

Original Article

Inflammatory characteristics in different types of nonallergic rhinitis

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Abstract: *Objective:* Nonallergic rhinitis (NAR) is classified on the basis of the eosinophil (EOS) level. Our study aimed to describe the inflammatory characteristics in different types of nonallergic rhinitis. *Methods:* In our study, 12 patients with allergic rhinitis (AR), 10 patients with nonallergic rhinitis with eosinophilia syndrome (NARES), 12 patients with nonallergic rhinitis without eosinophilia, and 11 healthy participants were enrolled from May 2016 to July 2016. We assessed the inflammatory characteristics by using skin prick testing (SPT) and determined the nasal/serum cytokine levels and regulatory T cells (Tregs) in the peripheral blood. *Results:* The nasal IL-4 (42.27 ± 22.10 vs. 24.93 ± 8.90) and IL-17 (25.51 ± 5.11 vs. 16.80 ± 4.92) levels, as well as the serum IL-4 ($49.87 [24.76-101.35]$ vs. $20.92 [9.40-29.87]$) and IL-17 ($31.07 [22.14-96.41]$ vs. $21.85 [18.55-26.46]$) levels were higher in the NARES group than in the control group ($P < 0.05$). The nasal IL-10 ($3.65 [3.45-4.03]$ vs. $4.16 [3.55-4.27]$) levels and the proportion of Tregs in the peripheral blood ($[5.0 \pm 1.8]\%$ vs. $[6.5 \pm 1.0]\%$) were lower in the NARES group than in the control group ($P < 0.05$). There were no significant differences between the AR group and the NARES group or between the NAR without eosinophilia group and the control group ($P > 0.05$). *Conclusions:* Nonallergic rhinitis was classified on the basis of the EOS level, and the two types of NAR had different pathogenesis of inflammation and inflammatory manifestations. Further, NARES and AR had similar pathogenesis of inflammation, which resemble the Th2 and Th17 inflammatory characteristics. Therefore, it would be more logical to determine the classification of rhinitis on the basis of EOS level.

Keywords: Allergic rhinitis, nonallergic rhinitis with eosinophilia, nasal douche, Th2 cell, Th17 cell, Treg cell

Introduction

Rhinitis is a type of chronic inflammation of the nasal mucosa, which is classified into allergic rhinitis (AR) and nonallergic rhinitis (NAR) that has no specific allergens. In the past decades, AR has been extensively studied by both domestic and international otolaryngologists [1, 2]. These investigators have concluded that AR is characterized by eosinophilia and hyper-reactive eosinophils (EOS). Mechanically, CD4⁺T cells are differentiated into Th1, Th2, Th17, and regulatory T cells (Tregs) according to different stimulators, and imbalances in Th1/Th2 cells and Treg/Th17 subpopulations contributes to the pathogenesis of AR. However, many questions remain regarding the diagnosis and treatment of NAR. Nonallergic rhinitis with eosinophilia syndrome (NARES), comprising NAR and

nasal EOS inflammation, is a clinical hyper-reactive syndrome characterized by eosinophilia in the nasal secretions, accounting for as many as 14% of cases of rhinitis patients [3]. The clinical syndrome of NARES, similar to AR, includes nasal obstruction, nasal itching, sneezing, and rhinorrhea. Patients with NARES have a large amount of EOS in the nasal secretions with no systemic manifestations (negative results on skin prick testing [SPT]); however, the pathogenesis of NARES is unclear; current studies have shown that NARES might be related to the nasal IgE [4, 5]. NARES is associated with bronchial asthma, nasal polyps, aspirin intolerance, and other diseases [6-8], but the mechanism underlying NARES has not been elucidated. In our previous research, we found NARES patients have suffer from nasal, lower airway and systemic EOS inflammation. And the degree

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of EOS inflammation is similar to AR patients [9]. EOS inflammation is mainly induced by hyperactivation of Th2 cells. Moreover, Th17 cells promote Th2 cell-driven eosinophilic airway inflammation [10]. On the basis of these findings, in the current study, NAR patients were classified as NARES group and NAR without eosinophilia group, we detect the nasal/serum cytokine levels and Tregs in the peripheral blood in the two groups; discuss the characteristics of inflammation in different types of NAR.

