Article

Discovery Medicine 2024; 36(185): 1260–1267
https://doi.org/10.24976/Discov.Med.202436185.116

Changing Characteristics of Treg/Th17 Cells in the Nasal Mucosa of a Mouse Model of Allergic Rhinitis and the Effect of Intervention with Anti-IL-17 Antibody

Haiyong Sun[1], Baoxu Liu[1], Wei Ma[1], Dejun Ji[1], Bing Guan[1,*]

[1]Department of Otolaryngology, Northern Jiangsu People's Hospital Affiliated to Yangzhou University, 225001 Yangzhou, Jiangsu, China
*Correspondence: 18051063666@yzu.edu.cn (Bing Guan)
Published: 20 June 2024

Background: The incidence rate of allergic rhinitis (AR) is on the rise, which seriously affects the quality of life, work efficiency, mental state of patients, and sleeping in AR sleep. This experiment aimed to investigate the changes in Treg/Th17 cells in the nasal mucosa of an AR mouse model and the intervention effect of an Anti-IL-17 antibody.

Methods: A mouse model of AR was induced by intraperitoneal ovalbumin (OVA) injection for sensitization and stimulation with nasal drops. The times of rubbing, sneezing, and symptomatology scores were counted and analyzed. Pathological damage to the nasal mucosa was observed by Hematoxylin-Eosin (HE) staining. Peripheral blood $CD4^+CD25^+CD127^-$ Treg cell levels and the content of Th17 cells were measured by flow cytometry (FCM). ELISA kits were used to detect the levels of relevant cytokines (IL-10 and TGF-β1) secreted by Treg cells. The intervention effect of Anti-IL-17 antibody was observed by giving Anti-IL-17 antibody treatment.

Results: The times of rubbing, sneezing, and symptomatology scores were significantly higher in mice in the OVA group than in the Control group ($p < 0.001$). The percentage of $CD4^+CD25^+CD127^-$ Treg cells in $CD4^+CD25^+$ T cells ($p < 0.05$) and the levels of IL-10 ($p < 0.001$) and TGF-β1 ($p < 0.001$) were significantly decreased. After OVA induction, the continuity of the nasal mucosa of mice was interrupted, the percentage of Th17 cells, IL-17, and IL-4 levels were increased, and IFN-γ levels were significantly reduced ($p < 0.001$). And protein expression of RORγt was significantly upregulated ($p < 0.001$). In addition, all of these results were reversed by Anti-IL-17 antibody treatment, significantly improving AR-related symptoms ($p < 0.05$).

Conclusion: Anti-IL-17 antibodies may regulate the body's immune response by promoting $CD4^+CD25^+CD127^-$ Treg cell differentiation, thereby ameliorating the symptoms associated with AR.

Keywords: allergic rhinitis; Anti-IL-17 antibody; immune response; inflammation level; Th17/Treg

Introduction

Allergic rhinitis (AR) is a chronic inflammatory disease of the inner layers of the nasal mucosa and is one of the world's most common chronic diseases caused by an immunoglobulin E (IgE)-mediated reaction to inhaled, characterized by sneezing, nasal congestion, and a runny nose [1]. AR poses a significant health burden on individuals as it affects the quality of life of patients and is associated with severe comorbidities, including asthma, sinusitis, and conjunctivitis [2]. AR is divided into seasonal AR, which is triggered periodically by outdoor allergens, especially grass, tree, or weed pollen, and perennial AR (PAR), in which symptoms persist year-round in response to persistent indoor allergens such as house dust mites (HDM), animal dander, insects and mold [3]. Currently, the mainstay of treatment is medication, including antihistamines and intranasal corticosteroids, but they provide only temporary relief and do not reduce symptoms in 30 to 60 percent of patients [4]. A minimal persistent inflammatory state is a key factor in recurrent AR episodes [5]. The pathological process of AR involves a variety of immune cells as well as immune factors, such as mast cells, eosinophils/basophils, T cells, histamine, prostaglandins, and interleukins [6,7].

Multiple inflammatory cells are involved in AR, with a complex and diverse structure of signaling pathway networks. T cells and inflammatory cytokines have been shown to play a key role in AR inflammation. IL-17 is a characteristic cytokine secreted by Th17 cells, and six members of the IL-17 family have been identified so far, including IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F [8]. Studies have found that IL-17 plays an essential role in inflammatory response, autoimmune diseases, and tumors [8,9]. Besides, as a newly discovered $CD4^+$ T cell subset, Th17 and T reg cells are closely related to allergic diseases. The imbalance of the proportion and function of Treg and Th17 cell subsets is an important link in the pathogenesis of AR [10,11]. However, the mechanism regarding the role of IL-17 in AR is unclear.

Therefore, in this experiment, we observed the change characteristics of Treg/Th17 cells in the nasal mucosa of mice by constructing an AR mouse model. The potential mechanism of action was explored by Anti-IL-17 antibody intervention treatment.

