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## Original Research Article

# Peripheral blood Th9 cells and eosinophil apoptosis in asthma patients

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### ARTICLE INFO

#### Article history:

Received 10 April 2014

Accepted 15 January 2015

Available online 28 January 2015

#### Keywords:

Allergic asthma

Th9 cells

Interleukin 9

Interleukin 5

Eosinophil apoptosis

### ABSTRACT

**Background and objective:** Th9 cells producing interleukin (IL) 9 are novel subset of CD4<sup>+</sup> T helper cells, which might contribute to airway inflammation in asthma. Moreover, the effect of IL-9 on eosinophils is still not fully understood. Study aim was to evaluate peripheral blood Th9 cells and eosinophil apoptosis in allergic asthma patients.

**Materials and methods:** Eighteen patients with allergic asthma and fourteen patients with allergic rhinitis were examined. Control group included sixteen healthy subjects. Allergic asthma and rhinitis patients did not use corticosteroids and antihistamines at least for 1 week. Peripheral blood eosinophils and CD4<sup>+</sup> cells were isolated by high density gradient centrifugation and magnetic separation. Th9 cells and apoptotic eosinophils were estimated by flow cytometer. Serum IL-9 and IL-5 concentration were determined by ELISA.

**Results:** Peripheral blood Th9 cells percentage was increased in allergic asthma group compared with allergic rhinitis and control group ( $0.74\% \pm 0.32\%$  vs.  $0.19\% \pm 0.10\%$  and  $0.15\% \pm 0.08\%$ , respectively,  $P < 0.05$ ). The same tendency was observed for IL-9 ( $P < 0.01$ ). Percentage of peripheral blood apoptotic eosinophils was decreased in allergic asthma and allergic rhinitis groups compared with control group ( $P < 0.05$ ). IL-9 concentration correlated with percentage of Th9 cells ( $r = 0.64$ ,  $P < 0.05$ ) and negatively with percentage of apoptotic eosinophils in allergic asthma group ( $r = -0.58$ ,  $P < 0.05$ ). Negative correlation was found between apoptotic eosinophils count and IL-5 concentration in allergic asthma group ( $r = -0.76$ ,  $P < 0.05$ ).

**Conclusions:** Patients with allergic asthma demonstrate increased peripheral blood Th9 cells count and serum IL-9, while eosinophil apoptosis is inversely related to IL-9 concentration.

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Peer review under responsibility of Lithuanian University of Health Sciences.



## 1. Introduction

Asthma is a chronic inflammatory disorder of the airways in which many cells and cell elements play a role and is characterized by chronic inflammation which is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing. Immune reactions in allergen induced airway inflammation are controlled by distinct subtypes of CD4+ T helper (Th) cells [1]. Initially, it was thought that many immune responses were strongly influenced by the interaction of Th1 and Th2 cells and by their cross-regulatory properties, in particular because of the production of cytokines interferon- $\gamma$  (IFN- $\gamma$ ) by Th1 cells and IL-4 by Th2 cells. In the last decade, Th17 cells, a subset of CD4+ T cells, has been broadly studied and is believed to be associated with the more severe asthma which is less responsive to corticosteroids. Human and mice Th17 cells secrete IL-17A, IL-17F, IL-22, IL-21 and have broad effects on many cell types. These cells induce the production of proinflammatory cytokines and chemokines which attract neutrophils to the site of inflammation and to antimicrobial peptides in order to strengthen host defense directly [2].

A new CD4+ population in a murine model was recently found [3,4]. Analysis of these cells suggested that they were not Th1, Th17 or inducible regulatory T-cell populations. It was concluded that they represented a new subset of T helper cells defined as Th9 cells. This CD4+ T-cell subset is induced to differentiate and produce high levels of IL-9 in murine models and in human by a combination of transforming growth factor- $\beta$  (TGF- $\beta$ ) and IL-4 [5]. IL-9 is a pleiotropic cytokine that has documented effects on lymphocytes, mast cells, and resident lung cells. IL-9 demonstrates proinflammatory activity in development of allergic airway inflammation in several mouse models [6,7] as well as in human models [8,9]. An important function attributed to IL-9 in lung physiology is induction of mucus production [10], goblet cell hyperplasia and other features of airway remodeling [7,11]. IL-9 promotes mast cell growth and function [11], have close functions to IL-13 (airway eosinophilia, mucus cell metaplasia) and prolongs survival of eosinophils during allergic airways inflammation [12].

