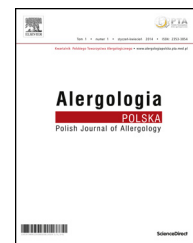


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Komentarz/Commentary

Early effects of ultra-rush wasp-venom immunotherapy on the expression of CD25 on CD4+ T cells



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ABSTRACT

To date, early effects of specific immune therapy on the parameters of adaptive immune responses in hymenoptera venom allergic patients remain unclear. Therefore, in this pilot study, we aimed to evaluate early changes in the levels of CD25 expression within CD4+ T cells occurring in the course of ultra-rush wasp venom immunotherapy. To this end, ten wasp venom-sensitive patients subjected to ultra-rush immune therapy were recruited for the study. CD4+ T cells and CD25 expression were assessed before the start of treatment, 24 h later and on day 120 with the use of monoclonal antibodies directed at CD4, CD25 (IL-2 receptor) by the means of multi-color flow cytometry. We have demonstrated non-significant decrease in the intensity of CD25 expression on the surface of CD4+ T cells at day 1 but not day 120 of specific immune therapy. Our study suggests that putative early immunosuppressive mechanisms of SIT are not related to dramatic changes in the expression of CD25 on CD4+ T cells.

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Introduction

Allergen-specific immune therapy (SIT) is the unique type of treatment that can induce tolerance to allergens in sensitized patients including those allergic to hymenoptera venom. Many of the long-term immune effects of SIT have traditionally been linked to modulation of CD4+ T cells, mainly those with regulatory phenotype characterized by the

expression of CD25 and/or FoxP3. Recently, hymenoptera venom SIT was shown to upregulate proliferation of regulatory T cells (Treg) (CD4+ cells with CD127-CD25+ phenotype, expressing Foxp3). Earlier experiments revealed that hymenoptera venom SIT enhanced numbers of circulating regulatory T cells. However, to date, less is known about the early effects of SIT on potential alterations of CD4+ T cells with CD25 expression. Thus, in the current study we aimed to evaluate the effects of ultra-rush wasp venom specific

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immune therapy on the intensity of CD25 expression on the surface of CD4⁺ T cells in first days and weeks of the treatment.

Materials and methods

Patients

Ten wasp venom-sensitive patients subjected to ultra-rush immune therapy with the use of Venomenhal Wespe (HAL Allergy) were recruited for the study. Peripheral blood mononuclear cells (PBMC) were isolated from EDTA-anticoagulated blood three times: up to 60 min before the first injection, 24 h later and on day 120.

Immunostaining

Staining of 150 µl blood samples was performed with the use of 5 µl of fluorochrome-labeled monoclonal antibodies: anti-CD4 FITC and anti-CD25 PE-Cy5 (BD Bioscience). Following incubation erythrocytes were lysed using Lysing Solution (BD Bioscience) and washed twice in phosphate-buffered saline (PBS, Biomed Lublin). Flow cytometric data were acquired on FACS Calibur (Becton-Dickinson, San Jose, CA, USA), and analyses were performed with the use of FlowJo software (Three Star Inc.). Unstained and fluorescence minus one (FMO) controls were used to establish proper gating of CD4⁺CD25⁺ cells (Fig. 1).

Statistical analysis

GraphPad Prism 5 software (GraphPad Software Inc., San Diego, CA, USA) and Wilcoxon matched pairs test (due to non-normal distribution) were used for statistical analysis of the data. Data were presented as frequency of specific markers within T cells, or as mean fluorescence intensity (MFI) of these markers within each CD4⁺ lymphocyte.

Table I – Tabular results of CD25 frequency and mean fluorescence intensity (MFI) within CD4⁺ T cells following specific immune therapy (SIT). Data presented as median values, and 25th and 75th percentile respectively in the brackets

	Day 0	Day 1	Day 120
CD25 ⁺	35.50	33.20	31.40
[% of CD4 ⁺ lymphocytes]	(28.30; 40.70)	(24.30; 37.30)	(17.65; 38.95)
CD25	11.90	10.50	9.27
[MFI in CD4 ⁺ lymphocytes]	(8.67; 12.80)	(7.96; 12.50)	(5.76; 12.45)

Results

First, we found that specific immune therapy did not cause significant changes in frequency of CD4⁺CD25⁺ T cells in treated patients (Fig. 2A, C; Table I). We observed only a non-significant decrease in the expression of CD25 on CD4⁺ T cells at day 1 (10.50) as compared to day 0 (11.90) ($p = 0.0674$ (Fig. 2B, C; Table I).

