

Response of Peripheral Blood Th17 Cells to Inhaled *Dermatophagoides pteronyssinus* in Patients with Allergic Rhinitis and Asthma

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Abstract

Background Recent studies have shown the importance of Th17 cells in the development of allergic airway diseases. We examined *Dermatophagoides pteronyssinus*-induced changes in peripheral blood Th17 cells to establish the importance of these cells in late-phase allergic inflammation in patients with allergic rhinitis (AR) and allergic asthma (AA).

Methods Eighteen patients with mild-to-moderate/severe persistent AR, 14 patients with intermittent- or mild-to-moderate persistent AA, and 15 healthy subjects (HS) were examined. All patients had positive skin test to *D. pteronyssinus*. Study subjects underwent bronchial challenge with *D. pteronyssinus*. The peripheral blood Th1, Th2, and Th17 cells were determined by flow cytometry 24 h before and 7 and 24 h after challenge. The serum IL-17 levels were determined by ELISA.

Results The percentage of Th17 cells and IL-17 levels was significantly higher in patients with AR and AA compared with HS before and after challenge. Twenty-four hours after challenge, the percentage of Th17 cells

increased significantly in patients with AA compared with baseline values. The IL-17 levels rose markedly in patients with AR and AA after challenge. Moreover, 24 h after challenge, the percentage of Th17 cells and IL-17 levels were significantly higher in patients with AA than those with AR.

Conclusions Percentages of peripheral blood Th17 cells and serum IL-17 levels were found to be higher in patients with AR and AA. An increase in the percentage of Th17 cells following challenge shows that Th17 cells may have an important role in the development of late-phase allergen-induced inflammation.

Keywords Allergic rhinitis · Allergic asthma · Bronchial challenge · *Dermatophagoides pteronyssinus* · Th17 cell · IL-17

Introduction

Allergic rhinitis and allergic asthma are chronic inflammatory airway diseases induced by allergen and regulated by T cells. T helper (Th) cells are heterogeneous because of their expression of cell surface molecules and different sets of cytokines that determine their functional activity and role in the pathogenesis of various chronic inflammatory diseases. Th2 cells activated by the allergen and their cytokine interleukins (IL)-4, IL-5, and IL-13 are critical for the induction and maintenance of allergic airway inflammation [1]. In contrast, Th1 cells mediate inflammation in tissues by the expression of interferon γ (IFN- γ) and play a role in the protection against intracellular microbes as well as lead to the development of autoimmune diseases [2]. In healthy subjects, Th1 and Th2 cells are in a balance with each other. There is evidence that synthesis of cytokines by

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allergen-activated Th2 cells inhibits Th1 cytokine synthesis. Th1 cells by secreting IFN- γ inhibit the proliferation and differentiation of basophils, mastocytes, and eosinophils, whose activities are controlled by Th2 cells [3].

Another subset of effector Th cells defined as Th17 cells produces IL-17 family cytokines (IL-17A, IL-17F, IL-22), which are involved in the recruitment, activation, and migration of neutrophils [4]. Data suggest that IL-17 produced by Th17 cells plays an important role in the pathogenesis of different chronic inflammatory processes, such as rheumatoid arthritis, autoimmune blistering skin diseases, psoriasis, inflammatory bowel diseases, and multiple sclerosis [5–7]. It is known that eosinophils play an important role in allergic inflammation; however, a growing body of evidence suggests that Th1 and Th17 cells also may be involved in the development of allergic airway inflammation, particularly in patients with severe forms of the disease [8]. Recent studies using mouse asthma models have shown that Th17 cells and their cytokines, such as IL-17A and IL-17F, are involved in antigen-induced neutrophil recruitment as well as the enhancement of Th2 cell-mediated eosinophil recruitment into the airways [9]. Previous studies with asthmatic patients have shown the increased levels of Th17 cytokines in lung tissue, sputum, bronchoalveolar lavage fluid, and correlation of IL-17 with airway hypersensitivity as well as asthma severity [10–12].

The present study was designed to evaluate the changes of Th17 cells and their cytokines after stimulation with *Dermatophagoides pteronyssinus* allergen to investigate the role of these cells in allergen-induced inflammation in patients with allergic rhinitis and asthma.

Patients and Methods

A total of 47 nonsmoking adults (25 men and 22 women; mean age, 30 ± 2 years) were examined: 18 patients with mild-to-moderate/severe persistent allergic rhinitis, defined according to the ARIA criteria [13], 14 patients with intermittent- or mild-to-moderate persistent allergic asthma, defined according to the GINA criteria [14], and 15 healthy subjects who comprised the control group. The patients were recruited from the Department of Pulmonology and Immunology, Hospital of the Lithuanian University of Health Sciences, Kaunas. The study protocol was approved by the Regional Biomedical Research Ethics Committee of the Lithuanian University of Health Sciences, and each participant gave his or her informed, written consent.

