

Inflammatory profiles in nasal mucosa of patients with persistent vs intermittent allergic rhinitis

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Keywords

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Abbreviations

AA, arachidonic acid; AR, allergic rhinitis; IAR, intermittent allergic rhinitis; PER, persistent allergic rhinitis; ECP, eosinophil cationic protein; Treg, T regulatory; HE, haematoxylin-eosin; T-bet, T-box transcription factor; FOXP3, forkhead box P3; RORC, retinoic acid-related orphan receptor C; LTC₄/D₄/E₄, leukotriene C₄/D₄/E₄; PGD₂, prostaglandin D₂.

Abstract

Background: To date there is little information on the inflammatory profiles of patients suffering from persistent (PER) and intermittent allergic rhinitis (IAR). Also, it is not clear whether differences exist in eosinophilic inflammation and/or T-helper cell sub-populations and their markers. The aim of this study was to primarily evaluate the inflammatory profiles of patients with moderate/severe PER and IAR.

Methods: Inferior nasal turbinate tissue was obtained from 12 PER, 12 IAR and 12 nonallergic nonrhinitic (control) patients, and symptoms (visual analogue scales, VAS) and impairment of life was monitored. All tissues were assessed for eosinophil and mast cell numbers by immunohistochemistry; IL-5, ECP and IgE concentrations by immunoassay; mRNA for transcription factors GATA-3, T-bet, FOXP3 and RORC by quantitative real-time polymerase chain reaction; and IgE-induced release of LTC₄/D₄/E₄ and PGD₂ *in vitro*.

Results: Eosinophils and mast cells were significantly increased in patients with PER and patients with IAR compared to control subjects; by patients with PER demonstrating even significantly greater increase of both cell types than patients with IAR. Similarly, ECP IL-5, GATA-3 mRNA expression and IgE-induced release of LTC₄/D₄/E₄ and PGD₂ from mast cells were significantly increased in patients with PER compared to patients with IAR. In contrast, the expression of T-bet, FOXP3 or RORC mRNA was not significantly different in the PER, IAR or control patients.

Conclusion: The findings from the present study suggest that PER is characterized by a significantly greater eosinophilic and predominantly Th2 cell-mediated nasal inflammatory profile compared to IAR.

Allergic rhinitis (AR) is a common disabling inflammatory disease, which affects between 10–40% of the global population (1). Epidemiological studies predominantly from Europe and the USA have demonstrated that AR affects about 23–30% of the population in Europe (2, 3) and about 10–30% of adults and up to 40% of children in the USA (4). More recently, one cross-sectional, population-based

study has indicated that the prevalence of AR ranges from 8.7% to 24.1% amongst the population in China; of which 25.6% of the patients were diagnosed with persistent AR (PER) and 74.4% with intermittent AR (IAR) (5). Similarly, another study in children demonstrated that the adjusted prevalence of AR was 10.8% in 3- to 6-year-old children in Wuhan City, China; with the prevalence being significantly higher in males than in females (13.0% vs 7.7%, $P < 0.05$) (6). Although not a life-threatening condition, AR imposes a substantial health and economic burden on affected individuals and society alike (7, 8). Clinically, AR is characterized by bothersome symptoms including rhinorrhoea, nasal congestion/obstruction, sneezing and nasal itch, and nowadays more commonly classified as 'intermittent' (IAR) or 'persistent' allergic rhinitis (PER), based on the frequency and duration of symptoms regardless of the nature of allergen exposure (3).

Mechanistic studies have demonstrated that the symptoms of AR result as a consequence of allergen-induced release and/or synthesis of a variety of pro-inflammatory mediators (including histamine, cytokines, arachidonic acid metabolites, chemokines, adhesion molecules, etc.) from a variety of inflammatory cells (particularly mast cells, eosinophils and T-lymphocytes) (3, 9, 10). There is increasing evidence of a correlation between allergen-specific IgE, nasal mast cell/eosinophil numbers and/or their mediators and the severity of symptoms in seasonal (11, 12) and perennial allergic rhinitis (13, 14). Similarly, the role of the T-lymphocytes, particularly the association between Th2 T-cells and increased infiltration of the nasal mucosa with eosinophils, increased expression of cytokines influencing eosinophil and mast cell/basophil activity, and modulation of B-cell activity in IgE isotype switching and synthesis is well documented in AR (3, 15). However, one study has recently demonstrated that allergic T-cell activation is not limited to a Th2 profile, but rather that allergen-stimulated T cells are able to produce IFN- γ at baseline, and during the pollen season, there is an increase in IFN- γ and a decrease in IL-13-producing T-cells, which results in a bias towards Th1/Th2 ratio (16). Moreover, recent evidence suggests that there are significant differences in nasal Th2 cytokine and related marker profiles of Caucasian and Asian (Chinese) patients with chronic rhinosinusitis with nasal polyps (17) and nasal polyposis (18); diseases traditionally characterized by persistent eosinophilic inflammation of the nasal and paranasal mucosa. Thus, while nasal polyp samples from Caucasian patients were characterized by eosinophilic inflammation (17, 18) and a significant increase in Th2 cytokines, samples from the Chinese patients were biased towards neutrophilic inflammation and a significant increase in Th1/Th17 cell pattern (17).

