# Lymphocytes subpopulations in asthmatic children

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***Abstract***

***Background:*** *Atopic asthma is driven and maintained by an array of cells from peripheral blood and airways. It is suggested that the key role play T cells, especially their CD4 positive subpopulation. CD8+, memory and naïve lymphocytes might be also involved in the pathogenesis of asthma.*

***Aim:*** *The goal of the study was to assess difference between relative number of in lymphocytes subpopulations from peripheral blood between asthmatic and healthy children.*

***Material and methods:*** *Twenty-five individuals aged 8.13 ±3.08 years old, 21 boys and 4 girls, suffering from atopic asthma and allergic rhinitis, shortlisted for specific immunotherapy, served as studied group. Fifteen healthy individuals, aged 9.83 ±3.37 years old, 7 boys and 8 girls, served as a control group. The flow cytometric assessment of CD2, CD4, CD8, CD45RA and CD45RO antigens expression on cells from peripheral blood collected to tubes containing EDTA was performed with Cytomics FC500 flow cytometer (Beckman Coulter).*

***Results:*** *In the group of asthmatic children 75.6% (68.59; 79.3) of CD2+ T cells were identified, whereas 79.36% (77.62; 82.34) of CD2pos cells were detected in control group, p = 0.006. There was no difference in the percentage of CD4+ and CD8+ T cells between analyzed groups. Children suffering from asthma had significantly lower percentage of memory cells compared with control group, 26.83% (24.93; 34.22) vs. 39.04% (32.78; 41.65), p = 0.004 respectively. Groups did not differ in percentage of naïve PBMC.*

***Conclusions:*** *Lack of differences between percentages of CD4+ and CD8+ T cells might suggest, that both populations are involved in asthma pathogenesis and their absolute number change simultaneously. Low CD45RO+ cells percentage could reflect successful treatment and adequate control of asthmatic inflammation.*

***Key words:*** *lymphocytes, asthma, T cells, memory cells, naïve cells.*

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## Introduction

Atopic asthma is still an serious health problem. It is estimated that about 300 mln people around the world suffer from bronchial asthma. The prevalence of the disease is estimated at 1-18% of population, depending on the geographic region. The number of children diagnosed as having asthma is still increasing [1, 2]. It could be associated with growing knowledge about the disease symptoms among parents and first care health practitioners as well as better diagnostic procedures available for physicians [2, 3].

An array of cells is involved in asthma pathogenesis and development. The main role play leukocytes from peripheral blood, cells from tissues located in airways and their interactions. Airways' inflammation, disturbance of immunological balance between lymphocytes from peripheral blood and characteristic symptoms are observed in the course of asthma. The key role in development of the disease is attributed to T cells, especially CD4 positive T helper cells. The role of CD8 positive T cells in asthma progress is still discussed. Similarly, the participation of memory and naïve lymphocytes in the course of disease is still studied [4, 5].

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The main goal of this study was to evaluate percentages of CD4+ and CD8+ T cells in peripheral blood of asthmatic children and healthy children as well as the percentage of naïve (CD45RA+) and memory (CD45RO+) lymphocytes.

## Material and methods

### Study group

Twenty-five individuals aged 8.13 ±3.08 years old, 21 boys and 4 girls, suffering from atopic asthma and allergic rhinitis, sensitized to grass pollen and/or *Dermatophagoides pteronyssinus* allergens, confirmed by skin prick test, shortlisted for specific immunotherapy, served as study group. No one of analyzed subjects has been treated with systemic corticosteroids for 4 weeks before blood collection.

Fifteen healthy individuals, aged 9.83 ±3.37 years old, 7 boys and 8 girls, served as a control group. Healthy individuals were characterized by negative quantitative IgE test and negative history of asthma. They were chosen from children undergoing routine periodical health screening with no systemic illness or recent respiratory disease. The experiments were approved by the Ethics Commission of Medical University of Warsaw. Blood was collected with parents approval. Characteristic of enrolled children is presented in Table 1.

### Flow cytometry

Fifty microlitres of peripheral blood collected to tubes containing EDTA was transferred to cytometric tubes. To each tube 10 µl of suitable monoclonal antibody was added. Antigens CD2, CD4 and CD8 were identified on the cells, after staining with monoclonal antibodies (Beckman Coulter) antiCD2-PE, antiCD4-FITC and antiCD8-ECD. CD45RA and CD45RO antigens expression on mononuclear cells was analyzed after staining with monoclonal antibodies antiCD45RA-PE and CD45RO-FITC. Appropriate isotypic control based on monoclonal antibodies antiIgG1-PE, antiIgG1-FITC and antiIgG1-ECD was performed for each sample before proper test.