It is well known that activated eosinophils play a major effector role in asthma pathogenesis. In the lung, eosinophils can potentially perform a number of functions, including antigen presentation, and secretion of cytokines (including IL-13, IL-5 from preformed stores, TGF- $\beta$ , and osteopontin), chemokines (such as CCL-11, CCL22, matrix metalloproteinases, granule mediators e.g., erythropoietin and major basic protein), as well as leukotrienes (LTC<sub>4</sub>, LTB<sub>4</sub>) [13]. Some data suggest that IL-9 may potentiate in vivo eosinophil function by increasing their survival and IL-5-mediated differentiation and maturation [14]. In addition, eosinophil is delayed by a variety of exterior ligands such as IL-5, IL-10, granulocyte macrophage colony stimulating factor (GM-CSF) and retinoic acid [15].

There are few data, concerning Th9 cells investigations in human. It is believed that Th9 cells and IL-9 participate in allergic airway inflammation and has an impact on eosinophil's survival. Therefore the aim of our study was to

investigate peripheral blood Th9 cells, IL-9 and eosinophil apoptosis in stable allergic asthma patients. Allergic rhinitis and healthy subjects groups were used as comparative groups.

## 2. Materials and methods

### 2.1. Subjects

A total of 48 nonsmoking adults (15 men and 33 women) were examined: 18 patients with intermittent or mild-to-moderate persistent allergic asthma, defined according the GINA criteria [1], 14 patients with mild-to-moderate persistent allergic rhinitis, defined according to the ARIA criteria [16], and 16 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 (BE-2-23), and each participant gave his/her informed written consent.

Patients with allergic rhinitis and asthma had a clinical history of the disease for  $\geq 1$  year, current symptoms, and positive results of skin prick test ( $\geq 3$  mm) with *Dermatophagoides pteronyssinus* (*D. pteronyssinus*), birch pollen allergens or five grass mixture allergens. All patients were not using inhaled, nasal or oral steroids at least 1 month before visits, short-acting  $\beta_2$  agonists at least 12 h and long-acting  $\beta_2$  agonists at least 48 h prior the lung function test, antihistamines and antileukotrienes – 7 days before skin prick test and prior the lung function test. None of the patients had a history of smoking. Baseline forced expiratory volume in 1 s (FEV<sub>1</sub>) was more than 70% of the predicted value in all patients. All healthy subjects were nonsmokers, without symptoms of rhinitis or asthma, with normal findings of spirometry, and all showed negative results of skin prick test.

### 2.2. 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*, *Dermatophagoides farinae*, cat and dog dander, 5 mixed grass pollen, birch pollen, mugwort, *Alternaria*, *Aspergillus* and *Cladosporium*. Temoin 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  $\geq 3$  mm [17].

### 2.3. Lung function testing

Pulmonary function was tested using a pneumotachometric spirometer "CustovitM" (Custo Med, Germany). Baseline forced expiratory volume in 1 s (FEV<sub>1</sub>), forced vital capacity (FVC), and FEV<sub>1</sub>/FVC ratio were recorded as the highest of three reproducible measurements. The results were compared with the predicted values matched for age, body height, and sex according to the standard methodology [18].

#### 2.4. Measurement of airway responsiveness to methacholine

Airway responsiveness was assessed as changes in airway function after challenge with inhaled methacholine using a reservoir method [19]. Methacholine was nebulized into a 10-L reservoir with a pressure nebulizer (Pari Provocation I; Pari, Starnberg, Germany). Aerolized methacholine was inhaled through a one-way valve at 5-min intervals starting with 15- $\mu$ g methacholine dose and doubling it until a 20% decrease in FEV<sub>1</sub> 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 FEV<sub>1</sub> from the baseline value. The provocative dose of methacholine causing a  $\geq$ 20% fall in FEV<sub>1</sub> (PD<sub>20</sub>) was calculated from the log dose–response curve by linear interpolation of two adjacent data points.

#### 2.5. Peripheral blood collection and isolation of eosinophils

Peripheral blood samples for eosinophil isolation and apoptosis measurement were collected into sterile vacutainers with ethylenediaminetetraacetic acid (EDTA). Polymorphonuclear leukocytes (PMN) were isolated by high density gradient centrifugation. The whole blood was layered on Ficoll-Paque PLUS (GE Healthcare, Finland) and centrifuged at 1000  $\times$  g for 30 min at room temperature. PMN were separated by hypotonic lysis of erythrocytes and eosinophils were separated using magnetic eosinophil isolation Kit (Miltenyi Biotec, USA). Isolated eosinophils were diluted in cell culture RPMI 1640 media (Biological Industries, Israel) at a final concentration of 2  $\times$  10<sup>6</sup>/mL. The viability of eosinophils was checked by flow cytometry and it always was  $>$ 95%.