It is not clear whether differences also exist in eosinophilic inflammation and/or T-helper cell sub-populations and their markers, in Chinese vs Caucasian subjects suffering from PER or IAR. The aim of this study was therefore firstly to investigate the inflammatory cell and mediator profiles; with particular emphasis on the T-cell-mast cell-eosinophil axis; and secondly to investigate their relationships with sensitization to IgE and symptom severity in patients with PER and IAR.

Methods

Patients and study design

A total of 36 male and female subjects (24 patients with AR and 12 control subjects) presenting for nasal surgery at the Department of Oto-Rhino-Laryngology of the West China Hospital of SiChuan University, China, were recruited into the study. Surgery was performed to treat septal deviations with additional turbinate hypertrophy (conchotomy) in all control and IAR patients, and in nine of the patients with PER; three patients with PER had therapy-resistant enlarged inferior turbinates without septal deviation as reason for surgery.

In the case of AR group, a diagnosis of AR was based upon the concordance between a typical history of allergic symptoms and diagnostic tests; including skin prick test and the Phadiatop test (Phadia, Uppsala, Sweden) to detect allergen-specific IgE antibodies against common inhalant allergens. All patients with AR were further classified as suffering from PER ($n = 12$) or IAR ($n = 12$), according to the Allergic Rhinitis and its Impact on Asthma (ARIA) criteria (3, 19). Prior to surgery, a physician assessed the severity of AR symptoms, as being 'mild' or 'moderate/severe' on a 0–10 cm VAS scale. A questionnaire covered three questions on impairment of sleep, daily activities, or work and school performance because of the AR. Patients were asked to rate each item on a scale of 0–5 (in which 0 = not problem, 1 = very mild problem, 2 = mild or slight problem, 3 = moderate problem, 4 = severe problem and 5 = problem as 'bad as it can be'). If the score of any item was more than 2, this patient's QoL was considered impaired.

Diagnosis of co-morbid asthma was made on the basis of lung function and challenge tests where applicable, and all subjects were asked to stop using oral or nasal corticosteroids for 4 weeks and antibiotics for 2 weeks, prior to surgery.

Nasal turbinate samples were treated further as necessary for assessment of specific outcome parameters by immunohistochemistry, immunoassay, real-time polymerase chain reaction (PCR) and IgE-induced synthesis of metabolites of arachidonic acid (AA) *in vitro*.

The study was approved by the local Ethical Committee on Human Experimentation of the West China Hospital, Sichuan University, and informed written consent was obtained from all patients before collecting any samples for experimental purpose.

Assessment of eosinophil and mast cells by immunohistochemistry

Eosinophil counts and mast cell tryptase were evaluated in samples processed by standard immunohistochemistry techniques. Briefly, the tissues were fixed in formalin (Kelon, Chengdu, China) and embedded in paraffin. Paraffin wax sections of 4–5 μm thickness were air dried for 24 h at 37°C and then deparaffinized and hydrated, prior to staining. In the case of samples to be evaluated for eosinophils, the samples were stained with haematoxylin–eosin. The

stained sections were washed in two changes of acidified water, dehydrated in three changes of 100% ethanol and mounted in Permount™ Mounting Medium (Bioss, Beijing, China), before analysis by microscopy at 400× magnification, using an Olympus CX-40 microscope.

In the case of samples to be evaluated for mast cell tryptase, the samples were incubated with 1% mouse anti-human mast cell tryptase monoclonal antibody (DaKo Cytomation, CA, USA) for 45 min at 30°C following, washed with tris-buffered saline (TBS) for 10 min, the samples were incubated with EnVision™ (DaKo Cytomation, CA, USA) for 45 min at 30°C. The sections were counterstained with Mayer's haematoxylin and mounted as above and examined under a light microscope.

