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Review report of the doctoral dissertation „Determination of the spatial structure and comparative analysis of selected inhaled allergens and their complexes with antibodies" by Tomasz Osiński.

The submitted by Tomasz Osiński doctoral dissertation " Determination of the spatial structure and comparative analysis of selected inhaled allergens and their complexes with antibodies" presents analysis of 3D structure of three important aeroallergens derived from three different allergenic sources.

Allergic diseases triggered by aeroallergens, such as allergic asthma or allergic rhinitis are the most frequent chronic diseases all round the world. Their prevalence has been steadily increasing since the first reports on their prevalence were made. Aeroallergens belong to different, structurally and functionally unrelated proteins derived from different sources including plants, fungi or animals. The common feature of those proteins is their ability to trigger immunoglobulin E (IgE) immune response in susceptible humans. The effector phase of the immediate immune response depends on the recognition of a 3D structure of allergenic epitopes by human IgE paratopes. The interaction is crucial for triggering mast cell degranulation with all the consequences related to the mediators released by those cells. Understanding the processes involved in IgE-allergen interaction may help in creation of hypoallergenic proteins which can be used for allergen immunotherapy. Moreover, elucidation of individual epitopes shared by different proteins may explain the commonly encountered in clinical practice phenomenon of allergen cross-reactivity. Therefore the efforts of a comprehensive evaluation of 3D structure of common aeroallergens undertaken by

the author seem to be of the utmost importance in understanding processes which lead to allergen-IgE interactions.

The dissertation is divided into several sections including introduction, materials and methods, results, discussion and references. It contains 15 tables, 30 figures and more than 200 updated references. In the first section the author outlines the significance of allergic diseases in the modern world and presents basic mechanisms concerning IgE-allergen interactions. The main allergenic sources which are known triggers in allergic asthma patients including house dust mites, cockroach and molds are also characterized. Bearing in mind the extremely high IgE affinity to an antigen (allergen) one can clearly appreciate the role which IgE-allergen interaction plays in amplification of the effector cell (mast cells/basophils) activation and facilitated antigen presentation leading to perpetuation of allergic response. The introduction helps to understand the approach used by the author who evaluated structure of three individual allergens trying to elucidate specific and unique structural and molecular patterns which may be responsible for their "allergenicity".

The original study consists of three subprojects. The methods and results have been already published in top-ranked, peer-reviewed scientific journals. Therefore, herein I would like to only briefly refer to the findings which in my opinion are crucial from clinical point of view.

The selected allergens include Der p 1 from the house dust mite *Dermatophagoides pteronyssinus*, Bla g 4 from the cockroach *Blatella germanica* and Alt a 1 from the mold *Alternaria alternata*. They are all major allergens which trigger IgE response in more than 50% of patients allergic to the individual allergenic sources. Moreover, the exposure to the selected allergenic sources is closely associated with development of asthma and with triggering of asthma symptoms in allergic patients. The selected proteins, however represent different functional and structural protein classes. Der p 1 belongs to cysteine proteases, Bla

g 4 to lipocalins while Alt a 1 represents a class of mold proteins which function has not been clearly determined yet. Although heterogenic from structural or functional point of view they are characterized by high immunogenicity and allergenicity making them potential targets for allergen immunotherapy. Since ability to trigger IgE immune response is not a simple derivative of a protein content in a given allergenic source it seems that functional and/or structural characteristics of an individual protein play a crucial role in that process. Moreover, each of the selected allergens share some structural homology with other more or less closely phylogenetically related proteins and those structural similarities may be responsible for IgE cross-reactivity. Analysis of structural similarities between the described allergens and the phylogenetically related proteins allowed for demonstration of crucial structures of individual molecules involved in: 1. binding of specific antibodies; 2. binding of specific ligands; and 3. dimerization process. In the first subproject the author analyzed structure of binding sites of species specific and cross-reactive antibodies which enabled for demonstration of molecular patterns responsible for specificity of individual epitopes. The specificity of antibody binding to the studied Der p 1 epitopes is associated with overrepresentation of some amino acids such as tyrosine in the allergen epitope which binds directly to antibody paratopes. Moreover, the author clearly demonstrated that subtle changes in the epitope structure resulted from substitution of only few amino acids leads to profound effect on antibody binding. In the second subproject comparison of ligand-allergen interaction in the functionally related lipocalins allowed for demonstration of molecular structures of Bla g 4 responsible for binding of tyramine. In addition, comparison of 3D structure of Bla g 4 with Per a 4, which on one hand shares only 21% sequence identity but on the other hand cross-reacts with anti-Bla g 4 IgE allowed for description of plausible IgE epitopes. Finally, in the third subproject, structure of Alt a 1 as a unique beta-barrel protein was described. Moreover, demonstration of structures and molecular patterns responsible for

dimerization and plausible regions responsible for IgE binding were shown. Those different approaches to evaluate structurally unrelated proteins which common feature is triggering IgE response in susceptible humans shed some light on understanding of immediate hypersensitivity reactions, cross-reactivity, and present some avenues for modification of individual allergens to obtain hypoallergenic vaccines for immunotherapy.

I have also several comments which should be addressed while defending the doctoral thesis.

1. Why the 4C1, 10B9 and 5H8 anti-Der p 1 antibodies were chosen for analysis of epitope-paratope interaction?
2. Do epitopes of 4C1, 10B9 and 5H9 reflect naturally occurring IgE epitopes?
3. Could dimerization of Der p 1 or Bla g 4 be demonstrated or expected based on their structure?
4. Can a simple chemical modification of Der p 1 epitopes which impact immunoglobulin binding be inferred from the presented data?
5. Lipocalins are ubiquitous proteins. Do plausible IgE epitopes in the Bla g 4 share any structural similarities with human lipocalins?

Page 78 line 13 Instead of "Hum s TL" should be Hom s TL, where Hom s is an abbreviation of *Homo sapiens*.

In summary, application of modern biochemical techniques including macromolecular crystallography with comprehensive analysis of structure similarities between related molecules allowed for demonstration of unique patterns of 3D structure of the selected aeroallergens which are crucial for antibody or ligand binding as wells as for formation of

homodimers. Those findings indicate potential targets for allergen modification in order to create hypoallergenic proteins with possible application in immunotherapy of allergic diseases.

In my opinion Tomasz Osiński doctoral dissertation fulfills all the requirements for obtaining PhD degree. Therefore I request to allow Tomasz Osiński to continue for a public defense of the thesis presented in the dissertation. Assuming the high scientific level and a potential practical impact of the discoveries presented in the dissertation I would like to recommend to consider this dissertation as an outstanding.

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