Materials and Methods

Animals

50 Specific pathogen Free (SPF)-grade male BALB/c mice (7 weeks old, weighing about 22 g) were housed in a laminar flow chamber of specific pathogen-free (SPF) grade at a constant temperature of 25 °C, with 12 h of day and night illumination and an ad libitum diet. All animals were purchased from Skibbes Biotechnology Company (Anyang, China).

Model Construction and Animal Grouping

After basal sensitization by intraperitoneal injection of 200 µL of phosphate buffer solution (PBS) (Thermo Scientific, C0221A, Waltham, MA, USA) containing 25 µg of ovalbumin (OVA) (Sigma, 138831-86-4, St. Louis, MO, USA) and 2 mg of aluminium hydroxide on days 1, 8, and 15, the mice were given nasal drops using 40 µL of PBS containing 1000 µg of OVA (20 µL/side) on day 22, and the AR model was created by the nose drops 1 week later.

Firstly, the mice were divided into control and OVA groups to assess changes in mice with AR. Subsequently, mice were divided into control, OVA, and OVA+Anti-IL-17 Antibody groups (n = 10/group) to assess the intervention effect of Anti-IL-17 (Abcam, ab79056, Shanghai, China). After modeling, the mice in the OVA group were given an equal dose of normal saline nasal drops. The mice in the OVA+Anti-IL-17 Antibody group were intranasally administered with IL-17 antibody (1:50, 30 µL/side) 30 min before each nasal drip. The mice in the control group were given the same amount of normal saline instead of intraperitoneal injection, with nasal drip lasting for 7 days. After blood collection, the mice were immediately euthanized (Sodium pentobarbital, 150–300 mg/kg intraperitoneal injection), and the nasal mucosa was taken for HE staining.

Symptomatologic Observation

After the last nasal stimulation, the animals were observed for 30 min and scored according to the following criteria: 1 to 5 times of rubbing was scored as 1 point, 6 to 15 times was scored as 2 points, >15 times was scored as 3 points; 1 to 3 sneezing was scored as 1 point, 4 to 10 was scored as 2 points, >10 was scored as 3 points; 1 point was scored for sneezing into the anterior nostrils, 2 points for sneezing out of the anterior nostrils, and 3 points for sneezing over the whole surface of the face. If the total score exceeded 5 points, the modeling was successful.

Sample Collection

At the end of the experiment, the blood was collected from eyeballs, left at room temperature for 1 h, centrifuged at 3000 r/min for 10 min, and then the separated serum was stored at –80 °C for reservation. After blood collection, mice were immediately euthanized by cervical dislocation, and the nasal mucosa was separated and stored at –80 °C.

Hematoxylin-Eosin (HE) Staining

Bilateral nasal mucosal tissues removed by isolation were cleaned and fixed overnight in 4% paraformaldehyde (Nantong Jiangtian Chemical Co., Ltd., 30525-89-4, Nantong, China). Fixed tissues were taken, paraffin-embedded, and cut into 5 µm thick tissue sections for HE staining. Sections were dewaxed and dehydrated, stained with hematoxylin (Wuhan Doctoral Biology Company, C0105S, Wuhan, China) for 5 min, rinsed tissues under running water, stained with eosin for 3 min, rinsed again under running water, dehydrated with gradient ethanol, clarified with xylene, sealed with neutral gum, and air-dried. The morphology of nasal mucous tissue was observed, and the images were captured under a light microscope (Leica, DM2700 M, Weztlar, Germany).

Flow Cytometry

0.1 mL of anticoagulated venous blood was put into the flow cytometer sampling tube. For Tregs and Th17 cells, 10 µL of CD4 - FITC (Wuhan Sanying Biotechnology Co., Ltd., 65143, Wuhan, China) and CD4 - APC (Abcam, ab210347, Shanghai, China) were added, respectively, left at room temperature and protected from light for 30 min. 0.1% Triton X - 100 was added into the above sample tube; the cells were acted on for 15 min, and the cells were washed. CD127 - Treg antibody (Abcam, ab259806, 1:1000, Shanghai, China) was added to samples, and the cells were placed at room temperature and protected from light for 30 min. The isotype control tubes were set up at the same time; the cells were lysed and then detected by flow cytometry (Pudi Biotechnology Co., Ltd., Becton Dickinson FACSCalibur, Shanghai, China). Finally, the cells were analyzed using CellQuest software (Version: 4.1, BD Biosciences, Franklin Lake, NJ, USA).

