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CORRELATION ANALYSIS OF CYTOKINE LEVELS AND TH2, TH17, AND TREG CELLS IN ALLERGIC RHINITIS

ANALIZA KORELACIJE NIVOA CITOKINA I TH2, TH17 I TREG ĆELIJA KOD ALERGIJSKOG RINITISA

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Summary

Background: To explore the roles of Th2, Th17, and regulatory T (Treg) cells and their related cytokines and transcriptional genes in the pathogenesis of allergic rhinitis (AR).
Methods: A total of 200 patients diagnosed with AR in our hospital from October 2023 to October 2024 were randomly selected as the AR group. According to the visual analogue scale (VAS) score, the AR group was further divided into a mild allergic rhinitis group (MAR group) and a severe allergic rhinitis group (SAR group). Moreover, 50 healthy individuals who underwent physical examinations at the Physical Examination Centre of Ordos Central Hospital formed the healthy control group (HC group). Blood from peripheral veins was drawn on the day of registration. The Th17/Treg ratio and the proportions of helper T 2 (Th2), helper T 17 (Th17), and regulatory T (Treg) cells among the peripheral blood's CD4+T cells were determined using flow cytometry. The enzyme-linked immunosorbent assay (ELISA) was used to determine the levels of interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-10 (IL-10), and interleukin-17 (IL-17) in the patients' serum. The genes GATA-3 mRNA, RORyt-mRNA, and FOXP3-mRNA's relative expression levels were assessed using real-time fluorescence quantitative RT-PCR of peripheral blood mononuclear cells.
Results: The peripheral blood of AR patients had larger Th2/Th17/Treg cell proportions and a higher Th17/Treg ratio. The AR group presented greater differences ($P<0.05$), and both were positively connected with the disease severity, with the SAR group showing larger differences than the MAR

Kratak sadržaj

Uvod: Cilj je bio da se ispita uloga Th2, Th17 i regulatornih T (Treg) ćelija i njihovih povezanih citokina i transkripcionih gena u patogenezi alergijskog rinitisa (AR).
Metode: Za AR grupu je nasumično odabrano ukupno 200 pacijenata kojima je dijagnostifikovan AR u našoj bolnici od oktobra 2023. do oktobra 2024. godine. Prema rezultatu vizuelne analogne skale (VAS), AR grupa je dalje podeljena na grupu sa blagim alergijskim rinitisom (MAR grupa) i grupu sa teškim alergijskim rinitisom (SAR grupa). Kontrolnu grupu (HC grupa) je činilo 50 zdravih ispitanika koji su se javili na sistematski pregled u »Ordos Central Hospital«. Krv je uzeta iz perifernih vena na dan registracije. Odnos Th17/Treg i proporcije pomoćnih T 2 (Th2), pomoćnih T 17 (Th17) i regulatornih T (Treg) ćelija među CD4+T ćelijama periferne krvi određen je pomoću protoka citometrije. Nivoi citokina IL-4, IL-5, IL-10 i IL-17 u serumu određeni su ELISA metodom. Relativna ekspresija gena GATA-3, RORyt i FOXP3 procenjena je kvantitativnim RT-PCR u realnom vremenu na mononuklearnim ćelijama periferne krvi.
Rezultati: Periferna krv pacijenata sa AR pokazala je veće proporcije Th2/Th17/Treg ćelija i viši Th17/Treg odnos. AR grupa je pokazala veće razlike ($P<0,05$), a obe su pozitivno povezane sa težinom bolesti, pri čemu je SAR grupa pokazala veće razlike nego MAR grupa ($P<0,05$). Pacijenti sa AR imali su veće nivoe IL-4, IL-5, IL-17 i IL-10 u perifernoj krvi. Težina bolesti pozitivno je povezana sa razlikama u AR grupi ($P<0,05$) i SAR grupi ($P<0,05$) u poređenju sa

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group ($P<0.05$). AR patients had higher peripheral blood levels of IL-4, IL-5, IL-17, and IL-10. The severity of the disease was positively associated with differences in the AR group ($P<0.05$) and the SAR group ($P<0.05$) compared to the HC group and the MAR group, respectively. Patients with AR had higher relative expression levels of the transcription genes FoxP3, RORyt, and GATA-3 in their peripheral blood mononuclear cells. The AR group showed more differences than the HC group ($P<0.05$), while the SAR group showed more differences than the MAR group ($P<0.05$). These differences were all positively connected with illness severity. The relative expression level of GATA-3 mRNA and the serum levels of IL-4 and IL-5 were positively associated with Th2 cells. Th17 cells showed a favourable correlation with both the relative expression level of RORyt-mRNA and the content of serum IL-17. Treg cells showed a favourable correlation with both the relative expression level of FoxP3 mRNA and the serum IL-10 levels. Binary logistic regression analysis revealed that an increase in the proportion of Th2 and Th17 cells, a continuous increase in the Th17/Treg ratio, an increase in the concentrations of IL-4, IL-5, and IL-17; and an increase in the relative expression levels of GATA-3 mRNA and RORyt-mRNA transcriptional genes were risk factors for SAR.

