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ORIGINAL ARTICLE

Imbalanced serum levels of resolvin E1 (RvE1) and leukotriene B4 (LTB4) in patients with allergic rhinitis

Ramin Lotfi[1,2] · Akram Davoodi[2] · Seyed Hamidreza Mortazavi[3] · Ali Gorgin Karaji[4] · Hanieh Tarokhian[4] · Alireza Rezaiemanesh[4] · Farhad Salari[4]

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Abstract

Timely and successful resolution of acute inflammation plays a crucial role in preventing the development of chronic airway inflammation in allergic rhinitis (AR). This study intends to assess the serum levels of pro-inflammatory leukotriene B4 (LTB4), anti-inflammatory mediators, including resolvin E1 (RvE1), RvD1, IL-10, and TGF-β, besides mRNA expression level of G-protein coupled receptor 120 (GPR120) and peroxisome proliferator-activated receptor-γ (PPAR-γ) receptors in peripheral blood leukocytes of AR patients. Thirty-seven AR patients and thirty age- and gender-matched healthy subjects were enrolled in this study. The serum levels of LTB4, RvE1, RvD1, IL-10, and TGF-β were measured using enzyme-linked immunosorbent assay (ELISA) technique, and the mRNA expression level of GPR120 and PPAR-γ was assessed by the real-time PCR method. The serum levels of RvE1 and LTB4 were significantly higher in patients with AR than in healthy subjects ($P < 0.01$ and $P < 0.0001$, respectively). However, a significantly lower ratio of RvE1 and RvD1 to LTB4 was found in patients with AR relative to healthy subjects ($P < 0.05$ and $P < 0.0001$, respectively). Likewise, the serum levels of both IL-10 and TGF-β cytokines were significantly reduced in patients with AR compared to healthy subjects ($P < 0.01$ and $P < 0.0001$, respectively). Furthermore, the mRNA expression of PPAR-γ was significantly lower in patients with AR than in healthy subjects ($P < 0.05$). Our findings indicate that imbalanced pro-resolving lipid mediator RvE1 and pro-inflammatory LTB4 might contribute to the defective airway inflammation-resolution and subsequent progression toward chronic inflammation in AR patients.

Keywords Allergic rhinitis · Resolvin E1 · Chronic inflammation · PPAR-γ · Leukotriene B4

Abbreviations

AR	Allergic rhinitis
AHR	Airway hyperresponsiveness
RvE1	Resolvin E1
IgE	Immunoglobulin E
LTB4	Leukotriene B4
AA	Arachidonic acid
PPAR-γ	Peroxisome proliferator-activated receptor-γ
GPR120	G-protein coupled receptor 120
SPMs	Specialized pro-resolving lipid mediators
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
IL-10	Interleukin-10
TGF-β	Transforming growth factor-β
EDTA	Ethylenediamine tetraacetic acid
PBMCs	Peripheral blood mononuclear cells
ELISA	Enzyme-linked immunosorbent assay
qRT-PCR	Quantitative real-time polymerase chain reaction

✉ Farhad Salari
f.salari@kums.ac.ir

[1] Lung Diseases and Allergy Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran

[2] Student Research Committee, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran

[3] Department of Pediatrics, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran

[4] Department of Immunology, School of Medicine, Kermanshah University of Medical Sciences, PO-Box: 6714869914, Kermanshah, Iran

Introduction

Allergic Rhinitis (AR) is a common chronic inflammatory disease of the upper airways, in which a genetically susceptible individual begets an aberrant immune response against typically innocuous environmental substances [1, 2]. Although acute inflammation is a host-protective response against invading pathogens and/or injurious agents, emerging evidence indicates that persistent uncontrolled or unresolved inflammation is a crucial event in the pathogenesis of numerous chronic inflammatory disorders, including cancer, autoimmune diseases, metabolic syndromes, cardiovascular diseases, asthma, and rhinitis [3–5].

The omega-6 fatty acid arachidonic acid (AA)-derived leukotrienes (LTs) play critical roles in inducing acute inflammatory responses as the potent chemoattractant and activator for immune cells [6, 7]. In this regard, elevated levels of LTB4 have been reported in the nasal cavity and exhaled breath condensate (EBC) of patients with AR, as well as sputum, urine, bronchoalveolar lavage fluid (BALF), arterial blood samples, and EBC in patients with asthma [8–16].

