

# Specific sublingual immunotherapy decreases spontaneous and stimulated basophils degranulation. Preliminary studies

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## Abstract

**Background:** Atopic asthma is a chronic inflammatory disease. The symptoms of asthma and allergy are caused by histamine and numerous cytokines released by basophils degranulated after IgE cross-linking on the cell surface. It is postulated that specific immunotherapy can reverse disturbed immunoregulation as well as suppress basophil activation. Sublingual immunotherapy is postulated to be effective in atopic asthma, although information about its influence on cellular response is limited. The follow-up of the therapy might be measured by basophil's activation markers e.g. CD203c antigen. Its expression on the cells surface can be measured by flow cytometry.

**Aim:** The aim of the study was to evaluate CD203c expression on resting and stimulated basophils before and after twelve months sublingual immunotherapy.

**Materials and Methods:** Venous blood from 6 asthmatic children aged of  $8 \pm 2.4$  years old was analyzed. Antigen CD203c expression on basophils was measured by Allergen Kit (Beckman Coulter) with Cytomics FC500 flow cytometer (Beckman Coulter). Patients were allergic to *Dermatophagoides pteronyssinus* antigens (positive skin prick tests) and were examined before and after twelve months of sublingual immunotherapy (Staloral 300, France). CD203c expression was measured before and after stimulation with specific allergens (Stallergenes, France).

**Results:** Level of idiopathic activation (presence of CD203c on basophils before allergen stimulation measured by mean fluorescence of fluorescent dye bound with cell surface antigen) in patients before specific immunotherapy was  $7.85 \pm 5.17$  MCF units, whereas after a year of immunotherapy the result was  $4.22 \pm 1.14$  MCF,  $p < 0.05$ .

After specific allergen's stimulation presence of CD203c was detected on  $42.26 \pm 5.12\%$  basophils, whereas after one year of sublingual immunotherapy expression of CD203c was detected on  $25.52 \pm 12.8\%$  cells,  $p < 0.05$ .

**Conclusions:** Based on the preliminary results it can be concluded that sublingual immunotherapy is efficient in desensitization of basophils from *D. pteronyssinus* allergic patients.

**Key words:** sublingual immunotherapy (SLIT), CD203c, basophils, flow cytometry, *Dermatophagoides pteronyssinus*.

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## Introduction

Basophils and mast cells are the main effector cells responsible for IgE-dependent reactions. High affinity receptors for IgE immunoglobulin (FcεRI) are present on the

surface of those cells. When receptors bind IgE cross-linked with allergens, intracellular chains cross over and the cell degranulate. A large panel of mediators including cytokines and histamine, is released into extracellular space due to

degranulation process. In addition, changes in the expression of basophil surface's molecules are observed: some antigens appear *de novo* (CD63, CD107a), and other increase their expression (CD13, CD203c). The CD203c antigen is one of the most important marker of basophil's activation. This protein (ectonucleotide pyrophosphatase/phosphodiesterase 3, E-NPP3, PD-I $\beta$ , B10, gp130<sup>RB13-6</sup>) belongs to type I phosphodiesterase/nucleotide pyrophosphatase family [1]. Its physiological function is unknown. The CD203c was found exclusively on basophils and mast cells. Its expression increases after basophil's degranulation and therefore is a reliable marker of basophil's activation in IgE-dependent process. Resting basophils are characterized by low expression of this protein and the expression rapidly increases when activated [2, 3].

The only effective causative treatment of atopic diseases is immunotherapy [4]. Subcutaneous immunotherapy (SCIT) was first described in late 60's of XX century [5]. The method is based on injection of allergens in increased concentration to desensitize effector cells against specific antigens [6]. Unfortunately SCIT dosage is associated with possibility of anaphylactic reactions to allergens and has to be delivered in hospital [4]. Sublingual immunotherapy (SLIT) is an alternative to subcutaneous route of delivery. There is a few information about acute systemic reactions after SLIT [7], this route of delivery is patient friendly (especially in childhood) and can be administered at home [4]. Clinical efficacy of SLIT was already proved, but there is still limited number of studies about its influence on basophils activation process.