Subjects and methods

Subjects

The study was conducted between May 2016 and July 2016, and included 12 patients with NARES (age: 38.85 ± 12.01 , male:female = 6:6), 10 patients with NAR without eosinophilia (age: 36.63 ± 11.81 , male:female = 5:5), 12 patients with AR (age: 34.38 ± 12.49 , male:female = 5:7), and 11 healthy participants (age: 35.42 ± 11.23 , male:female = 5:6). Exclusion criteria included: 1) systemic disease, and 2) nasal sinus diseases such as nasosinusitis, deviation of nasal septum, nasal polyps, etc. There were no statistical differences regarding age, sex, and smoking history among the patients in the four groups ($P > 0.05$). All subjects provided written informed consent.

AR and NAR were defined according to the latest published diagnostic criteria [11]. AR patients presented: 1) two or more symptoms of rhinitis including sneezing, nasal itching, rhinorrhea, and nasal obstruction; and 2) positive SPT. Patients with NARES exhibited: 1) two or more symptoms of rhinitis including sneezing, nasal itching, rhinorrhea, and nasal obstruction; 2) negative SPT; 3) EOS $> 2.58/200$ High power field (HP) in nasal lavages [10]. NAR without eosinophilia was defined as: 1) two or more symptoms of rhinitis including sneezing, nasal itching, rhinorrhea, and nasal obstruction; 2) negative SPT; and 3) EOS $< 2.58/200$ HP in nasal lavages [12].

Methods

We obtained the medical histories and performed SPT, nasal lavage, and blood examination for rhinitis patients; they were required to

suspend treatment with antihistamines, glucocorticoids, and cold remedies for one week before these tests.

SPT: SPT was performed with the international standard allergens (Alutard® SQ, ALK-Abello A/S, Hoersholm, Denmark) including 13 types of allergens: house dust mites, dust mites, tropical mites, dog hair, cat hair, pollen group I, pollen group IV, German cockroach, American cockroach, felon herb, ragweed, mold group I, and mold group IV. The species included in pollen group I were the plane tree, poplar, willow, and elm. Pollen group IV included spider brake, ghee timothy, darnel, and pasture grass. Included in mold group I were *Alternaria tenuis*, *Chaetomium globosum*, mixed *Cladosporium*, and *Fusarium verticillioides*. Mold group IV included blue mold, *Penicillium expansum*, and *Penicillium notatum*. The prick test was performed by a professional who adhered strictly to the protocol received with the ALK prick-test solution. The test results were obtained 15 min after the prick. The test was considered positive if there was a pale yellow skin papule with surrounding erythema.

Nasal douche test: Saline nasal irrigation was performed using a syringe, and 10 mL of irrigation solution (warm 0.9% normal saline) was injected into the middle and lower nasal meatus (irrigation was considered thorough if there was saline running out from the other nostril). The irrigation fluid was recollected with a funnel, and irrigation with the fluid in the funnel was repeated three times and lasted for 5 min. Next, we drew out the irrigation fluid and asked the patient to blow his/her nose gently to ensure that the remaining fluid was completely collected in the funnel. Then, 5 mL of the irrigation fluid was used for testing. After that, 20 μ L of the cell sediments were smeared onto a slide and the slide was observed under a microscope at 200 HP. The total number of inflammatory cells (e.g., EOS, macrophages, lymphocytes, and neutrophils) was determined. Then, the number of each inflammatory cell/200 HP was calculated. The absolute value of EOS was determined, and 0.4 mL of the supernatants was subjected to a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) for measurement of IFN- γ , IL-4, IL-17, and IL-10 according to the manufacturer's instructions (Elabscience Biotechnology Co., Ltd., Bethesda, MD, USA).

