ORL

ORL 2020;82:257–267
DOI: 10.1159/000507865
Received: January 9, 2020
Accepted: March 29, 2020
Published online: July 29, 2020

Research Article

Interleukin-17A Expression Correlated with the Prognosis of Chronic Rhinosinusitis with Nasal Polyps and the Anti-Interleukin-17A Effect in a Murine Nasal Polyps Model

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Keywords
Interleukin-17A · Chronic rhinosinusitis with nasal polyps · Murine nasal polyps model · Matrix metalloproteinase-9

Abstract
Objective: To investigate the expression of interleukin-17A (IL-17A) in patients with chronic rhinosinusitis with nasal polyps (CRSwNP) and to analyze its effect on prognosis and to explore the role and mechanism of anti-IL-17A effect in vivo by establishing a murine nasal polyps (NP) model. ***Methods:*** Patients with CRSwNP who underwent endoscopic sinus surgery and matched control subjects were collected. We investigated IL-17A expression in human NP tissues using immunohistochemistry and analyzed their clinical features, including Lund-Mackay computed tomography scoring (LMCS) before surgery, Lund-Kennedy endoscopic scoring (LKES) before surgery (LKES B), LKES 6 months after surgery (LKES A), and reduction of LKES (LKES R). Then, after establishing the murine NP model to detect the expression and correlation of IL-17A and matrix metalloproteinase-9 (MMP-9) in nasal tissue, we studied nasal lavage fluid and serum by PCR and enzyme-linked immunosorbent assay in vivo. Anti-IL-17A treatment was administered in the murine NP model to confirm the function of IL-17A during the pathogenic processes. ***Results:*** IL-17A expression was upregulated in NP tissues from patients with CRSwNP compared with control subjects ($p < 0.001$). The number of IL-17A$^+$ cells was significantly negatively correlated with LKES R in patients with CRSwNP ($p < 0.01$). However, there was no significant correlation between IL-17A and LMCS or LKES B (all $p < 0.05$).

J.-C. Huang and X.-H. Chen contributed equally to this work.

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Further, IL-17A and MMP-9 were more abundant in nasal mucosa of the murine NP model compared with that of control mice (all $p < 0.05$), and severe polypoid lesions were apparently observed in murine NP models. Anti-IL-17A treatment downregulated the mRNA and protein expression of MMP-9 in nasal mucosa and reduced the number of polypoid lesions in the murine NP model (all $p < 0.05$). ***Conclusion:*** Our results suggest that IL-17A plays a crucial role and may affect the prognosis of CRSwNP. Anti-IL-17A treatment may reduce the formation of polypoid lesions through inhibition of MMP-9 expression.

Introduction

Chronic rhinosinusitis (CRS) is one of the most common chronic diseases, with an estimated prevalence of 8% in China [1] and 10.9–11.9% in Europe and the US [2, 3]. CRS not only seriously affects the quality of a patient's life, but also consumes a great number of medical resources. It has been reported that in the US, the direct medical expenses for CRS were as high as 8.6 billion dollars per year, and the indirect costs were as high as 12.8 billion dollars [4]. As defined in the European Position Paper on Rhinosinusitis and Nasal Polyps 2012 (EPOS 2012) [5], CRS is currently divided into CRS with nasal polyps (CRSwNP) and CRS without nasal polyps. Our previous study found that 44.4% of patients with CRSwNP patients had recurrence after 1 year of standardized treatment [6]. One study reported that 33.7% of patients with CRSwNP showed difficult-to-treat CRS after 1 year of standardized treatment [7]. Another study reported that a total of 55.3% of patients with CRSwNP experienced recurrence within 2 years after surgery [8]. In addition, some research reported that the recurrence rate of CRSwNP reached 60–70% within 18 months after surgery [9]. However, the pathogenesis of CRSwNP still remains unclear. Therefore, further research on the pathogenesis of CRSwNP is necessary to discover potential therapeutic targets.

CRSwNP is mainly characterized by increased levels of matrix metalloproteinases (MMPs) and prominent edema formation in the extracellular matrix (ECM) [10, 11]. MMPs constitute a family of zinc- and calcium-dependent proteinases that play a key role in the degradation of the ECM [12]. The imbalance of MMPs to their tissue inhibitors of metalloproteinases can lead to the formation of edema, which is one of the main mechanisms of the remodeling of CRSwNP [13]. Previous studies reported that MMPs may be important potential therapeutic targets for CRSwNP [14].