Patients with allergic rhinitis and allergic asthma had a clinical history of the disease for ≥ 1 year, current symptoms, and positive results of skin prick test (≥ 3 mm) with *D. pteronyssinus*. All patients were instructed to refrain

from using inhaled or nasal as well as oral steroids at least 1 month before visits, short-acting β_2 agonists at least 12 h, and long-acting β_2 agonists at least 48 h before the lung function test, and antihistamines and antileukotrienes 7 days before the skin prick test. None of the patients had a history of smoking. Baseline forced expiratory volume in 1 s (FEV₁) was more than 70 % of the predicted value in all patients. None of the patients had any clinically significant allergy to other allergens, such as pollen or cat and dog dander. All healthy subjects were nonsmokers, without symptoms of rhinitis or asthma, with normal findings of spirometry, and all showed negative results of the skin prick test.

Skin Prick Testing

All patients were screened for allergy by the skin prick test using standardized allergen extracts (Stallergenes S.A., France) for the following allergens: *D. pteronyssinus*, *D. farinae*, cat and dog dander, mixed grass pollen, birch pollen, mugwort, cockroach *Alternaria*, *Aspergillus*, and *Cladosporium*. Phenolated glycerol-saline was used for a negative control, and histamine hydrochloride (10 mg/mL) was used for a positive control. Skin testing was read 15 min after application. The results of skin prick test were considered positive if the mean wheal diameter was ≥ 3 mm [15].

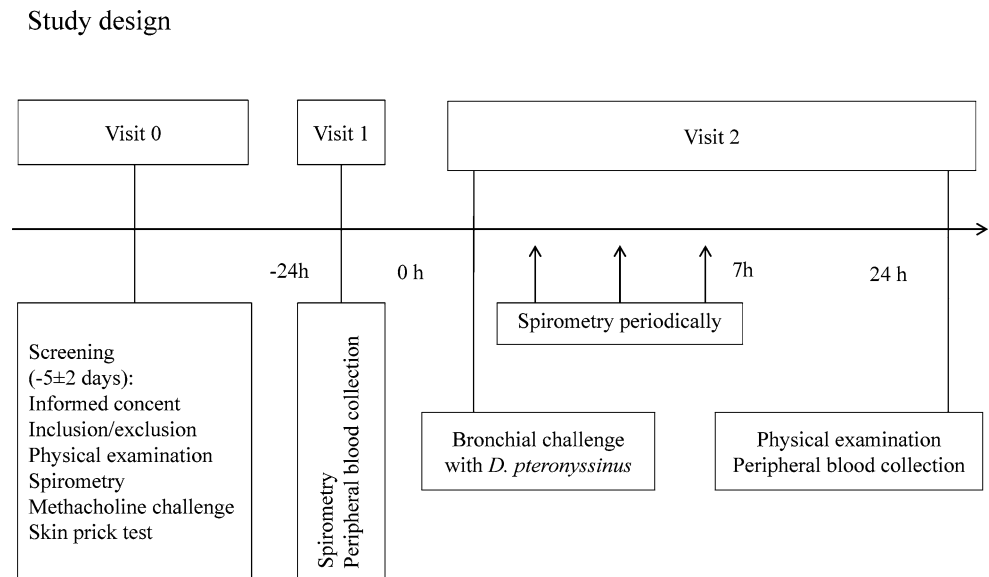
Study Design

On a screening visit (V0), all subjects were informed about participation in the study, informed written consent was obtained, inclusion/exclusion criteria were verified, and also physical examination, spirometry, responsiveness to methacholine, and skin prick test were performed. Twenty-four hours before bronchial challenge with *D. pteronyssinus* (V1), spirometry was performed and peripheral blood was collected; these data were used as baseline values.

Bronchial challenge with *D. pteronyssinus* was performed at 8:00 in the morning, and spirometry was reassessed every 10 min within the first hour and then every hour for subsequent 6 h. Physical examination and peripheral blood collection were repeated 7 and 24 h after bronchial challenge with *D. pteronyssinus* (V2). The study design is presented in Fig. 1.

Lung Function Testing

Pulmonary function was tested by using a pneumotachometric spirometer “CustovitM” (Custo Med, Germany). Baseline forced expiratory volume in 1 s (FEV₁), forced vital capacity (FVC), and FEV₁/FVC ratio were recorded as the highest of three reproducible measurements. The

Fig. 1 Study design

results were compared with the predicted values matched for age, body height, and sex according to the standard methodology [16].

Measurement of Airway Responsiveness to Methacholine

Airway responsiveness was assessed as changes in airway function after challenge with inhaled methacholine using a reservoir method [17]. Methacholine was nebulized into a 10-L reservoir with a pressure nebulizer (Pari Provocation I; Pari, Starnberg, Germany). Aerosolized methacholine was inhaled through a one-way valve at 5-min intervals starting with 0.015-mg methacholine dose and doubling it until a 20 % decrease in FEV₁ from the baseline or the total cumulative dose of 3.87 mg was achieved. The bronchoconstricting effect of each dose of methacholine was expressed as a percentage of decrease in FEV₁ from the baseline value. The provocative dose of methacholine causing a ≥ 20 % fall in FEV₁ (PD₂₀) was calculated from the log dose-response curve by linear interpolation of two adjacent data points.

