The influence of sublingual immunotherapy on lymphocytes' subpopulations from children suffering from controlled bronchial asthma

OLGA CIEPIELA[1], ANNA ZAWADZKA-KRAJEWSKA[2], IWONA KOTUŁA[1], URSZULA DEMKOW[1]

[1]Department of Laboratory Diagnostics and Clinical Immunology of Developmental Age, Medical University of Warsaw, Warsaw, Poland
[2]Department of Pediatric Pneumonology and Allergology, Medical University of Warsaw, Warsaw, Poland

Abstract

Background: *Specific immunotherapy is a causative, effective asthma treatment. Its clinical efficacy has been already proven, but the influence of sublingual immunotherapy (SLIT) on peripheral blood leukocytes is still investigated.*

Aim: *The aim of the study was to assess the influence of one year sublingual immunotherapy on the frequency of T cells' subsets and naïve and memory lymphocytes.*

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 study group. Tests were performed before and after twelve months of specific sublingual immunotherapy. Nineteen children (76%), 18 boys and 1 girl, were examined before and after one year SLIT with Staloral 300 composed individually for each patient. The assessment of CD2, CD4, CD8, CD45RA and CD45RO antigens expression on cells from peripheral blood collected to tubes containing EDTA was performed with the use of Cytomics FC500 flow cytometer (Beckman Coulter).*

Results: *CD4+ to CD8+ T cells ratio increased significantly after one year of sublingual immunotherapy. Before SLIT CD4/CD8 was of 1.53 (1.30; 1.70), whereas after one year of therapy 1.58 (1.51; 1.84), p = 0.0464. Although, no differences were found in the frequency of CD4+ and CD8+ T cells. The percentage of naïve and memory cells in peripheral blood of asthmatics before and after one year of allergen-specific treatment did not change either.*

Conclusions: *One year of SLIT influenced the immunological system of asthmatic children causing increase of CD4/CD8 T cells ratio.*

Key words: *asthma, sublingual immunotherapy (SLIT), lymphocytes, flow cytometry.*

(Centr Eur J Immunol 2011; 36 (4): 243-247)

Introduction

Specific immunotherapy is a causative, effective asthma treatment. Since subcutaneous immunotherapy (SCIT) has been found as partially questionable due to its side effects, sublingual immunotherapy (SLIT) arouse wide interest. SLIT very seldom causes acute systemic reactions [1]. Vaccine can be administered at home and the procedure of drug intake is clear and simple. Clinical efficacy of SLIT has been already proven, but its influence on peripheral blood leukocytes is still investigated [2-8].

The influence of specific immunotherapy on immune system is complex. It results in the restoration of immunological balance between T helper cells and decreased synthesis of allergen-specific IgE. It is suggested that effect of both SCIT and SLIT depends on desensitization, but induction phase of the tolerance is different [9].

The allergens administered by sublingual immunotherapy are transferred through sublingual mucosa and are incorporated to mucosa-associated lymphoid tissue (MALT) [6]. Subsequently allergens are presented to dendritic cells, which in response produce IL-12 promoting differentiation

Correspondence: Olga Ciepiela, PhD, Department of Laboratory Diagnostics and Clinical Immunology of Developmental Age, Medical University of Warsaw, Marszałkowska 24, 00-576 Warszawa, phone number/fax number +48 22 629 65 17, e-mail: olga.ciepiela@wum.edu.pl

of Th0 cells to T helper 1 lymphocytes. T helper 1 cells release IFN-γ, which restrains Th2 cells proliferation. In the consequence restoration of immunological balance is observed. Furthermore, IFN-γ triggers IgG4 synthesis and inhibits IgE synthesis by allergen-specific B cells [10].

The aim of the study was to assess the influence of one year sublingual immunotherapy on immunological system of children suffering from atopic asthma, especially the frequency of T cells' subsets as well as the presence of naïve and memory lymphocytes.