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Table 1. Comparison of cytokine levels in each group

Group	Nasal IFN- γ	Nasal IL-4	Nasal IL-17	Nasal IL-10	Serum IFN- γ	Serum IL-4	Serum IL-17	Serum IL-10
AR group	12.15 (8.65~17.80)	40.35 \pm 18.92	23.85 \pm 5.40	4.44 \pm 0.56	358.44 \pm 197.31	33.71 (22.52~103.96)	29.34 (22.43~73.54)	3.73 (3.57~4.30)
NARES group	28.89 (10.91~127.07)	42.27 \pm 22.10	25.51 \pm 5.11	3.97 \pm 0.68	367.83 \pm 202.09	49.87 (24.76~101.35)	31.07 (22.14~96.41)	3.65 (3.45~4.03)
NAR without eosinophilia group	7.92 (7.67~45.85)	25.62 \pm 12.48	18.48 \pm 4.91	4.31 \pm 0.48	494.05 \pm 180.44	31.15 (24.28~42.99)	19.84 (17.68~23.87)	3.76 (3.47~4.67)
Control group	8.98 (7.88~14.90)	24.93 \pm 8.90	16.80 \pm 4.92	4.80 \pm 1.32	372.08 \pm 228.49	20.92 (9.40~29.87)	21.85 (18.55~26.46)	4.16 (3.55~4.27)

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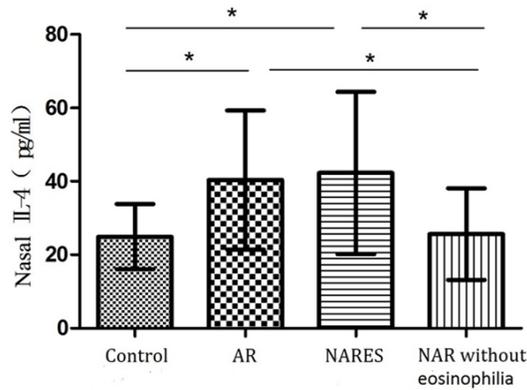


Figure 1. Comparison of nasal IL-4 in each group (* $P < 0.05$).

Detection of $CD4^+CD25^+FOXP3^+Treg$ in the peripheral blood involved the following procedure: 50 μ L of blood was incubated with 10 μ L of CD_4 antibody and 10 μ L of CD_{25} antibody for 15 min in the dark. Then, 1 mL hemolysin was added and the mixture was centrifuged at 1500 rpm for 5 min; the supernatants were discarded and the sediments were washed using phosphate-buffered saline (PBS). After centrifugation at 1500 rpm for 5 min, the supernatants were discarded and the sediments were incubated in 2 mL of Solution A (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) for 10 min in the dark. Then, the solution was centrifuged at 1500 rpm for 5 min, the supernatants were discarded and 500 μ L of Solution C (Becton, Dickinson and Company) was added. After centrifugation at 1500 rpm for 5 min, the sediments were washed in 2 mL PBS and then subjected to centrifugation at 1500 rpm for 5 min. The sediments were incubated with 10 μ L FOXP3 antibody for 30 min in the dark; then, the solution was mixed in 2 mL PBS and subjected to centrifugation at 1500 rpm for 5 min. The ratio of $CD4^+CD25^+FOXP3^+Treg/CD4^+T$ was determined using a FACSCalibur (Becton, Dickinson and Company).

