VIA MEDICA

PRACA ORYGINALNA

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
Head: Prof. U. Demkow, MD, PhD
[2]Department of Paediatric Pneumonology and Allergology, Medical University of Warsaw
Head: Prof. M. Kulus, MD, PhD

The influence of sublingual immunotherapy on several parameters of immunological response in children suffering from atopic asthma and allergic rhinitis depending on asthma features

Wpływ rocznej immunoterapii podjęzykowej na wybrane parametry odpowiedzi immunologicznej u dzieci chorych na astmę atopową i alergiczny nieżyt nosa o różnej manifestacji klinicznej

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

Abstract

Introduction: The clinical efficacy of sublingual immunotherapy (SLIT) has already been proven and is known to be high. Its influence on the immunological system of patients suffering from bronchial asthma was also examined. However, it is still unclear how the polysensitisation, coexistence of other atopic disease and asthma treatment step influence the response to treatment with specific immunotherapy. Herein we evaluate the impact of one-year SLIT on selected markers of immunological response depending on different individual and clinical factors of children suffering from atopic asthma and allergic rhinitis.

Material and methods: Twenty-five patients aged 8.1 ± 3.1 years (range 5–15 years), 21 boys and 4 girls, suffering from asthma and allergic rhinitis with polysensitisation to seasonal and non-seasonal allergens, shortlisted for SLIT, were included in the study. Th1 cell and Th2 cell percentages, Bcl-2 expression in T cells, and basophil activation after allergen challenge (house dust mite and/or grass pollen antigen in solution used for skin prick tests) in peripheral blood were measured using flow cytometry. The association between clinical features of asthma and the influence of SLIT on immunological parameters was evaluated with exact Fisher test.

Results: No association between the influence of one-year sublingual immunotherapy on immunological system and patients' age, polysensitisation, asthma treatment step, or coexistence of any other atopic diseases was observed. However, an increase of the Th1 percentage in children sensitised against more than three allergens was found more often (at the limit of statistical significance) than in the group of children sensitised against three or less allergens.

Conclusions: Based on our results, we cannot point to any subgroup isolated in the study, in which the response of the immunological system to sublingual immunotherapy is more satisfactory than any other. Nevertheless, the increase of Th1 cells may be more specific for polysensitised children.

Key words: asthma, basophil activation, immunological system, sublingual immunotherapy, T helper cells

Pneumonol. Alergol. Pol. 2014; 82: 503–510

Address for correspondence: Olga Ciepiela, PhD, Department of Laboratory Diagnostics and Clinical Immunology of Developmental Age, Medical University of Warsaw, Marszalkowska 24, 00–576 Warsaw, Poland, tel./fax: +48 22 629 65 17, e-mail: olga.ciepiela@wum.edu.pl
DOI: 10.5603/PiAP.2014.0067
Praca wpłynęła do Redakcji: 8.01.2014 r.

ISSN 0867–7077

Streszczenie

Wstęp: Kliniczna skuteczność immunoterapii podjęzykowej została już gruntowanie potwierdzona i udokumentowana. Badano również jej wpływ na układ odpornościowy chorych na astmę oskrzelową. Jednak do tej pory nie wyjaśniono zależności pomiędzy występowaniem alergii wieloważnej, współwystępowaniem innych chorób alergicznych i stopniem terapii astmy a wpływem prowadzonej alergenowo-swoistej immunoterapii podjęzykowej. Celem niniejszej pracy była ocena wpływu rocznej immunoterapii podjęzykowej na wybrane parametry odpowiedzi immunologicznej w zależności od cech osobniczych i klinicznych cech choroby u pacjentów chorych na astmę i alergiczny nieżyt nosa.

Materiały i metody: Do badania zakwalifikowano 25 dzieci w wieku 8,1 ± 3,1 roku (5–15 lat), 21 chłopców i 4 dziewczynki, chorych na astmę oskrzelową i alergiczny nieżyt nosa z alergiami poliwalentnymi sezonowymi i niesezonowymi, które były zakwalifikowane do alergenowo-swoistej immunoterapii podjęzykowej. Odsetek limfocytów pomocniczych Th1 i Th2, ekspresję antygenu Bcl-2 w limfocytach T oraz aktywację bazofilów pod wpływem swoistych alergenów (zawiesiny antygenów roztoczy kurzu domowego i/lub pyłków traw do testów skórnych) badano metodą cytometrii przepływowej. Zależności pomiędzy badanymi parametrami oceniano dokładnym testem Fishera.