Tubes containing blood mixed with monoclonal antibodies were incubated for 30 minutes in dark and in room temperature (RT). After incubation erythrocytes were lysed with lysing solution (BD Pharm Lyse – Becton Dickinson), dissolved 5 times in distilled water. Tubes were mixed and incubated for 15 minutes in dark in RT. After lysing tubes were washed twice with 3 ml 0.9% NaCl and centrifuged every time. After washing cells stained with monoclonal antibodies were dissolved in 1 ml of saline. Obtained material was analyzed with flow cytometer Cytomics FC500 (Beckman Coulter). The first cytogram show peripheral blood leukocytes divide to main populations basis of size and granularity of the cells (FS – forward scatter, SS – side scatter) (Fig. 1). Further analysis was performed on the cells from A gate (peripheral blood mononuclear cells – PBMC). Cells expressing analyzed antigens were visualized on the presented cytograms (Figs. 2 and 3).

### Statistical analysis

Results are presented as median (Q1, Q3). Statistical analysis was performed using the Mann Whitney U test. A $p$ value of less than 0.05 was considered significant.

**Table 1.** The characteristics of children from study and control groups

| Parameter | Children suffering from asthma | Control group |
|---|---|---|
| Age | 8.13 ±3.08 (5-15) years old | 9.83 ±3.37 (5.5-14.5) years old |
| Gender | | |
| boys | $n = 21$ | $n = 7$ |
| girls | $n = 4$ | $n = 8$ |
| Bronchial asthma (%) | 100% | 0% |
| Allergic rhinitis (%) | 100% | 0% |
| Allergen-specific IgE (> 0,7 kU/l) | | |
| Grass pollen | 88% | 0% |
| *Artemisia vulgaris* (Wormwood pollen) | 20% | 0% |
| *Betula verrucosa* (Birch pollen) | 56% | 0% |
| *Dermatophagoides pteronyssinus* | 28% | 0% |
| *Dermatophagoides farinae* | 28% | 0% |
| The family history of atopic diseases | 52% | 0% |

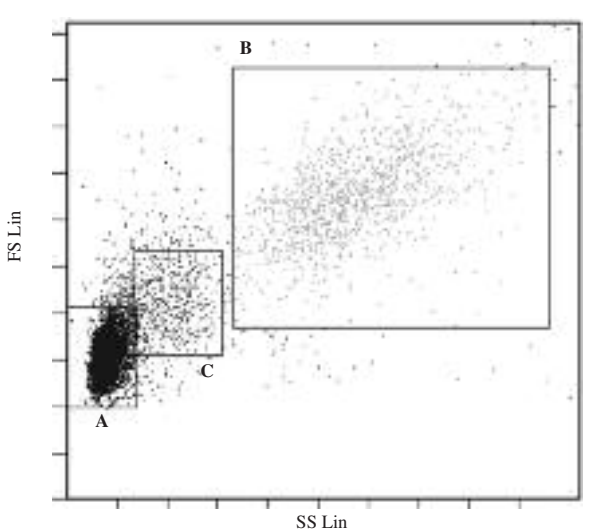



**Fig. 1.** The layout of peripheral blood leukocytes in basic FS/SS flow cytogram. Gate A contains lymphocytes, gate B – granulocytes and gate C – monocytes