Statistical analysis

Data were analyzed by SPSS 13.0 (IBM Corp., Armonk, NY, USA). If data were proven to be normally distributed, they were presented as ($\bar{x} \pm s$) and analyzed by one-way analysis of variation (ANOVA) followed by the least significant difference (LSD) test; otherwise, data were presented as median (interquartile range) and analyzed by the Kruskal-Wallis test followed by

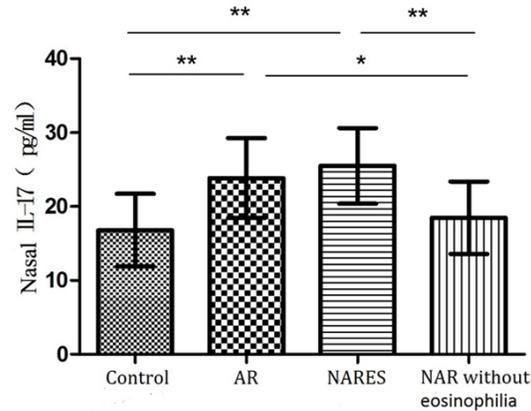


Figure 2. Comparison of serum IL-4 in each group (* $P < 0.05$, ** $P < 0.01$).

the Nemenyi test. A value of $P < 0.05$ was considered statistically significant.

Results

Eosinophils in nasal lavages

Compared with the control group (0.00 [0.00~0.15]) and the NAR without eosinophilia group (0.03 [0.00~1.43]), the eosinophils in nasal lavages were obviously increased in the NARES group (7.15 [4.58~17.50], $P < 0.05$) and the AR group (6.65 [3.13~30.30], $P < 0.05$). There no obvious differences between the AR group and the NARES group ($P > 0.05$), and between the NAR without eosinophilia group and the control group ($P > 0.05$).

Nasal and serum cytokine profile

Cytokines in the nasal lavage fluid and serum in the different groups (AR group, NARES group, NAR without eosinophilia group, and control group) are listed in **Table 1**. Compared with the control group, the cytokines including IFN- γ , IL-4, and IL-17 in the nasal lavage fluid and IL-4 and IL-17 in the serum were obviously increased in the NARES group ($P < 0.05$), while the IL-10 content in the nasal lavage fluid was decreased in the NARES group ($P < 0.05$). The expression of cytokines exhibited no obvious differences between the AR group and the NARES group ($P > 0.05$), and between the NAR without eosinophilia group and the control group ($P > 0.05$).

With regard to IFN- γ , its expression in the nasal lavage fluid was higher in the NARES group than in the control group and the NAR, without eosin-

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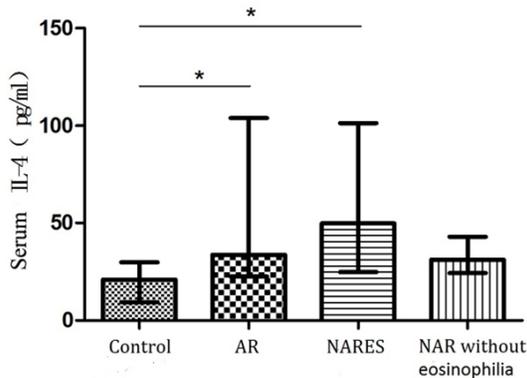


Figure 3. Comparison of nasal IL-17 in each group (* $P < 0.05$).

ophilia group ($P < 0.05$); there were no significant differences in IFN- γ expression in the other groups ($P > 0.05$). The levels of serum IFN- γ exhibited no obvious differences among the four groups ($P > 0.05$).

The expression of IL-4 in the nasal lavage fluid was higher in the AR and NARES groups than in the control and NAR without eosinophilia groups ($P < 0.05$); IL-4 in the nasal lavage fluid showed no obvious differences between the AR and NARES groups, and between the control and NAR without eosinophilia groups ($P > 0.05$) (**Figure 1**). Compared with the control group, serum IL-4 was increased in the AR and NARES groups ($P < 0.05$), while IL-4 showed no differences in the other groups ($P > 0.05$) (**Figure 2**).