All samples were assessed for positively stained cells in 10 random fields by two independent observers, blinded to the clinical status of the patients from whom the samples were obtained, and expressed as the total number of cells observed in the 10 fields.

Assessment of IL-5, ECP and IgE levels by immunoassay

Freshly obtained tissue specimens were snap frozen in liquid nitrogen and stored at -80°C until further analysis. For homogenization, tissues were weighed and suspended in 1.0 ml of 0.9% NaCl supplemented with protease inhibitor cocktail tablets per 0.1 g tissue. The samples were homogenized, using a Braun homogenizer at 1500 g for 5 min, and then centrifuged at 3000 g for 10 min at 4°C. Following centrifugation, the supernatants were collected and stored at -80°C until, assay for IL-5 using commercially available ELISA kits (Quantikine ELISA, R&D Systems, Minneapolis, MN, USA) and IgE and ECP using the UNICAP system (Phadia, Uppsala, Sweden).

Assessment of GATA-3, T-box transcription factor (T-bet), Forkhead box P3 (FOXP3) and Retinoic acid-related orphan receptor C (RORc) mRNA by quantitative real-time PCR

mRNA levels of the transcription factors GATA-3, T-bet, FOXP3 and RORc were determined by means of real-time PCR. Snap frozen tissue samples were placed in liquid nitrogen and thoroughly ground with a mortar and pestle and homogenized with Lysis Buffer (QIAGEN GmbH, Hilden, Germany). Total RNA was purified using the RNeasy kit (QIAGEN GmbH, Hilden, Germany) following manufacturer's instructions. A weight of 0.5 µg of total RNA was then reverse transcribed to generate cDNA with the PrimeScript RT Reagent Kit (Takara Biotechnology Co., Ltd., Dalian, China) as instructed by the supplier. cDNA equivalent to 40 ng total RNA was used to perform quantitative PCR. Amplification reactions were performed on an iCycler iQ Real-Time PCR Detection System (BioRad Laboratories, CA, USA) by using their specific primer sequences (20). PCRs processed with SYBR Premix Ex Taq II kit (Takara Biotechnology Co., Ltd., Dalian, China) and PCR protocol consisted of one cycle at 95°C for 10 s followed by 40 cycles at 95°C for 5 s and at 60°C for 45 s. The expression of three

housekeeping genes actin beta, hydroxymethylbilane synthase and elongation factor 1 was used to normalize for transcription and amplification variations among samples after a validation with geNorm software (Ghent University, Ghent, Belgium) (20)(11). The relative expression units of each gene per 20 ng of cDNA sample were determined by using the qBase program (version 1.3.5; Ghent University, Ghent, Belgium), which consists of a collection of Microsoft Excel sheets that automatically analyse real-time quantitative PCR data, combining the Δ CT relative quantification model with PCR efficiency correction and multiple reference gene normalization.

IgE-induced synthesis of leukotriene (LT) C₄/D₄/E₄ and prostaglandin D₂ (PGD₂) from nasal tissue *in vitro*

Fresh samples from patients with PER and IAR were suspended in 10 ml Roswell Park Memorial Institute 1640 (RPMI 1640) tissue culture medium (Sigma-Aldrich, Bornem, Belgium), containing 2 mM L-glutamine (Invitrogen, Merelbeke, Belgium), antibiotics (50 IU/ml penicillin and 50 µg/ml streptomycin) (Invitrogen) and 0.1% BSA (Bovine Serum Albumin; Sigma) on collection. The tissue was cut thoroughly and the suspension passed through a mesh to obtain tissue fragments of approximately 0.9 mm³ in size. The fragments were weighed and resuspended at 0.04 g tissue/1 ml fresh culture medium and pre-incubated for 1 h at 37°C, in a 5% CO₂/air atmosphere in the presence of 1 µg/ml human myeloma IgE (Calbiochem, VWR International, Leuven, Belgium). Following three washings with fresh culture medium, the tissue fragments were resuspended at 0.04 g tissue/1 ml culture medium and 0.5 ml of each suspension dispensed per well, of a 48 well tissue culture plate (BD Falcon, VWR, Leuven, Belgium). Either culture medium (negative control) or a 10 µg/ml solution of ϵ -chain-specific anti-human IgE antibody (Dako Belgium N.V., Heverlee, Belgium) was added into each well and the suspension incubated at 37°C, 5% CO₂ for 30 min. At the end of incubation, the samples were centrifuged at 1300 rpm for 10 min and the supernatants were separated and stored immediately at -20°C until analysis of LTC₄/D₄/E₄ and PGD₂.