Bronchial Challenge with Allergen

A bronchial challenge was performed with a *D. pteronyssinus* allergen (Stallergenes SA, France) at different concentrations (0.01, 0.1, 1.0, 10, 33.3 IR/mL) using a KoKo DigiDoser nebulizer (Sunrise Medical, Somerset, PA) [18]. A freeze-dried lyophilized allergen was diluted with non-phenol saline. The reaction was considered positive if a fall in FEV₁ ≥ 20 % from baseline was achieved. Spirometry was reassessed every 10 min within the first hour and then

every hour for subsequent 6 h for safety reasons in case of late asthmatic reaction occurrence.

Evaluation of Cytokine Production in CD4 + T cells

Peripheral blood mononuclear cells were isolated from heparinized venous blood on Ficoll-Paque gradient and resuspended in RPMI 1640 (Sigma-Aldrich, USA) containing L-glutamine autologous plasma. Two million cells per milliliter were activated for 5 h at 37 °C with phorbol-12-myristate-13-acetate (50 ng/mL; Sigma-Aldrich) and ionomycin (1 µg/mL; Invitrogen, USA) in the presence of protein transport inhibitor (BD GolgiStop™, BD Biosciences, USA) to avoid cytokine secretion. After the activation, the cells were washed two times with stain buffer (fetal bovine serum [FBS], BD Biosciences) and fixed with cold BD Cytofix™ fixation buffer (BD Biosciences) for 10 min at room temperature. After the fixation, the cells were washed twice and stored in stain buffer at 4 °C for up to 24 h before the staining procedure. Permeabilization of the fixed cells was performed by adding BD Perm/Wash™ buffer (BD Biosciences) and incubating the cells for 15 min at room temperature. The cells were stained by adding 20 µL of human Th1/Th2/Th17 phenotyping cocktail (BD Biosciences) containing human CD4 PERCP-CY5.5, human IL-17A PE, human IFN- γ FITC, and human IL-4 APC in each tube and incubating for 30 min at room temperature in the dark. Finally, the cells were washed twice with Perm/Wash™ buffer and suspended in stain buffer (FBS) before flow cytometric analysis. Flow cytometry was performed on a FACSCanto flow cytometer (BD Biosciences). Results are reported as percentages of CD4 + T cells producing a definite cytokine.

Detection of IL-17 in Serum

Serum IL-17 and IL-5 levels were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (BioSource S.A., Belgium and BD Biosciences, respectively). The minimum detectable dose was 2 pg/mL for IL-17 and 4 pg/mL for IL-5. The concentration of IL-17 and IL-5 in the samples was determined by comparing the optical density values of the samples to the standard curve. The peripheral blood cell analysis was performed on an automated hematology analyzer (Sysmex XE-5000, Japan).

Statistical Analysis

Statistical analysis was performed by using the Statistical Package for Social Sciences, version 17.0 for Windows (SPSS 17.0). The normality assumption of data was verified with the Shapiro-Wilk test. All data that were normally distributed are presented as mean and standard error of the mean (SEM). The data that did not follow a normal distribution were expressed as median and interquartile range (IQR).

Due to a skewed distribution of the variable, nonparametric tests were used. The Kruskal–Wallis test was used to evaluate statistical differences between both groups of patients and control group. If significant differences were detected, differences between two independent groups were determined by the Mann–Whitney *U* test.

Comparisons of the changes in data before and after bronchial challenge in each group were evaluated for statistical significance at each time point with the Friedman test and followed by the Wilcoxon test for paired analyses. For multiple comparisons, the Bonferroni method was used to adjust *P* values. For normally distributed variables,

differences between groups were explored using one-way ANOVA followed by a post hoc analysis using the Bonferroni test (equal variances assumed) or Games-Howell test (equal variances not assumed). Comparisons of the allergen-induced changes (before and after bronchial challenge) in dependent samples were analyzed with repeated measures analysis of variance (ANOVA).

Statistical significance was assumed at a *P* value of <0.05.

Results

Characteristics of Studied Subjects

A total of 47 nonsmoking adults (25 men and 22 women; mean age, 30 ± 2 years) were examined: 18 patients with allergic rhinitis, 14 with allergic asthma, and 15 healthy subjects. The demographic and clinical characteristics of the subjects are presented in Table 1. There were no significant age and gender differences comparing the groups. All patients were sensitized to *D. pteronyssinus* detected by the skin prick test. The mean wheal diameter induced by *D. pteronyssinus* was similar in both the groups of patients. The baseline FEV₁ (%) was significantly lower in the allergic asthma group (98 ± 9.9 ; $P < 0.05$) compared with the allergic rhinitis group (114 ± 13.2). Provocative dose of methacholine causing a 20% decrease in FEV₁ (PD₂₀) was estimated for four patients with allergic rhinitis. Bronchial challenge with *D. pteronyssinus* provoked early bronchoconstriction in 13 patients with allergic asthma. Only one patient with allergic asthma did not develop early bronchoconstriction after bronchial challenge with *D. pteronyssinus*. A maximum percentage fall in FEV₁ after bronchial challenge was significantly greater in