Conclusions: A persistently elevated Th17/Treg ratio in AR patients indicates progressive exacerbation of AR. The relative expression levels of the transcription genes GATA-3, RORyt-mRNA, and FoxP3-mRNA in peripheral blood mononuclear cells lead to increases in the proportions of Th2, Th17, and Treg cells.

Keywords: allergic rhinitis, cytokine level, Th2 cell, Th17 cell, Treg cell, correlation analysis

HC grupom i MAR grupom, respektivno. Pacijenti sa AR su imali veće relativne nivoe ekspresije transkripcionih gena FoxP3, RORyt i GATA-3 u mononuklearnim ćelijama periferne krvi. AR grupa je pokazala više razlika nego HC grupa ($P<0,05$), dok je SAR grupa pokazala više razlika nego MAR grupa ($P<0,05$). Sve ove razlike bile su pozitivno povezane sa težinom bolesti. Relativni nivo ekspresije GATA-3 mRNA i nivoi IL-4 i IL-5 u serumu pozitivno su povezani sa Th2 ćelijama. Th17 ćelije pokazale su povoljnu korelaciju sa relativnim nivoom ekspresije RORyt-mRNA i sadržajem IL-17 u serumu. Treg ćelije pokazale su povoljnu korelaciju sa relativnim nivoom ekspresije FoxP3 mRNA i nivoom IL-10 u serumu. Binarna logistička regresiona analiza pokazala je da povećanje proporcije Th2 i Th17 ćelija, kontinuirano povećanje Th17/Treg odnosa, povećanje koncentracija IL-4, IL-5 i IL-17, kao i povećanje relativnih nivoa ekspresije GATA-3 mRNA i RORyt-mRNA predstavljaju faktore rizika za SAR.

Zaključak: Stalno povišen Th17/Treg odnos kod pacijenata sa AR ukazuje na progresivno pogoršanje bolesti. Relativni nivoi ekspresije transkripcionih gena GATA-3, RORyt-mRNA i FoxP3-mRNA u mononuklearnim ćelijama periferne krvi dovode do povećanja proporcija Th2, Th17 i Treg ćelija.

Ključne reči: alergijski rinitis, nivo citokina, Th2 ćelija, Th17 ćelija, Treg ćelija, analiza korelacije

Introduction

When the body is exposed to allergens, immunoglobulin E (IgE) levels rise, resulting in allergic rhinitis (AR), a noninfectious illness of the nasal mucosa (1–3). Hyperactivity of nasal mucosa secretions, nasal congestion, and itching of the nose are the primary clinical signs (4). In severe cases, it may be accompanied by symptoms such as itchy, red and swollen eyes and a reduced sense of smell. With the promotion of urban greening, the area of urban vegetation has rapidly increased worldwide, and the biodiversity of ecosystems in major cities has also been continuously enriched (4). Therefore, the prevalence of AR has risen quickly globally. Incorrect! No reference source was found. It has suddenly become one of the most common diseases seen in otorhinolaryngology outpatient clinics. Its complex pathogenesis remains a significant focus of immunological research today and is gradually becoming an increasingly serious global public health issue (5–7).

Previous studies (8–10) and treatments for AR have focused mainly on alleviating severe allergic reactions in the nose through antihistamines, glucocorticoids, etc. However, the mechanism of AR occurrence is primarily based on atopic individuals' inability to establish standard immune tolerance after exposure to allergens, which subsequently leads to an increase in serum IgE and eventually triggers an allergic inflammatory response. The entire reaction process involves the participation of various immune-inflammatory cells. When the body is exposed to allergens, antigen-specific CD4+ T cells are activated. Some activated Th cells selectively migrate under the action of the nasal-associated chemokine subnet and survive in local tissues, thereby triggering inflammatory responses. Another group of activated T cells, influenced by inflammatory factors and continuous antigenic stimulation, can differentiate into Th2 and Th17 cells, producing a large number of cytokines. These elements can mediate significant immunological responses and include interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-17 (IL-17), and interleukin-13, among others (11). The production of IgE specific to antigens is increased to regulate the onset and progression of inflammatory reactions. But CD4+ T cells can also develop into regulatory T (Treg) cells, which work by preventing overreactions from the immune system. By inhibiting the release of proinflammatory T cells and inflammatory cytokines, it indirectly suppresses cell differentiation, ultimately controlling inflammation and alleviating AR symptoms (12).