The levels of IL-17 in the nasal lavage fluid were higher in the AR and NARES groups than in the control and NAR without eosinophilia groups ($P < 0.05$); the IL-17 levels in the nasal lavage fluid exhibited no differences between the AR and NARES groups and between the control and NAR without eosinophilia groups ($P > 0.05$) (**Figure 3**). Serum IL-17 levels in the AR and NARES groups were higher than those in the control and NAR without eosinophilia groups ($P < 0.05$); while serum IL-17 levels did not differ between the AR and NARES groups or between the control and NAR without eosinophilia groups ($P > 0.05$) (**Figure 4**).

IL-10 in the nasal lavage fluid was decreased in the NARES group compared with the control group ($P < 0.05$), while there were no differences among the other groups ($P > 0.05$). Serum IL-10 levels in the AR and NARES groups were

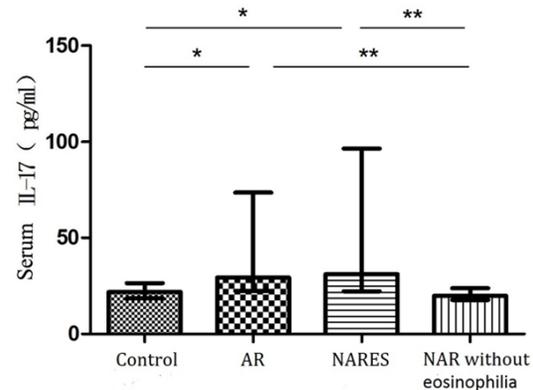


Figure 4. Comparison of serum IL-17 in each group (* $P < 0.05$, ** $P < 0.01$).

reduced compared with the control group, while there were no differences in expression of serum IL-10 among the four groups ($P > 0.05$).

CD4⁺CD25⁺FOXP3⁺Treg cells in the peripheral blood

The ratio of CD4⁺CD25⁺FOXP3⁺Treg cells to CD4⁺T cells in the peripheral blood was lower in the NARES group (5.0 ± 1.8)% than in the control group (6.5 ± 1.0)% and the NAR without eosinophilia groups (6.6 ± 2.0)% ($P < 0.05$); the ratio in the AR group (4.5 ± 1.3)% was decreased compared with that in the control group (6.5 ± 1.0)% and the NAR without eosinophilia group (6.6 ± 2.0)% ($P < 0.01$). However, there were no differences between the AR and NARES groups or between the control and NAR without eosinophilia groups ($P > 0.05$). **Figure 5** shows the flow cytometry scatter plot (right upper quadrant indicates the CD4⁺CD25⁺FOXP3⁺Treg cells, which were obviously less frequent in the AR and NARES groups than in the other two groups).

Discussion

Previous studies have shown that imbalances of Th1/Th2 cells and Treg/Th17 cells comprise the critical abnormal immune mechanism underlying AR. Th1 cells mainly secrete IFN- γ and TNF- β , and participate in the cellular immune response. In patients with AR or asthma, there is excessive differentiation of Th0 cells into Th2 cells, which makes Th2 cells the predominant cell type. Th2 cells primarily secrete IL-4, IL-5, and IL-13, and play a role in humoral immunity; among these cytokines, B