Discussion

In this pilot study, we intended to evaluate early changes in CD25 (IL-2R α) expression within CD4⁺ T cells in the course of wasp venom ultra-rush desensitization therapy. Notably, CD25 is considered to be one of the surface markers of regulatory CD4⁺ T cells. Quite unexpectedly and in some contrast to other reports, we did not observe dramatic changes in the levels of CD25 on circulating CD4⁺ T cells. These findings, however, do not oppose the well-established role of regulatory T cells in the induction of immune tolerance in hymenoptera venom allergy. Firstly, CD25 is not only a marker of regulatory T cells but also activated T cells. Secondly, the putative changes in CD4⁺CD25⁺ T cells in the

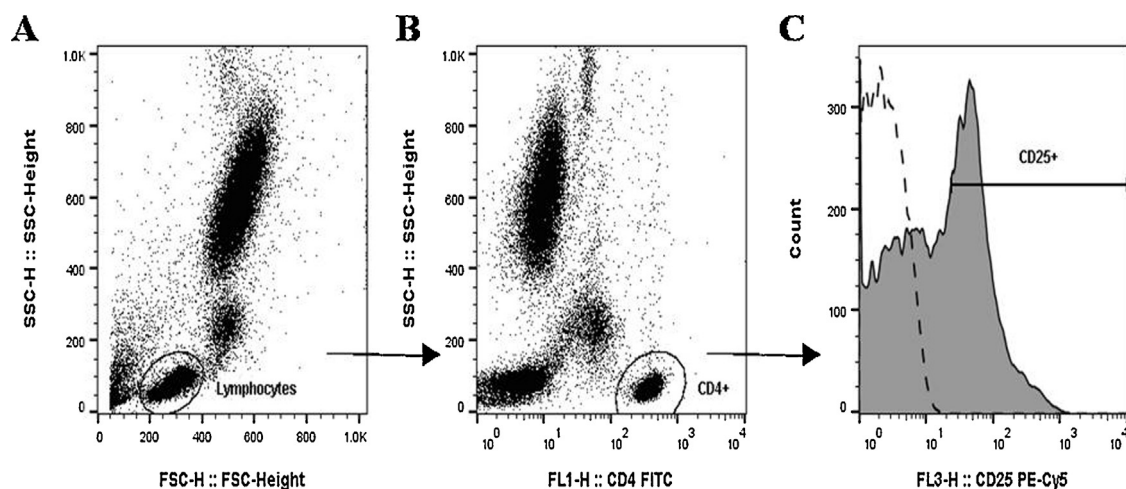


Fig. 1 – Gating strategy to distinguish CD4⁺CD25⁺ lymphocytes, based on forward/side scatter properties (FSC/SSC) (A) and expression of CD4 (T cells marker) (B) and CD25 (IL-2R α) (C). Histogram with dashed lines represents fluorescence minus one (FMO) control