The resolution of acute inflammation (also known as catabasis) is a biochemically active process that occurs in overlapping phases governed by the spatial and temporal production of omega-3 and omega-6 fatty acids-derived specialized pro-resolving lipid mediators (SPMs) or immunoresolvents. Interestingly, immunoresolvents are unique since they exert both anti-inflammatory and pro-resolving properties without suppressing the immune response [3–5, 17]. According to previous studies, an imbalance between immunoresolvents and pro-inflammatory LTs is linked to several chronic inflammatory disorders [18–21]. As the most well-known member of immunoresolvents, resolvins have the two major series, including E- and D-series, which are biosynthesized from the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), respectively [6, 22–24]. A growing body of evidence has indicated that resolvins exert their anti-inflammatory and immunomodulatory functions through inhibiting the generation of pro-inflammatory mediators and regulating leukocytes trafficking to the site of inflammation. Furthermore, resolvins have been demonstrated to induce the production of suppressive cytokines, such as interleukin-10 (IL-10) and transforming growth factor-β (TGF-β). Previous studies have shown the potential of resolvin E1 (RvE1) and RvD1 or their agonists to facilitate the inflammation-resolution in murine models of inflammatory diseases, including asthma, contact hypersensitivity, atopic dermatitis, peritonitis, and acute lung injury [22, 25–32].

Apart from the anti-inflammatory actions of omega-3 fatty acids-derived mediators, omega-3 fatty acids also exert anti-inflammatory functions via binding to cell surface G-protein coupled receptor 120 (GPR120) and intracellular peroxisome proliferator-activated receptor-γ (PPAR-γ) receptors [33]. Indeed, PPAR-γ activation can promote tissue catabasis through inhibiting the recruitment of polymorphonuclears (PMNs) and M1 macrophages into the site of inflammation and subsequent production of pro-inflammatory cytokines. Furthermore, PPAR-γ and its agonists increase the production of IL-10 and TGF-β [34, 35]. Several studies have demonstrated the beneficial anti-inflammatory effects of PPAR-γ and its agonists in murine models of allergic rhinitis, asthma [36–41], and human asthma [42].

Given the crucial roles of omega-3 fatty acids-derived immunoresolvents and omega-3 receptors in preventing the development of chronic airway inflammation and subsequently promoting inflammation-resolution, this study was conducted to investigate the serum levels of LTB4, RvE1, RvD1, IL-10, and TGF-β, besides the mRNA expression level of GPR120 and PPAR-γ in peripheral blood leukocytes of patients with allergic rhinitis and healthy subjects.

Materials and methods

Study design, participants, and ethical considerations

This cross-sectional study was conducted on subjects referring to the Allergy Clinic of Dr. Mohammad Kermanshahi Hospital in Kermanshah province, Iran. A total of 37 patients with allergic rhinitis who were diagnosed by a specialist physician via clinical examination and based on validated criteria for the presence of nasal symptoms [43] were enrolled in this study. Patients with allergic rhinitis were formerly under treatment with topical intranasal steroids and/or nasal decongestants, but at the sampling time, none of the participants had taken any medication for at least the past 72 hours. The frequency of allergic rhinitis symptoms in patients was evaluated using the Rhinitis Quality of Life Questionnaire (RQLQ). Furthermore, a validated semi-quantitative food-frequency questionnaire (FFQ) having 168 items, which was prepared by a trained nutritionist and includes a list of foods that are commonly consumed by Iranians, was used to assess the usual dietary intake. All participants were asked to report the number of times they consumed a single serving of each food item over the past year on a daily basis, weekly, or monthly. Using this FFQ, the information on total omega-3, EPA, DHA, and total omega-6 intake was estimated [44]. Moreover, 30 age- and gender-matched healthy subjects lacking any background of inflammatory disorders were selected as the control group. The study protocol was in accordance with the Declaration

of Helsinki and was performed with approval from the Biomedical Ethics Committee of the Kermanshah University of Medical Sciences (Ethical code: IR.KUMS.REC.1396.8). All participants received comprehensive information regarding the goals and procedures of the study and then signed the informed consent form.