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA kits were used to detect the levels of IL-10 (Thermo Fisher Scientific, JL20242, Waltham, MA, USA), TGF-β1 (Thermo Fisher Scientific, E-EL-0162c, Waltham, MA, USA), IL-17 (Thermo Fisher Scientific, PI550, Waltham, MA, USA), IL-4 (Thermo Fisher Scientific, JL20266, Waltham, MA, USA) and IFN-γ (Thermo Fisher Scientific, Abs510007, Waltham, MA, USA) in mouse serum. The experimental steps were carried out strictly according to the kit instructions. Finally, the Optical density (OD) value of each sample was detected at

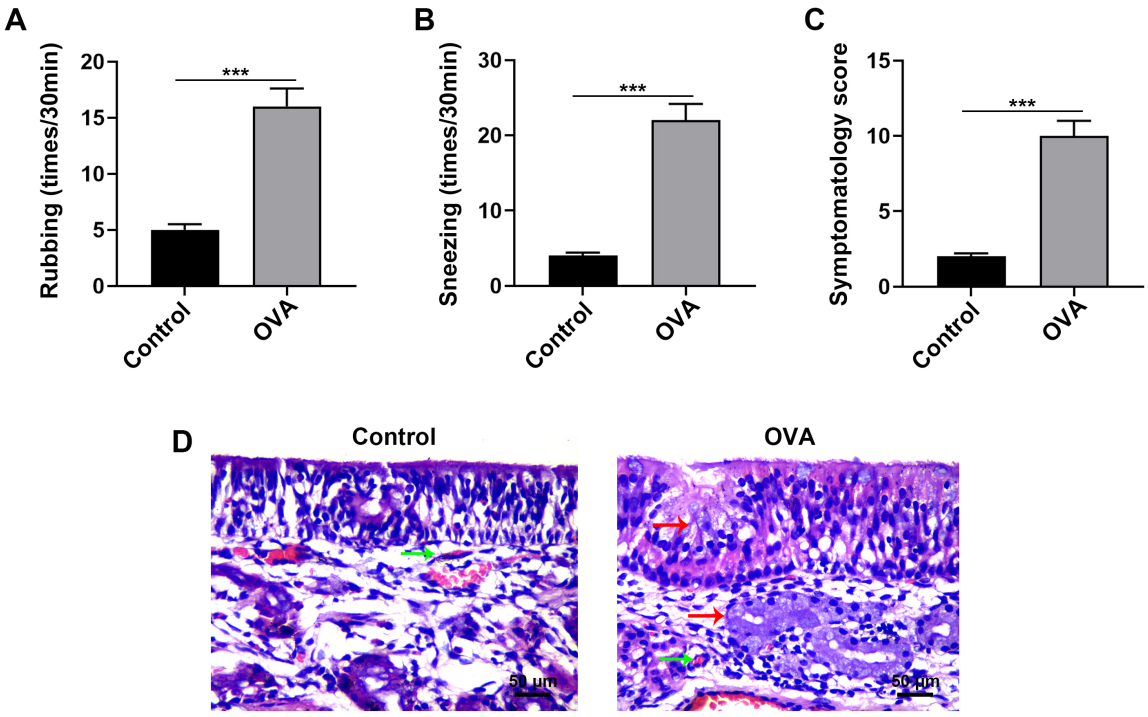

Fig. 1. Morphological and pathological damage in mice with allergic rhinitis (AR). (A) Times of nose rubbing in mice. (B) Times of sneezing. (C) Symptomatology score. (D) Tissue pictures of pathological damage to the nasal mucosa. Red arrows represented goblet cells. The green arrow represented eosinophils. ***$p < 0.001$. n = 10. OVA: ovalbumin.

the wavelength of 450 nm in the enzyme marker. Analyzed ELISA results using an enzyme-linked immunosorbent assay reader (Varioskan LUX, Thermo Fisher Scientific, VL0000D0, Waltham, MA, USA).

Western Blot

Nasal mucosal tissues were sheared and ground homogenized in a sterile environment, pre-cooled Radio Immunoprecipitation Assay (RIPA) lysis buffer was added, and total tissue protein was extracted. The Bicinchoninic Acid Assay (BCA) method (Beyotime, 23225, Shanghai, China) is used for protein concentration. Aliquots of each group of protein samples were taken and mixed with 5 × Loading buffer, and the proteins were separated by 10% SDS-PAGE for 2 h. The proteins were electrotransferred to polyvinylidene difluoride (PVDF) membranes for 1 h. The membranes were closed with 5% skimmed milk powder at room temperature for 2 h. The membranes were washed with TBST. Primary antibody RORγt (Merck, 531369, 1:1000, Darmstadt, Germany) was added to the membrane and co-incubated overnight at 4 °C. On the next day, the primary antibody was discarded, the membrane was washed with TBST, and HRP-labelled goat anti-rabbit IgG (Beyotime, WLA023, 1:5000, Shanghai, China) was added, incubated for 1 h at room temperature. The membrane was rewashed with TBST. Droplets of Enhanced chemiluminescence (ECL) (Beyotime, P0018S, Shanghai, China) were used to develop the color. A gel imaging system (Thermo Fisher Scientific, Tanon 5200, Waltham, MA, USA) was used to take pictures, and software (Image-ProPlus 6.0, Media Cybernetics, Rockville, MA, USA) was used to analyze the grey values of the bands. GAPDH (Beyotime, AG019, 1:2000, Shanghai, China) was used as the internal reference protein, and the relative protein expression was recorded as the grey values of the target protein band/GAPDH band.

