An Experimental Study on the Remodeling of Nasal Mucosa in Mouse Model of Allergic Rhinitis

Tao Wang#, Rui Zheng#, Peng Li, Xue-kun Huang, Jian-cong Huang, Qin-tai Yang*

Department of Otorhinolaryngology-Head and Neck Surgery, The Third Affiliated Hospital, Sun Yat-Sen University, Guang Zhou 510630, China

# Tao Wang and Rui Zheng contributed equally to this work. They are joint first authors.

*Corresponding author: Qin-Tai Yang, Department of Otorhinolaryngology-Head and Neck Surgery, The Third Affiliated Hospital, Sun Yat-Sen University, Guang Zhou 510630, China (Email: yang.qt@163.com)

Abstract: Objective: The aim of this study was to observe the remodeling of nasal mucosa and the expression of cytokines IL-4 and INF-γ in a mouse model of allergic rhinitis (AR), and investigate the role of nuclear factors T-bet/GATA-3 ratio in the immune imbalance in the AR mouse model established in this study as well. Methods: Thirty BALB/c mice, randomly divided into control and treatment group, were used for establishing the ovalbumin (OVA)-sensitized AR mouse model. Hematoxylin and eosin stain (H&E stain) was employed to observe the change in catarrhal symptoms, ELISA was used for detect IL-4 and INF-γ in serum and Western blot to detect the expression of T-bet and GATA-3 in nasal mucosa. Results: Remarkable tissue remodeling was observed in nasal mucosa of the AR mouse model. In comparison with the control group, the ovalbumin-sensitized mice exhibited significant epithelial exfoliation, goblet cell hyperplasia, squamous epithelial tissue conversion, epithelial necrosis, lamina propria and submucosa gland hyperplasia, dilation of blood vessels, tissue edema, and the characteristic eosinophil. The contents of IL-4 and INF-γ were determined as (15.95±4.09) and (6.44±1.15) pg/ml in serum of treatment group respectively, and (7.13±1.65) and (11.37±2.97) pg/ml in serum of control group respectively. Statistical analysis indicated that IL-4 content in treatment group was significantly higher than that in control group (P<0.05), while INF-γ content was significantly lower (P<0.05). The expression ratio of T-bet/GATA-3 in nasal mucosa of control group was (0.61±0.18), significantly higher than that in treatment group (0.12±0.02) (P<0.05). Conclusion: There exists remarkable tissue remodeling in nasal mucosa of AR mouse model, which probably relates to the downstream Th1/Th2 cell immune imbalance and IL-4/IFN-γ dyscrasia caused by the abnormal expression of transcription factors T-bet/GATA-3.

KEYWORDS: Allergy rhinitis; Tissue remodeling; GATA-3; T-bet; Animal model

Allergy rhinitis (AR) is the clinical symptom of allergic systemic disease affecting the nose. The nasal mucosa with allergic inflammation has similar tissue remodeling with bronchial asthma mucosa, which is the main cause of the further chronicity and aggravating refractory treatment of AR [1]. The imbalance of Th1/Th2 cell ratio and function is the uppermost dysimmunity of AR [2]. T-bet and GATA-3 are the candidate genes of allergic airway inflammation, which are respectively for the upstream transcription factors of Th1 and Th2 cells. Their expressions have been found closely related with the polarization and balance of Th1/Th2 cells [3]. In the present study, we at-
tempted to establish a mouse model of allergic rhinitis, observe the secretion of cytokines IL-4 and INF-γ in peripheral blood of AR mouse model and the remodeling of nasal mucosa, and investigate the expression of upstream transcription factors T-bet/GATA-3 in nasal mucosa and their relativity.

Materials and Methods

Experimental animals and reagents

Experimental animals: Thirty 4—8-week-old specific pathogen free BALB-c mice each weighing 18—22 grams, half male and half female, were purchased from experimental center of Sun Yat-sen University (China). These mice were randomly divided into treatment and control group with 15 mice each via sortition.