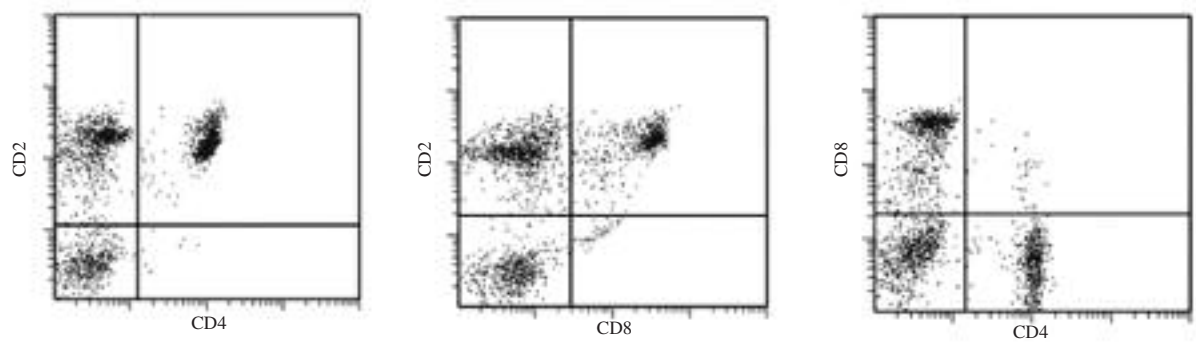



**Fig. 2.** The visualization of CD2, CD4 and CD8 positive cells within peripheral blood mononuclear cells. Analysis was performed in A gate from Figure 1

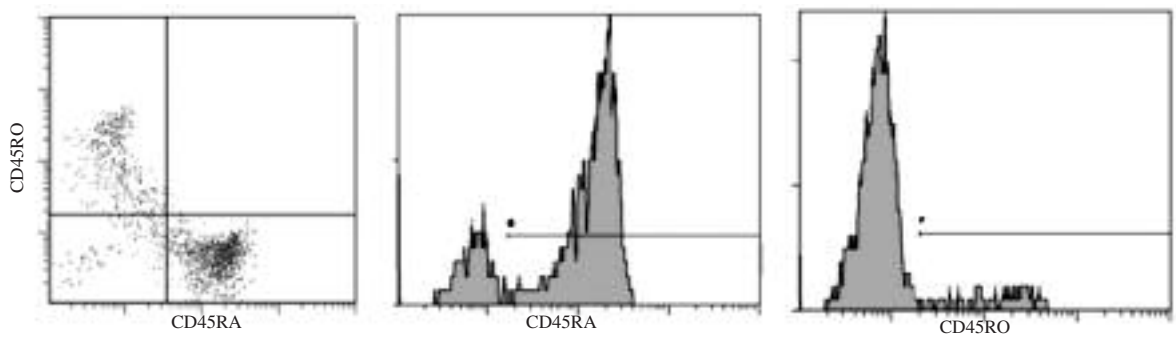



**Fig. 3.** Visualization of CD45RA and CD45RO positive cells included to A gate from Figure 1

## Results

### CD2 positive cells

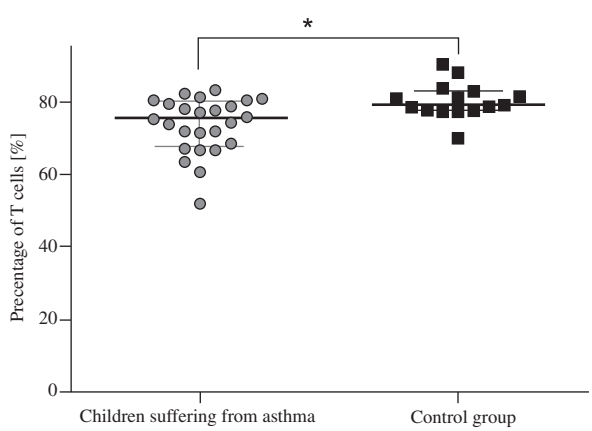
75.6% (68.59; 79.3) of CD2+ T cells were identified in study group, whereas 79.36% (77.62; 82.34) of CD2pos cells were detected in control group, $p = 0.006$ (Fig. 4).

### CD4 positive cells

In the group of children suffering from atopic asthma 33.94% (29.17; 37.54) CD4+ T cells were detected. In the group of healthy children 37.75% (33.18; 41.76) CD4+ T cells were identified, $p = 0.09$ (Fig. 5).