#### 2.6. Detection of cytokine in serum

The serum cytokine levels were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions: the minimum detectable dose of IL-9 was 0.1 ng/mL (Abcam, USA) and for IL-5 was 5 pg/mL (Abcam, USA).

The peripheral blood cell analysis was performed on an automated hematology analyzer (Sysmex XE-5000, Japan).

#### 2.7. Apoptosis assay

Isolated eosinophils were resuspended in the annexin-binding buffer (pH 7.4) containing 50 mM HEPES, 700 mM NaCl, 12.5 mM CaCl<sub>2</sub> (Invitrogen, USA) and incubated with fluorescein isothiocyanate-labeled (FITC)-annexin V (Invitrogen, USA) and propidium iodide (PI) for 15 min at room temperature in the dark. After the incubation, apoptosis was analyzed by flow cytometry using the CellQuest software (BD Biosciences, USA). Apoptotic cells were quantified as the percentage of the total population that was positive for FITC, but negative for PI. Necrotic cells were positive for PI.

#### 2.8. Evaluation of cytokine production in CD4+ T cells

Peripheral blood mononuclear cells (PBMC) were isolated by high density gradient and CD4+ T cells separated using CD4+ T

Cell Isolation Kit (Miltenyi Biotec, USA). Two million cells/mL were activated for 6 h at 37 °C with phorbol-12-myristate-13-acetate (PMA) (50 ng/mL; Sigma–Aldrich, USA) and ionomycin (1  $\mu$ g/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 2 times with BD Pharmingen™ stain buffer (BD Biosciences) and fixed with cold BD Cytofix™ fixation buffer (BD Biosciences, USA) for 10 min at room temperature. After the fixation, the cells were washed twice. Permeabilization of the fixed cells was performed by adding BD Perm/Wash™ buffer (BD Biosciences, USA) and incubating the cells for 15 min at room temperature. The cells were stained by adding human CD4-A488, human IL-9-PerCP-Cy5.5 (Th9), human IFN $\gamma$ -PE-Cy7 (Th1), human TGF $\beta$ 1-PE (Treg) and IL-4-PerCP-Cy5 (Th2) and incubated 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 FACSCalibur flow cytometer (BD Biosciences, USA). Th9 cells were defined as CD4+ IL-9+ IFN $\gamma$ - TGF $\beta$ 1- IL-4- IL17-.

#### 2.9. Statistical analysis

Statistical analysis was performed by using the Statistical Package for Social Sciences, version 20.0 for Windows (SPSS 20.0). The normality assumption of data was verified with the Shapiro–Wilks test. All the data that were normally distributed are presented as mean and standard deviation (SD).

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. Spearman rank test was used to assess relationships between measurements. Statistical significance was assumed at *P* value of  $<$  0.05.

### 3. Results

#### 3.1. Characteristics of studied subjects

A total of 48 nonsmoking adults (15 men and 33 women; mean age 31  $\pm$  9 years) were examined. 18 patients with intermittent or mild-to-moderate persistent allergic asthma, 14 patients with mild-to-moderate persistent allergic rhinitis, and 16 healthy subjects who comprised the control group were examined. There were no significant age and gender differences comparing the groups. Twenty-one patients were sensitized to *D. pteronyssinus*; 5 patients, to birch pollen allergens, and 6 patients, to five grass mixture allergens. The mean wheal diameter induced by allergen was similar in both groups of patients. Demographic and clinical data of study subjects are presented in Table 1. There was no significant baseline FEV<sub>1</sub> (% of predicted) difference in all group. A provocative dose of methacholine causing a 20% decrease in FEV<sub>1</sub> (PD<sub>20</sub>) was estimated to 13 allergic asthma patients and one patient with allergic rhinitis. An absolute eosinophil count in the peripheral blood of patients with