Inclusion criteria

The inclusion criteria for the patient group were being within the age range of 15–60 years old, being discerned with allergic rhinitis, and the ability to complete and sign the informed consent form.

Exclusion criteria

Having other acute and chronic inflammatory disorders, like infectious or autoimmune diseases, taking corticosteroids or immunotherapy during the past month, having other types of rhinitis and other allergic diseases, like asthma, at the same time.

Blood sample collection

The blood specimens were collected in the clot tubes. Subsequently, serum samples were instantly separated for measuring the lipid mediators and cytokines. For molecular assessments, using the Ficoll-Paque density gradient media, peripheral blood mononuclear cells (PBMCs) were isolated from the blood samples collected in vacuum tubes prefilled with the anticoagulant ethylenediamine tetraacetic acid (EDTA). Furthermore, using an optical microscope, eosinophils count and percentage for all participants was determined following the preparation of peripheral blood smears.

Measurement of serum levels for total IgE, LTB4, RvE1, RvD1, IL-10, and TGF-β

Serum levels of total IgE, LTB4, RvE1, RvD1, IL-10, and TGF-β were evaluated using the enzyme-linked immunosorbent assay (ELISA) kits (Padtan Elm, MyBioSource, and Eastbiopharm, respectively) according to the manufacturer's guidelines.

RNA extraction, cDNA synthesis, and qRT-PCR

Total RNA was isolated from PBMCs by RNA extraction kit (Favorgen Biotech Corp., Taiwan) based on the manufacturer's instructions. The extracted RNA was then reverse-transcribed to cDNA by cDNA synthesis kit (Favorgen Biotech Corp., Taiwan) according to the manufacturer's recommendations. All samples were kept at − 70 °C for subsequent analysis. Quantitative real-time PCR (qRT-PCR) was done using the Light cycler 96 device (Roche, Germany) and by SYBR green (Amplicon, Odense, Denmark) master mix. Primers were designed with Beacon Designer™ Software (Primer Biosoft, USA). Primer sequences for PPAR-γ were; PPAR-γ forward 5′- GGATGTCTCATAATGCCA TCAGG-3′ and PPAR-γ reverse 5′- GGTCAGCGGACT CTGGATT-3′. Also, GPR120 primer sequences were as follow; GPR120 forward 5′- GCCCACCATTCCTGGAGAG-3′ and GPR120 reverse 5′- CCTTGATGCCTTTGTGATCTG TAA-3′. The values were normalized based on the expression of GAPDH as a housekeeping gene using the following primers: GAPDH forward 5′- GACCCCTTCATTGACCTC AAC-3′ and GADPH reverse 5′- GATCTCGCTCCTGGA AGATG-3′. All qRT-PCR reactions were accomplished in a final volume of 15 µL comprising 7.5 µL SYBR green master mix, 5.5 µL deionized water, 1 µL cDNA, and 0.5 µL of each forward and reverse primer. The conditions of each real-time PCR reaction as follow; 1- pre-incubation: 95 °C for 180 s, 2- two-step amplification for 40 cycles: 95 °C for 15 s and 60–61 °C for 20 s, 3- melting: 95 °C for 10 s, 65 °C for 60 s, and 97 °C for 1 s, 4- cooling: 37 °C for 30 s. All reactions were performed in duplicate to avoid and reduce the false positive and negative results caused by the user's technical errors. The relative gene expression differences between controls and patients were calculated using the Pfaffl method $(R=(E_{target})^{\Delta Ct\ target\ (control-sample)}/(E_{Ref})^{\Delta Ct\ Ref(control-sample)})$ [45].

Statistical analysis

Statistical analyses and graph drawing were performed by SPSS software version 16.0 (SPSS, Chicago, IL, USA) and GraphPad Prism software version 6 (GraphPad Software, La Jolla, California, USA). The data normality was evaluated by the 1-sample Kolmogorov-Smirnov (K-S) test. Independent sample *T* test and Mann-Whitney *U*-test were utilized for parametric and non-parametric data analysis, respectively. A P value < 0.05 was considered as statistically significant. In all statistical analyses, results are expressed as mean ± standard error of the mean (SEM).