Material and methods

Study group

Twenty-five individuals aged 8.13 ±3.08 years, 21 boys and 4 girls, suffering from atopic asthma and allergic rhinitis, sensitized to grass pollen and/or *Dermatophagoides pteronyssinus* allergens, confirmed with skin prick test, shortlisted for specific immunotherapy were enrolled. Tests were performed before and after twelve months of specific sublingual immunotherapy. Blood samples from 19 children (76%), 18 boys and 1 girl, were examined after one year SLIT. No one of analyzed subjects has been treated with systemic corticosteroids for 4 weeks before blood collection. Patients were treated with Staloral 300 (Stallergens) according to the manufacturer instructions. Lower doses had been administered to one patient due to low tolerability of the drug. The experiments were approved by the Ethics Commission of Medical University of Warsaw. Blood was collected after informed consent was obtained from parents/patients.

Flow cytometry

Fifty microlitres of peripheral blood collected to tubes containing EDTA was transferred to cytometric tubes. 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 were analyzed within mononuclear cells with usage of monoclonal antibodies antiCD45RA-PE and antiCD45RO-FITC. Appropriate isotypic control with monoclonal antibodies anti-IgG1-PE, antiIgG1-FITC and antiIgG1-ECD was performed for each sample before proper test. To each tube 10 µl of suitable monoclonal antibody was added.

Blood mixed with monoclonal antibodies was incubated for 30 minutes in dark 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 kept for 15 minutes in dark in RT. After lysing cells 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 0.9% NaCl. Obtained material was analyzed with the use of flow cytometer Cytomics FC500 (Beckman Coulter). The proper analysis was performed on cells from gate containing small mononuclear cells, mainly lymphocytes. The examples of flow cytograms with stained peripheral blood mononuclear cells (PBMC) are presented in Figure 1.

Statistical analysis

Results are presented as median (Q1, Q3). Statistical analysis was performed using the Wilcoxon matched pair test. A p value of less than 0.05 was considered significant.

Results

CD2 positive cells

In the group of children suffering from bronchial asthma before treatment 75.6% (68.59; 79.63) of T cells was detected, whereas after one year of sublingual

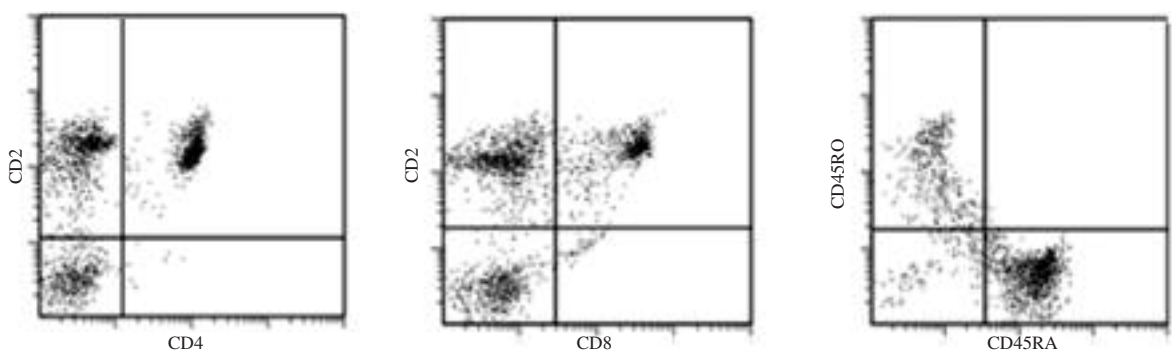


Fig. 1. Flow cytograms presenting T cells, naïve and memory peripheral blood mononuclear cells

immunotherapy 79.42% (72.89; 81.71) T cells was identified, $p = 0.19$ (Fig. 2).

CD4 positive cells

In the group before treatment 33.94% (29.17; 37.54) of CD4 positive T cells were identified. After one year of SLIT 36.3% (34.23; 39.21) CD4+ lymphocytes were detected, $p = 0.48$ (Fig. 3).

CD8 positive cells

Twenty-four percent (20.3; 26.00) of CD8+ T cells were detected in group before immunotherapy, while in the group after one year of SLIT 22.94% (19.74; 25.42) CD8+ T cells were observed, $p = 0.22$ (Fig. 4).