Table 1 Demographic and clinical characteristics of the subjects

Characteristic	Healthy subjects (n = 15)	Patients with allergic rhinitis (n = 18)	Patients with allergic asthma (n = 14)
Age (years), mean $\pm$ SEM (range)	30 ± 3 (22–45)	30 ± 1 (20–36)	34 ± 4 (21–50)
Sex (male/female), n	6/9	13/5	6/8
Wheal diameter induced by <i>D. pteronyssinus</i> (mm), mean $\pm$ SEM (range)	0	7.3 ± 0.9 (3–15)	6.1 ± 0.7 (4–11)
FEV ₁ (% of predicted), mean $\pm$ SEM (range)	104.9 ± 13.3 (79–114)	114.0 ± 13.2 (97–141)	$98.0 \pm 9.9^{\dagger}$ (82–116)
PD ₂₀ (mg), geometric mean (range)	–	2.75^a (1.0–3.9)	0.36 (0.1–0.7)
Maximal decrease in FEV ₁ after bronchial challenge with <i>D. pteronyssinus</i> (%), mean $\pm$ SEM (range)	5.5 ± 3.2 (2.7–8.9)	6.5 ± 2.4 (2.5–9.0)	$33.8 \pm 15.6^{* \dagger}$ (9.7–55.3)

FEV₁ forced expiratory volume in the first second; PD₂₀ provocative dose of methacholine causing a 20 % fall in FEV₁

* $P < 0.05$ versus healthy subjects

† $P < 0.05$ versus patients with allergic rhinitis

^a PD₂₀ was estimated only for four patients with allergic rhinitis

patients with allergic asthma compared with patients with rhinitis (33.8 ± 15.6 vs. 6.5 ± 2.4 ; $P < 0.05$) and healthy subjects (5.4 ± 3.5 ; $P < 0.05$). In patients with allergic rhinitis, bronchial challenge with *D. pteronyssinus* provoked nasal symptoms, but it did not cause bronchoconstriction. The absolute eosinophil count in the peripheral blood of patients with allergic rhinitis and asthma was significantly higher 24 h later after allergen challenge (0.3 ± 0.1 and 0.4 ± 0.1 , respectively; $P < 0.05$). The percentage of eosinophils in the peripheral blood increased significantly in patients with allergic asthma 24 h after bronchial challenge (6.4 ± 0.7 ; $P < 0.05$) compared with the baseline values (Table 2).

Subsets of Th Cells in Patients with Allergic Rhinitis and Asthma Before and After Bronchial Challenge with *D. pteronyssinus*

The percentage of Th17 cells was significantly higher in patients with allergic rhinitis and asthma compared with

Table 2 Changes in number of peripheral blood cells before and after bronchial challenge with *D. pteronyssinus* in patients with allergic rhinitis and asthma and healthy subjects

Characteristic	24 h before	7 h after	24 h after
Patients with allergic rhinitis			
Leukocytes, $\times 10^9/L$	5.6 ± 0.4	7.2 ± 0.5	6.2 ± 0.4
Eosinophils, %	3.5 ± 0.6	3.6 ± 0.6	5.5 ± 1.1
Eosinophils, $\times 10^9/L$	0.3 ± 0.1	0.3 ± 0.1	$0.3 \pm 0.1^*$
Neutrophils, %	56.9 ± 2.1	55.2 ± 1.7	54.6 ± 1.8
Neutrophils, $\times 10^9/L$	3.3 ± 0.3	4.1 ± 0.3	3.4 ± 0.3
Lymphocytes, %	31.5 ± 2	32.8 ± 1.6	31.7 ± 1.9
Lymphocytes, $\times 10^9/L$	1.9 ± 0.1	2.4 ± 0.1	1.9 ± 0.1
Patients with allergic asthma			
Leukocytes, $\times 10^9/L$	5.8 ± 0.4	7.1 ± 0.4	5.9 ± 0.3
Eosinophils, %	5.8 ± 1.2	4.5 ± 0.9	$6.4 \pm 0.7^\dagger$
Eosinophils, $\times 10^9/L$	0.3 ± 0.3	0.3 ± 0.3	$0.4 \pm 0.1^*$
Neutrophils, %	53.4 ± 3.3	55 ± 3.2	51.1 ± 2
Neutrophils, $\times 10^9/L$	3.2 ± 0.3	4 ± 0.5	3 ± 0.3
Lymphocytes, %	32 ± 2.4	31.8 ± 2.3	33.8 ± 1.9
Lymphocytes, $\times 10^9/L$	1.9 ± 0.1	2.2 ± 0.1	2 ± 0.1
Healthy subjects			
Leukocytes, $\times 10^9/L$	4.9 ± 0.7	6.4 ± 0.5	5.6 ± 0.6
Eosinophils, %	3.3 ± 1.6	2.4 ± 0.6	2.7 ± 1.3
Eosinophils, $\times 10^9/L$	0.2 ± 0.03	0.3 ± 0.1	0.2 ± 0.1
Neutrophils, %	53.1 ± 4.5	56.2 ± 1	57.2 ± 5.3
Neutrophils, $\times 10^9/L$	3.6 ± 1	3.1 ± 0.9	3.4 ± 1.1
Lymphocytes, %	33.9 ± 3.4	32.1 ± 1.1	30.9 ± 3.9
Lymphocytes, $\times 10^9/L$	1.7 ± 0.4	1.6 ± 0.5	1.6 ± 0.8