Our previous study found that MMP-9 is highly expressed in CRSwNP, and it has been confirmed in vitro that interleukin-17A (IL-17A) upregulated MMP-9 expression in nasal epithelial cells and might play a crucial role in tissue remodeling of CRSwNP [15]. However, no in vivo experimental studies have been performed on related animals. IL-17A, commonly referred to as IL-17, is a proinflammatory cytokine that has been associated with the pathology of numerous inflammatory and autoimmune diseases such as allergic rhinitis [16], asthma [17], rheumatoid arthritis [18], psoriasis [19], and cancer [20]. As reported, treatment with anti-IL-17 or IL-17RA reduced joint inflammation, cartilage destruction, and bone erosion in a collagen-induced arthritis mouse model as well as in human synovium and bone explants [21, 22]. Hueber et al. [23] confirmed that AIN457 (a human antibody to IL-17A) treatment reduced the area and severity index of psoriasis. However, whether anti-IL-17A treatment plays a role in CRSwNP has still not been reported. Therefore, the purpose of the present study was to explore the expression of IL-17A and its impact on prognosis in CRSwNP as well as to establish a murine nasal polyps (NP) model to detect the expression of IL-17A and evaluate the anti-IL-17A effect on NP formation in this model.

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Table 1. Characteristics and clinical features of the study subjects

	Control	CRSwNP
Total subject number	12	21
Sex, male/female	9/3	13/8
Age, years	28±11	43±15
Atopy	4 (33.33%)	14 (66.67%)
Asthma	0	1 (4.76%)
Aspirin sensitivity	0	0
LMCS	–	13.24±4.50
LKES B	–	10.57±1.72
LKES A	–	5.62±2.99
LKES R	–	4.90±2.59

Values are presented as *n*, *n* (%), or mean ± SD. CRSwNP, chronic rhinosinusitis with nasal polyps; LKES A, Lund-Kennedy endoscopic scoring 6 months after surgery; LKES B, Lund-Kennedy endoscopic scoring before surgery; LKES R, reduction of Lund-Kennedy endoscopic scoring; LMCS, Lund-Mackay computed tomography scoring.

Subjects and Methods

Patients and Samples

Patients were selected from the Department of Otorhinolaryngology of The Third Affiliated Hospital of Sun Yat-Sen University (Guangzhou, China). Inferior turbinate or uncinated process samples from patients without sinus disease undergoing septoplasty or optic nerve decompression were collected as controls (*n* = 12). The diagnosis of CRSwNP was based on the guidelines of the EPOS 2012 [5] (*n* = 21). NP from patients with CRSwNP were obtained during endoscopic sinus surgery. Subjects who had autoimmune diseases, cystic fibrosis, aspirin intolerance triad, unilateral rhinosinusitis, allergic fungal rhinosinusitis, or immotile ciliary disease were excluded. None of the patients had used oral corticosteroids for at least 2 months or topical application for at least 1 month prior to surgery. Each sample was stored in liquid nitrogen or fixed in 10% neutral buffered formalin. The atopic status of the subjects was evaluated for determination of immunoglobulin E antibodies against common aeroallergens by blood sampling. Asthma was diagnosed by an allergist based on medical history and lung function analysis. All patients in the CRSwNP group underwent computed tomography (CT) scanning before surgery. The Lund-Mackay CT scoring (LMCS) system was used to evaluate the severity of their sinus disease. The Lund-Kennedy endoscopic scoring (LKES) system was used to evaluate the severity of inflammation and the outcome of treatment. All patients were submitted to a nasal endoscopy examination before (LKES B) and 6 months after surgery (LKES A). The reduction of LKES (LKES R) was the difference between LKES B and LKES A scores. More detailed characteristics and clinical features of subjects are provided in Table 1.

Immunofluorescence Staining

The human tissue samples stored in liquid nitrogen were embedded in optimal cutting temperature compound. Serial cryosections were cut into 5-μm sections and fixed in acetone. Sections were incubated at 4 °C overnight with antibodies of human IL-17A (Santa Cruz Biotechnology Inc., USA). Secondary antibodies (Life Technology, USA) were incubated for 1 h at ambient temperature, and then 4′,6-diamidino-2-phenylindole (DAPI) nucleic acid stain (Life Technology) was applied for 10 min. Sections were examined at a magnification of 400× in a Nikon microscope (Eclipse E600; Nikon). All sections were examined by two independent blinded investigators. The number of IL-17A$^+$ cells in five random fields was counted from each tissue specimen.