Concentrations of LTC₄/D₄/E₄ and PGD₂ were measured in tissue supernatants obtained after the stimulations using ELISA kits for LTC₄/D₄/E₄ (Oxford Biomedical Research, Nuclilab BV, Ede, the Netherlands) and PGD₂ (Cayman Chemicals, Ann Arbor, MI, USA) following the instructions of the manufacture.

Statistical analysis

Statistical analysis was performed with SPSS 11.0 (SPSS, Inc, Chicago, IL, USA). Data are expressed in box-and-whisker plots. Baseline variables were analysed using a one-way ANOVA test, and significance of any difference between the AR and control groups was assessed using the Wilcoxon test (for paired comparisons) and the Mann-Whitney *U* 2-tailed test (for unpaired comparisons). *P*-values of <0.05 were considered as statistically significant. In detail,

the Wilcoxon test was used for between-group comparisons in release of LTC₄/D₄/E₄ and PGD₂, the Mann–Whitney *U* 2-tailed test was used for others between-group comparisons.

Results

Patient characteristics

The demographic and baseline clinical characteristics of subjects in the three groups from which nasal samples were collected are shown in Table 1. All groups were comparable in terms of age, female/male ratio and atopy. As expected, there were significant differences in individual nasal symptom scores for PER vs IAR and control groups, with patients with PER being significantly more symptomatic than both IAR and control patients. Significant differences in symptom score VAS results were also reflected in a more severe impact of the disease on sleep, daily activities and school/work performance. Similarly, all groups were significantly different with regard to serum total IgE and specific IgE-d₁ (Dermatophagoides pteronyssinus 1) levels; by patients with PER again showing significantly greater concentrations compared to IAR and control patients. One patient in the PER group was also diagnosed with co-morbid asthma.

Eosinophils and mast cells

Assessment of eosinophil (haematoxylin-eosin stained cells) and mast cell (tryptase stained cells) numbers indicated that both these cell types were significantly higher in the PER and

IAR groups compared with the control group (Fig. 1). Comparison between PER and IAR groups further showed eosinophils and mast cells to be significantly increased in patients with PER compared to patients with IAR ($P < 0.0001$ and $P = 0.001$, respectively). Of interest, there was a significant correlation between 'VAS nasal symptoms' and counts for eosinophils and mast cells ($r = 0.607$, $P = 0.003$; and $r = 0.584$, $P = 0.018$, respectively). No other correlations were detected.

Similarly, assessment of ECP demonstrated that the concentration of this mediator was also significantly higher in patients with PER compared to IAR and control patients, with no significant differences between IAR and control patients (Fig. 1).

IL-5 and IgE

Figure 2 shows the concentrations of IL-5 and IgE measured in tissues of PER, IAR and control patients. Both IL-5 and total IgE levels were significantly increased in patients with PER compared with IAR ($P = 0.002$ for both) and control patients ($P = 0.015$ and $P < 0.0001$, respectively); with no significant differences between IAR and control patients.

Expression of GATA-3, T-bet, FOXP3 and RORc mRNA

Figure 3 shows the mRNA expressed for GATA-3, T-bet, FOXP3 and RORc in the nasal tissue of PER, IAR and control patients. GATA-3 was significantly upregulated in patients with PER compared with IAR ($P = 0.003$) and