Nevertheless significant changes in the frequency of CD4+ and CD8+ T cells were not detected after one year of SLIT, significant increase of CD4/CD8 ratio was observed. In the group before SLIT the ratio was 1.53 (1.30; 1.70), whereas after one year of treatment 1.58 (1.51; 1.84), $p = 0.0464$ (Fig. 5).

CD45RA positive mononuclear cells

In the group of asthmatics before immunotherapy 64.37% (54.12; 71.93) of CD45RA+ PBMC were identified, whereas after one year of SLIT 62.7% (59.04; 70.23) naïve PBMC were detected, $p = 0.92$.

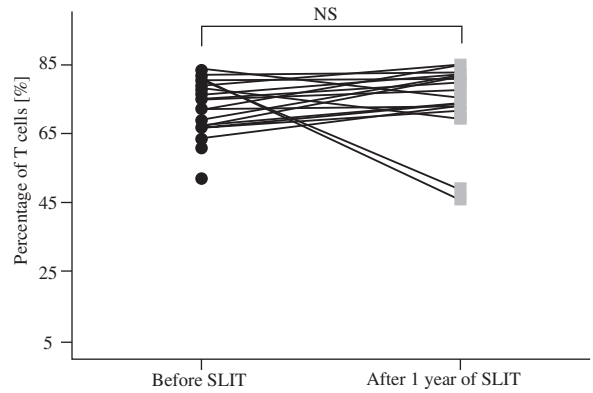
CD45RO positive mononuclear cells

26.83% (24.93; 34.22) of CD45RO+ cells were detected in the group before treatment, while after one year of SLIT 35.77% (27.05; 39.83) of memory lymphocytes were found, $p = 0.34$.

Discussion

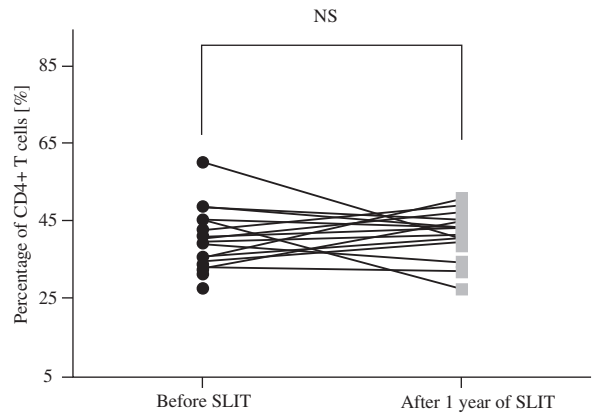
The main goal of asthma therapy is to achieve and sustain control of the disease as well as reduce inflammatory process within airways. The only effective causative treatment of atopic diseases is immunotherapy. Sublingual immunotherapy is based on administration of allergens in increased doses to desensitize effector cells against specific antigens [11, 12]. Its clinical effectiveness is already proved, but its impact on peripheral blood cells involved in asthma development is still under investigations. In the present study authors examined the impact of SLIT on peripheral blood T lymphocytes and their naïve and memory subpopulations.

Sublingual immunotherapy lasting for one year did not influence neither the frequency of CD4 positive lymphocytes nor CD8+ T cells. However the ratio CD4/CD8 after treatment increased significantly. It partially stays in line with observation made by Antúnez *et al.* They examined the impact of two years subcutaneous and sublingual immunotherapy on immunological system of children sensitized to house dust mite allergens. They observed significant increase of CD4+ cells after treatment, not noticed in the present study [9]. Additionally, they postulate that SLIT does not affect the frequency of activated lymphocytes expressing CD25 antigen. Dissimilar observation was made by these authors regarding mechanism of action of SCIT. They discovered increase of activated CD4+ lymphocytes and decrease of CD8+ lymphocytes after subcutaneous immunotherapy [9]. Opposite, other authors examining the impact of SCIT on peripheral blood lymphocytes from adults sensitized to house dust mite allergens, observed no change after 6 month of SCIT in the frequency of CD4+ nor CD8+ lymphocytes. Moreover, opposite to us, no