Results

Studied groups

This study investigated 37 patients with AR and 30 healthy subjects. There was no significant difference between patients with AR and healthy subjects in terms of age, gender, as well as lipid profile in dietary intakes. Demographic information and dietary intake of omega-6 and

Table 1 Demographic information and dietary intake of omega-6 and omega-3 for all participants and the frequency of AR symptoms in the patient group

Variables	Allergic rhinitis (AR) patients (n = 37)	Healthy subjects (n = 30)	*P* value
Gender (female/male)	24/13	20/10	0.296
Mean age, yr (SEM)	35.18 ± 1.74	34.96 ± 2.33	0.840
BMI (Mean ± SEM)	25.47 ± 0.71	24.22 ± 0.78	0.842
Asthma history	–	–	
The mean duration of illness, yr	6	–	
The frequency of AR symptoms			
Sneezing (%)	91.7	–	
Rhinorrhea (%)	94.4	–	
Nasal obstruction (%)	91.7	–	
Nasal itching (%)	86.1	–	
Eye itching (%)	66.7	–	
Watery eyes (%)	70	–	
FFQ			
Total omega-3 (gr)	1.62 ± 0.166	1.98 ± 0.31	0.37
EPA (gr)	0.033 ± 0.006	0.038 ± 0.015	0.42
DHA (gr)	0.11 ± 0.019	0.13 ± 0.046	0.45
Total omega-6 (gr)	20.34 ± 1.88	19.85 ± 1.54	0.75

yr years, *SEM* standard error of the mean, *BMI* body mass index, *FFQ* food-frequency questionnaire

omega-3 for all participants, as well as the frequency of AR symptoms in patients, are shown in Table 1.

Percentage count of peripheral blood eosinophils

The percentage count of peripheral blood eosinophils in patients with AR was significantly higher than that in healthy subjects ($P < 0.0001$) (Fig. 1).

Serum levels of total IgE, LTB4, RvE1, RvD1, IL-10, and TGF-β

The mean serum levels of total IgE were slightly higher in patients with AR than that in healthy subjects; however, no significant difference was found between the two groups (Fig. 2a). Moreover, LTB4 serum levels were significantly higher in patients with AR compared to healthy subjects ($P < 0.0001$) (Fig. 2b). The serum levels of RvE1 was also significantly higher in patients with AR than in healthy subjects ($P = 0.0018$) (Fig. 2c). However, no significant difference in the serum levels of RvD1 was observed between AR patients and healthy subjects (Fig. 2d). The serum levels of IL-10 in patients with AR were significantly lower than those in healthy subjects ($P = 0.0047$) (Fig. 2e). Moreover, the serum levels of TGF-β in AR patients were significantly lower compared to healthy subjects ($P < 0.0001$) (Fig. 2f).

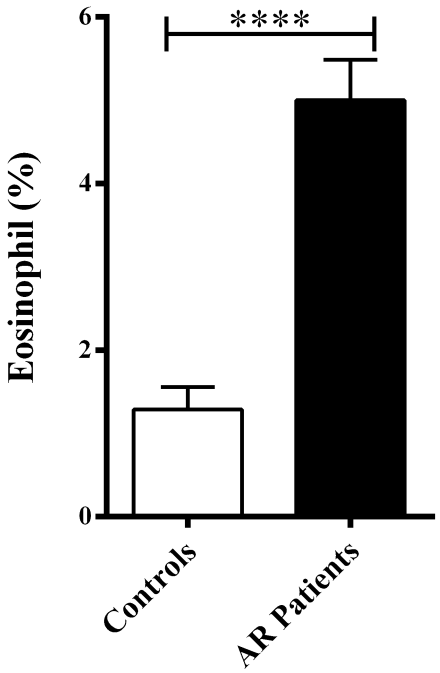


Fig. 1 The percentage count of peripheral blood eosinophils in patients with AR and healthy subjects. A significantly higher percentage count of peripheral blood eosinophils was observed in patients with AR than that in controls. ****$P < 0.0001$