**Table 1 – Demographic and clinical characteristics of study subjects.**

Characteristics	Patients with allergic asthma N = 18	Patients with allergic rhinitis N = 14	Healthy subjects N = 16
Age (years), mean ± SD (range)	28 ± 9	31 ± 10	27 ± 8
Sex (male/female), n	10/7	4/10	1/15
Wheal diameter induced by allergen (mm), median ± SD (range)	6 ± 1.2 (4-8)	5.5 ± 1.8 (4-11)	-
Sensibilisation to <i>D. pteronyssinus</i> /birch/5 grass mixture allergen, n	13/3/2	9/3/2	-
PD <sub>20</sub> (mg), geometric mean (range)	0.38 (0.25-0.54)	0.52 <sup>a</sup>	-
FEV <sub>1</sub> (% of predicted), mean ± SD	98 ± 15	105 ± 10	102 ± 12

<sup>a</sup> N = 1 because metacholine challenge test provoked bronchoconstriction only to one allergic rhinitis patient.

**Table 2 – Peripheral blood cells in patients with allergic asthma, allergic rhinitis and healthy subjects.**

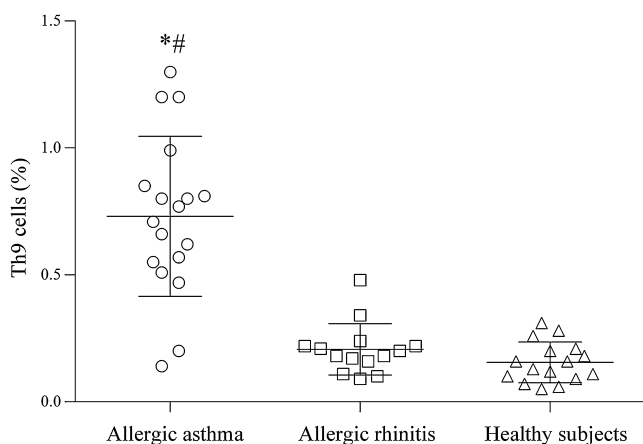
Characteristics	Patients with allergic asthma	Patients with allergic rhinitis	Healthy subjects
Leukocytes, ×10 <sup>9</sup> /L	6.84 ± 1.78	5.41 ± 1.02	6.01 ± 1.49
Eosinophils, %	4.8 ± 2.1 <sup>a</sup>	3.6 ± 3.0	3.8 ± 2.6
Eosinophils, ×10 <sup>9</sup> /L	0.30 ± 0.13	0.21 ± 0.21	0.18 ± 0.21
Neutrophils, %	53.0 ± 7.4	52.9 ± 4.5	52.0 ± 7.1
Neutrophils, ×10 <sup>9</sup> /L	3.65 ± 1.41	2.86 ± 0.56	3.19 ± 1.11
Lymphocytes, %	33.7 ± 5.3	31.8 ± 8.9	34.4 ± 7.9
Lymphocytes, ×10 <sup>9</sup> /L	2.24 ± 0.44	1.84 ± 0.35	2.11 ± 0.50

<sup>a</sup> P < 0.01 vs. patients with allergic rhinitis and healthy subjects.

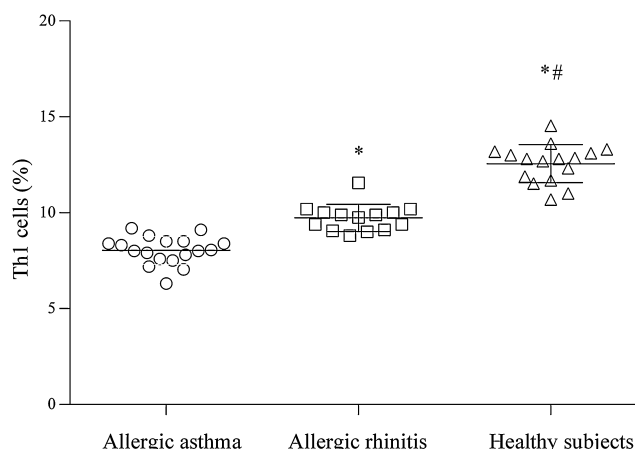
allergic asthma was significantly higher ( $P < 0.05$ ) compared with the patients with allergic rhinitis and healthy subjects (Table 2).