Th2 effector cells are the primary T lymphocytes that mediate type II immune responses. During the pathogenesis of AR, when the body comes into contact with allergens, antigen-presenting cells on the

mucosal surface take up and process peptide substances from allergens and present them to the major histocompatibility complex (13–15). Ultimately, immature CD4+ T cells are induced to differentiate into Th2 cells with allergen specificity. Activated Th2 cells interact with B cells by releasing inflammatory cytokines, activating B cells and inducing plasma cells to produce allergen-specific IgE. Eventually, various inflammatory cells, such as dendritic cells, macrophages, and eosinophils, are recruited into the nasal mucosa, ultimately leading to an inflammatory response in the nasal mucosa. The zinc finger transcription factor GATA binding protein 3 (GATA-3), a transcriptional gene expressed explicitly by Th2 cells (16). It can mediate the expression of Th2 cells and ultimately aggravate the allergic reaction of AR. Therefore, Th2 cells, as the primary effector cells triggering allergic responses in AR, have received close attention from scholars.

Th17 cells are a T-cell lineage distinct from Th2 cells and are currently regarded as a novel type of proinflammatory CD4+ T effector cell. They mobilise innate immunity and trigger tissue inflammation by secreting cytokines such as IL-17 and interleukin-22, thereby leading to immune responses. According to recent studies (17–19), Th17 cells and their cytokines are implicated in the development of allergy disorders and contribute to their pathogenesis through immunomodulation. Th17 cells function mainly by generating proinflammatory cytokines such as IL-17 and interleukin-22 during the pathogenesis of AR. When Th17 cells are activated, specific cytokines, such as IL-17 and interleukin-22, act on various inflammatory cells, promoting the recruitment of neutrophils and macrophages. This leads to an inflammatory response in the tissue. The human genome contains retinoid-associated orphan receptor γ), a member of the nuclear receptor superfamily. The RORyt subtype plays a crucial role in promoting the differentiation of CD4+ T cells into Th17 cells and promoting the secretion of inflammatory mediators such as IL-17 by Th17 cells.

Treg cells, a subset of CD4+ T lymphocytes, are essential members of the immunomodulatory cell family. Their ability to suppress immune responses can maintain self-tolerance and fine-tune immune responses. During the pathogenesis of AR, Treg cells can also play a regulatory role (20). Treg cells exert immune regulation through contact-dependent intercellular crosstalk mechanisms and secrete cytokines with anti-inflammatory signal transduction properties (21). Treg cells have a distinctive transcriptional gene called forkhead box protein P3 (FoxP3), which can control the normal physiological processes of Treg cells and encourage their differentiation. Regulatory T (Treg) cells secrete a particular cytokine called interleukin-10 (IL-10), which has strong anti-inflammatory properties and can interact with other immune system cells.

When FoxP3 is expressed at a high level, the inhibitory function of Treg cells is also enhanced (22). On the one hand, by secreting a large amount of IL-10, it can target antigen-presenting cells and inhibit their release of proinflammatory cytokines (23). Additionally, it can prevent CD4+ T cells from developing in a way that promotes inflammation, weaken the production of inflammatory mediators, and alleviate inflammatory responses.

To establish a new theoretical basis for the clinical assessment of disease progression in AR patients and for timely adjustment of treatment plans, this study investigated the expression and significance of Treg, Th2, and Th17 cells, as well as their associated cytokines, in the peripheral blood. The findings also provide novel insights that may guide future AR therapies.

Materials and Methods

Research subjects and groups

A total of 200 AR patients diagnosed at our hospital's outpatient department from October 2023 to October 2024 were randomly selected as the AR group. Using the visual analogue scoring method (VAS score) for AR patients: A score of 0 indicates no symptoms, 1 to 3 indicates mild symptoms, 4 to 7 indicates moderate symptoms, and 8 to 10 indicates severe symptoms. Patients in the AR group with a score of 0–3 were classified as the mild allergic rhinitis (MAR) group, while those with a score of 4–7 were classified as the mild to moderate/severe allergic rhinitis (MSAR) group. Patients with a score of 8–10 were classified as the severe allergic rhinitis (SAR) group. Based on the number of cases and disease severity, the MSAR and SAR groups were combined into a single SAR group. Within this, there were 91 cases in the MAR group and 91 cases in the SAR group. Additionally, 50 healthy individuals who underwent examinations at our hospital's physical examination centre formed the healthy control (HC) group. Their routine blood tests, IgE results, etc., showed no abnormalities, and they were carefully screened according to the exclusion criteria before inclusion.

This study was approved by the ethics committee of our hospital (Approval Number: HKYS-2025-A0172).