Data are expressed as mean $\pm$ SEM

* $P < 0.05$ versus healthy subjects; $^\dagger P < 0.05$ compared with the baseline values

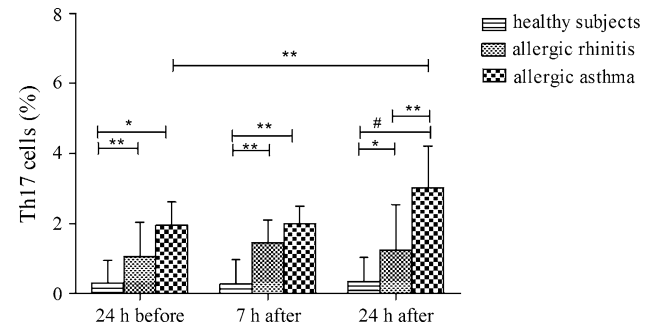


Fig. 2 Percentage of Th17 cells among peripheral blood CD4 + T cells in patients with allergic rhinitis and asthma and healthy subjects before and after bronchial challenge with *Dermatophagoides pteronyssinus*. Data are expressed as median (IQR); * $P < 0.05$; ** $P < 0.01$; # $P < 0.001$

healthy subjects 24 h before (1.1 % and 1.9 % vs. 0.3 %; $P < 0.01$ and $P < 0.05$, respectively) bronchial challenge as well as 7 h (1.5 % and 2 % vs. 0.3 %; $P < 0.01$) and 24 h after (1.2 % and 3 % vs. 0.3 %; $P < 0.05$ and $P < 0.001$, respectively) bronchial challenge (Fig. 2). The percentage of Th17 cells in the allergic asthma group significantly increased 24 h after bronchial challenge compared with the baseline values ($P < 0.01$), although no changes in the percentage of Th17 cells were documented after 7 hours. Twenty-four hours after bronchial challenge, the percentage of Th17 cells was significant higher in the allergic asthma group ($P < 0.01$) compared with the rhinitis group. However, no significant difference in the percentage of Th17 cells was observed in patients with allergic rhinitis after bronchial challenge.

The percentage of Th2 cells was significantly higher in patients with allergic rhinitis and asthma compared with healthy subjects 24 h before bronchial challenge (2.8 % and 3.7 % vs. 1.5 %; $P < 0.01$ and $P < 0.001$, respectively) as well as 7 h (2.9 % and 4 % vs. 1.5 %; $P < 0.05$ and $P < 0.001$, respectively) and 24 h (3.8 % and 5 % vs. 1.4 %; $P < 0.001$) after bronchial challenge (Fig. 3). The percentage of Th2 cells increased significantly 24 h after bronchial challenge compared with the baseline values in patients with allergic rhinitis ($P < 0.05$) and asthma ($P < 0.05$). In patients with allergic asthma, a significantly higher percentage of Th2 cells was documented 24 h ($P < 0.001$) after bronchial challenge compared with patients in the allergic rhinitis group.

The percentage of Th1 cells was significantly lower in patients with asthma compared with healthy subjects 24 h before bronchial challenge (8.4 vs. 12 %; $P < 0.01$; Fig. 4). Twenty-four hours after bronchial challenge the percentage of Th1 cells was significantly lower in patients with allergic rhinitis and asthma (8.3 % and 5.3 % vs. 13 %; $P < 0.01$ and $P < 0.05$, respectively) compared with the healthy subjects group.

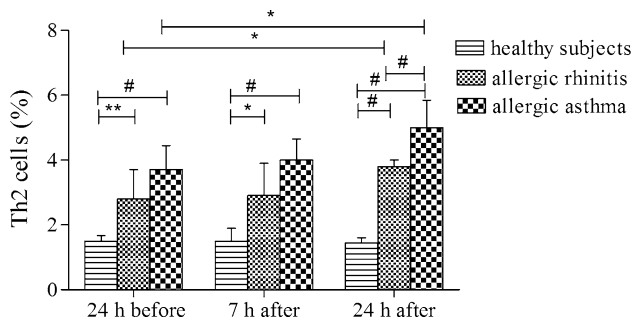


Fig. 3 Percentage of Th2 cells among peripheral blood CD4 + T cells in patients with allergic rhinitis and asthma and healthy subjects before and after bronchial challenge with *Dermatophagoides pteronyssinus*. Data are expressed as median (IQR); * $P < 0.05$; ** $P < 0.01$; # $P < 0.001$