Table 1 Patient characteristics and symptom scores

Parameter	Control	Persistent allergic rhinitis (PER)	Intermittent allergic rhinitis (IAR)	ANOVA (LSD- <i>t</i> test)		
No.	12	12	12	0.035‡		
Age (y; mean(range))	29.4 (20.9–37.8)	18.6 (18–21.5)	21.7 (18–37.6)			
Onset of allergic rhinitis (AR)(Y)		4.1 (2–8)	11.2 (10.5–12.5)			
Female/male sex	5/7	4/8	4/8			
Asthma	0/12	1/12	0/12			
Total IgE in serum(kU/l)	35 (8.2–103)	220 (42.9–481)	65 (12.8–218)	<0.0001†	0.019‡	0.038§
SIgE-d1 in serum(kU/l)	0.175 (0.175–0.175)	16.1 (18.5–41.9)	1.2 (0.175–6.4)	<0.0001†	0.005‡	0.043§
SIgE-g6 in serum(kU/l)	0.175 (0.175–0.175)	0.175 (0.175–0.175)	0.175 (0.175–0.175)			
Impairment of sleep*	0 (0–0)	4.08 (4–5)	3.20 (3–4)	<0.0001†	<0.0001‡	<0.0001§
Impairment of daily activities*	0 (0–0)	4.58 (4–5)	3.25 (3–4)	<0.0001†	<0.0001‡	<0.0001§
Impairment of work and school*	0 (0–0)	4.33(4–5)	2.17 (1–3)	<0.0001†	<0.0001‡	<0.0001§
Positive Phadiotop result	0/12	12/12	12/12			
Visual analogue scales (VAS) score nasal symptoms	2.0 (1.6–2.2)	6.5 (6.1–7.2)	4.1 (3.1–4.2)	<0.0001†	0.042‡	0.025§
VAS score nasal congestion	1.9 (1.5–2)	6.3 (6–6.9)	4.2 (3.7–4.5)	<0.0001†	<0.0001‡	<0.0001§
VAS score rhinorrhoea	0 (0–0)	4.5 (3.5–6.8)	1.6 (1.1–3.1)	<0.0001†	<0.0001‡	0.046§
VAS score sneezing	0 (0–0)	5.2 (4.1–6.6)	1.5 (1–2.7)	<0.0001†	<0.0001‡	0.023§
VAS score nasal itch	0 (0–0)	3.9 (2.5–5.1)	1 (0.4–1.8)	<0.0001†	0.001‡	0.322§
VAS score loss of smell	0 (0–0)	1 (0–6.4)	0.6 (0–3.5)	0.06†	0.223‡	0.452§

The severity of symptoms was evaluated by VAS scores for nasal congestion, rhinorrhoea, sneezing, nasal itch and loss of smell.

SIgE-d1, specific IgE to Dermatophagoides pteronyssinus 1; SIgE-g6, specific IgE to grass pollen.

*Data are expressed as mean and mini-maximum, all others as medians and interquartile ranges. †Control vs PER; ‡PER vs IAR; §Control vs IAR.

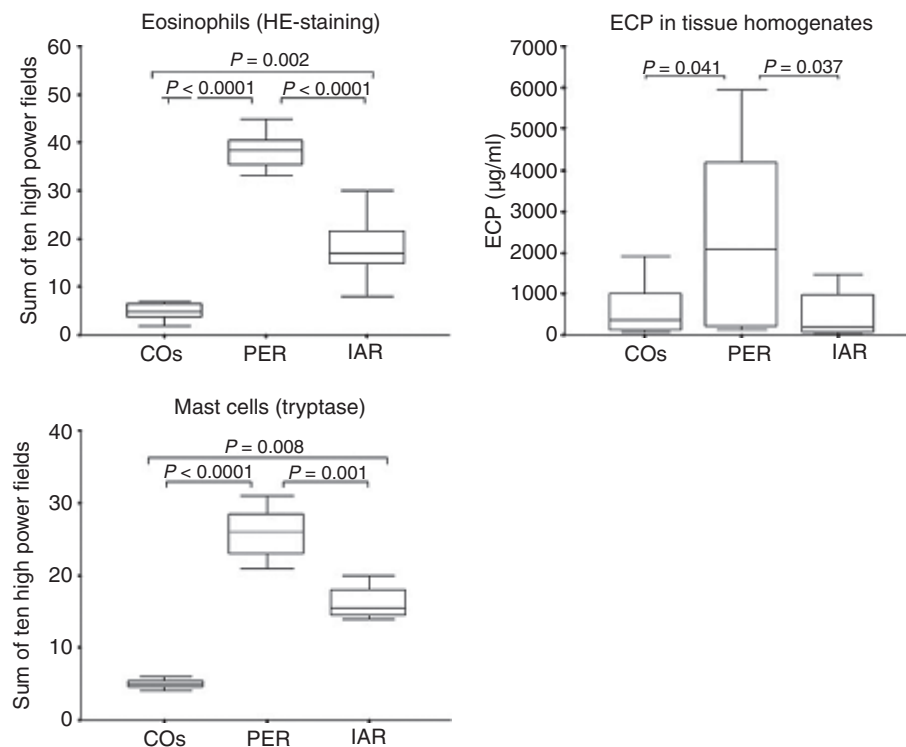


Figure 1 Eosinophil and mast cell numbers and eosinophil cationic protein (ECP) concentration measured in nasal tissues of control, patients with persistent allergic rhinitis (PER) and intermittent

allergic rhinitis (IAR). All values are reported as medians and interquartile ranges; significance level was set at a value of 0.05.