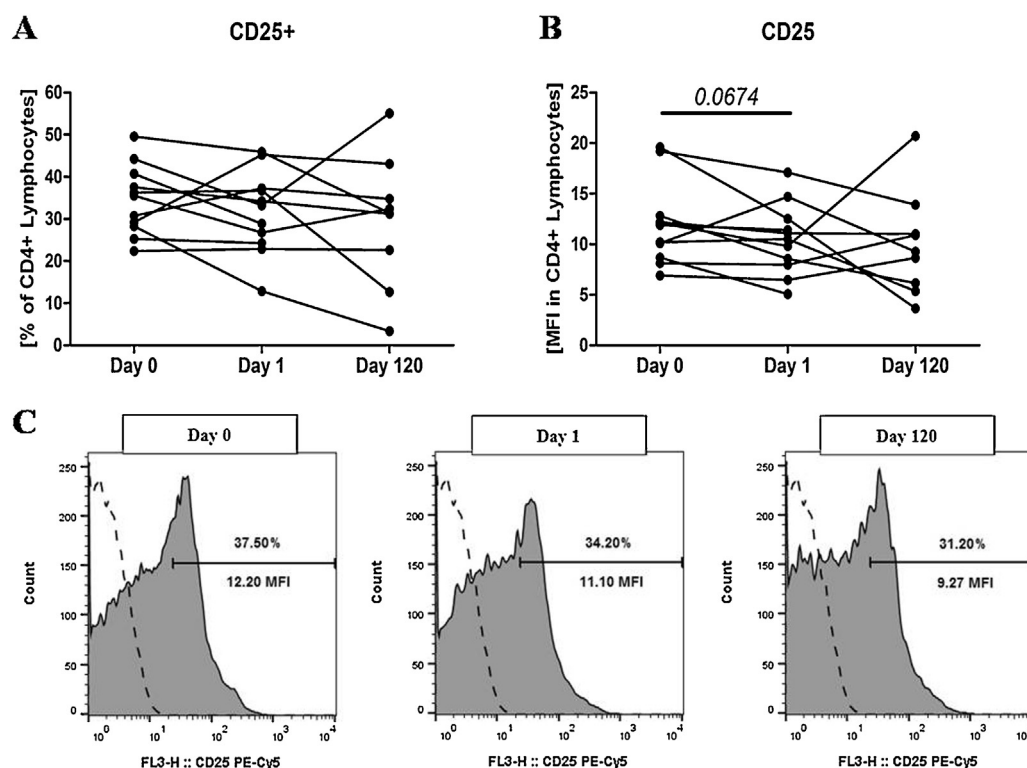


Fig. 2 – Effects of specific immune therapy on frequency of CD4+CD25+ T cells (A), and mean fluorescence intensity (MFI) of CD25 within CD4+ T cells (B), with corresponding sample histograms (C)

course of immune therapy can occur not necessarily in the beginning but in later stages of the treatment. Pereira-Santos and colleagues demonstrated that bee and wasp venom desensitization therapy led to significant increase of CD4+CD25+ T cells as late as 6 months after the start of treatment. Moreover, in some concert with our findings, these authors did not find significant changes in the levels of another activation marker, namely HLA-DR in the course of SIT. Indeed, the immune-related mechanisms of SIT are very complex and warrant continuous studies. Altogether, our study suggests that putative early immunosuppressive mechanisms of wasp venom SIT cannot necessarily be related to significant changes in the expression of CD25 on CD4+ T cells.

Authors' contributions/Wkład autorów

According to order.

Conflict of interest/Konflikt interesu

None declared.

Financial support/Finansowanie

None declared.

Ethics/Etyka

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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FURTHER READING / LITERATURA DODATKOWA

- [1] Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy and immune tolerance to allergens. *World Allergy Organ J* 2015;8(1):17.
- [2] Jutel M, Kosowska A, Smolinska S. Allergen immunotherapy: past, present, and future. *Allergy Asthma Immunol Res* 2016;8(3):191–197.
- [3] Fujita H, et al. Mechanisms of allergen-specific immunotherapy. *Clin Transl Allergy* 2012;2(1):2.
- [4] Caramalho I, et al. Bee venom enhances the differentiation of human regulatory T cells. *Allergy* 2015;70(10):1340–1345.

- [5] Pereira-Santos MC, et al. Expansion of circulating Foxp3+ D25bright CD4+ T cells during specific venom immunotherapy. *Clin Exp Allergy* 2008;38(2):291-297.
- [6] Miyara M, Sakaguchi S. Human FoxP3(+)CD4(+) regulatory T cells: their knowns and unknowns. *Immunol Cell Biol* 2011;89(3):346-351.
- [7] Kaczorowski M, Jutel M. Human T regulatory cells: on the way to cognition. *Arch Immunol Ther Exp (Warsz)* 2013;61(3):229-236.
- [8] Rosenblum MD, Way SS, Abbas AK. Regulatory T cell memory. *Nat Rev Immunol* 2016;16(2):90-101.
- [9] Nittner-Marszalska M, et al. Influence of specific immunotherapy on the activity of human T lymphocyte Kv1.3 voltage-gated potassium channels in insect venom allergic patients. *J Membr Biol* 2011;242(1):23-29.
- [10] Jutel M, et al. Mechanisms of allergen-specific immunotherapy and novel ways for vaccine development. *Allergol Int* 2013;62(4):425-433.