The aim of the study was to evaluate idiopathic and stimulated CD203c expression on basophils before and after twelve months of specific sublingual immunotherapy.

## Materials and Methods

Six individuals aged of  $8 \pm 2.4$  years old suffering from atopic disease which was confirmed with skin prick test (positive to *Dermatophagoides pteronyssinus* allergens), shortlisted for specific immunotherapy, served as studied group. Neither of analyzed subjects was treated with antihistamine drugs or oral corticosteroids. 1 ml blood taken by venipuncture in tubes containing EDTA was collected. Tests were performed before and after twelve months of specific sublingual immunotherapy. Patients were taking Staloral 300 in concentration of 300 IR/ml as often as prescribed by physician. The experiments were approved by the Ethics Commission of Medical University of Warsaw. Blood was collected with parents approval.

### Basophil's activation tests

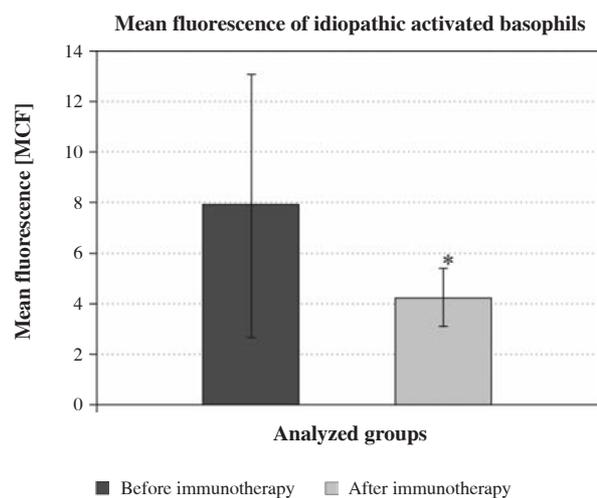
All tests were carried out within two hours from blood collection. Anticoagulated blood was used for complete blood count analysis. Residual sample (400  $\mu$ l) was used for basophil's activation test. CD203c induced expression was evaluated using the Allergenicity Kit (Beckman Coulter)

according to the manufacturer's instructions. Allergens (Stallergenes, France) used for the test were prepared in 1:500 dilution of concentration used for skin prick tests in phosphate buffered saline (PBS) [8].

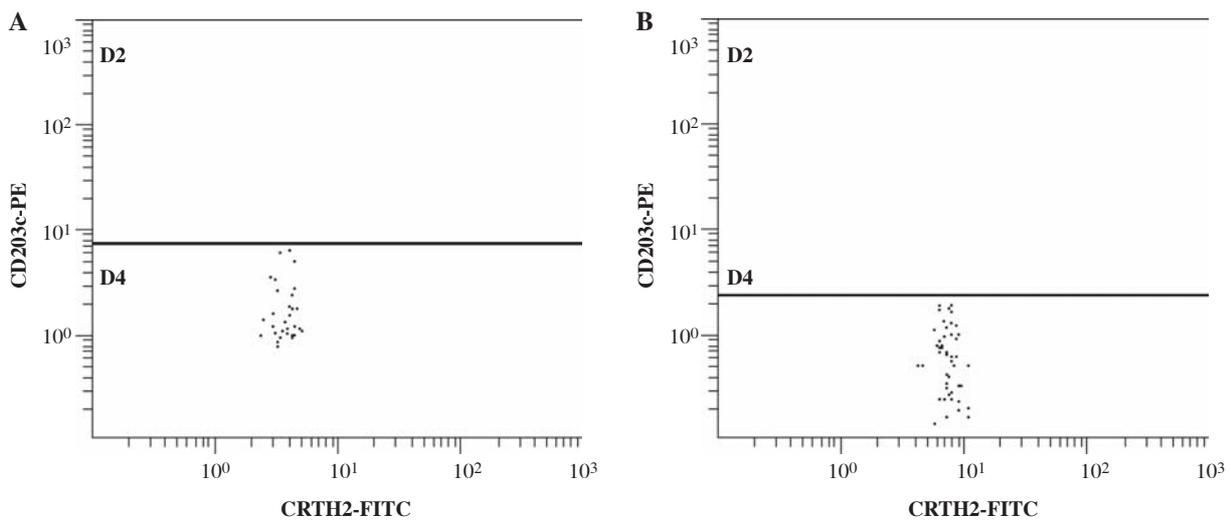
Briefly, EDTA-anticoagulated peripheral blood aliquots (100  $\mu$ l) stained with 20  $\mu$ l of mixture of monoclonal antibodies (CRTH<sub>2</sub>-FITC, CD203-PE, CD3-PC7) and Activation solution (100  $\mu$ l) were stimulated (37°C) for 15 min with 20  $\mu$ l of optimal dilution of allergens; antibody directed against the high affinity IgE receptor (Fc $\epsilon$ RI) (Beckman Coulter) was used as positive control and PBS as a negative control. After incubation the reaction was stopped with Stop solution. Erythrocytes were lysed with Lysing solution for 10 minutes in room temperature (RT). Suspension was centrifuged (5 min, 300 g) after lysing, washed with PBS, once more centrifuged and resuspended in 500  $\mu$ l Fixative solution. Leukocytes were analyzed using a five-color flow cytometer (Cytomics FC500, Beckman Coulter). During acquisition basophils were selected as CD203c positive/CRTH2 high/CD3 negative population using FL1/FL2 and SS/FL5 dot plots. In negative control threshold for positivity was set at less than 5% of activated cells according to the literature data [1, 9]. In positive control sensitivity for IgE dependent reaction was verified. Threshold for positive reaction was settled at less than 15% of activated cells, according to literature data [1, 10] and investigators studies' results.