Wyniki: Nie zaobserwowano istotnych zależności pomiędzy wpływem immunoterapii podjęzykowej na układ odpornościowy pacjentów a wiekiem, występowaniem alergii wieloważnej, stopniem terapii astmy i współwystępowaniem innych chorób alergicznych. Wzrost odsetka limfocytów pomocniczych Th1 pod wpływem immunoterapii podjęzykowej obserwowano nieco częściej u dzieci uczulonych na więcej niż 3 alergeny niż u pozostałych.

Wnioski: Na podstawie badań przeprowadzonych przez autorów pracy nie można wskazać żadnej grupy pacjentów, u których można się spodziewać silniejszej mobilizacji układu odpornościowego jako odpowiedzi na prowadzoną immunoterapię podjęzykową u dzieci chorych na astmę i alergiczny nieżyt nosa. Wzrost odsetka limfocytów pomocniczych Th1 może występować częściej u dzieci z alergią poliwalentną.

Słowa kluczowe: astma, aktywacja bazofilów, układ odpornościowy, immunoterapia podjęzykowa, limfocyty pomocnicze

Pneumonol. Alergol. Pol. 2014; 82: 503–510

Introduction

Allergen-specific immunotherapy is an effective asthma treatment, acting on the cause rather than the symptoms of the disease. Subcutaneous immunotherapy (SCIT) has been found to bring about serious side effects. An alternative treatment, sublingual immunotherapy (SLIT), causes acute systemic reactions very rarely, and its administration is simple and safe even for children. A drug can be administered at home without supervision of an allergologist. The clinical efficacy of SLIT has already been proven, and it was found to be high. Its influence on the immunological system of patients suffering from asthma has also been examined several times [1–8].

Poor asthma control and severity of the disease have an influence on asthma symptoms and the quality of the patient's life [9]. It was observed that the airways of patients with uncontrolled asthma contain more inflammation-associated cells than do airways of patients with controlled disease [10–12]. It is still unclear how polysensitisation, coexistence of other atopic disease, and asthma treatment step influence the response to specific immunotherapy. In the present paper, subgroups of paediatric patients were examined to assess whether there is any relation between clinical features of asthma and the response of the immunological system to the allergen-specific sublingual immunotherapy administered for one year. Selected analysed parameters included Th1 and Th2 cell percentages (with regard to classic asthma cause aetiology), and apoptotic status of Th1 and Th2 cells measured by Bcl-2 expression (as it is suggested that prolonged Th2 cell life and shortened Th1 cell life may depend on apoptosis resistance or susceptibility, respectively). Assessment of Th1 and Th2 Bcl-2 positive cell percentages was interesting with regard to our previously presented results, i.e. one-year sublingual immunotherapy significantly influenced an increase of Bcl-2 expression in Th1 cells [13]. Assessment of basophils activation was performed due to their role as effector cells in allergic response to specific allergens in sensitised individuals.

The aim of this paper was to evaluate the impact of one-year SLIT on selected markers of immunological response with regard to the dependence of different individual and clinical factors of children suffering from asthma and allergic rhinitis.

Material and methods

Studied group

Twenty-five patients aged 8.1 ± 3.1 years (5–15 years olds), 21 boys and 4 girls, were included in the study. The patients (100%) suffered from controlled atopic bronchial asthma and

allergic rhinitis. In the study group 15 children (60%) had perennial allergic conjunctivitis, 8 (32%) suffered from atopic dermatitis, 4 (16%) had food allergy, and 1 (4%) had otitis media with effusion. Atopy was confirmed by skin prick test and/or the presence of allergen-specific IgE (asIgE). The exact characteristics of the enrolled children are presented in Table 1. Table 2 shows the frequency of specific IgE of at least class II in the examined population. Two children were allergic only to *Dermatophagoides pteronyssinus and Dermatophagoides farinae* antigens, another

Table 1. Characterisation of enrolled children with regard to skin-prick tests, specific IgE results, and vaccine content