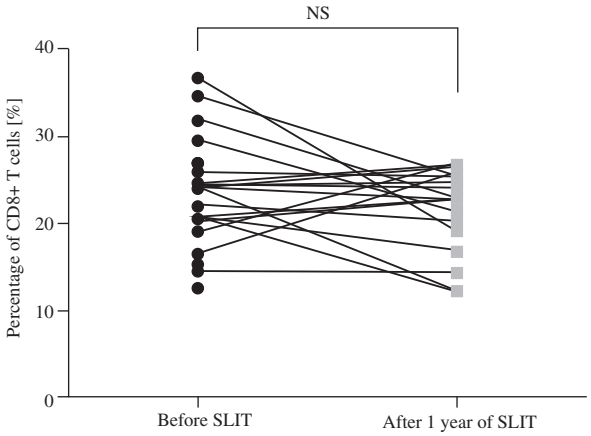
NS – not significant

Fig. 2. The frequency of T cell in peripheral blood in children suffering from asthma before and after one year of sublingual immunotherapy (SLIT)



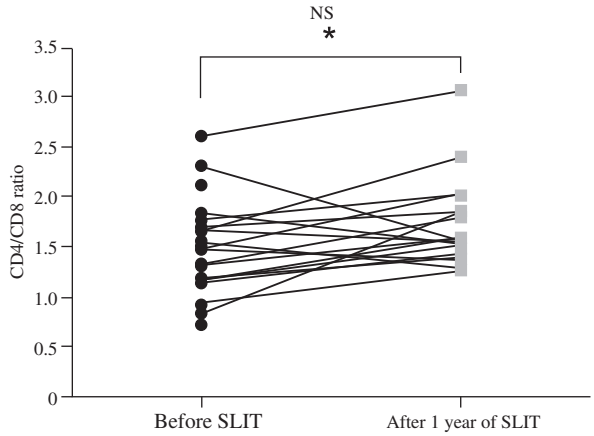
NS – not significant

Fig. 3. The frequency of CD4+ T cell in peripheral blood in children suffering from asthma before and after one year of sublingual immunotherapy (SLIT)



NS – not significant

Fig. 4. The frequency of CD8+ T cell in peripheral blood from children suffering from asthma before and after one year of sublingual immunotherapy (SLIT)



NS – not significant

Fig. 5. Comparison of CD4/CD8 ratio in peripheral blood from children suffering from asthma before and after one year of sublingual immunotherapy (SLIT)

changes in CD4/CD8 ratio was observed [13]. It should be emphasized that only 10 patients were enrolled in the described study [9]. The observed difference may results from the different age of enrolled patients, since Sade *et al*. examined adults subjects [13], and their immunological system may react differently than in children. Abovementioned authors postulate, that allergen-specific immunotherapy influence on the T cell number with specific TCR (T cell receptor) on the cells' surface. It is suggested that TCR plays a key role in the pathogenesis of atopic diseases. The presence of specific TCR on T helper cells might modify their response to alloantigens [13]. The difference between results obtained by us and other research groups might have source in the duration of treatment. It should be emphasized that no changes in analyzed parameters was observed after 6 month of treatment [13], decrease of CD4/CD8 ratio after 1 year of SLIT and increase of CD4+ T cells percentage as well as increase of CD4/CD8 ratio after 2 years of sublingual immunotherapy [9].

In the present study no impact of SLIT on CD45RA+ and CD45RO+ peripheral blood mononuclear cells was observed. We suggest that one year sublingual immunotherapy will not influence on memory nor naive cells percentage. Other authors emphasize that increase of CD45RA+ cells frequency in asthmatic patients is a desirable effect, and may be achieved for instance after auxiliary supplementation with probiotics [14]. There is no other study regarding potential SLIT impact on naïve or memory cells from peripheral blood of asthmatics. Our research is original and may have impact on the understanding of the influence of SLIT on immunological system of children suffering from atopic asthma. On the other hand, it is postulated that T cell epitope immunotherapy might cause the decrease in the frequency of allergen-specific memory lymphocytes [15]. We did not isolate allergen-specific lymphocytes nor identify their surface antigens. All CD45RA+ and CD45RO+ PBMC were examined. Probably allergen-specific subpopulation of memory T cell is very small, and subtle changes of these cells might not be visible in total lymphocytes population.