### Statistical analysis

Results are presented as a mean  $\pm$  SD. Statistical analysis was performed using the Wilcoxon matched pair test. A p value of less than 0.05 was considered significant.



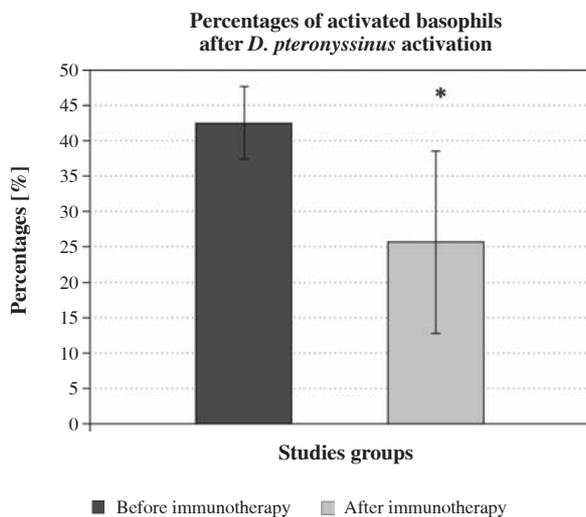
**Fig. 1.** Mean fluorescence of spontaneous activated basophils before and after one year sublingual immunotherapy. Results are statistically relevant,  $p < 0.05$



**Fig. 2.** Spontaneous basophil activation is decreased after twelve months immunotherapy. A. Level of spontaneous activation before immunotherapy; B. Level of spontaneous activation after immunotherapy (example)

**Results**

The number of resting basophils with presence of CD203c antigen on cell surface was counted before allergen stimulation. The mean fluorescence of fluorochrome bound to CD203c was measured. In patients before specific immunotherapy mean fluorescence of spontaneously activated basophils was  $7.85 \pm 5.17$  MCF units, whereas after the year of immunotherapy the result was  $4.22 \pm 1.14$  MCF,  $p < 0.05$  (Fig. 1).