**3.2. Peripheral blood subsets of CD4+ cells**

The percentage of peripheral blood Th9 cells was significantly higher in the allergic asthma group compared with the allergic rhinitis group and healthy subjects ( $0.74\% \pm 0.32\%$  vs.  $0.21\% \pm 0.10\%$ ,  $0.16\% \pm 0.08\%$ , accordingly;  $P < 0.05$ ) (Fig. 1). The percentage of Th1 cells was significantly lower in the patients with allergic asthma and allergic rhinitis compared with the healthy subjects ( $8.03\% \pm 0.73\%$  and  $9.73\% \pm 0.70\%$  vs.  $12.57\% \pm 0.99\%$  accordingly;  $P < 0.001$ ) (Fig. 2). Compared with the



**Fig. 1 – Percentage of Th9 cells among peripheral blood CD4+ cells in patients with allergic asthma, allergic rhinitis and healthy subjects \*P < 0.05 vs. healthy subjects; #P < 0.05 vs. allergic rhinitis.**

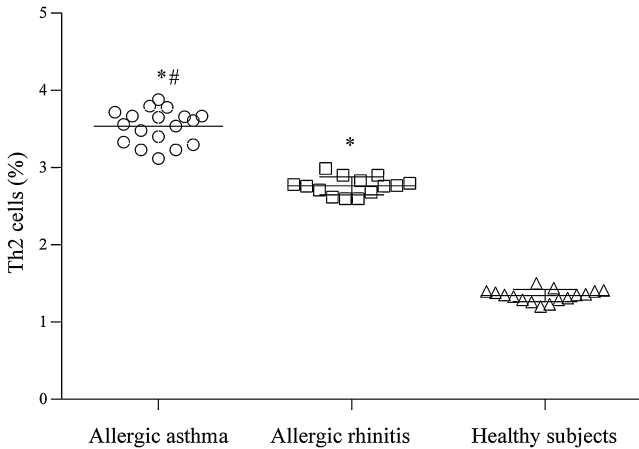


**Fig. 2 – Percentage of Th1 cells in patients with allergic asthma, allergic rhinitis and healthy subjects \*P < 0.05 vs. allergic asthma; #P < 0.05 vs. allergic rhinitis.**

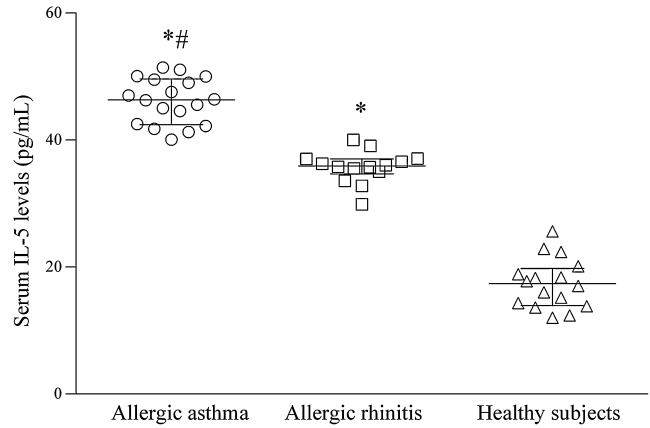
healthy subjects, the percentage of Th2 cells was significantly higher in the patients with allergic asthma and those with allergic rhinitis ( $3.53\% \pm 0.22\%$  and  $2.76\% \pm 0.12\%$  vs.  $1.34\% \pm 0.08\%$ , accordingly;  $P < 0.001$ ) (Fig. 3). An increased Th9/Th1 ratio in the allergic asthma and allergic rhinitis groups was found ( $P < 0.01$ ) (Table 3). The Th9/Th2 ratio was significantly lower in the patients with allergic asthma compared to their counterparts with allergic rhinitis ( $P < 0.01$ ).

**3.3. Peripheral blood IL-9 and IL-5 levels and eosinophil apoptosis**

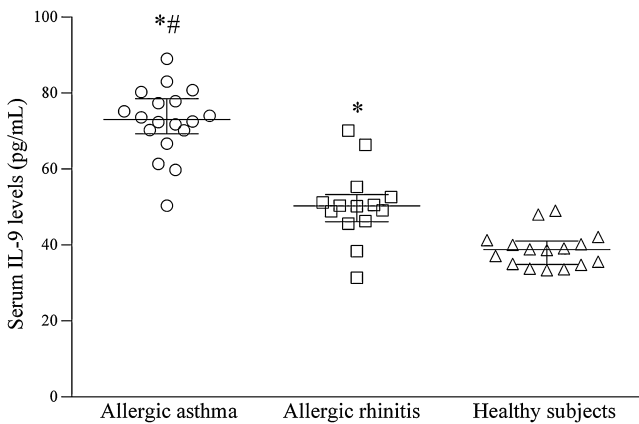
Compared with the healthy subjects, serum IL-9 levels were significantly higher in the patients with allergic asthma and