Finally, it can be concluded that difference in the frequency of T cells' subpopulation after one year of SLIT may not be visible after one year of observation and longer treatment is needed to deeply influence the immune system.

Acknowledgments

This project is co-financed by the PhD grant 1WW/NK1D/09 funded by Medical University of Warsaw and Mazovia PhD Scholarship (2008-2009) funded by Mazovia Voivodeship, Polish Government.

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

References

1. Akkoc T, de Koning PJ, Rückert B, et al. (2008): Increased activation-induced cell death of high IFN-gamma-producing T(H)1 cells as a mechanism of T(H)2 predominance in atopic diseases. J Allergy Clin Immunol 121: 652-658.
2. Bahceciler NN, Arikan C, Taylor A, et al. (2005): Impact of sublingual immunotherapy on specific antibody levels in asthmatic children allergic to house dust mites. Int Arch Allergy Immunol 136: 287-294.
3. Bohle B, Kinaciyan T, Gerstmayr M, et al. (2007): Sublingual immunotherapy induces IL-10-producing T regulatory cells,

allergenspecific T-cell tolerance, and immune deviation. J Allergy Clin Immunol 120: 707-713.
4. Burastero SE, Mistrello G, Falagiani P, et al. (2008): Effect of sublingual immunotherapy with grass monomeric allergoid on allergen-specific T-cell proliferation and interleukin 10 production. Ann Allergy Asthma Immunol 100: 343-350.
5. Cosmi L, Santarlasci V, Angeli R, et al. (2006): Sublingual immunotherapy with Dermatophagoides monomeric allergoid down-regulates allergen-specific immunoglobulin E and increases both interferon gamma and interleukin-10-production. Clin Exp Allergy 36: 261-272.
6. Leatherman BD, Owen S, Parker M, et al. (2007): Sublingual Immunotherapy: Past, present, paradigm for the future? A review of the literature. Otolaryngol Head Neck Surg 136 (Suppl 3): S1-20.
7. Marcucci F, Sensi L, Frati F, et al. (2001): Sublingual tryptase and ECP in children treated with grass pollen sublingual immunotherapy (SLIT): safety and immunologic implications. Allergy 56: 1091-1095.
8. Silvestri M, Spallarossa D, Battistini E, et al. (2002): Changes in inflammatory and clinical parameters and in bronchial hyperreactivity asthmatic children sensitized to house dust mites following sublingual immunotherapy. J Investig Allergol Clin Immunol 12: 52-59.
9. Antúnez C, Mayorga C, Corzo JL, et al. (2008): Two year follow-up of immunological response in mite-allergic children treated with sublingual immunotherapy. Comparison with subcutaneous administration. Pediatr Allergy Immunol 19: 210-218.
10. Ott H, Sieber J, Brehler R, et al. (2009): Efficacy of grass pollen sublingual immunotherapy for three consecutive seasons and after cessation of treatment: the ECRIT study. Allergy 64: 179-186.
11. www.ginaasthma.com – Global Strategy for Asthma Management and Prevention, updated 2009.
12. Zawadzka-Krajewska A. Astma oskrzelowa. In: Choroby układu oddechowego u dzieci. Kulus M (ed.). ABC Walters Kluwer Business, Lublin 2010; 268-308.
13. Sade K, Kivity S, Levy A, Fireman E (2003): The effect of specific immunotherapy on T-cell receptor repertoire in patients with allergy to house-dust mite. Allergy 58: 430-434.
14. Gutkowski P, Madaliński K, Grek M et al (2010): Effect of orally administered probiotic strains Lactobacillus and Bifidobacterium in children with atopic asthma. Centr Eur J Immunol 35: 233-238.
15. Verhoef A, Alexander C, Kay AB, Larché M (2005): T Cell Epitope Immunotherapy Induces a CD4+ T Cell Population with Regulatory Activity. PLoS Med 2: e78. doi:10.1371/journal.pmed.0020078.