Murine NP Model

BALB/c mice (4 weeks of age, 20–22 g; Beijing Vital River Laboratory Animal Technology Co., Ltd. Inc.) were categorized into one control and two experimental groups: a normal saline-instilled group (control group), the NP model group (NP group), and the NP model group treated with anti-IL-17A (R&D Systems, Minneapolis, MN, USA) (NP+anti-IL-17A group). Briefly, mice in the experimental groups were intraperitoneally injected with 50 μg of ovalbumin (OVA) (Sigma, St. Louis, MO, USA) and 5 mg of $A1(OH)_3$ dissolved in

1 mL of normal saline every other day for six consecutive times. From day 12, mice were intranasally administered 5% OVA diluted in 40 μL of phosphate-buffered saline daily for 1 week. Thereafter, mice were intranasally administered 5% OVA three times a week until day 102. On day 47, the experimental groups had a Merocel sponge placed in the left nasal cavity, and the sponge was dripped with staphylococcal enterotoxin B (SEB) once a week for 8 weeks. The NP and NP+anti-IL-17A groups were respectively administered isotype immunoglobulin G (purified normal rabbit immunoglobulin G; R&D Systems; 50 μg per mouse) and anti-IL-17A (R&D Systems; 50 μg per mouse) weekly intraperitoneally from day 49 to day 102 before OVA instillation referring to other literature [24, 25]. Control mice were not sensitized but administered isotype immunoglobulin G intraperitoneally from day 49 to day 102 weekly. On day 102, mice were euthanized to obtain tissues and blood for further assay. Each group consisted of 6 mice; 3 of them were used for real-time quantitative PCR, while the nasal mucosae of the remaining 3 mice were used for histological analysis.

Histological Analysis

For histological analysis, the heads of mice were removed and fixed in 4% paraformaldehyde and subsequently decalcified, embedded in paraffin, and sectioned coronally at 5-μm thickness. Samples were stained with hematoxylin and eosin for mucosal lesions. Polypoid lesions were defined as distinct mucosal elevations with inflammatory cell infiltration around microcavities.

Real-Time Quantitative PCR

The total RNA of the mouse sinus mucosal tissue was taken and stored in RNAiso Plus (TaKaRa Biotechnology, Dalian, China) and reverse-transcribed to cDNA with the PrimeScript RT reagent kit (TaKaRa Biotechnology). The average gene transcript levels were then normalized to that of β2 microglobulin. The reaction was performed using an ABI 7500 FAST instrument (ABI, Foster City, CA, USA) with the SYBR Premix Ex Taq kit (TaKaRa Biotechnology). Relative gene expression was done by the $2^{-\Delta\Delta CT}$ method.

ELISA

Blood was taken from the eyeball of the mouse, left standing for 2 h, and then centrifuged to obtain serum. Nasal lavage was performed as previously described [26]. After partial tracheal resection, the needle was inserted into the posterior choana through the tracheal opening. Next, the choana was lightly perfused with 1 mL of phosphate-buffered saline, and liquid was collected from the nostrils. IL-17A and MMP-9 levels in nasal lavage fluid and serum were measured by commercial ELISA kits (CUSABIO, Wuhan, China). All protocols were carried out according to the manufacturers' recommendations.

Statistical Analysis

Statistical analysis was performed using IBM SPSS 20 (SPSS, Chicago, IL, USA) and GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA, USA) software. Data were presented as mean ± standard error of the mean or median (25th–75th percentiles). For statistical analysis, the Student *t* test or the Mann-Whitney U test (two-tailed) was used for between-group comparisons. Pearson or Spearman correlation was used to determine variable relationships. $p < 0.05$ was considered statistically significant.

Results

Expression of IL-17A in Nasal Samples

To detect the IL-17A$^+$ cells in the samples of different groups, immunofluorescence analysis was used. Representative images showed that IL-17A was located in the cytoplasm and cell membrane, and IL-17A$^+$ cells were significantly more expressed in CRSwNP than in the control group (Fig. 1a). A semiquantitative analysis of immunofluorescence staining showed that the number of IL-17A$^+$ cells was significantly increased in nasal tissues from patients with CRSwNP compared with control subjects ($p < 0.001$) (Fig. 1b).

Correlation between IL-17A Expression and Clinical Features in CRSwNP

In patients with CRSwNP, the LMCS score before operation was 13.24 ± 4.50; the LKES B and LKES A scores were 10.57 ± 1.72 and 5.62 ± 2.99, respectively. The LKES R score was