**Fig. 3.** Percentages of activated basophils after stimulation with *D. pteronyssinus* antigens in atopic patients before and after one year immunotherapy. Results are statistically relevant,  $p < 0.05$

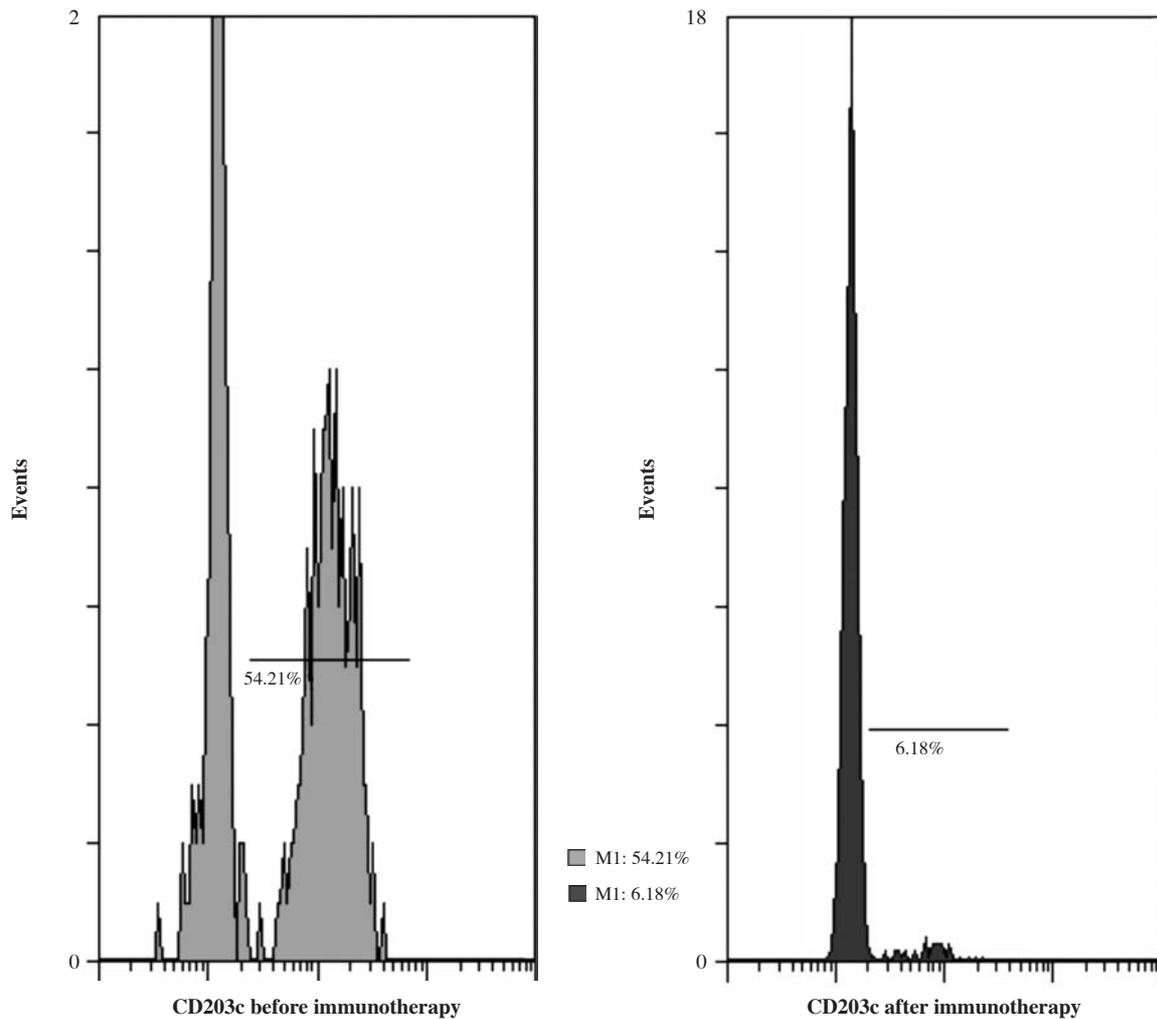
Average change is presented in Fig. 2. The D2 regions contain less than 5% of cells, as it is established for negative control.

The number of basophils with CD203c presence was evaluated to assess patients sensitivity to *D. pteronyssinus* antigens stimulation. Presence of CD203c was detected on  $42.26 \pm 5.12\%$  basophils after incubation with specific allergens, whereas after one year of sublingual immunotherapy presence of CD203c was detected on  $25.52 \pm 12.8\%$  cells,  $p < 0.05$ .