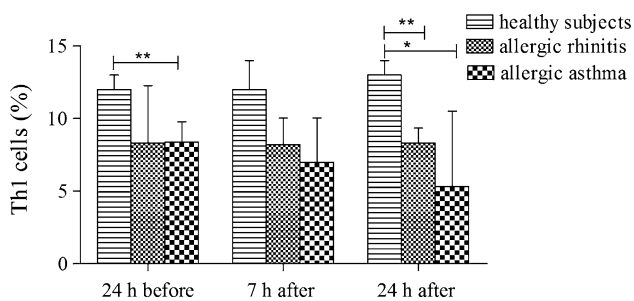


Fig. 4 Percentage of Th1 cells among peripheral blood CD4 + T cells in patients with allergic rhinitis and asthma and healthy subjects before and after bronchial challenge with *Dermatophagoides pteronyssinus*. Data are expressed as median (IQR); * $P < 0.05$; ** $P < 0.01$

Previously described changes in the percentages of Th17 and Th1 cells resulted in an increased Th17/Th1 ratio (Table 3). In patients with allergic rhinitis and asthma compared with healthy subjects, this ratio was significantly higher 24 h before (0.14 and 0.22 vs. 0.02; $P < 0.05$) as well as 7 h (0.18 and 0.29 vs. 0.02; $P < 0.05$) and 24 h after (0.17 and 0.5 vs. 0.04; $P < 0.05$) bronchial challenge. In patients with allergic asthma, the Th17/Th1 ratio increased significantly 24 h after bronchial challenge compared with the baseline value ($P < 0.05$). Twenty-four hours after bronchial challenge, the Th17/Th1 ratio was significantly higher in patients with asthma compared with their counterparts with allergic rhinitis ($P < 0.05$). The percentage of Th17 cells before and after bronchial challenge increased in parallel with the percentages of Th2 cells; therefore, no changes in the Th17/Th2 ratio were observed. The Th1/Th2 ratio was significantly lower in patients with allergic rhinitis and asthma compared with healthy subjects 24 h before bronchial challenge (3.93 and 2.47 vs. 8.67; $P < 0.05$) as well as 7 h (2.66 and 1.63 vs. 8; $P < 0.05$) and 24 h (2.28 and 1.26 vs. 8.3; $P < 0.05$) after bronchial challenge.

Table 3 Th17/Th1, Th17/Th2, and Th1/Th2 ratios in healthy subjects and patients with allergic rhinitis and asthma before and after bronchial challenge with *Dermatophagoides pteronyssinus*

Characteristic	24 h before	7 h after	24 h after
Patients with allergic rhinitis			
Th1/Th2 ratio	3.93 (2.46) [†]	2.66 (2.28) [†]	2.28 (1.6) [†]
Th17/Th1 ratio	0.14 (0.11) [†]	0.18 (0.21) [†]	0.17 (0.14) [†]
Th17/Th2 ratio	0.51 (0.54)	0.42 (1.01)	0.51 (0.41)
Patients with allergic asthma			
Th1/Th2 ratio	2.47 (1.52) [†]	1.63 (1.32) [†]	1.26 (1.07) [†]
Th17/Th1 ratio	0.22 (0.23) [†]	0.29 (0.28) [†]	0.50 (0.33) ^{†#}
Th17/Th2 ratio	0.45 (0.71)	0.54 (0.42)	0.6 (0.38)
Healthy subjects			
Th1/Th2 ratio	8.67 (1.66)	8.0 (2.08)	8.3 (1.83)
Th17/Th1 ratio	0.02 (0.07)	0.02 (0.08)	0.04 (0.05)
Th17/Th2 ratio	0.17 (0.65)	0.14 (0.72)	0.44 (0.36)

Data are expressed as median (IQR)

* $P < 0.05$ compared with the baseline values

[†] $P < 0.05$ vs. healthy subjects

$P < 0.05$, patients with allergic asthma vs. patients with allergic rhinitis

IL-17 Levels Before and After Bronchial Challenge in Patients with Allergic Rhinitis and Asthma

The serum IL-17 levels in patients with allergic rhinitis and asthma were significantly higher compared with healthy subjects 24 h before bronchial challenge (4.2 and 4.7 vs. 2.1 pg/mL; $P < 0.001$) as well as 7 h (4 and 5.7 vs. 1.4 pg/mL; $P < 0.001$) and 24 h (7 and 11.6 vs. 0.6 pg/mL; $P < 0.001$) after bronchial challenge (Fig. 5). There was no difference in the serum IL-17 levels 7 h after bronchial challenge in patients with allergic rhinitis and asthma, but a significant increase in the serum IL-17 levels was observed 24 h after bronchial challenge compared with the baseline values ($P < 0.01$ and $P < 0.001$, respectively). A considerably higher serum IL-17 level was found 24 h after bronchial challenge in patients with allergic asthma compared with those with rhinitis ($P < 0.01$).