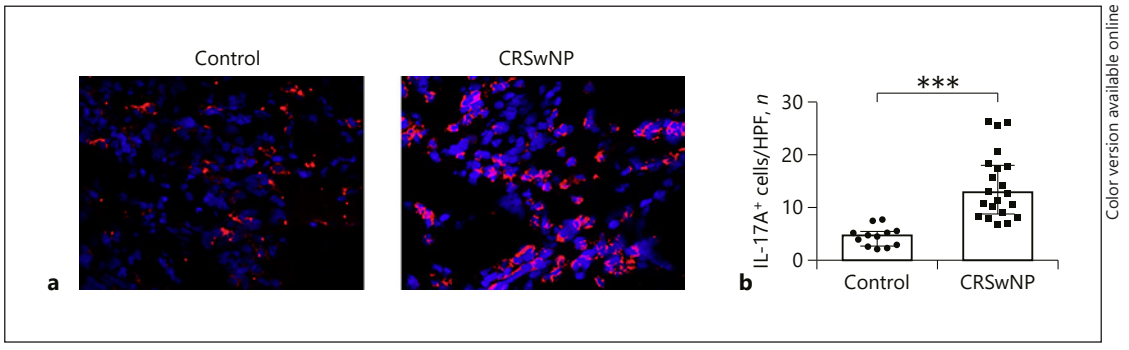


Fig. 1. Expression of IL-17A in nasal samples. **a** Representative images of IL-17A$^+$ cells from control subjects and patients with CRSwNP by immunofluorescence (magnification ×400). **b** IL-17A$^+$ cells in nasal tissue were counted semiquantitatively. *** $p < 0.001$. CRSwNP, chronic rhinosinusitis with nasal polyps; HPF, high-power field; IL-17A, interleukin-17A.

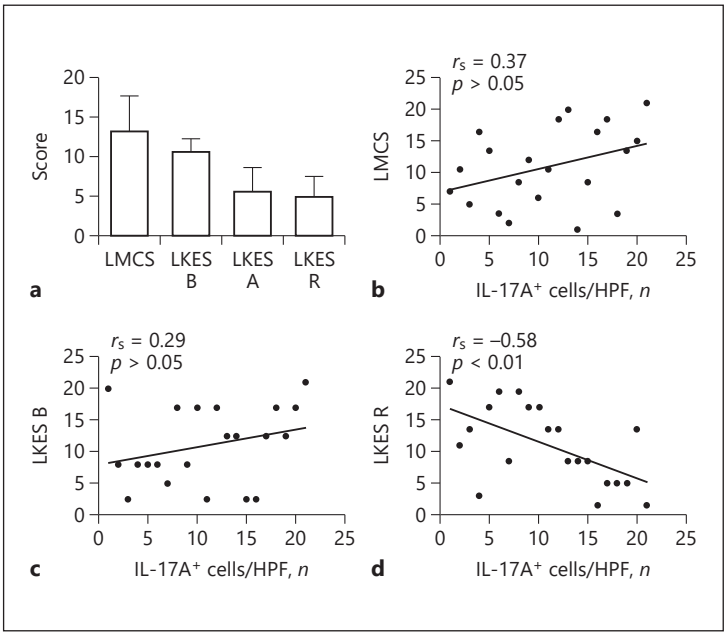


Fig. 2. Correlation between IL-17A expression and clinical features in patients with CRSwNP. **a** Clinical characteristics in patients with CRSwNP. **b–d** Correlation between numbers of IL-17A$^+$ cells from immunofluorescence assay and LMCS (**b**), LKES B (**c**), and LKES R (**d**) in matched patients with CRSwNP. CRSwNP, chronic rhinosinusitis with nasal polyps; HPF, high-power field; IL-17A, interleukin-17A; LKES A, Lund-Kennedy endoscopic scoring 6 months after surgery; LKES B, Lund-Kennedy endoscopic scoring before surgery; LKES R, reduction of Lund-Kennedy endoscopic scoring; LMCS, Lund-Mackay computed tomography scoring.

4.90 ± 2.59 (Fig. 2a; Table 1). Furthermore, we found that the number of IL-17A$^+$ cells in the immunofluorescence study was significantly negatively correlated with LKES R (r_s = –0.58, p = 0.006) (Fig. 2d), suggesting that IL-17A may be one of the factors affecting the prognosis of CRSwNP. However, there were no significant correlations between IL-17A and LMCS (r_s = 0.37, $p > 0.05$) (Fig. 2b) or between IL-17A and LKES B (r_s = 0.29, $p > 0.05$) (Fig. 2c).