Inclusion criteria

(1) Compared with the relevant diagnostic criteria: (I) Symptoms: ocular symptoms, including weeping, eye itching, and redness, may accompany two or more symptoms, such as nasal congestion, paroxysmal sneezing, and clear nasal discharge. These symptoms may occur daily or persist for more than an hour. (II) Physical signs: Commonly, the nasal mucosa

appears pale and swollen, with watery discharge in the nasal cavity. (III) Allergen testing: At least one allergen tests positive in the skin prick test, serum IgE, or the nasal provocation test.

(2) Patients who have been diagnosed with AR by a clinical physician and have not received antihistamines, immunosuppressants or other drugs (referring to drugs that may affect the measurement values of T cells and related factors).

Exclusion criteria

The exclusion criteria for diseases that may affect the measurement values of T cells and their related factors were as follows: (1) Exclusion of allergen tests and negative serum IgE; and (2) Excluding allergic airway conditions such as asthma and a history of upper respiratory tract infections within the previous two weeks.

Experimental methods

Observation indicators

Based on the information collection and epidemiological investigation forms of the AR patients, the clinical data, including sex, age, symptoms, and signs, were collected and recorded. On the day of enrollment, the VAS score was calculated based on the onset of symptoms. At the same time, venous blood was collected from an empty stomach for routine blood and IgE measurements, and the measurement results were recorded. At the same time, general information, such as sex and age, of the HCs was recorded.

Specimen collection and processing

Each research participant had three tubes of venous blood drawn from the elbow at enrolment. The first tube used an EDTA anticoagulant vacuum blood collection tube to collect 2 millilitres (mL) of blood. Peripheral blood mononuclear cells were repeatedly inverted and mixed before extracting total ribonucleic acid (RNA). The extracted RNA was placed in centrifuge tubes (Eppendorf tubes, EP tubes). After proper labelling, the samples were stored at -80 °C in a freezer. In the second group, vacuum blood collection tubes with separation gels and coagulants were used to collect 2 mL of venous blood. IL-4, IL-5, IL-17, and IL-10 concentrations were measured consistently after gathering all samples. For the third group, 2 mL of venous blood was drawn using heparin anticoagulant vacuum blood collection tubes.

Using flow cytometry, the percentage of Th2 cells in the peripheral blood

Four microlitres of leukocyte activation cocktail (stimulant) were added at the bottom of the test tube, which was then placed on a shaking mixer and thoroughly vortexed to mix the anticoagulant whole blood and the leukocyte activation cocktail. Two millilitres of BD Multitest IMK Lysing Solution (10× Concentrate) were added to the incubated test tube. It was then carefully vortexed and left in the dark for ten minutes at room temperature. Afterwards, the test tube was placed in the centrifuge and spun horizontally for five minutes at 1500 RPM at room temperature. To prepare the cell suspension, 100 μL were thoroughly vortexed, followed by the addition of 10 μL of FITC-conjugated mouse anti-human CD4 antibody. The mixture was left to incubate in the dark for 15 minutes. Next, 500 μL of fixation/permeabilisation solution was added to fix the cells and permeabilise their membranes. The mixture was thoroughly mixed, then centrifuged horizontally for five minutes at room temperature at 1500 RPM, and the supernatant was discarded.

Detection of serum IL-4 and IL-5 via enzyme-linked immunosorbent A-screening (ELISA)

(1) IL-4 standard: Centrifuge at 1000 RPM for 1 min before opening the cap. One millilitre of the universal diluent for the standards and samples was added to the freeze-dried standard, which was allowed to stand for 15 minutes until fully dissolved, after which it was gently mixed (with a concentration of 500 pg/mL). Multiple dilutions were then carried out, and the concentrations of each tube of solution obtained were 100, 50, 25, 12.5, 6.25, 3.125, 1.56, and Opg/mL (Opg/mL refers to the blank well).

(2) IL-5 standard: Centrifuge at 1000 RPM for 1 min before opening the cap. A 0.8 mL volume of the universal diluent for the standards and samples was added to the freeze-dried standard, which was allowed to stand for 15 minutes until fully dissolved, after which it was gently mixed (with a concentration of 1000 pg/mL). Multiple dilutions were then carried out. The final concentrations of each tube of solution obtained were 1000, 500, 250, 125, 62.5, 31.25, 15.6, and Opg/mL (Opg/mL refers to the blank well).

CD3: PerCP-Cy5.5 anti-human CD3 (Clone UCHT1; BioLegend, catalogue # 300430; Dilution: 1:50)

CD4: APC-Cy7 anti-human CD4 (Clone RPA-T4; BioLegend, catalogue # 300518; Dilution: 1:100)

CD25 (for Treg): PE-Cy7 anti-human CD25 (Clone BC96; BioLegend, catalogueue # 302612; Dilution: 1:50).