Lp	asIgE, class II–VI	Positive result of skin trick test	Content of SLIT vaccine
1.	Rye, timothy, *D. pteronyssinus, D. farinae*, birch, alder	*D. pteronyssinus, D. farinae,* birch, alder, grass pollen, grain pollen, hazel, rye	Staloral 300 grass pollen/rye
2.	Rye, timothy	Grass pollen, rye	Staloral 300 grass pollen/rye
3.	Timothy, birch, dog's fur, rye	Hazel, birch, alder, grass pollen, dog's fur, rye	Staloral 300 grass pollen/rye
4.	Timothy, rye, birch	Hazel, birch, alder, grass pollen, mugwort, rye, grain pollen, plantain, beech, *D. farinae*	Staloral 300 grass pollen/rye
5.	Rye, timothy, birch, alder, hazel	Rye, grass pollen, birch, alder, hazel, grain pollen	Staloral 300 grass pollen/birch
6.	Rye, grass pollen	Rye, grass pollen, grain pollen	Staloral 300 grass pollen/rye
7.	Hazel, birch, alder, grass pollen, rye, plantain, *D. farinae, D. pteronyssinus*, feathers, guinea pig's, dog's, cat's, hamster's, rabbit's fur, penicyline, *Aspergillus, Cladosporium*	Hazel, birch, alder, grass pollen, rye, plantain, grain pollen, *D. farinae, D. pteronyssinus, Aspergillus, Cladosporium*	Staloral 300 grass pollen/rye
8.	Rye, grass pollen	Hazel, birch, alder, grass pollen, rye, grain pollen	Staloral 300 grass pollen/rye
9.	Hazel, birch, alder, grass pollen, rye, *D. farinae, D. pteronyssinus*	Hazel, birch, alder, grass pollen, grain pollen, rye, *Alternaria*	Staloral 300 alder/hazel/birch/grass pollen
10.	Rye, timothy, birch, alder	Birch, alder, grass pollen, grain pollen, rye	Staloral 300 grass pollen/rye
11.	Rye, timothy, *D. farinae, D. pteronyssinus*	*D. farinae, D. pteronyssinus,* grass pollen, rye	Staloral 300 *D. pteronyssinus/D. farinae*
12.	*D. farinae, D. pteronyssinus*	*D. farinae, D. pteronyssinus*	Staloral 300 *D. pteronyssinus/D. farinae*
13.	*D. farinae, D. pteronyssinus*	*D. farinae, D. pteronyssinus*	Staloral 300 *D. pteronyssinus/D. farinae*
14.	Rye, grass pollen	Rye, grass pollen	Staloral 300 grass pollen/rye
15.	Rye, grass pollen	Rye, grass pollen, grain pollen, cat's fur	Staloral 300 grass pollen
16.	Rye, timothy	Rye, grass pollen	Staloral 300 grass pollen/rye
17.	Hazel, birch, alder	Hazel, birch, alder, grass pollen	Staloral 300 alder/hazel/birch/grass pollen
18.	Dog's fur, grass pollen	Dog's fur, grass pollen	Staloral 300 grass pollen
19.	Hazel, birch, alder, grass pollen, rye, plantain, mugwort, dog's fur	Hazel, birch, alder, grass pollen, grain pollen, rye, plantain, mugwort, dog's fur	Staloral 300 grass pollen/rye
20.	Hazel, birch, alder, mugwort, timothy, rye	Hazel, birch, alder, grass pollen, mugwort, grain pollen, rye	Staloral 300 alder/hazel/birch/grass pollen
21.	Rye, grass pollen, mugwort	Rye, grass pollen, *Alternaria*	Staloral 300 grass pollen/rye
22.	Hazel, birch, alder, timothy	Hazel, birch, alder, grass pollen, grain pollen, rye	Staloral 300 grass pollen/rye
23.	Hazel, birch, alder, mugwort, timothy, rye, dog's fur, cat's fur	Hazel, birch, alder, grass pollen, grain pollen, rye, mugwort	Staloral 300 alder/hazel/birch/grass pollen
24.	Hazel, birch, alder, rye, grass pollen	Hazel, birch, alder, grass pollen, grain pollen, rye	Staloral 300 grass pollen/rye
25.	Hazel, alder, *D. farinae, D. pteronyssinus*, *Alternaria, Cladosporium,* hamster's fur	–	Staloral 300 *D. pteronyssinus/D. farinae*

Table 2. The frequency of specific IgE in the studied group

Allergen	Number of patients
Birch pollen	14 (56%)
Black alder pollen	15 (60%)
Hazel pollen	12 (48%)
Grass pollen	22 (88%)
Rye pollen	19 (76%)
Antigens of house dust mite *D. pteronyssinus*	7 (28%)

8 were sensitised against grass and tree pollens. Nine of the included children were sensitised against seasonal and non-seasonal allergens (i.e. grass pollens and animal fur, tree pollens, and house dust mites). Thirteen children (52%) had family history of atopic asthma. Patients were treated chronically with:

— leukotriene receptor antagonists — 11 (44%),
— antihistamine drugs — 24 (96%),
— inhalational corticosteroids — 15 (60%),
— intranasal steroids — 11 (44%),
— short-acting beta agonists — 25 (100%),
— long-acting beta agonists — 5 (20%).