Statistical Analyses

Statistical analyses were performed using SPSS 23.0 (SPSS Inc., Chicago, IL, USA) software, and the data for the measurement information were expressed as mean ± standard deviation ($\bar{x} \pm s$); the *t*-test was used for comparison between two groups, and Analysis of Variance (ANOVA) was used for comparison of means among multiple groups. Tukey's post hoc test for ANOVA. Differences were considered statistically significant at $p < 0.05$.

Results

Changes in Morphological and Pathological Damage in Mice with AR

Nose rubbing, sneezing, and symptomatology scores were significantly higher in mice in the OVA group than in the Control group ($p < 0.001$, Fig. 1A–C). In addition, the pathological damage of the nasal mucosa was detected, and

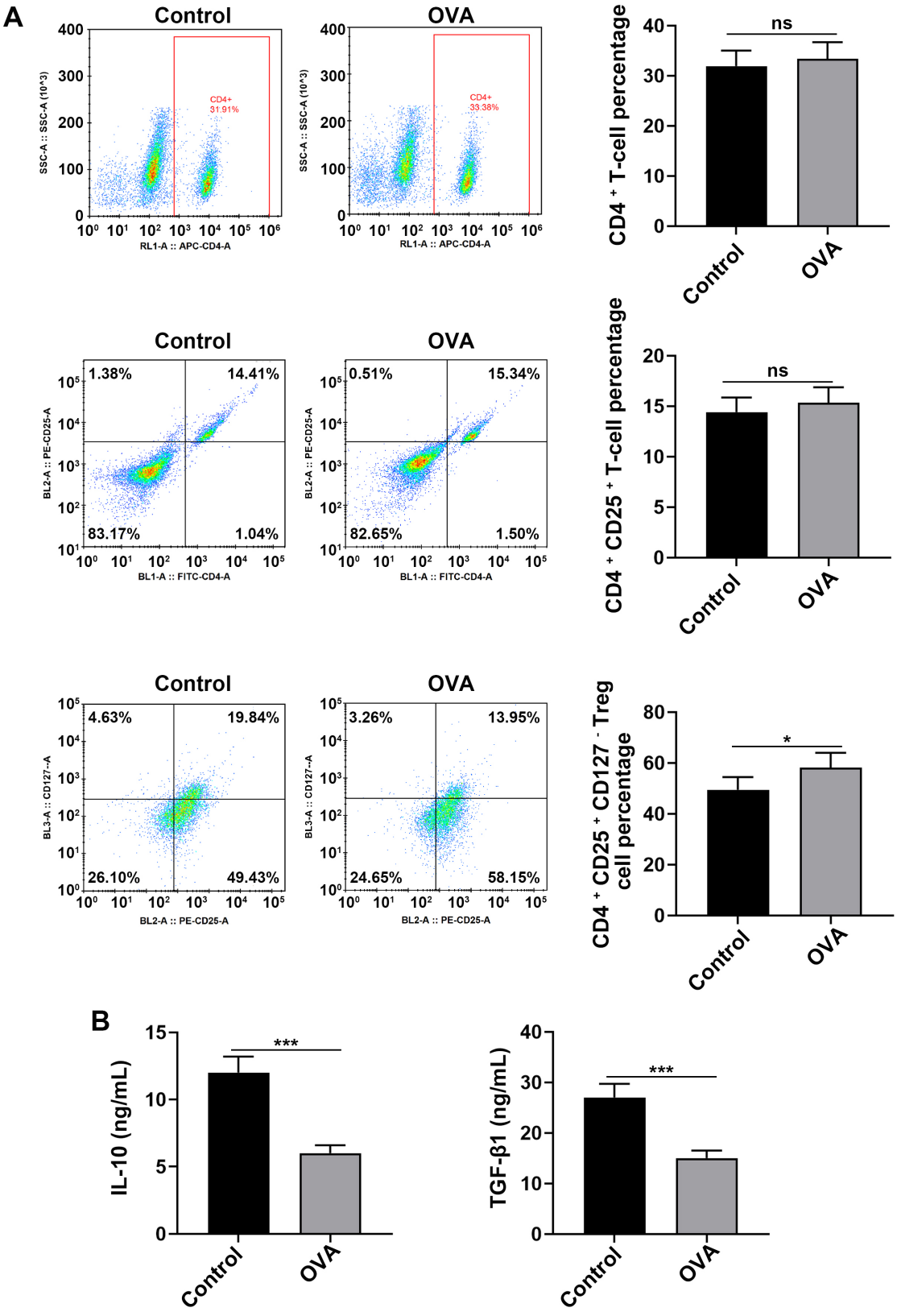

Fig. 2. Decreased Treg expression of AR mice. (A) $CD4^{+}CD25^{+}CD127^{-}$ Treg cell levels in peripheral blood were detected by flow cytometry. (B) ELISA kits were used to detect the levels of IL-10 and TGF-β1 in serum, which was related to Treg cells. $^{ns}p > 0.05$, $*p < 0.05$, $***p < 0.001$. n = 10.