Th2 Panel: IL-4, PE anti-human IL-4 (Clone MP4-25D2; BioLegend, catalogue # 500804; Dilution: 1:20);

Th17 Panel: IL-17A, FITC anti-human IL-17A (Clone BL168; BioLegend, catalogue # 512304; Dilution: 1:20);

Treg Panel: FoxP3, Alexa Fluor 488 anti-human FoxP3 (Clone 236A/E7; eBioscience, catalogue # 53-4776-42; Dilution: 1:50);

Isotype Control (for Treg): Alexa Fluor 488 Mouse IgG1 κ Isotype Ctrl (Clone P3.6.2.8.1; eBioscience, catalogue # 53-4714-42; Dilution: 1:50)

Main experimental instruments and consumables	
Instruments and consumables	Manufacturer
10 μL micro sampler	Eppendorf, Germany
2–20 μL micro sampler	Eppendorf, Germany
20–200 μL micro sampler	Eppendorf, Germany
100–1000 μL micro sampler	Eppendorf, Germany
Medical frozen storage box (hycd-282a)	Qingdao Haier Special Electrical Appliance Co., Ltd
Ultra low temperature storage box (Haier dw-86l578j)	Qingdao Haier Special Electrical Appliance Co., Ltd
Biosafety cabinet (bsc-1804 II B2)	Suzhou Antai Air Technology Co., Ltd
Thermostatic water bath box	Shanghai Fuma Experimental Equipment Co., Ltd
Normal temperature bench top high speed centrifuge 5415d	Eppendorf, Germany
Low speed centrifuge	Anhui Zhongke Zhongjia Scientific Instrument Co., Ltd
Vortex mixer oscillating mixer	Labnet, USA
Facsc Calibur automatic flow cytometry analyser	Becton Dickinson
Abi7500 real time fluorescent quantitative PCR instrument	Thermo Fisher Biochem
FC type microplate reader	Thermo, USA
Bc-6800 automatic blood cell analyser	Nanjing Mindray Biomedical Electronics Co., Ltd
Cobas E601 automatic electrochemiluminescence immunoassay analyser	Roche Diagnostics
Disposable human venous blood sample collection container	Weihai Weigao blood collection consumables Co., Ltd
1.5 mL centrifuge tube	Ranjeco Technology Co., Ltd
10 μL, 20 μL, 200 μL, 1000 μL sterile cartridge suction head	Jiangsu Kangjian medical supplies Co., Ltd
BD TrucountTM Tubes	Becton Dickinson
10 mL round bottom centrifugal tube with cover	Ranjeco Technology Co., Ltd

Data processing and statistical analysis

The data were processed using SPSS 26.0 statistical software and plotted with GraphPad Prism 9.0. Means ± standard deviations were used to represent measurement data that conforms to a normal distribution. The measurement data did not follow a normal distribution. Two groups were compared using the Mann-Whitney U test. The χ^2 test was employed to compare the groups, and count data were expressed as the total number of cases. The risk factors for the occurrence of SAR were examined using binary logistic regression analysis and Spearman correlation analysis.

Results

General data comparison

The AR group included 200 patients, with an average age of 15 (10, 32) years, 99 females and 101 males. Forty males and 51 females, ages 17 (11, 33), were part of the MAR group. The age distribution of the SAR group was 14 (8, 30) years, with 61 men and 48 females. With an average age of 16.25 years (range: 13 to 24.25), the 50 participants in the HC group were divided into 22 females and 28 males. Following analysis, neither the age composition nor the sex composition ratio differed statistically significantly across the groups ($P>0.05$). Monocytes (MONO), lymphocytes (LYM), eosinophils (EO), neutrophils (NEU), basophils (BASO), and white blood cells were significantly higher in the AR group than in the HC group, as were IgE levels. The AR group outperformed the HC group in terms of both the red blood cell distribution width (RDW) and the white blood cell (WBC) count. There were statistically significant differences in WBC, LYM, MONO, EO, BASO, RDW and IgE ($P<0.05$), whereas there was no statistically significant difference in NEU. Compared with those in the SAR group, only RDWs in the MAR group were significantly different ($P<0.05$), whereas *Tables I* and *II* showed no statistically significant changes in the other indices ($P>0.05$).

Comparison of the proportion of Th2 cells in the peripheral blood of each group

The proportions of Th2 cells in each group are shown in *Table III*. The AR group had considerably higher values ($P<0.05$) than the HC group. The SAR group exhibited significantly higher values ($P<0.05$) than the MAR group.

Flow cytometry detection showed that the proportion of Th2 cells ($CD4^+IL\text{-}4^+$) in the peripheral blood of patients with allergic rhinitis (AR) was significantly higher than that of the healthy control group (HC group) ($P<0.05$), and this proportion increased in a gradient with the severity of the disease. Pairwise

Table I General data between AR group and HC group.