Main reagents: Sensibiligen OVA (chemically pure grade V, A5503) and adjuvant aluminum hydroxide (13 mg/ml, A8222) were both purchased from Sigma Corporation of America. IL-4 and IFN-γ ELISA kits were purchased from R&D Systems, Inc. (USA). Anti-T-bet and anti-GATA-3 antibodies were purchased from Santa Cruz Biotechnology, Inc. (USA).

Establishment of AR model

Sensitization stage: Forty micrograms of OVA, following 2 mg of aluminum hydroxide, was dissolved in phosphate buffered saline (PBS), to a final volume of 200 μl. Thirty minutes after well mixed, it was used for intraperitoneal injection on the experimental mice for sensitization. The intraperitoneal injection was performed every other day and lasted for two weeks.

Excitation stage: One week after the last intraperitoneal injection (day 21), the experimental mice received 5% OVA challenges by atomizing inhalation using a nebulizer (Yuyue Multi-functional compressed-air Nebulizer 403A) for 30 min, then were subject to 20 μl of 40 mg/ml OVA solution for excitation via intranasal instillation. The excitation was performed every day and lasted for 7 days. Control group received 0.9% saline instead of OVA for intranasal instillation and atomizing inhalation (Fig. 1).

Investigated indices

(1) Symptom score. Within 30 min after the last intranasal sensitization, mice in both groups were observed for their nasal rubbing, sneezing and nasal discharge and scored as follows. Rhinocnesmus: gently nasal rubbing once or twice, 1 score; acutely rubbing nasal face, 3 scores; medially, 2 scores. Sneezing: 1—3 times, 1 score; 4—10 times, 2 scores; ≥11 times, 3 scores. Watery nasal discharge: flowing to anterior nares, 1 score; overstepping anterior nares, 2 scores; covering whole cheeks, 3 scores.

The quantitative scoring method of totaling itemized scores was adopted to analyze all above indices. The total score of ≥ 5 was considered as a threshold to indicate the successful establishment of mouse model of allergic rhinitis.

(2) Measurement of IL-4 and INF-γ contents in serum. Within 12 h after the last excitation, the mice were anaesthetized via celiac injection of chloral hydrate, subject to eyeball extirpating for sampling the peripheral blood. The blood sample was centrifuged at 3000 r/min for collecting the serum. ELISA was employed to detect the cytokines IL-4 and IFN-γ referring to the kit specification. The absorbance was taken at the wavelengths of 450 nm (INF-γ) and 490 nm (IL-4) in the enzyme-labeled instrument, which was converted to corresponding mass concentration presented as pg/L according to the standard curve.

(3) Pathological observation. After blood collection, the mice were sacrificed by cervical dislocation. The whole nasal cavity with osseous and nasal mucosa was carefully scissors, fixed in 4% paraformaldehyde buffer, decalcified with 5% methanoic acid, dehydrated and embedded in paraffin, then sectioned and stained with H&E. The sections were observed for nasal mucosa of allergic inflammation and tissue remodeling under light microscope.

(4) Western blot to measure T-bet and GATA-3 contents in nasal mucosa tissue. One hundred grams of nasal mucosa tissue was ground into homogenate in 600 μl of lysis buffer on ice. The homogenate was ultrasonic-treated, incubated on ice for 1 h and then centrifuged at 4℃ 150000 g/min for 15
min. The supernatant was taken for measuring the protein content using Bradford method. Fifty micrograms of protein was mixed with equal volume of loading buffer [100 mmol /L Tris-HCL (pH=6.8), 200 ml /L glycerol, 0.2 g/L bromphenol blue, 20g/L SDS, 200 mmol/L DTT], and separated on 100g /L polyacrylamide gel electrophoresis. The proteins on the electrophoresis pattern were transferred to nitrocellulose membrane, respectively incubated with anti-mouse antibodies of T-bet and GATA-3m (1:500) at room temperature for 2 h, followed by another 2 h of incubation with horseradish peroxidase-labeled Goat Anti-Mouse IgG (1:2000) at room temperature. Likewise, the membrane was also incubated with mouse anti-mouse GAPDH (1:4000) and then horseradish peroxidase-labeled Goat Anti-Mouse IgG (1:2000) each at room temperature for 2 h for the assay of GAPDH. The protein bands were assayed by using Chemiluminescence Quantitative Immunoassay Kit. The X-film with assayed bands was analyzed using image analysis system to get the accumulated absorbance of each band. The relative absorbance of T-bet, GATA-3 and GAPDH was compared for semi-quantitative PCR analysis.