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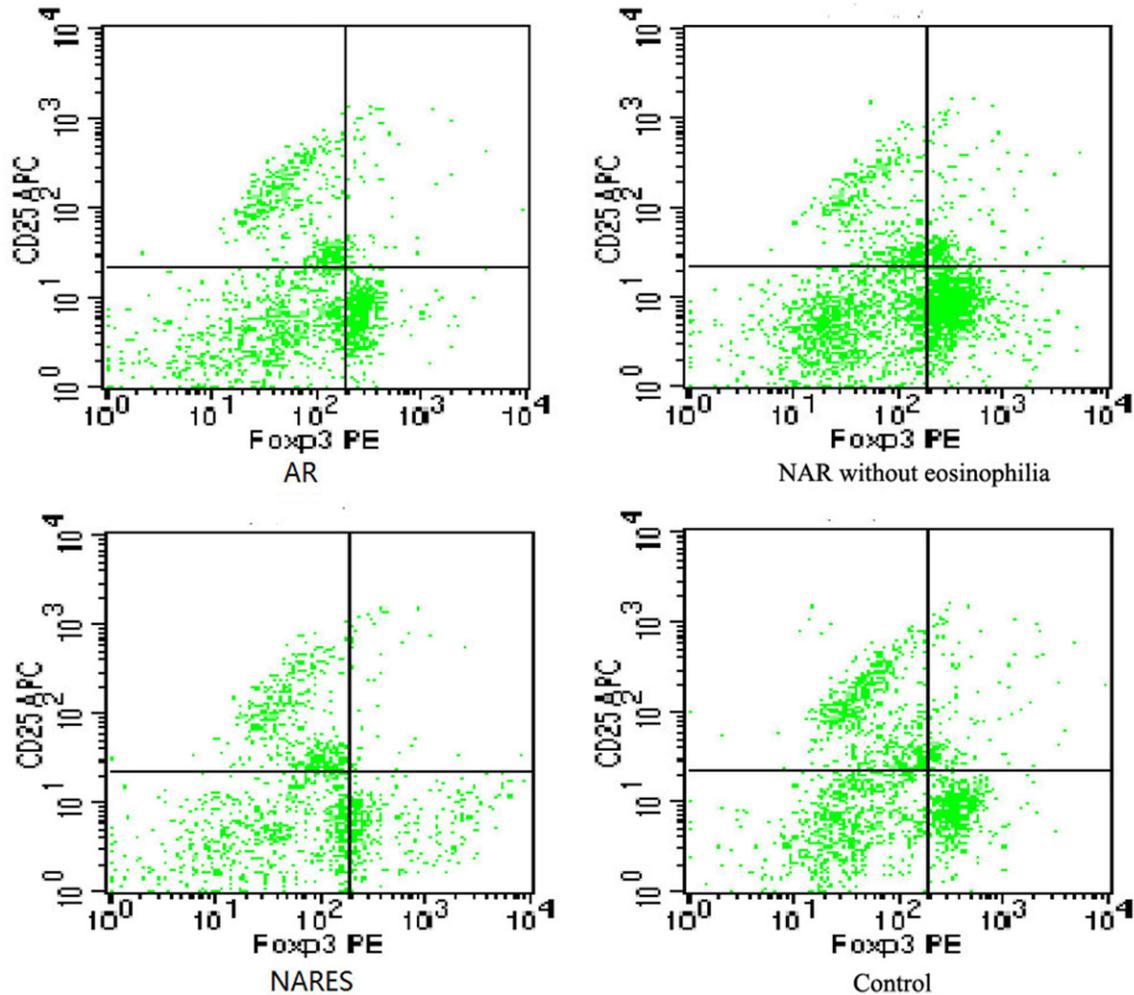


Figure 5. Comparison of flow cytometry scatter plot in each group.

lymphocytes produce IgE upon stimulation by IL-4 and IL-13, and IL-5 mediates EOS differentiation and migration. Th17 cells secrete IL-17, which plays a role in promoting inflammation and recruiting neutrophils. In addition, Th17 cells enhance Th2 cell-driven eosinophilic airway inflammation. Mechanically, Tregs inhibit the function of effector T cells through secretion of the immune suppressor IL-10 and TGF- β .

