, clinical and histological comparisons demonstrated that atopy might aggravate NP, although it didn't cause it. In addition, our results demonstrated the upregulated levels of IL-17A in atopic group compared to nonatopic group and significantly positive correlations between IL-17A and clinical, histological features. Thus, we propose that atopy may contribute to NP by stimulating the production of IL-17A. Nevertheless, our findings are not totally in accordance with those of a previous study³⁰, which showed a significant difference in presence of epithelial damage in polyp tissue between atopic and nonatopic patients. We think that the different results in two studies may be ascribed to trauma during surgery. In fact, it is difficult to completely omit artifacts caused by surgery.

However, the results of present study are partially in conflict with previous investigations. The expression of IL-17A was found not significantly different at the mRNA level among chronic rhinosinusitis without NP, chronic rhinosinusitis with NP, and controls³². Shi et al.³³ reported that the numbers of IL-17A positive cells in nasal polyps were significantly smaller than in the mucosa of control patients. These contrasting results might be ascribed to different clinical severity and regional disparity.

In summary, we have demonstrated that expression of IL-17A was significantly upregulated in NP patients and was more severe in atopic NP

patients. In addition, there were significantly positive correlations between IL-17A and clinical and histological features. Our study suggests an important role for IL-17A in NP. However, some more intensive studies are required to clarify these cellular mechanisms. Overall, we hope that present study would enable us to obtain a deeper insight into the pathogenesis of NP and provide a potential target for the treatment of NP.

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Disclosure Statement

The authors declare that no financial or other conflicts of interest exist in relation to the content of the article.

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