**Statistical analysis**

The data were presented as mean ± standard deviation (x ± s). Results of the control and treatment groups were compared using analysis of variance followed by t-test comparison of means. Statistical analysis was performed using SPSS 13.0 and P values of <0.05 were considered statistically significant.

**Results and analysis**

**Symptom score**

After excitation, 13 mice in the treatment group behaved frequent nasal rubbing using front claws, accompanied with sneezes and more watery nasal discharge. Most of these mice got a total score of ≥5, with the mean of 6.9, suggesting the successful establishment of AR mouse model. The mice in control group, however, just behaved gently nasal rubbing, less sneezes and no nasal discharge, which was scored as 2.3, less than 5 to indicate the success of model establishment. In this study, 86.7% (13/15) of the experimental mice achieved model establishment successfully, which concurrently have the upper and lower airway symptoms of both AR and asthma.

**Pathological analysis results**

Remarkable tissue remodeling was observed in nasal mucosa of the AR mouse model. In comparison with the control group, the ovalbumin-sensitized mice exhibited significant epithelial exfoliation, goblet cell hyperplasia, squamous epithelial tissue conversion, epithelial necrosis, lamina propria and submucosa gland hyperplasia, dilation of blood vessels, tissue edema, and the characteristic eosinophil. However, the nasal mucosa of mice in control group had intact epithelial structure and the epithelial cells arranged evenly. There was no lamina propria and dilation of blood vessels observed at submucosa, and just a few of inflammation cells (Fig. 2 a, b).

**Contents of IL-4 and INF-γ in serum**

The contents of IL-4 and INF-γ were determined as (15.95±4.09) and (6.44±1.15) pg/ml in serum of treatment group respectively, and (7.13±1.65) and (11.37±2.97) pg/ml in serum of control group respectively. Statistical analysis indicated that IL-4 content in treatment group was significantly higher than that in control group (P<0.05), while INF-γ content was significantly lower (P<0.05).

**Protein contents of T-bet and GATA-3 in nasal mucosa**

The expression of T-bet protein was determined as (0.52±0.18) in nasal mucosa of mice in control group, while significantly lower in treatment group (0.22±0.07) (P<0.05). GATA-3 protein was determined as (0.82±0.25) in nasal mucosa of mice in control group and as high as (1.74±0.46) in treatment group (Fig. 3a, b). The expression ratio of T-bet/ GATA-3 in nasal mucosa of control group was (0.61±0.18),

![Figure 2](image1.png) **Figure 2:** a, the tissue remodeling in nasal mucosa of the mice in treatment group; b, nasal mucosa of the mice in control group assuming normal form and no remarkable inflammation cells.

![Figure 6](image2.png) **Figure 6:** a, low expression of T-bet protein in nasal mucosa of mice in treatment group; b, high expression of GATA-3 protein in nasal mucosa of mice in treatment group. 1, control group; 2, treatment group.
significantly higher than that in treatment group (0.12±0.02) ($P <0.05$).