Establishment of a Murine NP Model

We used OVA/SEB and a Merocel sponge to induce the murine NP model (Fig. 3a). We confirmed the establishment of the murine NP model by testing relevant indicators. The number of polypoid lesions was apparently higher in the murine NP model than in control mice (Fig. 3b, c). Our results also showed that MMP-9 mRNA expression in mouse sinus mucosa tissue and MMP-9 protein expression in nasal lavage fluid were both increased in the murine NP model compared with control mice ($p < 0.001$, $p = 0.007$) (Fig. 3d, e). However,

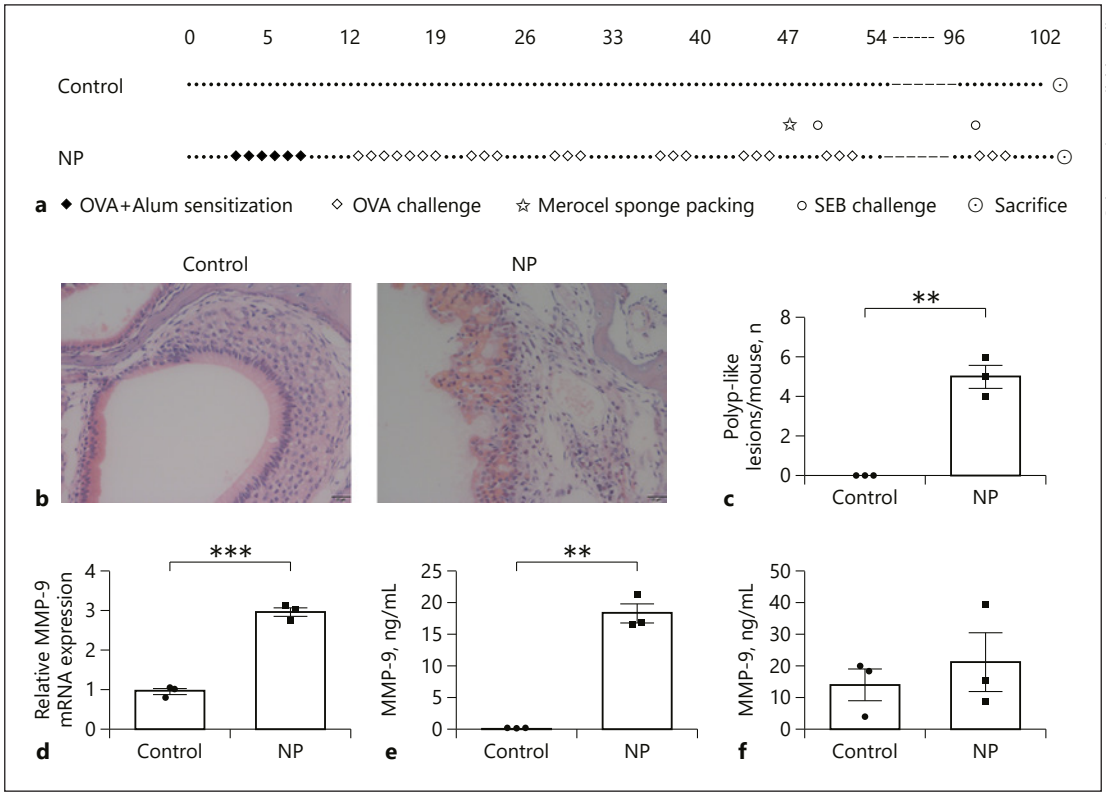


Fig. 3. Establishment of the murine NP model with NP lesions. **a** Protocol for generating the murine NP model. **b** Representative photographs of a polypoid lesion. **c** Comparison of numbers of NP lesions in different groups. **d** Relative mRNA expression levels of MMP-9 from each group were compared. **e** MMP-9 from mouse nasal lavage fluid was assayed by ELISA. **f** MMP-9 from mouse serum was also assayed by ELISA. We performed the murine model three times. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. MMP-9, matrix metalloproteinase-9; NP, nasal polyps; OVA, ovalbumin; SEB, staphylococcal enterotoxin B.

there were no statistical differences in MMP-9 protein levels in serum between them ($p > 0.05$) (Fig. 3f).

IL-17A Expression in the Murine NP Model

The murine NP model showed higher IL-17A mRNA expression in mouse sinus mucosa tissue ($p = 0.01$) (Fig. 4a) and higher IL-17A protein expression in nasal lavage fluid compared with that seen in control mice ($p = 0.027$) (Fig. 4b), whereas there were no statistical differences in IL-17A protein levels in serum between them ($p < 0.05$) (Fig. 4c).

Correlation Coefficient for the Association between IL-17A and MMP-9 in the Murine NP Model

To further study the relationship between the increasing IL-17A and MMP-9 expression in the murine NP model, we found that the protein expression of IL-17A positively correlated with MMP-9 from mouse nasal lavage fluid ($r_s = 0.829$, $p = 0.042$) (Fig. 5a), whereas no correlation from mouse serum was found ($r = 0.064$, $p = 0.904$) (Fig. 5b).