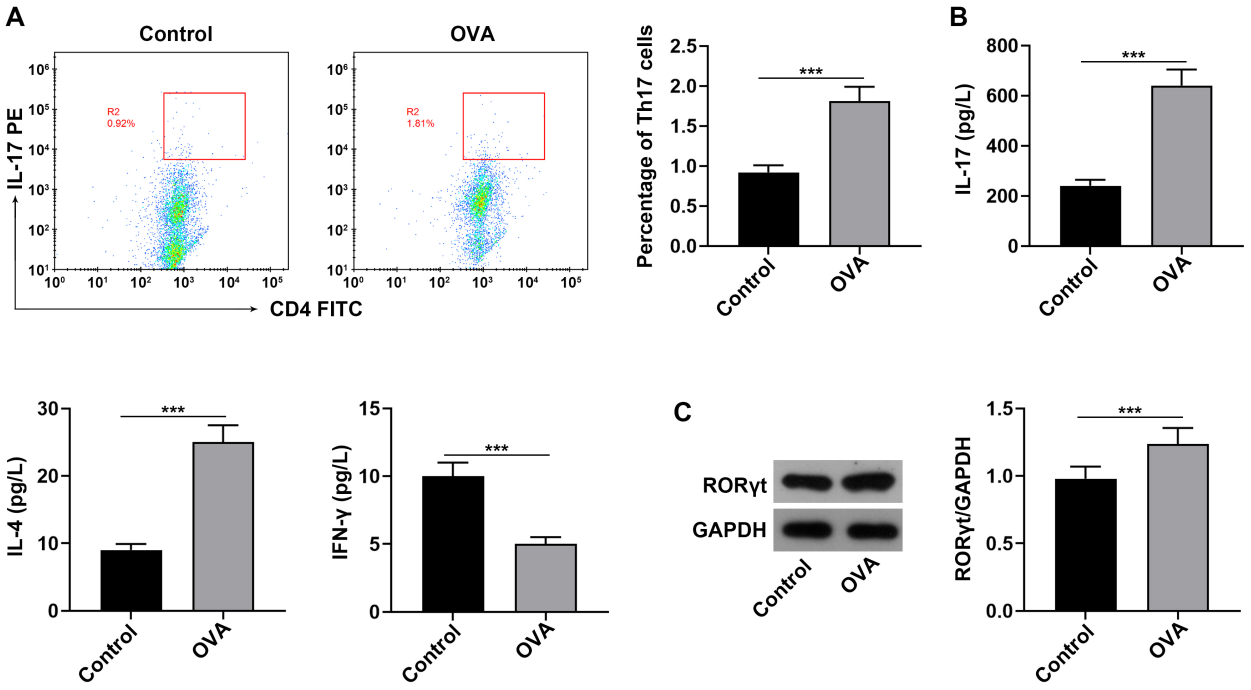

Fig. 3. Elevated Th17 of AR mice. (A) Flow cytometry was performed to determine the expression of Th17 cells in the peripheral blood of each group. (B) ELISA kits were used to evaluate IL-17, IL-4, and IFN-γ levels related to Th17 cells in serum. (C) Western blot assessment of RORγt level in nasal mucosa tissue. ***$p < 0.001$. n = 10.

it was found that the continuity of the nasal mucosa was interrupted in the OVA group compared to that of the Control group; the cilia were not arranged neatly, the cytoplasm of the columnar cells was abundant, and the subcutaneous sparse tissues of the glandular vesicle hyperplasia, vasodilatation, and eosinophils infiltration could be seen in more significant numbers (Fig. 1D).

Decreased Treg Expression of AR Mice

The difference in the percentage of $CD4^+$ T cells to lymphocytes in the OVA group compared to the Control group was not statistically significant ($p > 0.05$). The difference in the percentage of $CD4^+CD25^+$ T cells to $CD4^+$ T cells was not statistically significant ($p > 0.05$), whereas $CD4^+CD25^+CD127^-$ Treg cells accounted for a significantly higher percentage of $CD4^+CD25^+$ T cells ($p < 0.05$, Fig. 2A). In addition, IL-10 and TGF-β1 levels were significantly lower in the OVA group than in the Control group ($p < 0.001$, Fig. 2B).

Elevated Th17 of AR Mice

The expression of Th17 cells was significantly higher in the OVA group in contrast with the Control group ($p < 0.001$, Fig. 3A). Meanwhile, the serum levels of IL-17 ($p < 0.001$) and IL-4 ($p < 0.001$) were found to be significantly higher ($p < 0.001$), and IFN-γ level was found to be significantly lower ($p < 0.001$, Fig. 3B) in the OVA group in contrast with the Control group by ELISA. In addition, protein expression of Th17 cell transcription factor RORγt was significantly upregulated in the OVA group in contrast with the Control group ($p < 0.001$, Fig. 3C).