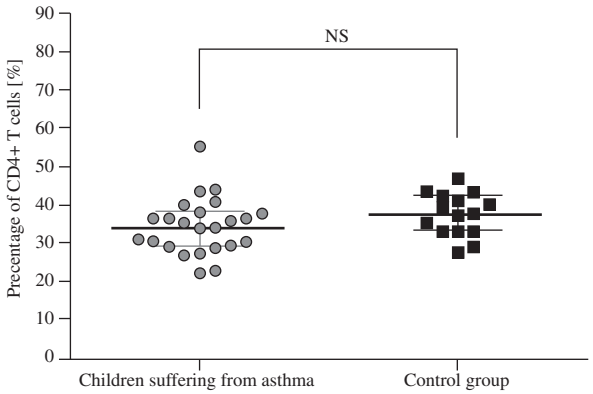
### CD8 positive cells

Twenty-four percent (20.3; 26.00) CD8 positive T cells were identified within study group, whereas in the control group 24.94% (20.72; 25.71) CD8+ T cells were detected, $p = 0.68$ (Fig. 6). The ratio between CD4 and CD8 positive T cells was also evaluated. Within the group of asth-



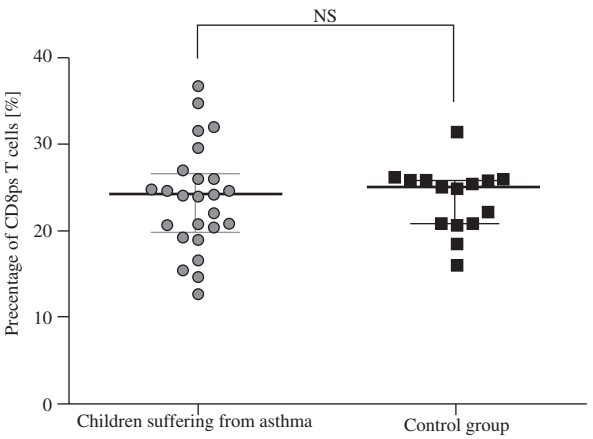

**difference is statistically significant*

**Fig. 4.** T cells percentage in peripheral blood of asthmatic and healthy children



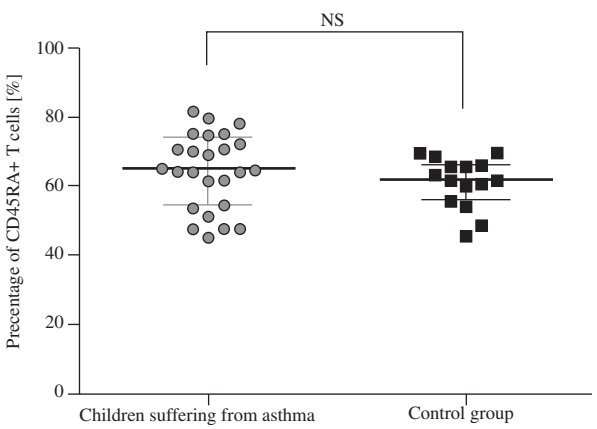

*NS – not significant*

**Fig. 5.** Percentages of CD4+ T cells in peripheral blood of asthmatic and healthy children



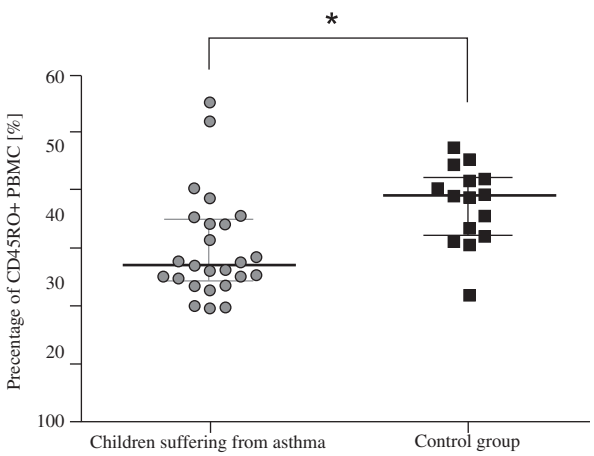

*NS – not significant*

**Fig. 6.** Percentages of CD8+ T cells in peripheral blood of asthmatic and healthy children




*NS – not significant*

**Fig. 7.** Percentages of CD45RA positive PBMC in peripheral blood of asthmatic and healthy children ns – not significant




**difference is statistically significant*

**Fig. 8.** Percentages of CD45RO positive PBMC in peripheral blood of asthmatic and healthy children

matic children CD4/CD8 ratio was 1.53 (1.30; 1.70), whereas within group of healthy children 1.59 (1.29; 1.92), $p = 0.56$

### CD45RA positive mononuclear cells

In the group of asthmatic children there was 64.37% (54.12; 71.93) of CD45RA+ mononuclear cells, while in the control group 61.45% (57.57; 65.52) of CD45RA cells were identified, $p = 0.22$ (Fig. 7).