The ratios of RvE1 and RvD1 to LTB4

The ratio of RvE1 to LTB4 (RvE1:LTB4) in patients with AR was significantly decreased than that in healthy subjects

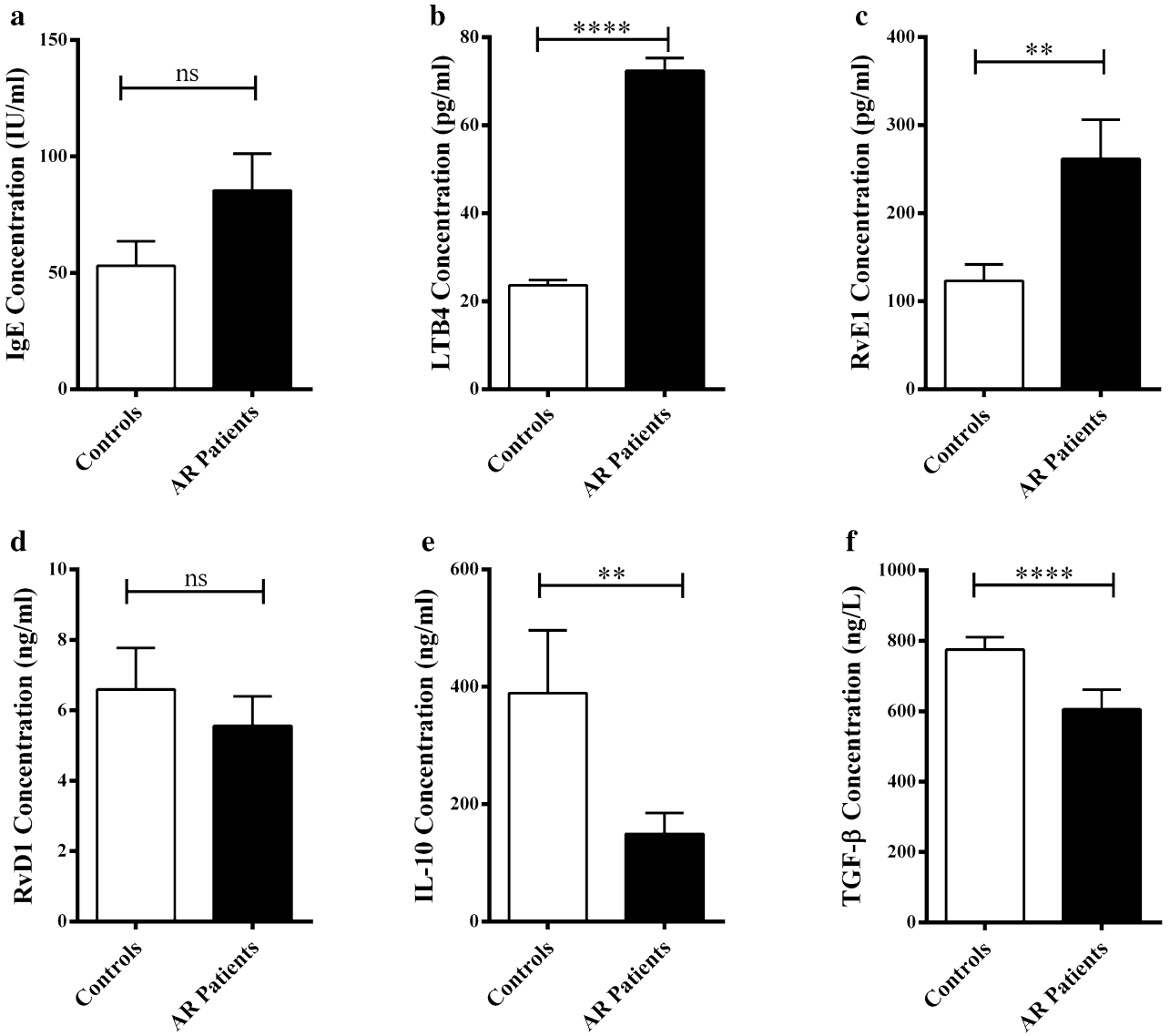


Fig. 2 Serum levels analysis **a** Serum level of total IgE in patients with AR and healthy subjects. No significant difference was found in the serum level of total IgE between patients with AR and healthy subjects. **b** LTB4 serum level in patients with AR and healthy subjects. LTB4 serum level in patients with AR was significantly higher than that in healthy subjects. **c** Serum level of RvE1 in patients with AR and healthy subjects. A significant difference was found in the serum level of RvE1 between patients with AR and healthy subjects. **d** Serum level of RvD1 in patients with AR and healthy subjects. No significant difference was found in the serum level of RvD1 between the two groups. **e** Serum level of IL-10 in patients with AR and healthy subjects. The serum level of IL-10 in patients with AR was significantly lower than that in healthy subjects. (f) TGF-β serum level in patients with AR and healthy subjects. The serum level of TGF-β between patients with AR and healthy subjects was statistically different and significant. ns = not statistically significant, $^{**}P < 0.01$, $^{****}P < 0.0001$