Anti-IL-17 Antibody Balances Th17/Treg Ratio of AR Mice

Next, we introduced an Anti-IL-17 antibody and found by HE staining that the continuity of nasal mucosa was interrupted in the OVA group compared to the control group, the cilia were not neatly arranged, and the subcutaneous sparse tissues of glandular vesicles were hyperplasia, vasodilatation, and more eosinophils infiltration could be seen. In contrast with the OVA group, the nasal mucosal continuity was still poor in the OVA+Anti-IL-17 Antibody group; however, the atrophy of goblet cells, glandular follicles, and dilated blood vessels significantly improved (Fig. 4B). The percentage of $CD4^+CD25^+CD127^-$ Treg cells as a percentage of $CD4^+CD25^+$ T cells was significantly lower in the OVA group than in the Control group ($p < 0.05$). The percentage of $CD4^+CD25^+CD127^-$ Treg cells as a percentage of $CD4^+CD25^+$ T cells was significantly increased in the OVA+Anti- IL-17 Antibody group compared to the OVA group ($p < 0.05$, Fig. 4A). Meanwhile, IL-10 ($p < 0.001$), TGF-β1 ($p < 0.001$), and IFN-γ ($p < 0.01$) levels were significantly lower in the OVA group, and Th17 cell expression ($p < 0.001$), IL-17 ($p < 0.001$) and IL-4 ($p < 0.001$) levels were significantly higher in the OVA group compared to the Control