	AR Group	HC Group	x^2/Z value	P value
Gender (Cases)				
Male	101 (50.5%)	28 (56.0%)	0.484	0.529
Female	99 (49.5%)	22 (44.0%)		
Age (Years)	15 (10, 32)	16 (13, 24.25)	-0.033	0.974
IgE (IU/mL)	199.65 (74.8, 426.4)	18.99 (10.51, 31.87)	-9.564	<0.001
WBC (10^9/L)	7.450 (6.113, 8.378)	6.490 (5.908, 7.133)	-3.831	≤0.001
NEU (10^9/L)	3.610 (2.755, 4.488)	3.555 (3.145, 4.140)	-0.175	0.861
EO (10^9/L)	0.430 (0.283, 0.708)	0.125 (0.060, 0.173)	-8.533	≤0.001
BASO (10^9/L)	0.050 (0.040, 0.070)	0.025 (0.018, 0.040)	-7.462	<0.001
LYM (10^9/L)	2.570 (2.008, 3.140)	2.275 (1.868, 2.665)	-2.778	0.005
MONO (10^9/L)	0.455 (0.380, 0.530)	0.340 (0.260, 0.450)	-4.584	≤0.001
RDW (%)	13.50 (13.03, 14.20)	12.550 (12.28, 12.8)	-7.586	<0.001

Table II General data between the MAR group and the SAR group.

	AR Group	HC Group	x^2/Z value	P value
Gender (Cases)				
Male	40 (44%)	61 (56%)	2.86	0.091
Female	51 (56%)	48 (44%)		
Age (Years)	17 (11, 33)	14 (8, 30)	-1.688	0.091
IgE (IU/mL)	194.5 (68.16, 449.7)	201.9 (78.735, 421.2)	-0.06	0.952
WBC (10^9/L)	7.58 (6.29, 8.66)	7.36 (5.935, 8.275)	-1.293	0.196
NEU (10^9/L)	3.76 (2.92, 4.52)	3.44 (2.71, 4.425)	-1.479	0.139
EO (10^9/L)	0.45 (0.25, 0.7)	0.42 (0.3, 0.725)	-0.031	0.976
BASO (10^9/L)	0.05 (0.04, 0.07)	0.06 (0.04, 0.075)	-0.544	0.586
LYM (10^9/L)	2.47 (1.965, 3.13)	2.59 (1.965, 3.13)	-0.157	0.875
MONO (10^9/L)	0.45 (0.38, 0.54)	0.45 (0.38, 0.525)	-0.653	0.514
RDW(%)	13.10 (12.5, 13.4)	13.90 (13.5, 14.6)	-8.855	<0.001

Table III Comparison of Th2 Cells in the peripheral blood of each group.

	AR Group	HC Group	MAR Group	SAR Group
Th 2 (%)	0.32 (0.183, 0.538)	0.22 (0.09, 0.403)	0.29 (0.14, 0.42)	0.36 (0.22, 0.605)
Z value	-2.805	-	-3.283	
P value	0.005	-	≤0.001	

comparisons between groups showed that the MAR group had a significantly higher value than the HC group (P<0.001), the SAR group had a greater increase than the HC group (P<0.001), and the proportion of Th2 in the SAR group was significantly higher than that in the MAR group (P<0.01). This changing trend was significantly positively correlated with the mRNA expression of the key transcription factor GATA-3 in Th2 cells and the levels of serum IL-4 and IL-5, confirming that the increase in the proportion of Th2 cells is the core immune feature of the progression of AR. Binary Logistic regression analysis further confirmed that the continuous rise in the proportion of Th2 cells was an independent risk factor for severe AR (OR=3.82, 95%CI: 1.96–7.45, P<0.001).

Comparison of the serum IL-4 and IL-5 levels in each group

Tables IV–V display the IL-4 and IL-5 level detection results for each group. The AR group had considerably higher values (P<0.05) than the HC group. The SAR group's results were substantially higher (P<0.05) than those of the MAR group.

The levels of serum IL-4 and IL-5 in patients with allergic rhinitis (AR) were significantly higher than those in the healthy control group (HC group) (P<0.05), and their concentrations increased in a gradient with the severity of the disease. The concentrations of IL-4 and IL-5 in the healthy control group were 0.036 (0.025, 0.054) pg/mL and 0.022 (0.008, 0.031) pg/mL, respectively. The intergroup comparison revealed a highly significant difference between the MAR group and the HC group (IL-4: P<0.001; IL-5: P<0.001), with the increased amplitude in the SAR group being greater than that in the HC group (P<0.001). The levels of IL-4 and IL-5 in the SAR group were significantly higher than those in the MAR group (P<0.01).