The change in the number of CD203c positive basophils stimulated with *D. pteronyssinus* in the samples from selected patient before and after twelve month immunotherapy is presented in Fig. 4. Before SLIT 54.21% of basophils were activated after allergen stimulation, whereas after immunotherapy only 6.18% of basophils degranulated under the influence of specific antigen's extract.

**Discussion**

There is an increased interest in SLIT all over the world. Sublingual immunotherapy, which can be administered at home and carry a very low risk of anaphylactic response as a side effect, is a promising alternative for subcutaneous immunotherapy [11]. It is postulated that SLIT is attractive option, especially for children. Its clinical efficacy has been reported recently. Improved quality of life, reduced drug intake (antihistamine and inhalant steroids) and decreased incidence of allergic reaction after SLIT was widely described [12]. Rodriguez-Perez et al. have summarized results of research in which effectiveness of 18 months SLIT is evident. Nevertheless this type of immunotherapy was effective only in grass pollen allergy, but not house dust



**Fig. 4.** Example of changes in CD203c expression on basophils stimulated with *D. pteronyssinus* antigens before and after immunotherapy

mite [13, 14]. Immunological effect of SLIT are evaluated rarely, especially in children's population.

Specific immunotherapy is proved to decrease total and specific IgE concentrations. While the specific IgG4 concentration increases, even up to 30-fold after one year of desensitization. It has to be pointed that only high dose SLIT caused satisfactory effect [15, 16]. Variety of immunological markers have been analyzed to prove efficacy of specific immunotherapy. Investigators tested serum concentration of ECP (eosinophil cationic protein), the marker of eosinophils degranulation, to evaluate decrease of eosinophils activity. The ECP concentrations were decreased after 6, 12 and 24 month of SLIT. Moreover eosinophil's binding to ICAM-1 particles was declined, which may explain decrease of number of asthmatic incidences. Reduction of IL-13, cytokine, released from Th2 lymphocytes, was observed as well as

increased Th1/Th2 ratio. It indicates that long lasting SLIT can influence immunoregulation [5]. On the other hand short-term SLIT, in spite of clinical efficacy, neither restores immunological balance in released cytokines profile nor alters levels of allergen-specific immunoglobulins E [17].

The goal of the present paper was to assess one year sublingual immunotherapy effect on CD203c basophil's activation marker. The paper is very original as no comparative data were found. Although it was described that histamine release (secondary to basophils degranulation) is reduced in patients after SCIT [5], no publication reporting histamine release changes after SLIT treatment was found.

Our findings are in the line with observations that one year long SLIT is clinically effective [18]. Reduction of resting and post-stimulatory basophils activation level may be related to clinical efficacy. Usually patients after SLIT more rarely

take antihistamine drugs or oral corticosteroids which suppress allergic response and as the result evaluation of their quality of life is raised comparing to placebo group [12]. Further analysis of the studied group will be performed.

The mechanism of sublingual immunotherapy is largely unknown. The allergens from the vaccinia cross mucosal barrier and are potentially directed to gut-associated lymphoid tissue (GALT) where are exposed to immunological system [5]. The contact leads to development of regulatory T cells secreting TGF- $\beta$  [11,19]. Moreover the exposure of allergens to immunological system causes increased production of specific IgG4. IgG4 immunoglobulin prevents allergen's binding to IgE, which is the direct mechanism of basophils degranulation's decrease. Immunotherapy influences also on B-cell production of IgA. Increase of IgA concentration enhances mucosal barrier and prevents allergen's penetration. It is also postulated that SLIT can sensitize Th2 cells to apoptosis, which might be the mechanism of restoring Th1/Th2 balance [20].

Our paper sheds light into mechanisms of the clinical efficacy of sublingual immunotherapy to *D. pteronyssinus* allergens on cellular level. As it is postulated that SLIT modifies immune response in different way than SCIT [21], further investigations are needed to assess influence of SLIT on immunological system.

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