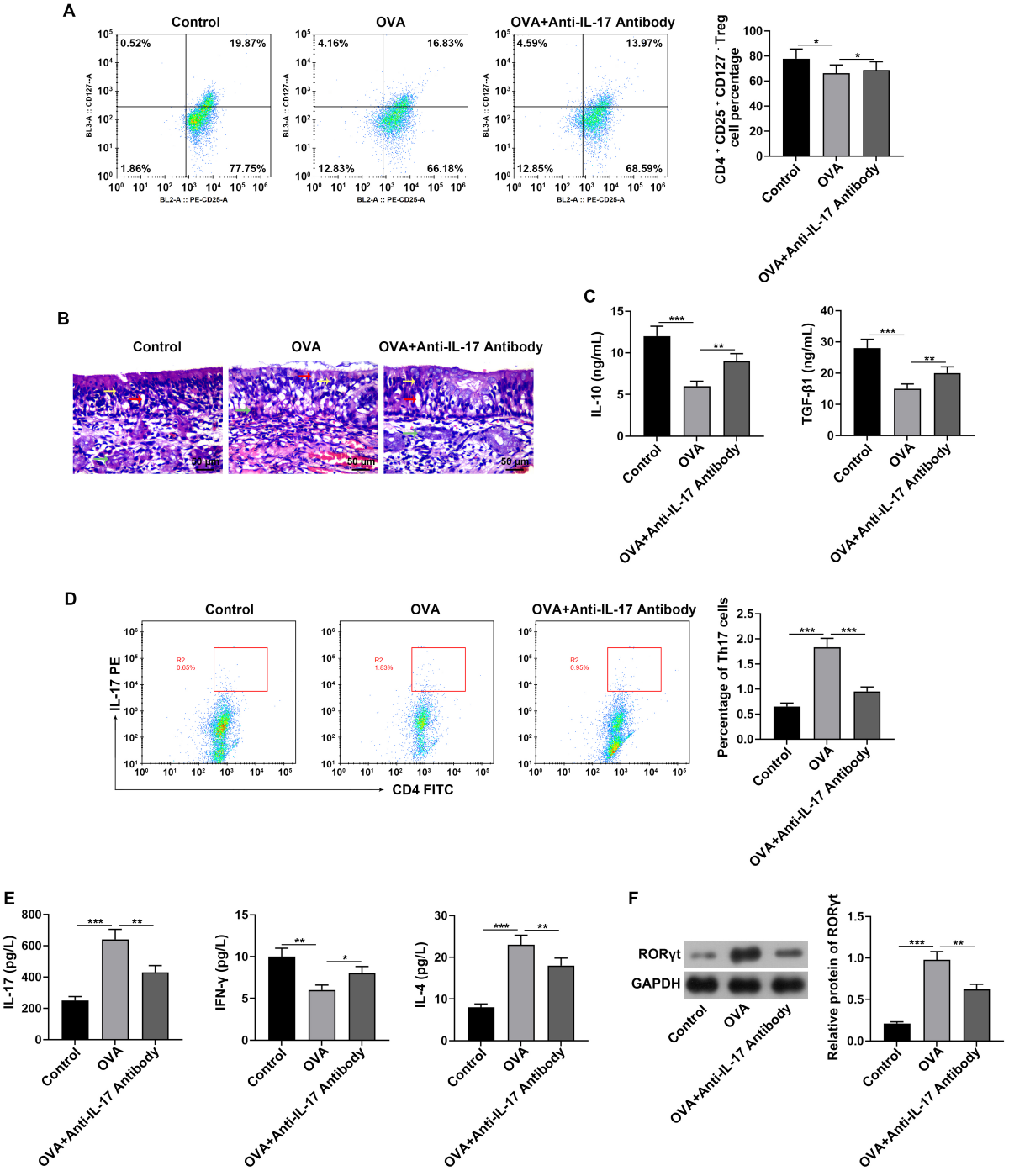

Fig. 4. Anti-IL-17 antibody balances Th17/Treg ratio in the nasal mucosa of AR mice. (A) $CD4^+CD25^+CD127^-$ Treg cell levels were detected in peripheral blood by flow cytometry. (B) Tissue pictures of pathological damage of nasal mucosa. Red arrows represented goblet cells; yellow arrows represented columnar cells, and green arrows represented eosinophils. (C) Levels of IL-10 and TGF-β1, the relevant cytokines secreted by Treg cells, were detected by ELISA kits in serum. (D) Flow cytometry was performed to determine the expression of Th17 cells in the peripheral blood of each group. (E) ELISA kits were used to evaluate the levels of IL-17, IL-4, and IFN-γ in serum. (F) Western blot assessment of RORγt level in nasal mucosa tissue. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. n = 10.

group. Compared with the OVA group, the levels of IL-10 ($p < 0.01$), TGF-β1 ($p < 0.01$), and IFN-γ ($p < 0.05$) were significantly increased in the OVA+Anti- IL-17 Antibody group (Fig. 4C), and the levels of Th17 cell expression ($p < 0.001$), IL-17 ($p < 0.01$) and IL-4 ($p < 0.01$) were significantly decreased (Fig. 4D,E). In addition, the RORγt protein level was significantly upregulated in the OVA group compared to the Control group ($p < 0.001$).

The RORγt protein level was significantly down-regulated in the OVA+Anti- IL-17 Antibody group compared to the OVA group ($p < 0.01$, Fig. 4F).

Discussion

AR is a type I hypersensitivity immune response, a common chronic allergic respiratory disease characterized by one or more nasal symptoms [12]. Currently, although AR treatment has been heavily investigated with a variety of medications (histamines, glucocorticoids, nasal decongestants, and mast cell stabilizers), long-term treatment side effects and relapse rates with these medications are increasing [13,14]. As a result, there is a growing need for new or alternative methods to improve allergy symptoms, and in-depth studies of the pathogenesis of allergic rhinitis are of great importance. In this study, by constructing an AR mouse model, we found that AR mice had elevated nose rubbing times, sneezing times, and symptomatology scores, decreased levels of Treg cells, IL-10, and TGF-β1, increased levels of Th17 cells, IL-17, and IL-4, and upregulated protein expression of RORγt. All reversed symptoms in AR mice after treatment with Anti-IL-17 antibodies. Nose rubbing and increased sneezing are an important clinical symptom of AR. Therefore, we first observed the symptoms in AR mice. Elevated nose rubbing, sneezing, and symptomatology scores were found in AR mice. This is similar to previous findings in mouse models that OVA-induced increased rubbing and sneezing [15].

An imbalance of Th17/Treg is thought to contribute to various autoimmune and inflammatory diseases [16–18]. It has been demonstrated that Treg cells can promote and maintain tolerance to allergens by modulating the immune response triggered by innate and adaptive allergens [19]. Treg cells can also exert anti-inflammatory effects by releasing IL-10 [20]. In this study, we found that AR mice reduced levels of Treg cells and increased levels of Th17 cells, significantly reduced levels of IL-10 and TGF-β1, and increased levels of IL-17 and IL-4. This is similar to previous findings that Treg cytokine IL-10 is downregulated and Th17 cytokine IL-17 is upregulated in AR mice [10]. TGF-β is a stimulator of Treg and Th17 cells [7]. Treg and Th17 cells play opposite roles in the inflammatory response, with Th17 cells inducing autoimmune tissue damage and inflammation. In contrast, Treg cells suppress autoimmunity and control adverse immune responses; Th17 cells mainly secrete IL-17, which has a strong pro-inflammatory effect and can induce the expression of pro-inflammatory cytokines and chemokines and matrix metalloproteinases, causing tissue cell infiltration and tissue destruction. Th17 cell levels and IL-17 levels are significantly increased in patients with AR. In addition, a study has found that the levels of Th17 and IL-17 in patients with allergic rhinitis changed significantly after taking drugs [21]. In this experiment, the Treg cytokine IL-10 was up-regulated, and the Th17 cytokine IL-17 was down-regulated after Anti-IL-17 antibody treatment. RORγ is a characteristic transcription factor required to differentiate Th17 cells into their major cytokine IL-17 [22]. In this study, the Anti-IL-17 antibody reduced the protein level of RORγt. These results suggest that IL-17 can alleviate AR symptoms by balancing Treg and Th17 cytokines. However, this study still has some limitations, and the pathway of action of the Anti-IL-17 antibody has not been investigated. We will further explore the mechanism of action of Anti-IL-17 antibodies in our future work.