($P = 0.0427$) (Fig. 3a). Similarly, the ratio of RvD1 to LTB4 (RvD1:LTB4) was meaningfully reduced in patients with AR relative to healthy subjects ($P < 0.0001$) (Fig. 3b).

mRNA expression of GPR120 and PPAR-γ

No statistically significant difference was found in the mRNA expression of GPR120 between AR patients and healthy subjects (Fig. 4a). However, the mean mRNA expression levels of PPAR-γ in patients with AR were significantly lower than that in healthy subjects ($P = 0.0426$) (Fig. 4b).

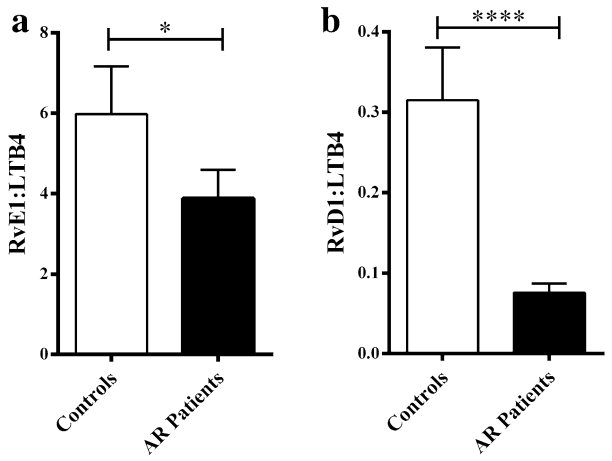


Fig. 3 Ratios analysis. **a** The ratio of RvE1 to LTB4 (RvE1:LTB4) in patients with AR and healthy subjects. RvE1:LTB4 was significantly lower in patients with AR compared to healthy subjects. **b** The ratio of RvD1 to LTB4 (RvD1:LTB4) in patients with AR and healthy subjects. RvD1:LTB4 in patients with AR was significantly lower than in healthy subjects. $*P < 0.05$, $****P < 0.0001$

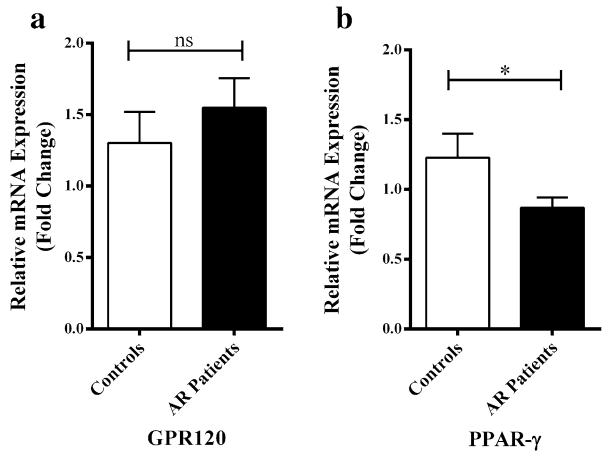


Fig. 4 mRNA expression analysis. **a** The GPR120 mRNA expression level in patients with AR and healthy subjects. No significant difference in the GPR120 mRNA expression level was found between patients with AR and healthy subjects. **b** PPAR-γ mRNA expression in patients with AR and healthy subjects. A significant difference was found in the PPAR-γ mRNA expression level between patients with AR and healthy subjects. ns = not statistically significant, $*P < 0.05$