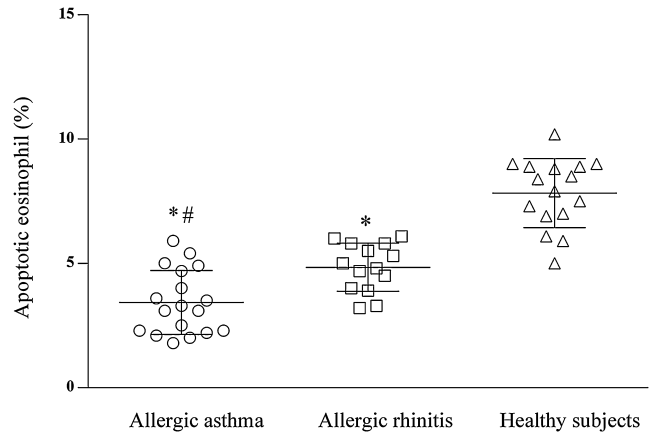
**Fig. 3 – Percentage of Th2 cells in patients with allergic asthma, allergic rhinitis and healthy subjects \*P < 0.05 vs. healthy subjects; #P < 0.05 vs. allergic rhinitis.**



**Fig. 5 – IL-5 levels in patients with allergic asthma, allergic rhinitis and healthy subjects \*P < 0.05 vs. healthy subjects; #P < 0.05 vs. allergic rhinitis.**



**Fig. 4 – IL-9 levels in patients with allergic asthma, allergic rhinitis and healthy subjects \*P < 0.05 vs. healthy subjects; #P < 0.05 vs. allergic rhinitis.**



**Fig. 6 – Eosinophil apoptosis in patients with allergic asthma, allergic rhinitis and healthy subjects \*P < 0.05 vs. healthy subjects; #P < 0.05 vs. allergic rhinitis.**

those with allergic rhinitis (72.6 ± 9.1 pg/mL vs. 50.5 ± 9.7 pg/mL, 38.8 ± 4.7 pg/mL, accordingly; P < 0.001) (Fig. 4). The same tendency was observed for serum IL-5 level in all groups (46.2 ± 3.6 pg/mL vs. 35.7 ± 2.5 pg/mL, 17.4 ± 3.9 pg/mL, accordingly; P < 0.001) (Fig. 5).

The percentage of peripheral blood apoptotic eosinophils was decreased in the patients with allergic asthma and allergic rhinitis compared with the healthy subjects (3.4% ± 1.3% and 4.9% ± 1.0% vs. 7.9% ± 1.4%, accordingly; P < 0.05) (Fig. 6).

**3.4. Associations between peripheral blood IL-9, IL-5 levels and eosinophil apoptosis**

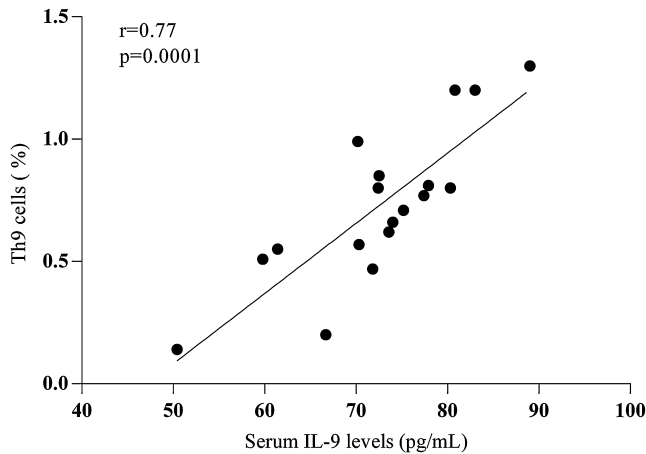
Serum IL-9 level significantly correlated with percentage of Th9 cells (r = 0.64, P < 0.05) (Fig. 7) and negatively with percentage of apoptotic eosinophils in the allergic asthma group (r = -0.58, P < 0.05) (Fig. 8). The negative correlation was found between apoptotic eosinophils count and IL-5 level in the allergic asthma group (r = -0.76, P < 0.05) (Fig. 9).