Anti-IL-17A Effect in the Murine NP Model

To investigate the therapeutic potential of blocking the effects of IL-17A in CRSwNP, we used a murine model of NP treatment with an anti-mouse IL-17A antibody (Fig. 6a). After

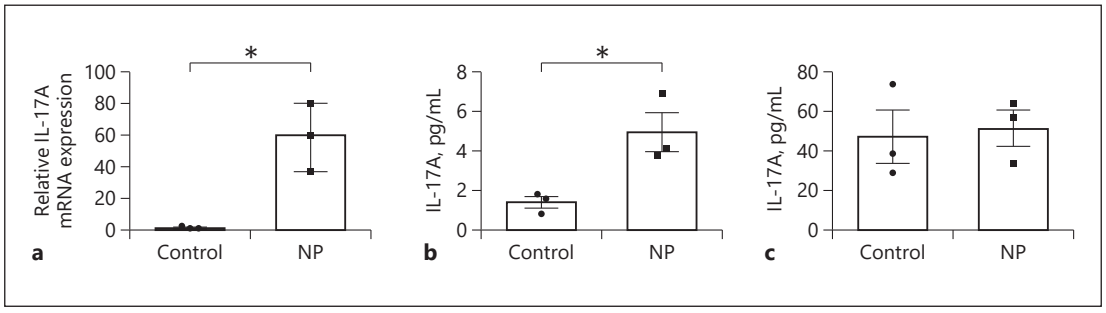
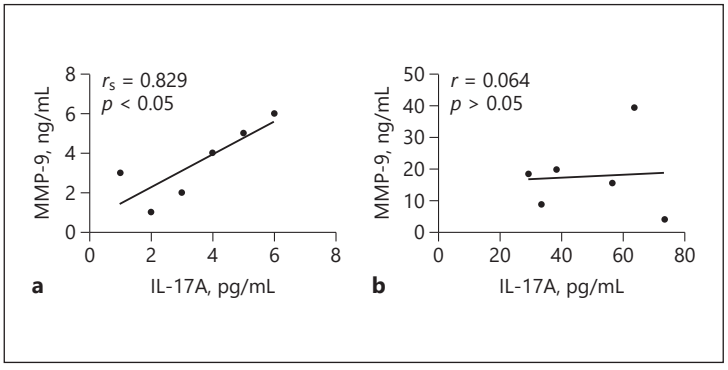


Fig. 4. IL-17A expression of the murine NP model. **a** Relative mRNA expression levels of IL-17A from each group were compared. **b** IL-17A from mouse nasal lavage fluid was assayed by ELISA. **c** IL-17A from mouse serum was also assayed by ELISA. We performed the murine model three times. * $p < 0.05$. IL-17A, interleukin-17A; NP, nasal polyps.

Fig. 5. Correlation coefficient for the association between IL-17A and MMP-9 in the murine CRS model. **a** Protein expression levels from mouse nasal lavage fluid were assayed by ELISA (Spearman correlation). **b** Protein expression levels from mouse serum were also assayed by ELISA (Pearson correlation). CRS, chronic rhinosinusitis; IL-17A, interleukin-17A; MMP-9, matrix metalloproteinase-9.



treatment with anti-IL-17A antibody, MMP-9 mRNA expression in mouse sinus mucosa tissue and MMP-9 protein expression in nasal lavage fluid were significantly decreased compared with those of NP mice (without anti-IL-17A) ($p = 0.001$, $p = 0.01$) (Fig. 6b, c). Moreover, anti-IL-17A treatment reduced the number of polypoid lesions in the murine NP model (Fig. 6d, e).

Discussion

At present, the pathogenesis of CRSwNP is still unclear, and it remains a challenging clinical entity with its propensity for recurrence after standardized treatment [7, 8]. CRSwNP affects quality of life and requires a large amount of medical resources [4]. Therefore, to further study the pathogenesis of CRSwNP and explore possible therapeutic targets has important clinical relevance.

IL-17A, commonly referred to as IL-17, is the most important member of the IL-17 cytokine family, and its main biological effect is to promote inflammatory response [27]. IL-17A is involved in the pathogenesis of a variety of inflammatory and autoimmune diseases and tumors, including allergic rhinitis [16], asthma [17], rheumatoid arthritis [18], psoriasis [19], and cancer [20]. Our study showed that the expression of IL-17A in nasal mucosa of CRSwNP patients was significantly higher than that in the control subjects by immunofluorescence, which was consistent with the findings of other scholars [28, 29],