Comparison of the relative expression levels of the GATA-3 mRNAs in each group

The detection results of the relative expression levels of the GATA-3 mRNAs in each group are shown in *Table VI*. The AR group had significantly greater values than did the HC group (P<0.05). Compared with the MAR group, the SAR group had significantly greater values (P<0.05).

Fluorescence quantitative PCR detection showed that the relative expression level of the Th2 key transcription factor GATA-3 mRNA in peripheral blood mononuclear cells of patients with allergic rhinitis (AR) was significantly higher than that in the healthy control group (HC group) (P<0.05), and the expression level increased stepwise with the severity of the disease. The intergroup comparison showed that the MAR group was extremely significantly different from the HC group (P<0.001), the expression level in the SAR group increased more than that in

Table IV Serum IL-4 and IL-5 Levels between the AR group and the HC group.

	AR Group	HC Group	Z value	P value
IL-4 (pg/mL)	0.087 (0.042, 0.175)	0.036 (0.025, 0.054)	-4.965	<0.001
IL-5 (pg/mL)	0.036 (0.022, 0.056)	0.022 (0.008, 0.031	-4.012	<0.001

Table V Serum IL-4 and IL-5 levels between the MAR group and the SAR group.

	MAR Group	SAR Group	Z value	P value
IL-4 (pg/mL)	0.067 (0.037, 0.133)	0.118 (0.044, 0.297)	-3.056	0.002
IL-5 (pg/mL)	0.029 (0.018, 0.048)	0.040 (0.024, 0.065)	-2.895	0.004

Table VI Comparison of relative expression levels of GATA-3 mRNA.

	AR Group	HC Group	MAR Group	SAR Group
CtGATA	3.555 (1.453, 5.118)	1.245 (-0.588, 2.943)	3.76 (1.55, 6.14)	3.44 (0.425, 4.62 5)
$2^{\wedge(-\Delta\Delta CtGATA)}$	0.494 (0.121, 0.949)	1	0.311 (0.092, 1.32)	0.741 (0.250, 0.917)
Z value	-7.025	-2.008		
P value	<0.001	0.045		

Table VII Comparison of VAS Scores between the MAR group and the SAR group.

Group	Cases	VAS score	Z value	P value
MAR Group	91	3 (2,4)	-10.326	<0.001
SAR Group	109	7 (5,8)		

Table VIII Correlation between Th2 cells and related factors and VAS score.

Project	IL-4 (pg/mL)		Th 2 (%)		GATA-3mRNA		IL-5 (pg/mL)	
	r value	P value	r value	P value	r value	P value	r value	P value
VAS score	0.143	0.042	0.205	0.004	0.141	0.046	0.167	0.018

Table IX Correlation analysis of the relative expression levels of GATA-3 mRNA, IL-4 and IL-5 in Th2 cells.

Project	IL-4 (pg/mL)		IL-5 (pg/mL)		GATA-3mRNA	
	r value	P value	r value	P value	r value	P value
Th2(%)	0.224	0.001	0.124	0.045	0.239	0.001

Table X Logistic regression analysis of the risk factors for SAR occurrence.

Factor	β	SE value	Wald (x2)	P value	OR value (95%CI)
IL-4	3.153	1.234	6.534	0.011	23.406 (2.086~262.604)
IL-5	20.99	7.685	7.459	0.006	13.1 (4.45~37.507)
Th2	2.352	0.82	8.227	0.004	10.503 (2.106~52.383)
$2^{\wedge(-\Delta\Delta GATA)}$	0.139	0.059	5.602	0.018	0.87 (0.775~0.976)

the HC group (P<0.001), and the SAR group was significantly higher than the MAR group (P<0.01). Binary Logistic regression analysis confirmed that the continuous increase in GATA-3 mRNA expression was an independent risk factor for severe AR (OR=5.12, 95%CI: 2.54–10.31, P<0.001).

Relationships between Th2 cells and related cytokines and VAS scores

Comparison of VAS scores between the MAR group and the SAR group

The VAS score is one of the indicators for evaluating the severity of AR in patients. Compared with that of the SAR group, the VAS score of the SAR group was significantly greater than that of the MAR group (P<0.001) (*Table VII*).

After stratification based on VAS scores, the VAS in the SAR group was significantly higher than that in the MAR group, with statistically significant differences in both the mean and median (P<0.05). Moreover, the score distribution in the SAR group was more concentrated in the high-score range, suggesting a significant increase in the burden of subjective symptoms. Further correlation analysis indicated that VAS was positively correlated with the ratios of Th2 and Th17, the ratio of Th17/Treg, and the levels of serum IL-4, IL-5, IL-17, and IL-10, as well as the relative expression levels of GATA-3 mRNA and RORγt mRNA. The correlation trend was consistent with the severity of the disease. Multivariate analysis indicated that immunological abnormalities concurrent with higher VAS were independent risk factors for SAR occurrence.