The serum levels of IL-5 were significantly higher in patients with allergic rhinitis and asthma compared with healthy subjects 24 h before bronchial challenge (32.2 and 42.1 vs. 16.3 pg/mL, $P < 0.001$ and $P < 0.01$, respectively) as well as 7 h (46.2 and 52.7 vs. 18.2 pg/mL, $P < 0.01$, respectively) and 24 h (50.7 and 65.2 vs. 18 pg/mL, $P < 0.001$ and $P < 0.01$, respectively) after bronchial challenge (Fig. 6). A significant increase in the serum IL-5 levels was observed 24 h after bronchial challenge in allergic rhinitis and asthma groups compared with the baseline values ($P < 0.01$ and $P < 0.05$, respectively). No significant differences in the IL-5 levels were documented

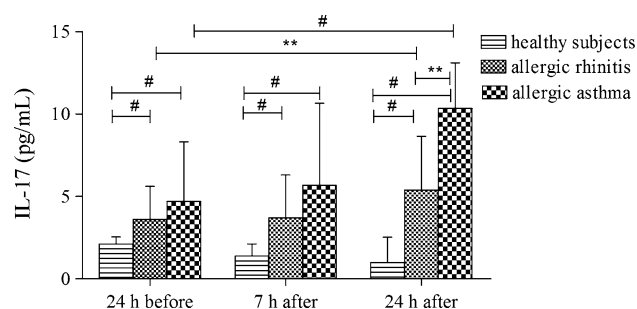


Fig. 5 Serum IL-17 levels in patients with allergic rhinitis and asthma and healthy subjects before and after bronchial challenge with *Dermatophagoides pteronyssinus*. Data are expressed as median (IQR); * $P < 0.05$; ** $P < 0.01$; # $P < 0.001$

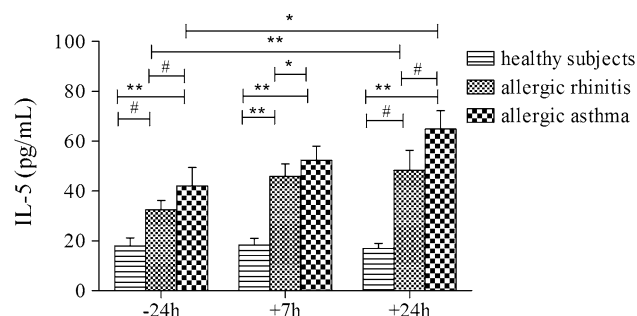


Fig. 6 Serum IL-5 levels in patients with allergic rhinitis and asthma and healthy subjects before and after bronchial challenge with *Dermatophagoides pteronyssinus*. Data are expressed as median (IQR); * $P < 0.05$; ** $P < 0.01$; # $P < 0.001$

in healthy subjects after bronchial challenge. The IL-5 levels were considerably higher in patients with asthma compared with those with rhinitis 24 h before ($P < 0.001$) as well as 7 and 24 h after bronchial challenge ($P < 0.05$ and $P < 0.001$, respectively).

Discussion

The present study showed that patients with allergic rhinitis and asthma had higher percentage of peripheral blood Th17 cells compared with healthy subjects. These data let us suggest that Th17 cells and their cytokines may be involved in the pathogenesis of allergic rhinitis and asthma. Bronchial challenge with *D. pteronyssinus* allergen was able to induce an increase in the percentage of peripheral blood Th17 cells in patients with allergic asthma 24 h after allergen challenge. This shows that Th17 cells appear to be important for allergen-induced inflammation.

Dermatophagoides pteronyssinus was chosen as the most common allergen causing allergic airway diseases. Regarding safety reasons, an accurate estimation of the severity of allergen-induced bronchial obstruction is essential. The data showed that bronchial hyperresponsiveness to methacholine

and skin sensitivity to house dust mites allergens predict the safe concentration of inhaled allergen causing an early asthmatic reaction [19]. Other authors demonstrated that not only the degree of nonspecific bronchial hyperresponsiveness, but also the type of allergen, contributed significantly to the prediction of the airway response following allergen inhalation [20]. Considering these results, we concluded that the model of bronchial challenge with an allergen is safe and useful for the investigation of asthma pathophysiology, early and late responses of lung function, and inflammatory parameters.

In our study, bronchial challenge with *D. pteronyssinus* was used to define the role of Th17 cells during the late-phase, allergen-induced, airway inflammation. This phase of allergic inflammation depends on the recruitment and activation of various inflammatory cells (eosinophils, neutrophils, T cells, and structural cells of the airways). It is known that stimulation of airways with allergen induces an increase in the number of inflammatory cells in peripheral blood [21]. Previously, mouse models of allergic asthma demonstrated that Th17 cells and their cytokine expression were regulated upon allergen exposure and represented relation between activation of these cells induced by allergen and local recruitment and activation of neutrophils [11]. Most studies on humans showed an important role of Th17 in the development of local allergic airway inflammation: the levels of Th17 cytokines in the lungs, sputum, and bronchoalveolar lavage fluids were elevated [22]. Few studies have investigated the role of Th17 cells in systemic inflammatory response. Therefore, in our study we analyzed a systemic component (particularly peripheral blood Th1, Th2, Th17 cells, and serum IL-17 levels) of late-phase, allergen-induced, airway inflammation in patients with allergic rhinitis and asthma.