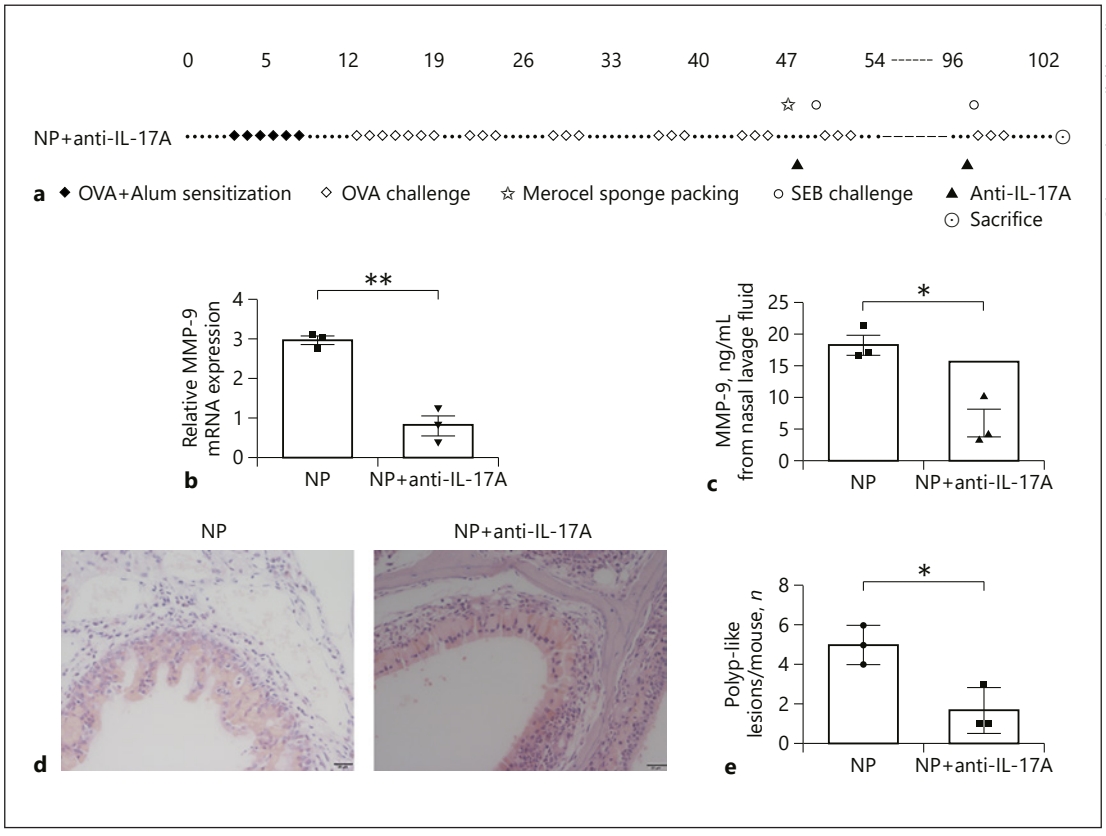


Fig. 6. Anti-IL-17A effect in the murine NP model. **a** Protocol for anti-IL-17A on the murine NP model. **b** Relative mRNA expression levels of MMP-9 from each group were compared. **c** MMP-9 from mouse nasal lavage fluid was assayed by ELISA. **d** Representative photographs of a polypoid lesion. **e** Comparison of numbers of NP lesions in different groups. We performed the murine model three times. * $p < 0.05$, ** $p < 0.01$. IL-17A, interleukin-17A; MMP-9, matrix metalloproteinase-9; NP, nasal polyps; NP+anti-IL-17A, nasal polyps model group treated with anti-IL-17A; OVA, ovalbumin; SEB, staphylococcal enterotoxin B.

suggesting that IL-17A is involved in the pathogenesis of CRSwNP. However, the relationship between IL-17A and patients' clinical features remains controversial. Makihara et al. [30] found a significant positive correlation between the radiological severity of sinusitis and the total number of IL-17A$^+$ cells in nasal tissue samples from patients with CRSwNP. Hu et al. [31] reported significant positive correlations between IL-17A expression in CRS and symptom severity, using endoscopic findings and CT appearance. However, Saitoh et al. [32] reported that CT findings and symptom scores were not correlated with the number of IL-17A$^+$ cells in CRS. Thus, we further linked with patients' clinical features into groups LMCS, LKES B, LKES A, and LKES R, and found that the number of IL-17$^+$ cells was not significantly associated with LMCS or LKES B. This phenomenon may be caused by the following reasons: the different premedication, the individual inflammatory factors and inflammatory cells, and the different stages when the patient received the surgery. However, we found that the number of IL-17A$^+$ cells was significantly negatively correlated with LKES R, suggesting that IL-17A may be one of the key factors affecting the prognosis of CRSwNP. High IL-17A levels might promote polypoid lesion formation and result in recurrence of CRSwNP.