### CD45RO positive mononuclear cells

26.83% (24.93; 34.22) of CD45RO+ PBMC were detected within group of children suffering from asthma, whereas 39.04% (32.78; 41.65) CD45RO pos cells were identified within control group, p = 0.004 (Fig. 8).

## Discussion

Atopic asthma is an inflammatory disease, driven and maintained mainly by T cells. CD4+ T cells play a key role

in development of bronchial atopic asthma. In healthy subjects the balance between CD4 positive T helper cells is observed. In asthmatic subjects T cells are persistently activated by array of allergens and the balance in immunological system is disrupted. The increase of Th2 cells percentage is observed – these cells are responsible for majority of processes causing bronchial hyperresponsiveness and development of atopic diseases [6].

The CD8 positive T cells are also involved in the pathogenesis of asthma. Increased ratio of these cells was detected in peripheral blood as well as in bronchoalveolar fluid of asthmatic subjects. According to experiment performed in rodents it is postulated that decrease of CD8 T cells percentage is associated with inflamed airways in asthma [7].

In the present study significantly lower percentage of T cells from peripheral blood of children suffering from asthma comparing with control group was detected. However, the counted median of T cells percentage in both analyzed groups was increased comparing with median values reported by Piątosa *et al*. [8]. There was no difference in CD4+ and CD8+ T cells percentage. Similarly, the ratio between CD4pos and CD8pos cells did not differ between analyzed groups. This observation stays in the line with data presented by other authors [9, 10]. Nevertheless no difference in percentage of analyzed cells between asthmatic and healthy subjects was detected, it is postulated that T cells from peripheral blood of people suffering from asthma highly express activation markers like CD25, VLA-1 and HLA-DR compared with cells isolated from blood of healthy subjects [9]. It is also suggested than percentage of CD8 positive T cells is positively correlated with severity of the disease. CD8+ T cells may differentiate into either T cytotoxic 1 (Tc1) or T cytotoxic 2 (Tc2), depending on the profile of released cytokines. In the course of asthma majority of Tc cells differentiate to Tc2 subpopulation. These cells release an array of cytokines similar to the profile released from Th2 cells, promoting asthma development [11]. No difference in the percentage of CD8 positive T cells between analyzed groups might result from the fact, that children suffering from asthma were treated properly and the inflammation was well controlled.

The percentage of memory lymphocytes (CD45RO positive PBMC) in the group of asthmatic children was significantly lower than that observed in the group of healthy individuals. This could be linked to the observations of Machura *et al.* These authors showed that the percentage of CD4+CD45RO+ T cells decrease in the course of asthma. Opposite, the increase of CD8+CD45RO+ cells is observed in asthmatic subject in comparison with healthy individuals. It is postulated that decrease of CD45RO positive T cells depends on corticosteroids intake [10]. Our observation does not confirm this hypothesis, as children enrolled to the study were receiving inhaled corticosteroids only periodically. Moreover the tests were performed at least 4 weeks after the last steroid intake.

Decrease of CD45RO+ cells percentage in asthmatic children compared with the control group do not stay in line with results obtained by Abdulamir *et al.* They found that there is no difference in CD45RO+ cells ratio between group of patients suffering from mild asthma and the group of healthy subjects. Additionally, they found increased expression of CD45RO on the lymphocytes from patients suffering from acute asthma [12]. Comparable results were obtained regarding to Th2 cells percentage expressing CD45RO antigen [13]. The increase of memory Th2 cells percentage along with the progress of the disease might justify their leading role as drivers of the inflammation in the course of asthma. As it was already mentioned, cytokines released by Th2 cells play a key role in asthma development. Low percentage of memory cells in peripheral blood of asthmatic children might be explained by the good level of asthma control in all analyzed subjects.

No difference in the percentage of CD4+, CD8+ and CD45RA+ lymphocytes between both analyzed groups was found. This observation is consistent with the results of Abdulamir *et al*. [12]. It is suggested that lack of the difference in the balance of CD4+ and CD8+ cells might argue that both population play a role in asthma development [12].

## Conclusions

The balance between CD4+ and CD8+ T cells in the course of mild asthma might suggest, that both populations are involved in its pathogenesis. Low frequency of CD45RO+ cells could reflect successful treatment and adequate control of asthmatic inflammation.

## Acknowledgments

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The authors declare no conflict of interest in relation to this article.

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