**Table 3 – CD4+ subtypes cells ratio in patients with allergic asthma, allergic rhinitis and healthy subjects.**

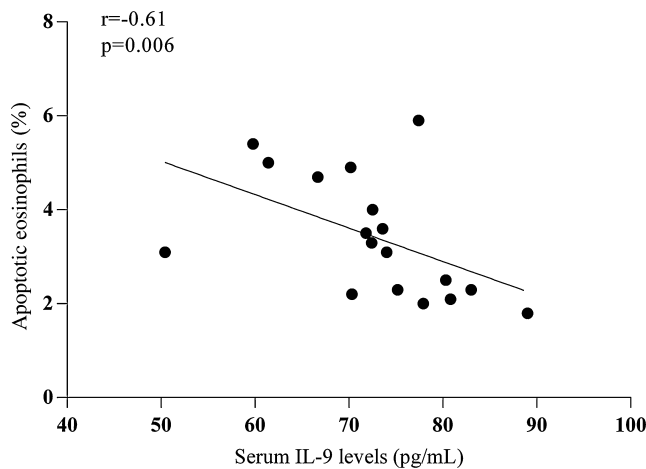
Characteristics	Patients with allergic asthma	Patients with allergic rhinitis	Healthy subjects
Th1/Th2 ratio mean ± SD	2.23 ± 0.09 <sup>a,b</sup>	3.51 ± 0.37 <sup>a</sup>	9.41 ± 1.08
Th9/Th1 ratio mean ± SD	0.09 ± 0.04 <sup>a,b</sup>	0.02 ± 0.01 <sup>a</sup>	0.01 ± 0.006
Th9/Th2 ratio mean ± SD	0.21 ± 0.09 <sup>a,b</sup>	0.08 ± 0.04 <sup>a</sup>	0.02 ± 0.06

<sup>a</sup> P < 0.01 vs. healthy subjects.

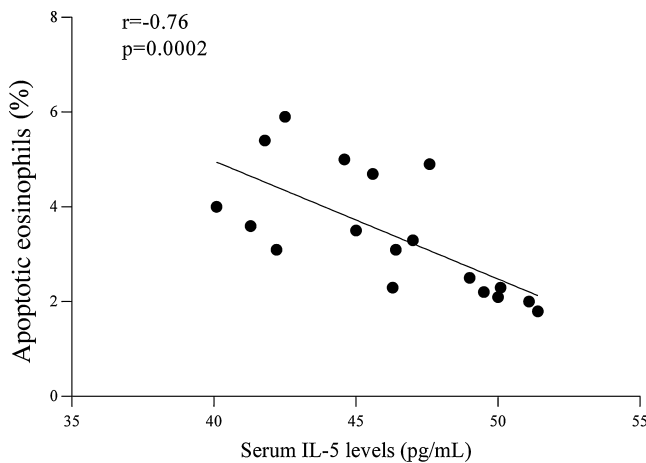
<sup>b</sup> P < 0.01 patients with allergic asthma vs. patients with allergic rhinitis.



**Fig. 7 – Correlation between the percentage of Th9 cells and the IL-9 level in patients with allergic asthma, allergic rhinitis and healthy subjects.**



**Fig. 8 – Correlation between the percentage of apoptotic eosinophils and the IL-9 level in patients with allergic asthma, allergic rhinitis and healthy subjects.**



**Fig. 9 – Correlation between the percentage of apoptotic eosinophils and the IL-5 level in patients with allergic asthma, allergic rhinitis and healthy subjects.**

#### 4. Discussion

In this study we investigated peripheral blood Th9 cells, IL-9 and eosinophil apoptosis in stable allergic asthma patients. It was demonstrated that allergic asthma patients present a higher percentage of peripheral blood Th9 cells compared to allergic rhinitis patients and healthy subjects. Further, we found an increased serum IL-9 level in allergic asthma and allergic rhinitis groups compared to healthy subjects. A significant negative correlation was estimated between serum IL-9 level and apoptotic eosinophil count in allergic asthma patient groups. These results present possible substantial role of Th9 cells and IL-9 in pathogenesis of allergic asthma.