In our study, the percentage of peripheral blood Th17 cells was found to be significantly higher in patients with allergic rhinitis and asthma. A study by Ciprandi et al. [23] reported higher frequencies of Th17 cells ex vivo stimulated with *D. farinae* allergen extract in patients with allergic rhinitis. Other study confirmed the existence of Th cells producing IL-17 in response to *D. farinae* extract in peripheral blood of the sensitized allergic asthmatics [24]. These data suggest that allergic patients have significantly higher percentages of peripheral blood Th17 cells than healthy subjects. In our study, an increase in the percentage of Th17 cells was found 24 h after bronchial challenge in patients with allergic asthma compared with patients with rhinitis. This may be explained by the fact that asthma and allergic rhinitis involves common inflammatory mediators that may contribute to both upper and lower airway inflammation, but allergic inflammation in allergic asthma is more expressed. It has been reported that a higher percentage of peripheral blood Th17 cells was found in

asthmatic patients in comparison with healthy subjects, and it tended to increase with severity of allergic asthma [25]. In our study, no significant difference in the percentage of peripheral blood Th17 cells in allergic rhinitis group after allergen challenge was found.

The main action of Th17 cells in allergic diseases is characterized by production of proinflammatory cytokines IL-17 and IL-22 [26]. IL-17 induces the expression of a wide range of cytokines and chemokines that have a positive impact on neutrophil recruitment and exacerbation of airway inflammation [27, 28]. In the present study, an increased level of IL-17 in serum from patients with allergic rhinitis and asthma compared with healthy subjects was documented. Bronchial challenge with *D. pteronyssinus* increased serum IL-17 levels 24 h after challenge in both the groups of patients, showing the same tendency of an increase as for Th17 cells after challenge. This demonstrated a systemic elevation of serum IL-17 levels as the result of allergen-induced activation of Th17 cells following late-phase allergic inflammation. Recent studies have shown that serum IL-17 levels are higher in patients with allergic rhinitis [29] and are associated with more severe forms of allergic asthma [30]. This let us suggest that IL-17 has an important role in the pathogenesis of allergic rhinitis and asthma.

In this study, also the higher percentages of Th2 cells in patients with allergic rhinitis and asthma compared with healthy subjects were reported, which increased significantly 24 h after allergen challenge. The percentage of Th2 cells was considerably higher in patients with allergic asthma 7 and 24 h after bronchial challenge compared with patients with allergic rhinitis. The results are in agreement with the data from other studies underlying the role of Th2 cells in allergic inflammation [31]. Th2 cell cytokines, such as IL-4, IL-5, IL-9, and IL-13, and immunoglobulin (Ig) E-mediated eosinophilic inflammation of the lungs have been recognized as key factors in the pathogenesis of asthma [1]. IL-5 activates eosinophils and enhances the maturation and migration of these cells into the site of allergic inflammation. Data from this study showed elevated serum IL-5 levels in patients with allergic rhinitis and asthma with a further increase in levels of this cytokine 7 and 24 h after bronchial challenge. The results are in agreement with the data from other studies that demonstrated increased serum IL-5 levels accompanied by an increased count and activation of eosinophils. Our study also demonstrated a higher percentage of peripheral blood eosinophils in patients with allergic rhinitis and asthma 24 h after allergen challenge (Table 2). Because of a parallel increase in the percentages of Th17 and Th2 cells before and after allergen challenge, the Th17/Th2 ratio did not change following the bronchial challenge. This supports the hypothesis that Th17 cells are no less important as

Th2 cells in the development of allergen-induced inflammation [32].

Our study demonstrated that the percentage of Th1 cells was lower in patients with allergic rhinitis and allergic asthma than in healthy subjects before and after allergen challenge. It is known that Th1 cells and their cytokines IFN- γ and IL-12 are involved in the protection against intracellular microbes and are associated with the development of autoimmune diseases. In allergic asthma, Th1 cell cytokines are associated with orchestration of monocytes and neutrophils. Both Th1 and Th2 are present in the lungs of asthmatic patients, and immune response to allergens in the pathogenesis of the disease is the result of a balance between these cells [33]. The baseline Th1/Th2 ratio was significantly lower in both groups of patients compared with healthy individuals and significantly decreased 24 h after bronchial challenge. These data support the previously described hypothesis that impaired balance of Th2 and Th1 cells may be important for development of allergic airway diseases.

Conclusions

Percentages of peripheral blood Th17 cells and serum IL-17 levels were higher in patients with allergic rhinitis and allergic asthma. An increase in the percentage of Th17 cells following bronchial challenge with *D. pteronyssinus* parallel to changes in Th2 cell subpopulation shows that Th17 cells may have an important role in the development of allergen-induced inflammation, especially late-phase allergen-induced inflammation, in patients with allergic rhinitis and asthma.

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Conflict of interest The authors declare that they have no conflict of interests.

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