STATEMENT

By: Assoc. Prof. Dr. Ivanka Georgieva Tsacheva,

Department of Biochemistry, Sofia University "St. Kliment Ohridski",
member of the scientific jury appointed by order No I-15/23.01.2025
of the Director of the Institute of Microbiology, Bulgarian Academy of Sciences

regarding the dissertation of NIKOLA RALCHEV RALCHEV entitled: "**Suppression of antigen-specific B lymphocytes through protein engineering molecules in hypersensitivity reactions**" for awarding the educational and scientific degree "PhD" in a professional direction 4.3. Biological Sciences, Scientific specialty "Immunology"
Research supervisor: Prof. Andrey Tchorbanov, PhD

Characteristics of the habilitation thesis

The dissertation of Nikola Ralchev is dedicated to a topic that represents a problem for more and more people nowadays, namely the clinical manifestation of house dust allergy as a type of hypersensitivity of the immune system caused by a combination of factors that include hereditary predisposition and modern living conditions. The latter are very important in triggering the allergic condition and underlie the frequency and severity of subsequent allergic episodes. Allergic reactions can be triggered in any individual at any time in his or her life and can unfortunately develop into chronic pathologies.

House dust mites (HDM) are the most common causes of respiratory allergies, causing allergic rhinitis and allergic asthma. One of the major allergen sources for HDM allergic patients worldwide is the microbe species *Dermatophagoides pteronyssinus* (Dpt). Allergens distributed in the environment by them have been identified, characterized as molecular structures and classified into three groups according to the range of distribution in sensitized individuals. Der p 1 is one of the major allergens to which more than 80% of Dpt-sensitized individuals react with allergy. The allergic reaction is accompanied by the appearance of IgE antibodies and the activation of B cells corresponding in specificity, which can be targeted for the creation of a selective therapeutic approach to eliminate them, and thus to reduce the titers of allergen-specific antibodies. Given that the approach to control allergic episodes is with symptomatic medications, once the patient has finished taking them, they are not protected from a new allergic attack the next time they encounter the allergen. In this sense, the creation of a therapeutic approach to cure, rather than just to relieve current symptoms, is a necessary, urgent task.

Nikola Ralchev presents a dissertation which covers 106 pages and which follows the classical structure - introduction, literature review, aim and objectives, materials and methods, results, discussion, conclusions and contributions.

The introduction is concise and focused on the problem at hand, making an excellent case for the subsequent experimental development. The literature review presents all aspects of the current understanding of the molecular mechanisms of hypersensitivity reactions and allergies in particular. The main sources of allergens in house dust, their allergenic properties as well as their

interaction with the immune system are discussed. The currently used therapeutic approaches - antihistamines, leukotriene antagonists, corticosteroids and allergen immunotherapy- are presented. The literature review also contains information on mouse models of HDM allergy. Information on the expression of two key receptors, CR1 and FcγRIIb, and their involvement in the mechanism of allergic symptomatology is presented.

Nicolas's literature review as a whole is an excellently balanced text in terms of content, topics covered and factual background, demonstrating his excellent awareness and ability to systematize and present in an essential form information from multiple sources regarding the issue at hand.

The underlying goal of the dissertation is to achieve selective suppression of allergen-specific B cells and a corresponding decrease in allergen-specific antibody production in two models, a humanized Rag2- γc- model and a BALB/c mouse model of chronic allergic inflammation.

To implement the overall design of the complex experimental design, the two mouse models were developed and one chimeric molecule based on human CR1 containing a peptide p52-71 corresponding in sequence to an identified epitope of the allergen Der p 1 and another chimeric molecule based on mouse FcγRIIb with the same epitope-bearing peptide were constructed.

The chapter Materials and Methods presents the preparative and analytical methods used - a wide range of molecular biological, immunological, biochemical, cellular, etc. The methods are described with precision, according to the style of scientific publications and with the necessary parameters and conditions that allow them to be reproduced by any interested experimenter. The methods are chosen appropriately to the tasks at hand and so as to provide experimental data obtained by fundamentally complementary approaches to support the same regularity.

The experiments resulted in the development of the two models and therapy with the corresponding chimeric molecule was conducted. In the humanized model, treatment with the human CR1-specific Dp52-71 chimera reduced the levels of allergic IgE antibodies, total protein and β-hexosaminidase in lung lavage fluid. It also achieved a reduction in the number of infiltrated human lymphocytes in the lung and suppression of total perivascular inflammation in the lungs of humanized mice.

In the chronic mouse model of house dust allergy, FcγRIIb-specific Dp52-71 chimeric molecules were found to bind to the FcγRIIb receptor on mouse B cells and were recognized by allergen-specific IgG1 antibodies. Allergen-specific IgG1 antibody levels were found to correlate with total protein and β-hexosaminidase activity in lung lavage fluid as well as $SiglecF^{high}$ $CD11c^{low}$ eosinophils in lungs. There was overexpression of FcγRIIb on the surface of CD19 IgE-positive B cells in the lungs of HDM-stimulated mice. Mouse chimera treatment decreased the levels of allergen-specific IgG1 antibodies in serum.

The experimental data in this chapter are presented by 17 high quality figures and the necessary statistical analysis. They are discussed comprehensively and in parallel with previously published data. The discussion once again demonstrates Nicolas's excellent theoretical background not only at the beginning of the experimental project, but also his significantly increased educational level

in the course of the work, as well as his developed ability to fully reflect on the results as a mature researcher. Two hundred literature sources are cited, covering an extended period of experimentation on the subject of allergies, from 1940 to 2024, which is indicative of diligence and responsibility in approaching the search for a solution to a defined scientific problem.

A total of 10 conclusions were formulated, four based on the results with the humanized model and six based on the results from the chronic mouse model. I accept the results as adequately representing the experimental data included in this dissertation.

The work of Nikola Ralchev also has contributions of a fundamental and scientifically applied nature, which I also accept as correctly formulated.

Scientific metrics related to the habilitation thesis

The results of the experimental work reflected in the dissertation are included in 2 publications. One of them is in a Q2 journal of 2022, the other is in a Q1 journal of 2023. In both publications Nikola Ralchev is the leading author and the scientific supervisor prof. Andrey Chorbanov is the corresponding author. The work has been presented by Nikola through posters and papers in 9 national and international scientific forums.

CONCLUSION

The dissertation is written in a high scientific style. The text in each chapter is biochemically sound and terminologically precise. The layout of the dissertation, text and figures, is excellent. The ambitious goal has been successfully realized both in terms of scientometrics and the maturation of a young mind into a well-rounded researcher. I would like to express my excellent impressions of this dissertation, which presents Nikola Ralchev as a promising young experimentalist with analytical and experimental skills of broad scope. The dissertation and the publications related to it exceed the requirements for obtaining the educational and scientific degree "Doctor".

Because of the above, I confidently give my positive assessment of the conducted research, the achieved results and contributions, and I propose to the honorable scientific jury to award the educational and scientific degree "PhD" to NIKOLA RALCHEV RALCHEV.

24.02.2025   Assoc. Prof. Dr. I. Tsacheva