EOS inflammation is mainly induced by the overactivation of Th2 cells. Furthermore, Th17 cells promote the Th2 cell-driven eosinophilic airway inflammation [10]. Our previous study found nasal, lower airway, and systemic EOS inflammation in NARES patients, and the extent of inflammation is similar to that found in AR [9]. The present study showed that the nasal

and serum IL-4 and IL-17 levels were increased and Tregs were reduced in AR and NARES patients, and activation of the Th2 and Th17 cells was observed in the two groups; moreover, the suppressive function of Tregs was inhibited. Meanwhile, there were no differences in the expression of cytokines secreted by Th2 and Th17 cells in the NAR without eosinophilia group. Levels of nasal IFN- γ were increased in the NARES group, partly due to the nasal cellular immune response in these patients. In addition, serum IL-10 levels were decreased in the NARES and AR groups compared with the control group, but no statistical differences were observed, most likely because of the small sample size in the study.

Powe et al. [4, 5] demonstrated that nasal mucosal IgE was increased in NAR and AR

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patients with no systemic Th2 responses in NAR. Thus, local IgE elevation induces the typically nasal allergic response in NAR patients with no systemic inflammation, a phenomenon termed “entopy”. Based on this, the concept of local allergic rhinitis (LAR) was introduced, and defined to include [4, 5] those patients with nasal Th2 inflammation (nasal specific IgE and/or positive reaction to nasal allergen stimulation) and no systemic allergic reactions. It has been shown that LAR accounts for as many as 47%-62.5% of NAR patients [13, 14], but previous studies were limited to examination of local inflammation in the nasal mucosa. In the present study, NAR patients were divided into NARES and NAR without eosinophilia groups, and systemic inflammation was observed. Compared with the control group, patients in the NARES group exhibited nasal and systemic Th2 and Th17 responses, similar to the findings in AR. NARES is not only a local nasal inflammation, but also a systemic disease, which is in contrast to LAR and “entopy”. However, the role of EOS in the pathogenesis of LAR, as well as the relationship between LAR and NARES, still needs further investigation.

It has been shown that NARES is related to aspirin intolerance, bronchial asthma, and nasal polyps [6-8]. Accumulating evidence suggests that NAR is a risk factor for asthma and chronic bronchitis [15, 16]. The inflammation in asthma is mainly regulated by Th2 and Th17 cell-mediated inflammation and by the reduced suppressive function of Tregs. The current study found that NARES patients exhibited both nasal and systemic inflammation mediated by Th2 and Th17 cells, reduction in the numbers of Tregs, and airway inflammation related to asthma, suggesting that NARES was a risk factor for lower airway diseases.

Compared to the NAR classifications according to pathological etiology, i.e., infectious, endocrinal, drug-induced, etc., our previous classification is based on the levels of EOS. NARES patients exhibit nasal, lower airway, and systemic EOS inflammation, similar to AR [9]. The present study further demonstrated that NARES was an inflammatory process regulated by Th2 cells, which promoted the differentiation and recruitment of EOS. Th17 cells and Tregs were involved in this process, which was similar to AR in regard to the pathogenesis and pathological changes. In contrast, the patho-

genesis of drug-induced or endocrinal NAR might be different from the eosinophilic inflammation.

In clinical practice, rhinitis is classified into AR and NAR based on “atopy”. Amin et al. classified asthma into atopic and nonatopic asthma (i.e., eosinophilic and non-eosinophilic) based on eosinophil infiltration. The former is characterized by Th2 cell-induced airway inflammation and increased infiltration of EOS, IL-4⁺ cells, IL-5⁺ cells, and mast cells, while the latter is characterized by non-Th2 cell-induced airway inflammation; further, the degree of asthma, epithelial damage, and steroid sensitivity are correlated with the pattern of asthma [17]. After classification of NAR based on EOS levels, our study suggested different pathogenesis in the two types of NAR; the inflammation induced by the Th2, Th17, and Treg cells was similar in the NARES and AR groups, suggesting the similarity in the pathogenesis of these diseases. Therefore, it would be more logical to classify rhinitis on the basis of EOS infiltration. But sometimes the peripheral blood level does not reflect the exact patients’ immunologic condition, we need further studies. And the differences in the degree of rhinitis and the steroid sensitivity in NARES and AR still will require further investigation.

Disclosure of conflict of interest

None.

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