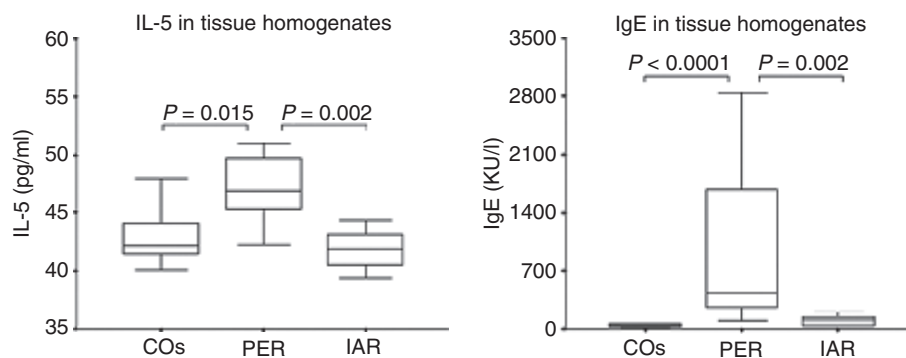


Figure 2 Concentrations of IL-5 protein and IgE measured in nasal tissues of control, patients with persistent allergic rhinitis (PER) and

intermittent allergic rhinitis (IAR). All values are reported as medians and interquartile ranges; significance level was set at a value of 0.05.

control patients ($P = 0.001$); with no significant differences between IAR and control patients. In contrast, the expression of T-bet, FOXP3 and RORc mRNA was not different between any patient groups (Fig. 3).

IgE-induced synthesis of LTC₄/D₄/E₄ and PGD₂ *in vitro*

Assessment of LTC₄/D₄/E₄ and PGD₂ after incubation for 30 min in culture medium alone showed that 0.045 (0.04–0.08) ng/ml and 0.037 (0.03–0.045) ng/ml LTC₄/D₄/E₄ and 82.87 (65.21–100.33) pg/ml and 53.38 (37.6–74.92) pg/ml PGD₂ were

released spontaneously from samples of patients with PER and IAR, respectively ($P = 0.101$; 0.181, for PER *vs* IAR). After correction for spontaneous release, the anti-IgE-induced release of LTC₄/D₄/E₄ and PGD₂ was found to be significantly greater from PER samples than from IAR samples ($P = 0.014$ and $P = 0.001$, respectively) (Fig. 4). Comparison of anti-IgE-induced *vs* spontaneous non-IgE-induced release of these mediators, however, demonstrated that anti-IgE-induced release of LTC₄/D₄/E₄ was significantly greater from only PER samples; whereas anti-IgE-induced release of PGD₂ was significantly greater from both IAR and PER samples, compared

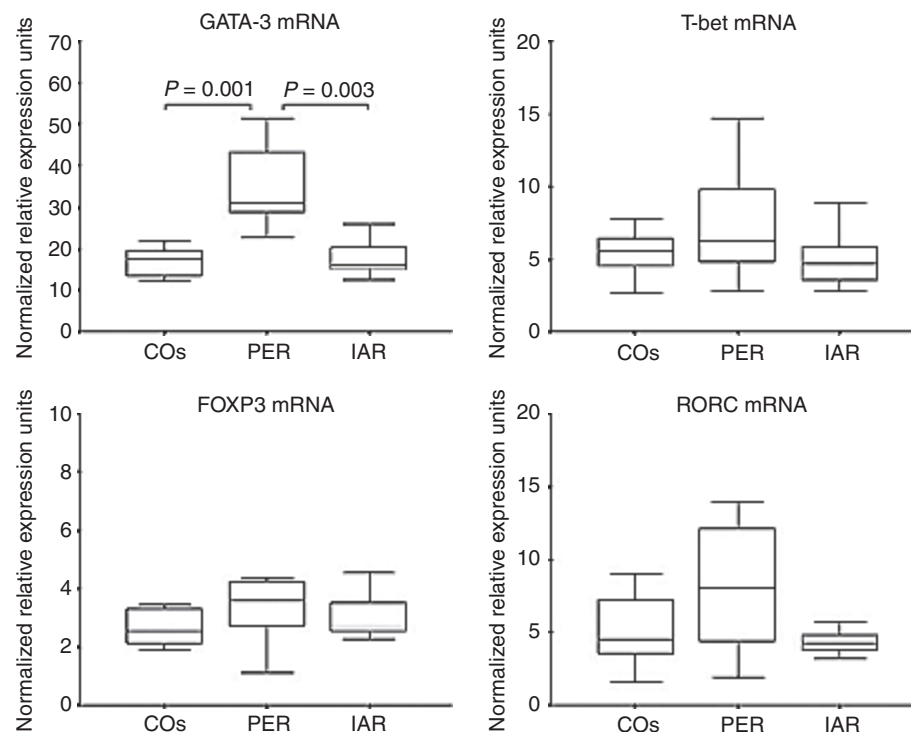


Figure 3 mRNA levels for GATA-3, forkhead box P3 (FOXP3), retinoic acid-related orphan receptor C (RORC) and T-bet transcription factors measured in nasal tissues of control, patients with persistent allergic rhinitis (PER) and intermittent allergic rhinitis (IAR).

mRNA concentrations were standardized against the levels of b-actin, hydroxymethylbilane synthase, and elongation factor 1 housekeeping genes and reported as medians and interquartile ranges. The significance level was set at a value of 0.05.