Recently a novel subset of helper T cells named Th9 cells was found in mice and humans. Th9 cells are proinflammatory, but appear to function in a broad spectrum of autoimmune diseases and allergic inflammation [20]. During our study Th9 cells were detected in adult human peripheral blood and we find a greater amount of Th9 cells in allergic asthma group than in allergic rhinitis group and healthy individuals. We believe our results are reliable because all patients were clinically stable and did use neither corticosteroids nor histamine antagonists at least 1 month prior to the study. Chang et al. [21] as well found that peripheral blood mononuclear cells isolated from asthmatic children generate greater numbers of Th9 cells in vitro when compared with nonasthmatic controls. Jones et al. [22] found significantly higher number of circulating Th9 cells in allergic individuals than in nonallergic ones. Further, circulating numbers of Th9 cells correlated with plasma IgE level. Several studies with murine asthma models have been performed. They found that Th9 cells differentiate in vitro by either TGF- $\beta$ 1/IL-4 or activin A/IL-4 do not differ in their function in vivo and can promote immune responses to allergen, especially the recruitment and activation of mast cells in the lungs [22]. After chronic exposure to *A. fumigatus* increased Th9 cell development in the lungs was seen [23]. It demonstrated for the first time Th9 cells polarization in a chronic allergen model in vivo and provided evidence that Th9 cells are pathogenically relevant for the development of allergic asthma. We did not found significant difference of Th9 count between allergic rhinitis and healthy individuals groups. It may be explained by the fact that asthma and allergic rhinitis involves common inflammatory cells and mediators which contributes to upper and lower airway inflammation, but allergic inflammation in allergic asthma is considerably stronger expressed.

Th9 cells are believed to be the main IL-9 producer. Findings of this new CD4+ T cell subset led to novel IL-9 investigations. A wide spectrum of functions in both hematopoietic and nonhematopoietic cells has been attributed to IL-9. Lung-selective overexpression of IL-9 results in spontaneous airway inflammation characterized by eosinophilia, mast-cell infiltration, enhanced mucus production and airway hyper-reactivity, which resembles the classical features of human asthma [6,24]. Administration of a neutralizing antibody to IL-9, however, ameliorates ovalbumin-induced airway inflammation in mice [25]. In our study we investigated allergic asthma and allergic rhinitis patient's serum IL-9 level. We found increased IL-9 level in those both groups compared with

healthy individuals, but no correlation estimated between Th9 cells count and serum IL-9 level in allergic rhinitis group. This might be because most of allergic rhinitis patients were mild. Several research groups have reported similar findings that IL-9 production is enhanced in the lungs of adult patients with chronic asthma [8,26] or that IL-9 production was significantly higher in atopic children than in non-atopic [21]. Chang et al. [21] investigated mice with PU.1 deficient T cells which developed normal Th2 responses in vivo, but exhibited attenuated allergic airway inflammation corresponding to decreased IL-9 and chemokine expression in peripheral T cells and in lungs as compared to wild type mice. These findings suggest that Th9 cells might be required for Th2 cells immunity and there is data supporting a role for IL-9 in allergic airway diseases.

Airway eosinophilia has been recognized as a predominant feature of allergic asthma and elevated numbers during the inflammation are often associated with the disease severity [27]. Increased eosinophil survival and decreased apoptotic death are believed to participate in the accumulation and the persistence of eosinophils in the asthmatic airways [28,29]. Temann et al. [24] reported marked eosinophilia in transgenic mice that overexpress IL-9 as well as Levitt et al. [30] presented eosinophilia in naive mice after recombinant IL-9 administration intratracheally. We found negative correlation between serum IL-9 and peripheral blood apoptotic eosinophil percentage as well as serum IL-5 and peripheral blood apoptotic eosinophil percentage in allergic asthma patients. IL-5 was originally discovered as an eosinophil colony-stimulating factor. The major cellular sources of IL-5 are Th2 cells after stimulation with antigens or with allergens and by mast cells upon stimulation with allergen/IgE complex. It is known that eosinophils themselves produce IL-5 as well. It is proved that IL-9 enhances IL-5 receptor expression and differentiation in eosinophils and so prolonging the survival of eosinophils. The expression of IL-9R $\alpha$  by mature human peripheral blood eosinophils was also demonstrated at the mRNA and protein level by Gounni et al. [14]. They found that recombinant human IL-9 inhibited in vitro human peripheral blood eosinophil apoptosis.

## 5. Conclusions

The existence of IL-9-secreting T cells in allergic disease leads to the question regarding the role of Th9 cells in human allergic asthma. Data from our study let us suggest that Th9 cells and their cytokine IL-9 are important in airway inflammation and may be involved in the pathogenesis of allergic asthma. Further investigations are required, and currently we are investigating Th9 cells response and these cells related cytokines in allergen-induced airway inflammation in patients with allergic asthma.

## Conflict of interest

The authors declare that they do not have any competing interests.

## Acknowledgments

The study was fully supported by the Research Council of Lithuania (No. LIG-08/2012).

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