To determine the effect of IL-17A in CRSwNP, we further established a murine NP model developed in previous studies [33, 34]. Many studies have reported that *Staphylococcus*

aureus exotoxins are linked with allergic diseases, and SEB is one of the *S. aureus* exotoxins commonly found in NP [35, 36]. Specific immunoglobulin E against SEB is more commonly seen in patients with CRSwNP [37]. Therefore, this murine NP model was generated by intranasal instillation of OVA and SEB after OVA sensitization. To better simulate the characteristics of patients with poor sinus drainage, we added a Merocel swelling sponge soaked with SEB to the left nasal cavity. In the study by Kim et al. [38], polypoid lesion was defined as distinct mucosal bulges with microcavity formation. In other studies the criteria for NP included a more elevated lesion than surrounding mucosal folds and inner microcavities [39, 40]. In our study, the murine NP model showed severe polypoid lesions, mixed inflammatory cells, and increasing expression of MMP-9, which is consistent with phenotypes of patients with CRSwNP. Furthermore, both mucosal tissues and nasal lavage fluid in these mice showed increasing IL-17A expression, whereas the expression of IL-17A in serum was not increased. Similar to that observed in our previous study [15], we found the markedly higher percentage of IL-17A$^+$ lymphocytes only in the nasal samples, but not in the blood from patients with CRSwNP. Therefore, CRS inflammation mediated by IL-17A is mainly confined to the mucosal compartment rather than a reflection of a systemic immune disorder. Thus, we consider that this murine NP model could represent the immunologic characteristics of human CRSwNP and be used for evaluating the role of IL-17A in the pathogenesis of this disease.

Tissue remodeling is the main pathological feature of chronic airway inflammation. The imbalance between MMPs, key factors of tissue remodeling and their tissue inhibitors, tissue inhibitors of metalloproteinases, leads to abnormal synthesis or degradation of the ECM, resulting in fibrosis or edema formation, which is one of the main mechanisms of CRS tissue remodeling [13]. Previous research found that CRSwNP is mainly characterized by prominent edema formation in the ECM and increasing levels of MMPs, especially MMP-7 and MMP-9 [10, 41, 42]. It was reported that MMP-9 is involved in airway tissue remodeling of chronic inflammatory diseases such as asthma and chronic obstructive pulmonary disease [43]. Besides, studies have found that MMPs may be important potential therapeutic targets for CRSwNP [14]. Our previous study found in in vitro experiments that IL-17A upregulated the expression of MMP-9 in nasal epithelial cells, and then promoted edema formation, resulting in the remodeling of CRSwNP [15].

In our animal experiments, there was no correlation with IL-17A and MMP-9 expression in serum, but we found IL-17A to be positively correlated with MMP-9 in nasal lavage fluid, suggesting that IL-17A orchestrated local inflammation in nasal mucosa and promoted the production of MMP-9 that leads to polypoid-like lesions. Thus, we next blocked the effects of IL-17A to investigate the therapeutic effect, and testified that anti-IL-17A treatment suppressed MMP-9 protein expression in nasal lavage fluid and downregulated MMP-9 mRNA expression in nasal sinus mucosa tissue, even reducing the number of polypoid lesions in a murine NP model, providing a new direction for the treatment of CRSwNP.

In summary, we showed that IL-17A was highly expressed in patients with CRSwNP and was significantly negatively correlated with LKES R, suggesting that IL-17A might be one of the factors affecting the prognosis of CRSwNP. IL-17A expression was more abundant in the murine NP model compared with control mice, and was positively correlated with expression of MMP-9 in nasal tissue samples. Anti-IL-17A treatment reduced the MMP-9 expression and the number of polypoid lesions in the murine NP model. Taken together, these findings suggest that IL-17A plays a crucial role and may affect the prognosis of CRSwNP. Anti-IL-17A treatment may reduce the formation of polypoid lesions by inhibiting MMP-9 expression, providing a novel therapeutic strategy to improve outcomes of patients with CRSwNP.

Statement of Ethics

This study was approved by the Ethics Committee of The Third Affiliated Hospital of Sun Yat-Sen University (No. [2013]2-9) and by the Animal Ethics and Welfare Committee of Jinan University. All participants provided written informed consent for publication.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

Funding Sources

This study was supported by grants from the National Natural Science Foundation of China (81371073, 81670913).

Author Contributions

J.-C. Huang and X.-H. Chen contributed equally as co-first authors. G.-H. Zhang and L.-H. Chang designed the research and are in charge of correspondence. J.-C. Huang and X.-H. Chen performed the experiments. Z.-Y. Wang helped carry out the experiments. J.-C. Huang and X. Li analyzed the data. J.-C. Huang, X.-H. Chen, and G.-H. Zhang wrote the manuscript. All the authors read and approved the final manuscript.

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