Exclusion criteria were a history of other immunological or haematological disorders, severe infectious diseases, or systemic corticosteroids administered less than four weeks before the blood collection.

Evaluation of all studied immunological parameters was performed at baseline and after 12 months of SLIT. As allergen-specific immunotherapy, Staloral 300 (Ewopharma AG, France) at a concentration of 300 index of biological reactivity/mL (IR/mL) was used (according to Tab. 1). The administration included an 11-day induction phase followed by a maintenance phase of 240 IR of allergen solution two times per week. Nineteen children were examined after 12.8 ± 2.6 months of SLIT (the parents of six children did not agree to test repetition). One child respond with major side effects in the maintenance phase (itching in the mouth, nausea, sneezing) and the scheme of his treatment was modified to administration of 30 IR of vaccine daily instead of 240 IR weekly, administered in two doses.

Flow cytometry

To evaluate the influence of one-year sublingual immunotherapy on the immunological system of children with asthma, selected parameters in peripheral blood were measured by flow cytometry. Th1 cell percentage was measured by evaluation of surface CCR5 antigen on CD4-positive lymphocytes, Th2 cell percentage was measured by evaluation of surface CRTH2 antigen on CD-4-positive lymphocytes. Change of Th1 and Th2 cell percentages within CD4 positive cells after one year of sublingual immunotherapy was recognised as an increase or decrease of appropriate T helper cell population. The intracellular expression of antiapoptotic Bcl-2 protein was measured in Th1 cells. Spontaneous basophil activation and activation after allergen challenge was evaluated by flow cytometric basophil activation test using CD203c antigen as a marker of cells activation. Allergens (Stallergenes, France) dedicated to skin-prick tests were used for the stimulation test (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* or/and grass pollens in concentration of 0.2 IR/mL in phosphate buffered saline). For children sensitised both to house dust mites and grass pollen, two basophil activation tests with different allergens were performed. Decreased number of CD203-positive basophils after allergen challenge studied before and after one year of SLIT was recognised as a decrease in stimulated basophil activation. Flow cytometric analysis was performed with a Cytomics FC500 flow cytometer (Beckman Coulter, USA).

Statistical analysis

The Wilcoxon Match Pair test was used to compare non-parametric data from patients before and after one year of SLIT. For analysis, percentages of T cells (Th1, Th2, Th1-Bcl-2+, and Th2-Bcl-2+ cells) and percentage of activated basophils were compared. A P value less than 0.05 was considered as significant for all statistical analyses. The association between clinical features of asthma and influence of SLIT on immunological parameters was evaluated with exact Fisher test.

Results

Association between children's age and response of immunological system after 12 months of sublingual immunotherapy

Children from the studied group were divided into two subgroups, dependent on the patient's age. Into the first subgroup, 13 children aged 5–10 years old were enrolled. To the second subgroup, 6 children aged 10–15 years old were enrolled. Results of analysis are presented in Table 3. No association between patients' age and response of immunological system after 12 months of sublingual immunotherapy was found.

Table 3. The association between immunological response after 12 months of SLIT and children's age

Analysed parameter	Age 5–10 years old n (%)	Age 10.5–15 years old n (%)	Statistical significance
Increase of Th1 cells percentage	9/13 (69%)	4/6 (66%)	p = 1
Decrease of Th2 cells percentage	7/13 (54%)	3/6 (50%)	p = 1
Increase of Th1 cells with intracellular expression of Bcl-2 percentage	9/13 (69%)	4/6 (66%)	p = 1
Decrease of spontaneous basophil activation	10/13 (77%)	4/6 (66%)	p = 1
Decrease of basophil activation after allergen challenge	*Number and percentage of tests* 12/20 (60%)	*Number and percentage of tests* 4/8 (50%)	p = 0.69

Table 4. The association between immunological response after 12 months of SLIT and asthma treatment step