Conclusion

In conclusion, it was shown that the Anti-IL-17 antibody exerts anti-inflammatory effects by increasing Treg cell levels and inhibiting Th17 cell levels. It has a positive impact on allergic inflammation in the AR mouse model. Anti-IL-17 antibody therapy may be a promising strategy for preventing and treating allergic airway diseases like AR.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

HYS has been involved in drafting the manuscript or revising it critically for important intellectual content. HYS and BG have made substantial contributions to the conception and design, or acquisition of data, or analysis and interpretation of data. BXL and WM have performed the research. DJJ has performed significantly in analysis and manuscript preparation. All authors have been involved in drafting the manuscript. All authors have contributed significantly to editorial changes of important content. All authors have read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, and approved by the ethics committee of Northern Jiangsu People's Hospital (2020KY-106).

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Bousquet J, Anto JM, Bachert C, Baiardini I, Bosnic-Anticevich S, Walter Canonica G, *et al*. Allergic rhinitis. Nature Reviews. Disease Primers. 2020; 6: 95.

[2] Siddiqui ZA, Walker A, Pirwani MM, Tahiri M, Syed I. Allergic rhinitis: diagnosis and management. British Journal of Hospital Medicine. 2022; 83: 1–9.

[3] Drazdauskaitė G, Layhadi JA, Shamji MH. Mechanisms of Allergen Immunotherapy in Allergic Rhinitis. Current Allergy and Asthma Reports. 2020; 21: 2.

[4] Meltzer EO, Blaiss MS, Derebery MJ, Mahr TA, Gordon BR, Sheth KK, *et al*. Burden of allergic rhinitis: results from the Pediatric Allergies in America survey. The Journal of Allergy and Clinical Immunology. 2009; 124: S43–S70.

[5] Canonica GW, Compalati E. Minimal persistent inflammation in allergic rhinitis: implications for current treatment strategies. Clinical and Experimental Immunology. 2009; 158: 260–271.

[6] Okubo K, Kurono Y, Ichimura K, Enomoto T, Okamoto Y, Kawauchi H, *et al*. Japanese guidelines for allergic rhinitis 2020. Allergology International. 2020; 69: 331–345.

[7] Lee GR. The Balance of Th17 versus Treg Cells in Autoimmunity. International Journal of Molecular Sciences. 2018; 19: 730.

[8] McGeachy MJ, Cua DJ, Gaffen SL. The IL-17 Family of Cytokines in Health and Disease. Immunity. 2019; 50: 892–906.

[9] Li X, Bechara R, Zhao J, McGeachy MJ, Gaffen SL. IL-17 receptor-based signaling and implications for disease. Nature Immunology. 2019; 20: 1594–1602.

[10] Van Nguyen T, Piao CH, Fan YJ, Shin DU, Kim SY, Song HJ, *et al*. Anti-allergic rhinitis activity of α-lipoic acid via balancing Th17/Treg expression and enhancing Nrf2/HO-1 pathway signaling. Scientific Reports. 2020; 10: 12528.

[11] Meng XT, Shi YY, Zhang H, Zhou HY. The Role of Th17 Cells and IL-17 in Th2 Immune Responses of Allergic Conjunctivitis. Journal of Ophthalmology. 2020; 2020: 6917185.

[12] Zhang Y, Lan F, Zhang L. Update on pathomechanisms and treatments in allergic rhinitis. Allergy. 2022; 77: 3309–3319.

[13] Akhouri S, House SA. Allergic Rhinitis. StatPearls Publishing: Treasure Island (FL). 2023.

[14] Ma Y, Yang XH, Tu Y, Athari SS. Survey the effect of drug treatment on modulation of cytokines gene expression in allergic rhinitis. Fundamental & Clinical Pharmacology. 2023; 37: 340–346.

[15] Ke X, Chen Z, Wang X, Kang H, Hong S. Quercetin improves the imbalance of Th1/Th2 cells and Treg/Th17 cells to attenuate allergic rhinitis. Autoimmunity. 2023; 56: 2189133.

[16] Yan JB, Luo MM, Chen ZY, He BH. The Function and Role of the Th17/Treg Cell Balance in Inflammatory Bowel Disease. Journal of Immunology Research. 2020; 2020: 8813558.

[17] Zhang S, Gang X, Yang S, Cui M, Sun L, Li Z, *et al*. The Alterations in and the Role of the Th17/Treg Balance in Metabolic Diseases. Frontiers in Immunology. 2021; 12: 678355.

[18] He X, Liang B, Gu N. Th17/Treg Imbalance and Atherosclerosis. Disease Markers. 2020; 2020: 8821029.

[19] Palomares O, Yaman G, Azkur AK, Akkoc T, Akdis M, Akdis CA. Role of Treg in immune regulation of allergic diseases. European Journal of Immunology. 2010; 40: 1232–1240.

[20] Yu H, Paiva R, Flavell RA. Harnessing the power of regulatory T-cells to control autoimmune diabetes: overview and perspective. Immunology. 2018; 153: 161–170.

[21] Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, *et al*. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature. 2006; 441: 235–238.

[22] Chaudhry A, Rudra D, Treuting P, Samstein RM, Liang Y, Kas A, *et al*. CD4+ regulatory T cells control TH17 responses in a Stat3-dependent manner. Science. 2009; 326: 986–991.