**Discussion**

A number of studies regarding the establishment of OVA-sensitized AR animal models have been reported, but their experimental environments and conditions vary among each other. Therefore, completely copying those protocols could barely establish stable AR model. Through the improvements in various model establishment methods, we have set up a relatively stable one as described in the experimental methods above. This model establishment method has a high success rate of approximately 90% and can concurrently induce the tissue remodeling and the upper and lower airway symptoms of both AR and asthma. In this study, we reported the tissue remodeling of nasal mucosa of the AR mouse model, manifested mainly as nasal mucosa incrassation, gland hyperplasia, tissue edema, dilation of blood vessels and a great deal of eosinophil. Amin et al. [4] reported that EOS increase in perennial AR animals is positively related with epithelial damage. In the nasal mucosa scraping smear of seasonal AR animals, goblet cells and EOS manifold remarkably, mastocytes increased and appeared degranulation during the flowering season [5]. Through long term observation on the nasal mucosa by allergen exposure in guinea pigs, She et al. [6] found that the ovalbumin-sensitized mice exhibited significant goblet cell hyperplasia, epithelial damage and deposition of extracellular matrix in the nasal septal mucosa and conchae. These classic characteristics of AR nasal mucosa are in accordance with the tissue remodeling in this study, while our model establishment method has the advantage of similar airway remodeling effect within shorter allergen exposure time.

It has been reported that Th1/Th2 immune imbalance and sthenic function of Th2 cell are the key causes of chronic airway inflammations like AR and asthma and the tissue remodeling [7]. IFN-$\gamma$ and IL-4 are the characteristic cytokines of Th1 and Th2, respectively. ELISA assay of IFN-$\gamma$ and IL-4 in peripheral blood of mouse showed that IL-4 in treatment group was enhanced while IFN-$\gamma$ was decreased remarkably. The increased IL-4/IFN-$\gamma$ ratio further confirmed that sthenic function of Th2 cell exists in AR mouse. Thus, through inhibiting the over-response of Th2 to adjust the differentiation of Th1 immune cells from Th0 cells, further to rectify Th1/Th2 imbalance, may be the key to cure allergic airway diseases.

At present, AR is considered as the appearance of systemic allergic inflammation at the nose. The systemic allergic inflammation feeds back to the initially diseased organ—nose, forms a vicious circle which further aggravates partial pathological changes, assuming clinical symptoms. The miss ratio of IL-4/IFN-$\gamma$ caused by immune imbalance of Th1 and Th2 cells is the pathological basis of allergic diseases. Differentiation of T cells involves a complex regulation network of some upstream specific transcription factors [8]. GATA-3 is a transcription factor specifically involving the differentiation of Th2 cells [9], and T-bet is specifically involving the differentiation of Th1 cells [10]. Bettelli et al. [11] found that T-bet-deficient mice are tended to autonomously develop to allergic diseases and gradually to immune imbalance, generating Th2 immune reaction. The interaction between T-bet and GATA-3 during Th cell differentiation plays important roles in airway immune pathology of asthma, and T-bet is the major factor affecting the occurrence of asthma [12]. In asthmatic patients, the transcription factor T-bet expressed poorly while GATA-3 expressed at high level, and the resultant miss ratio of T-bet/ GATA-3 promoted the airway remodeling [13]. These results confirmed that GATA-3 and T-bet play important roles in the pathogenic mechanism of asthma. Our results indicated that the expression of GATA-3 was up-regulated in nasal mucosa of mice in treatment group while T-bet was down-regulated. Abnormal expression of both T-bet/ GATA-3 and IL-4/IFN-$\gamma$, together with the tissue remodeling of nasal mucosa, are similar with the allergic lower airway diseases reported in these references.

There exist similar symptoms of AR occurrence and development. In nasal mucosa, high expression of GATA-3 and low expression of T-bet cause immune imbalance of downstream Th1/Th2 cells and further break the expression imbalance of cytokines IL-4/IFN-$\gamma$, manifesting clinical AR symptoms and tissue remodeling of nasal mucosa. GATA-3/T-bet expression imbalance is probably the genetic basis for AR occurrence and tissue remodeling of nasal mucosa, thus rectifying the imbalance may be the key to cure or control allergic rhinitis.

**References**