Analysed parameter	1 and 2 asthma treatment step n (%)	3 and 4 asthma treatment step n (%)	Statistical significance
Increase of Th1 cells percentage	8/11 (73%)	4/5 (80%)	p = 1.0
Decrease of Th2 cells percentage	8/12 (67%)	2/6 (33%)	p = 0.32
Increase of Th1 cells with intracellular expression of Bcl-2 percentage	7/11 (64%)	3/5 (60%)	p = 1.0
Decrease of spontaneous basophil activation	8/13 (61%)	6/6 (100%)	p = 0.13
Decrease of basophil activation after allergen challenge	*Number and percentage of tests* 11/19 (58%)	*Number and percentage of tests* 5/9 (56%)	p = 1.0

Association between asthma treatment step and response of immunological system after 12 months of sublingual immunotherapy

Children suffering from asthma were divided two subgroups, depending on asthma treatment step. Asthma treatment steps were classified in accordance with the Global Initiative for Asthma 2014 recommendations regarding necessity of reliever medication. Thirteen children with 1 and 2 asthma treatment steps were enrolled into the first subgroup; to the second subgroup, 6 children with 3 and 4 asthma treatment steps were enrolled. Results of analysis are presented in Table 4. No association between asthma treatment step and response of immunological system after 12 months of sublingual immunotherapy was found.

Association between asthma and allergic rhinitis coexistence with other allergic diseases and response of immunological system after 12 months of sublingual immunotherapy

The studied group was divided into two subgroups, depending on the allergic diseases that coexisted with atopic asthma and allergic rhinitis. Eleven children were enrolled into the first subgroup. They suffered from atopic asthma (AA), allergic rhinitis (AR), and allergic conjunctivitis (AC). Eight children were enrolled into the second group with AA, AR, and AC with coexistence of atopic dermatitis (AD), food allergy (FA) and others. Results of analysis are presented in Table 5. No association between analysed clinical features of asthma and response of immunological system after 12 months of sublingual immunotherapy was found.

Association between polysensitisation of children with asthma and response of immunological system after 12 months of sublingual immunotherapy

Children from the studied group were divided into two subgroups, depending on the number of positive reactions in allergen-specific IgE test. Into the first subgroup 10 children sensitized to three or fewer allergens were enrolled (only seasonal or only non-seasonal). Into second subgroup 9 polysensitised children (with more than three positive allergen-specific IgE results — for seasonal and non-seasonal allergens) were enrolled. Results of analysis are presented

Table 5. The association between immunological response after 12 months of SLIT and presence of diseases coexisting with asthma and allergic rhinitis

Analysed parameter	Children with AA, AR and AC n (%)	Children with AA, AR and AC + AD, FA and others n (%)	Statistical significance
Increase of Th1 cells percentage	6/11 (55%)	6/8 (75%)	p = 0.58
Decrease of Th2 cells percentage	3/11 (27%)	5/8 (63%)	p = 0.18
Increase of Th1 cells with intracellular expression of Bcl-2 percentage	5/11 (45%)	6/8 (75%)	p = 0.31
Decrease of spontaneous basophil activation	7/11 (64%)	7/8 (88%)	p = 0.34
Decrease of basophil activation after allergen challenge	*Number and percentage of tests* 10/15 (67%)	*Number and percentage of tests* 6/13 (46%)	p = 0.44

AA — allergic asthma, AR — allergic rhinitis, AC — allergic conjunctivitis, AD — atopic dermatitis, FA — food allergy

Table 6. The association between immunological response after 12 months of SLIT and presence of polysensitisation in children suffering from asthma

Analysed parameter	≤ 3 as IgE (number and percentage of children)	> 3 as IgE (number and percentage of children)	Statistical significance
Increase of Th1 cells percentage	4/9 (44%)	9/10 (90%)	p = 0.0572
Decrease of Th2 cells percentage	4/9 (44%)	5/10 (50%)	p = 1.0
Increase of Th1 cells with intracellular expression of Bcl-2 percentage	3/9 (33%)	3/10 (30%)	p = 1.0
Decrease of spontaneous basophil activation	6/9 (67%)	8/10 (80%)	p = 0.63
Decrease of basophil activation after allergen challenge	*Number and percentage of tests* 6/11 (55%)	*Number and percentage of tests* 11/17 (65%)	p = 0.70

in Table 6. No association between the number of positive reactions in as-IgE tests in children with asthma and the response of immunological system after 12 months of sublingual immunotherapy was found. However, an increase in the Th1 percentage in children sensitised against more than three allergens was found more often (at the limit of statistical significance) than in group of children sensitised against three or less allergens. As we have previously shown, an increase in Th1 cell percentage after one year of sublingual immunotherapy was significant for the entire studied group [13]. Based on the present analysis it can be concluded that an increase of Th1 cells is more specific for polysensitised children.