Discussion

Allergic rhinitis (AR) is a chronic inflammation of the upper airways, in which numerous cells and cellular elements play a role, especially T helper 2 (Th2) cells and their cytokines, like interleukin (IL)-4, IL-5, and IL-13 [46, 47]. Although evidence from animal models has disclosed the critical roles for omega-3 fatty acids-derived pro-resolving metabolites in regulating allergic airway inflammation, only limited studies are currently available in human allergic diseases. In the present study, we evaluated the importance of omega-3 fatty acids-derived metabolites, including RvE1 and RvD1, as well as omega-3 receptors in the pathogenesis of AR.

Resolvins are the most well-known omega-3 fatty acids-derived metabolites, acting as the spatial and temporal regulators of inflammatory responses in the airway [22, 48]. It has previously been shown that when given to a murine model of asthma intraperitoneally, RvE1 reduces airway recruitment of eosinophils and lymphocytes, level of IL-13 and ovalbumin (OVA)-specific immunoglobulin E (IgE) in both BALF and lung tissue, as well as mucus scores and AHR to inhaled methacholine [25]. Furthermore, RvE1 was reported to play a protective role in OVA sensitization, challenge, and post-allergen challenge phases against AHR and airway inflammation in murine models of asthma [49, 50]. In addition, treating asthmatic mice with RvE1 has been shown to strikingly suppress cytokine production by pulmonary macrophages through down-regulating the nuclear translocation of nuclear factor kappa B (NF-κB) p65 but no significant effect on lymphocytes [31]. In a murine model of atopic dermatitis, intraperitoneal administration of RvE1 suppressed the production of interferon-γ (IFN-γ) and IL-4, as well as IgE serum level. Additionally, RvE1 decreased the infiltration of eosinophils, mast cells, and T cells into skin lesions [28]. In the case of RvD1, experiments in murine models have shown that it may attenuate pulmonary inflammation of lipopolysaccharide- and ventilator-induced acute lung injury by suppressing NF-κB activation partly through a PPAR-γ activation-dependent mechanism [30, 51]. In the present study, we found that the serum levels of RvE1, but not RvD1, in patients with allergic rhinitis are significantly higher than those in healthy subjects. Studies using mice lacking LTB4 receptor-1 (BLT-1), a high-affinity receptor for LTB4, have shown the crucial roles of the LTB4-BLT1 pathway in the recruitment of allergen-specific $CD4^+$ and $CD8^+$ effector T cells producing IL-13 into the lung and development of airway inflammation and AHR [52, 53]. Interestingly, RvE1 can also bind to BLT-1, leading to the attenuation of LTB4-induced recruitment of the immune cells to the inflamed sites. Thus, RvE1 and LTB4 compete together for binding to BLT-1, and RvE1 acts indeed as an antagonist for BLT-1 [33]. In the present study, lower ratios of RvE1 and RvD1 to LTB4 indicate an imbalance between the productions of these mediators in patients with AR. These findings raise the possibility of LTB4 overcoming on RvE1 for binding to BLT-1 leading to defective airway inflammation-resolution and subsequent progression toward chronic airway inflammation. Altogether, it seems that increasing RvE1 is an indispensable effort of the immune system to actively resolve allergic airway inflammation. However, this effort

seems to be inadequate, most likely owing to higher serum levels of LTB4 than RvE1 and probable failure of RvE1 for binding and inhibiting BLT-1. Accordingly, final proving such a hypothesis requires further studies.

Notably, supplementation with omega-3 fatty acids has been shown to significantly increase the plasma concentration of RvE1 in healthy humans [54]. Here, using a validated FFQ, we found no significant difference in the dietary intakes of total omega-3, EPA, DHA, and total omega-6 fatty acids between patients with AR and healthy subjects.