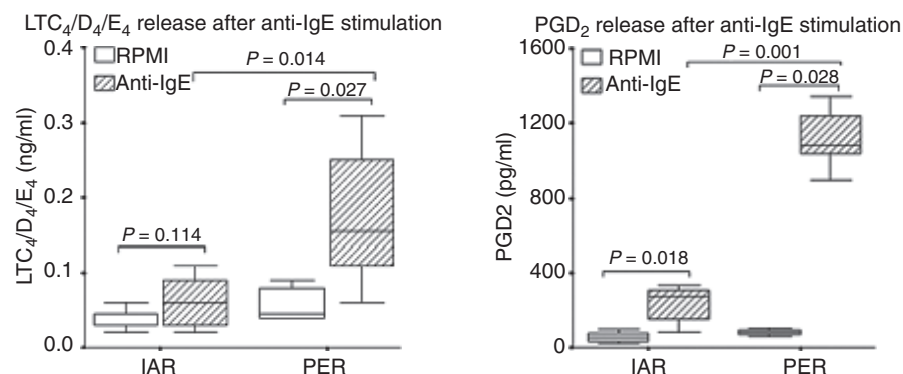


Figure 4 RPMI and anti-IgE-induced release of LTC₄/D₄/E₄ and PGD₂ from nasal tissues of patients with intermittent allergic rhinitis (IAR) and persistent allergic rhinitis (PER) *in vitro*. All values are

reported as medians and interquartile ranges, after correction for spontaneous release. The significance level was set at a value of 0.05.

with release of these mediators in the absence of anti-IgE (Fig. 4).

Discussion

The primary aim of this study was to determine whether PER and IAR patients were different with regard to their inflammatory cell and mediator profiles, which could possibly

explain any differences in the pathophysiology and severity of disease symptoms in these individuals. Our study has clearly demonstrated that while nasal mucosal eosinophils and mast cells were significantly increased in patients with both PER and IAR compared to control nonallergic, non-rhinitic patients, both these cell types were increased to an even greater and significant level in patients with PER than in patients with IAR. Counts of eosinophils and mast cells

significantly correlated with the VAS-data for nasal symptoms. Moreover, the patients with PER demonstrated significantly greater concentrations of ECP and IL-5 than that in patients with IAR, suggesting that the eosinophils were likely to be in a significantly greater state of activation in the patients with PER than in the patients with IAR. Indeed, our study also demonstrated that IgE and IgE-induced synthesis of LTC₄/D₄/E₄ and PGD₂ *in vitro* were also significantly increased in patients with PER compared to patients with IAR, suggesting that mast cells were also likely to be more easily activated in patients with PER than in patients with IAR. Furthermore, our study demonstrated that the expression of mRNA for GATA-3, but not the mRNA for T-bet, FOXP3 or RORC, was significantly upregulated in patients with PER compared to patients with IAR.

In fact, there is no significant difference between controls and IAR for both IL-5 and ECP. The number of eosinophils is, however, increased in IAR *vs* controls, which indicates the presence of eosinophils without their activation, as it also has been demonstrated recently for polyps (17).

To our knowledge, this is the first study to compare the inflammatory cell and mediator profiles in Asian (Chinese) patients suffering from PER or IAR and to provide data, which may be useful in the development of specific target treatment strategies for the management of these patients. The roles of eosinophils and mast cells as key effector cells in the etiology of IgE-mediated allergic airways disease, including AR and asthma, is well documented in several reviews (21–23). Although there is comparatively little information in the literature on the inflammatory profiles of patients suffering from PER, our findings for significant increases in the numbers of these cells and associated mediators (IL-5, ECP and LTC₄/D₄/E₄) in patients with PER are in accordance with the findings of several studies that have investigated persistent inflammation predominantly in patients with seasonal and perennial AR (3, 24), as well to a lesser extent in patients with PER (25). However, the findings from the present study of increased numbers of eosinophils and mast cells and the mediators associated with these cells suggest that those cells are likely to be in an activated state. Moreover, the findings for the significantly higher concentrations of serum and/or nasal total and specific IgE, ECP, IL-5 and LTC₄/D₄/E₄ measured in patients with PER than in patients with IAR are at least concordant with the significantly higher symptom scores for nasal congestion, rhinorrhoea, sneezing and nasal itching noted for patients with PER than for patients with IAR in this study. This is particularly likely as a combination of mediators synthesized and released by activated eosinophils, and mast cells are known to be involved in the development of the symptoms of AR during both the early-phase and the late-phase reactions (3, 11–14, 24, 25).