Discussion

The results of studies all over the world are not in agreement according to the effects of SLIT with regard to the dependence of the type of allergens to which studied groups are sensitised. Meta-analyses made in accordance with studies performed in children and adults indicated greater efficacy of SLIT in the group of patients sensitised to house dust mites than in groups sensitised to grass pollen or other seasonal allergens. On the other hand, another meta-analysis performed on the basis of studies in children showed than SLIT administered for treatment of pollen allergy is more effective than in house dust mite sensitisation [14]. There is also no compromise regarding administration of multi-allergen or single-allergen vaccination. Studies from all over the world show sufficient efficacy of SLIT with single allergens. Only a limited number of investigations have been performed in SLIT with multi-allergens. The usage of multi-allergen SLIT would be more eligible, since polysensitisation is more prevalent than sensitisation to a single allergen [15]. Studies performed by Emminger et al. showed that single-allergen SLIT indicates the same efficacy in patients suffering from monosensitisation as well as polysensitisation [16]. However, it

cannot be clearly defined which of those two methods for immunotherapy is more effective [14]. A study with 628 adult patients showed no difference in efficacy (measured with the Rhinocojunctivitis Total Symptom Score) of sublingual immunotherapy administered for two years between groups of patients suffering only from allergic rhinocojunctivitis (ARC) and ARC with coexistence of bronchial asthma. The same investigation also showed no difference in efficacy of SLIT in patients who were mono- and polysensitised [17].

We did not observe any difference in SLIT action in dependence of patients' age. However, it has been suggested that the efficacy of allergen-specific treatment is higher in adult patients than in children [18]. On the other hand, Compalati et al. and Han et al. did not find any difference in SLIT efficacy between children and adults [19, 20].

In the present study we analysed the impact of SLIT on the immunological system of patients with controlled asthma at different treatment steps. We did not find any difference in SLIT impact on immunological system between children with 1–2 steps of treatment compared to those with 3–4 asthma treatment steps. Other researchers studied the response to SLIT with regard to asthma symptoms intensity and found that allergic patients with more severe symptoms during the pollen season responded more intensely to sublingual immunotherapy than did patients with weaker symptoms [21, 22].

Our study confirms that there is no difference in the influence of sublingual immunotherapy on the immunological system of children with bronchial asthma and allergic rhinitis depending on age, coexistence of other atopic diseases, asthma treatment step, and type of allergy (mono- or polysensitisation). Based on our results we cannot point to any subgroup of children in whom response of immunological system to sublingual immunotherapy is more satisfactory than any other. The increase of Th1 percentage in children sensitised against more than three allergens was found more often than in the group of children sensitised against three or fewer allergens; nevertheless, the number of patients in the compared subgroups was probably too small to reach statistical significance. It should also be emphasised that the efficacy of SLIT and its impact on the immunological system is mainly dependent on the time (at least three years) and regularity of administration [8], as well as on the dosage of vaccine administered [17].

Acknowledgments

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

Conflict of interest

The authors declare no conflict of interest.

References:

1. Antunez C., Mayorga C., Corzo J.L., Jurado A., Torres M.J. Two year follow-up of immunological response in mite-allergic children treated with sublingual immunotherapy. Comparison with subcutaneous administration. Pediatr. Allergy Immunol. 2008; 19: 210–218.
2. Bahceciler N.N., Arikan C., Taylor A. et al. Impact of sublingual immunotherapy on specific antibody levels in asthmatic children allergic to house dust mites. Int. Arch. Allergy Immunol. 2005; 136: 287–294.
3. Bohle B., Kinciyan T., Gerstmayr M., Radakovics A., Jahn -Schmid B., Ebner C. Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergenspecific T-cell tolerance, and immune deviation. J. Allergy Clin. Immunol. 2007; 120: 707–713.
4. Burastero S.E., Mistrello G., Falagiani P. et al. Effect of sublingual immunotherapy with grass monomeric allergoid on allergen-specific T-cell proliferation and interleukin 10 production. Ann. Allergy Asthma Immunol. 2008; 100: 343–350.
5. Cosmi L., Santarlasci V., Angeli R. et al. Sublingual immunotherapy with Dermatophagoides monomeric allergoid down -regulates allergen-specific immunoglobulin E and increases both interferon gamma and interleukin-10-production. Clin. Exp. Allerg. 2006; 36: 261–272.
6. Leatherman B.D., Owen S., Parker M. et al. Sublingual Immunotherapy: Past, present, paradigm for the future? A review of the literature. Otolaryngol. Head Neck Surg. 2007; 136 (suppl. 3): S1–S20.
7. Ott H., Sieber J., Brehler R. et al. Efficacy of grass pollen sublingual immunotherapy for three consecutive seasons and after cessation of treatment: the ECRIT study. Allergy 2009; 64: 179–186.
8. Wahn U., Tabar A., Kuna P. et al. SLIT Study Group: Efficacy and safety of 5-grass pollen sublingual immunotherapy tablets in pediatric allergic rhinoconjunctivitis. J. Allergy Clin. Immunol. 2009; 123: 160–166.
9. Gonzalez-Barcala F.J., de la Fuente-Cid R., Tafalla M., Nuevo J., Caamaño-Isorna F. Factors associated with health-related quality of life in adults with asthma. A cross-sectional study. Multidiscip. Respir. Med. 2012; 7: 32. doi: 10.1186/2049--6958-7-32
10. He X.Y., Simpson J.L., Wang F. Inflammatory phenotypes in stable and acute childhood asthma. Paediatr. Respir. Rev. 2011; 12: 165–169.
11. Bime C., Nguyen J., Wise R.A. Measures of asthma control. Curr. Opin. Pulm. Med. 2012; 18: 48–56.
12. Al-Muhsen S., Johnson J.R., Hamid Q. Remodeling in asthma. J. Allergy Clin. Immunol. 2011; 128: 451–462.
13. Ciepiela O., Zawadzka-Krajewska A., Kotuła I., van Overveld F., Kulus M., Demkow U. Sublingual immunotherapy for asthma: affects T-cells but does not impact basophil activation. Pediatr. Allergy Immunol. Pulmonol. 2014; 27: 17–23.
14. Incorvaia C., Di Rienzo A., Celani C., Makrì E., Frati F. Treating allergic rhinitis by sublingual immunotherapy: a review. Ann. Ist. Super Sanita 2012; 48: 172–176.
15. Calderón M.A., Cox L., Casale T.B., Moingeon P., Demoly P. Multiple-allergen and single-allergen immunotherapy stra-

tegies in polysensitized patients: looking at the published evidence. J. Allergy Clin. Immunol. 2012; 129: 929–934.
16. Emminger W., Durham S.R., Riis B., Maloney J., Nolte H. The efficacy of single-grass-allergen-immunotherapy-tablet treatment in mono- and multi-sensitized allergic rhinitis patients: findings from a post hoc analysis. J. Allergy Clin. Immunol. 2009; 123 (suppl.): S75.
17. Didier A., Malling H.J., Worm M. et al. Optimal dose, efficacy, and safety of once daily sublingual immunotherapy with a 5-grass pollen tablet for seasonal allergic rhinitis. J. Allergy Clin. Immunol. 2007; 120: 1338–1345.
18. Di Bona D., Plaia A., Scafidi V. et al. Efficacy of sublingual immunotherapy with grass allergens for seasonal allergic rhinitis: a systematic review and meta-analysis. J. Allergy Clin. Immunol. 2010; 126: 558–566.
19. Compalati E., Passalacqua G., Bonini M., Canonica G.W. The efficacy of sublingual immunotherapy for house dust mites respiratory allergy: results of a GA2LEN meta-analysis. Allergy 2009; 64: 1570–1579.
20. Han D.H., Choi Y.S., Lee J.E. et al. Clinical efficacy of sublingual immunotherapy in pediatric patients with allergic rhinitis sensitized to house dust mites: comparison to adult patients. Acta Otolaryngol. 2012; 132 (suppl. 1): S88–S93.
21. Malling H.J., Montagut A., Melac M. et al. Efficacy and safety of 5-grass pollen sublingual immunotherapy tablets in patients with different clinical profiles of allergic rhinoconjunctivitis. Clin. Exp. Allergy 2009; 39: 387–393.
22. Viswanathan R.K., Busse W.W. Allergen immunotherapy in allergic respiratory diseases: from mechanisms to meta-analyses. Chest 2012; 141: 1303–1314.