G-protein coupled receptor 120 (GPR120), also known as free fatty acid receptor 4 (FFAR4), is a membrane-bound receptor for omega-3 fatty acids, playing potent anti-inflammatory functions [33]. GPR120 has been shown to be expressed at both the mRNA and protein levels in eosinophils isolated from the peripheral blood of non-obese individuals having mild eosinophilia. Also, stimulation of these eosinophils with GW9508, a synthetic GPR120 agonist, strikingly increased IL-4 secretion [55]. Moreover, there exists evidence indicating that GPR120 can boost airway relaxation and resolve airway inflammation [56]. In our study, no significant difference in the GPR120 mRNA expression level was observed between patients with AR and healthy controls.

Peroxisome proliferator-activated receptor-γ (PPAR-γ) is a functional ligand-activated transcription factor belonging to the nuclear hormone receptors superfamily. By signaling through PPAR-γ, omega-3 fatty acids mediate potent anti-inflammatory actions [17]. In the present study, PPAR-γ was found to be significantly reduced in patients with AR compared to healthy subjects. Recently, PPAR-γ has emerged as an important regulator of inflammatory responses, including within the airways [40]. Previous studies have reported contradictory results concerning the role of PPAR-γ in AR. The mRNA and protein expression levels of PPAR-γ were reported to be higher in the nasal mucosa of perennial allergic rhinitis (PAR) patients relative to healthy subjects, and PPAR-γ has been suggested to participate in chronic inflammation of PAR [57]. By contrast, another study indicated that the PPAR-γ mRNA expression is decreased in nasal polyps relative to the normal nasal mucosa. Furthermore, the PPAR-γ mRNA expression was decreased in patients with bilateral nasal polyposis after steroid treatment compared to before steroid treatment. However, the mRNA expression of PPAR-γ in the nasal biopsies did not show statistically significant differences between patients with AR and healthy subjects [58]. Moreover, it has been shown that the mRNA and protein expression levels of PPAR-γ are decreased in allergic asthma patients after allergen challenge compared to before allergen challenge. This study proposed an inverse association between PPAR-γ and airway inflammation [59]. In the present study, our finding on PPAR-γ was compatible with the previous studies showing the reduced mRNA expression level of PPAR-γ in allergic asthma and rhinitis [57, 58]. Moreover, PPAR-γ has been found to promote catabasis via enhancing the production of IL-10 and TGF-β cytokines [34, 35]. Interestingly, the serum levels for both IL-10 and TGF-β in the present study were also significantly decreased in patients with AR relative to healthy subjects.

Elevated serum levels of total IgE has been demonstrated in more than 90% of patients with allergic rhinitis [60]. In the present study, no statistically significant difference was found in total IgE serum levels between patients with AR and healthy subjects. Although elevated IgE titer is often considered as an indicating factor for allergic diseases, sometimes increased total IgE could be observed in the presence of parasitic infections. In addition, a low IgE titer with clear symptoms of allergy is not unusual [61, 62]. In this study, we found a significantly higher percentage count of peripheral blood eosinophils in patients with AR than Healthy subjects. However, studies have suggested that the nasal smear eosinophils display the best association with clinical and immunological parameters in allergic rhinitis. In this regard, IgE measurement simultaneously with counting the number of eosinophils in nasal discharges has been proposed [60, 62].

Conclusions

In conclusion, our findings reveal increased serum levels of pro-resolving lipid mediator RvE1 in allergic rhinitis patients, suggestive of an indispensable effort of the immune system to actively resolve allergic airway inflammation. However, this increment seems to be inadequate, most likely owing to higher serum levels of pro-inflammatory LTB4 than RvE1 and probably subsequent interfering signaling. Hence, an imbalance between RvE1 and LTB4 in patients with allergic rhinitis might contribute to the defective airway inflammation-resolution and subsequent progression toward chronic airway inflammation. Furthermore, we found a reduction of PPAR-γ gene expression and serum levels of IL-10 and TGF-β cytokines, which might implicate in impairment of catabasis and, thereby, contribute to the pathogenesis of allergic rhinitis. Therefore, these findings collectively suggest RvE1 and PPAR-γ as novel candidates for targeting therapy of allergic rhinitis and airway-associated inflammatory disorders.

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Author contributions FS planned and supervised the study. RL performed experiments, calculations, and manuscript writing. AD

participated in sample preparation. SHM as a specialist physician referred the patients for sampling. HT and AR critically revised the manuscript. All authors have read and approved the final version of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest regarding this manuscript.

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