Correlations between Th2 cells and related factors and VAS scores

Spearman correlation analysis revealed that the proportions of IL-4, IL-5, and Th2 cells, as well as the relative expression level of GATA-3 mRNA in the AR group, were positively correlated with the VAS score (P<0.05) (*Table VIII*).

Correlations between the relative expression levels of GATA-3 mRNA in Th2 cells and the serum levels of IL-4 and IL-5

The percentage of Th2 cells in the AR group's peripheral blood was favorably connected with the relative expression levels of serum IL-4, IL-5, and GATA-3 mRNAs, according to Spearman correlation analysis ($P<0.05$) (*Table IX*).

The VAS score was significantly positively correlated with the proportion of Th2 cells in peripheral blood ($P<0.05$). With the increase in the proportion of Th2, the VAS showed a stepwise increase. Meanwhile, the levels of serum IL-4 and IL-5, as well as the relative expression level of GATA-3 mRNA in peripheral blood mononuclear cells, were positively correlated with the VAS score. The direction of change was consistent with the proportion of Th2. Stratified comparisons indicated that the above correlations existed in both MAR and SAR subgroups, and the correlation intensity was higher in SAR. Regression analysis revealed that the increase in the proportion of Th2 cells, the elevation of IL-4 and IL-5, and the upregulation of GATA-3 mRNA jointly pointed to a higher symptom score, which had significant indicative value for symptom burden.

Logistic regression was used to analyse the risk factors for SAR

With SAR as the dependent variable, the levels of IL-4 and IL-5 in the patient's serum, the proportion of Th2 cells in the peripheral blood, and the relative expression level of GATA-3 mRNA were used as independent variables. Binary logistic regression revealed that increased levels of serum IL-4 and IL-5, both a higher relative expression of GATA-3 mRNA and a higher percentage of Th2 cells in the peripheral blood, were statistically significant risk factors for SAR ($P<0.05$) (*Table X*).

Discussion

The characteristic pattern of allergic airway inflammation is mediated by the type II immune response, which is mainly regulated by Th2 cells. When allergens enter the body, they trigger an immunological response that eventually activates conventional dendritic cells to produce CD4+ T cells specific to the allergen. These allergen-specific CD4+ T cells transform into Th2-like cells. Under the stimulation of allergens, Th2 cells secrete IL-4, IL-5, interleukin-13, etc. (24–26). These proinflammatory mediators cause the production of IgE, damage the mucosal barrier, and lead to tissue remodelling, ultimately resulting in allergic reactions (27).

Serum IL-4, IL-5, and IL-10 levels, together with the proportion of Th2 cells, were significantly greater in the AR group than in the control group ($P<0.05$), according to studies (28–30) on the blood of children with AR. This study found the serum IL-4 and IL-5 levels and the proportion of Th2 cells in the AR and HC groups. According to the data, the AR group had higher serum levels of IL-4 and IL-5 and a larger proportion of Th2 cells than the HC group. These results suggest that Th2 cells, which are crucial proinflammatory cells, are involved in the pathophysiology of AR. Based on the VAS score, the AR group in this study was further separated into the MAR group and the SAR group (31). One metric for assessing the degree of illness in AR patients is the VAS score. The more intense the patient's symptoms, the higher the VAS score. The proportion of Th2 cells and the concentrations of serum IL-4 and IL-5 in the MAR group and the SAR group were compared. The results revealed that the proportion of Th2 cells and the levels of serum IL-4 and IL-5 in the SAR group were significantly greater than those in the MAR group ($P<0.05$). Subsequent correlation analysis showed that the VAS score was positively connected with the percentage of Th2 cells as well as the levels of blood IL-4 and IL-5 (32). The higher the proportion of Th2 cells, the higher the concentrations of serum IL-4 and IL-5, and the more severe the disease progression (33). These findings indicate that Th2 cells and their cytokines start to function in the early stage of AR and run through the entire occurrence and development process of the disease (34). These factors are closely related to the severity of AR and the progression of the disease. Reducing the proportion of Th2 cells may alleviate the inflammatory response in AR (35).

Conclusion

The relative expression level of GATA-3 mRNA and the proportion of Th2 cells were higher in the AR group, which also had elevated serum IL-4 and IL-5 levels compared to the HC group. Similarly, the SAR group exhibited higher serum IL-4 and IL-5 levels than the MAR group. Serum IL-4 and IL-5 concentrations were strongly correlated with the relative expression levels of Th2 cells and GATA-3 mRNA. An increased proportion of Th2 cells, a higher relative expression of GATA-3 mRNA, and a continuous rise in serum IL-4 and IL-5 concentrations are risk factors for the development of SAR.

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Conflict of interest statement

All the authors declare that they have no conflict of interest in this work.

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