Taken individually, the differences in concentrations of specific mediators noted in nasal tissues of Chinese patients with PER and IAR in the present study provide additional pointers in the pathophysiology of disease not only in this ethnic group, but possibly patients with PER and IAR globally. As IL-5 is traditionally regarded as a Th2 cytokine, the significantly higher concentration of this mediator in

patients with PER suggests that there is predominantly a Th2 cell bias in these patients. Indeed, this is corroborated by the finding for also a significantly greater expression of GATA-3, a transcription factor specifically expressed in Th2 cells (26), in patients with PER compared to patients with IAR in the present study. Studies have demonstrated that GATA-3 plays a pivotal role in the differentiation of Th2 cells from uncommitted CD4⁺ lymphocytes (27) and is essential for the expression and release from Th2 cells of IL-4, IL-5 and IL-13, which mediate allergic inflammation (28). Furthermore, there is evidence the expression of GATA-3 is significantly increased in patients with AR compared with healthy controls (29, 30) and that there is further increase in GATA-3 in patients with AR, following nasal allergen challenge (31) or natural exposure during the pollen season (32).

Our finding for the lack of any significant difference in the expression of T-bet mRNA in patients with PER/AR and control patients is also in accordance with the findings of Malmh  ll et al.(30), who demonstrated that T-bet was not significantly different in the nasal mucosa of patients with AR compared with healthy controls, either before or during the pollen season. As T-bet has been shown to be important in the differentiation of Th1 cells (27, 31), our findings suggest that there is little or no Th1 cell bias in patients with either PER or IAR.

Our findings for the expression of FOXP3, a specific marker for regulatory T-cells (Treg)(32), however, are in contrast to the findings of Malmh  ll et al. (30), who demonstrated that the number of FOXP3⁺ cells in the nasal mucosa of patients with AR was significantly increased compared with healthy subjects. Other studies, however, have demonstrated that nasal secretions of patients with AR had significantly lower FoxP3 mRNA compared to nonallergic controls (33). Collectively, the contrasting findings from these studies clearly demonstrate that the expression of FOXP3 and Treg cell activity in AR and non-AR patients is likely to be dependent on several factors, one of which may be the extent of severity and duration of exposure to pollen allergen; because grass pollen (34) and house dust mite (35) immunotherapy were shown to significantly increase the numbers of nasal mucosal and blood Foxp3(+)-CD4(+)/CD25(+) cells, respectively, and these were associated with significant improvements in symptoms.

To our knowledge, the role of Th17-cell subset, which may potentially be involved in the pathogenesis of allergic disease (36), has not been investigated to date in PER, IAR, nor AR patients in general. In this regard, our studies have demonstrated that these cells are likely to be present in the nasal mucosa of patients with PER and IAR, as indicated by the expression of RORc mRNA in nasal samples from these patients. Although the levels of RORc appear to be higher in patients with PER, these are nevertheless not significant compared with either IAR or control patients, suggesting that the Th17 cells may not be involved in the pathogenesis of patients with PER or IAR. However, this needs to be confirmed in a larger cohort of patients than the one studied in the present study.

In this study on IAR and PER, both groups of patients mainly suffered from house dust mite allergy. Although the

groups were small and did not allow a stratification for onset of disease, allergen exposure, or a further characterization of the patients immune status, the severity and weekly duration of symptoms, the degree of impact on quality of life and the degree of inflammation at cellular and mediator level were different between IAR and PER. The reason for this difference also needs further study.

Conclusion

The findings from the present study suggest that IgE-mediated PER is characterized by a significantly exaggerated eosinophilic inflammation compared to IgE-mediated IAR. Moreover, also in Chinese patients, this appears to be predominantly Th2-cell and cytokine mediated in both PER and IAR,

with little or no influence of the Th1/Th17 cell axis, as that noted in Chinese patients suffering from chronic rhinosinuitis with nasal polyps.

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Disclosure of conflict of interest

None.

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