OPINION

from

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On

Dissertation for the award of the educational and scientific degree "Doctor", Professional field 4.3. "Biological Sciences",

Doctoral program, scientific specialty "Immunology"

Author: Nikola Ralchev Ralchev

Institute of Microbiology "Stefan Angelov", BULGARIAN ACADEMY OF SCIENCES

Subject: SUPPRESSION OF ANTIGEN-SPECIFIC B LYMPHOCYTES BY PROTEIN ENGINEERING MOLECULES IN REACTIONS OF HYPERSENSITIVITY

Form of doctoral studies: full-time

Scientific supervisors: Prof. Andrey Ivanov Chorbanov, PhD

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1**. Presentation of the procedure and the PhD student**

The set of materials presented in electronic form for the Doctoral Student (Nikola Ralchev Ralchev) is by Art. 74.1 of the procedure for acquiring the ONS "doctor" and with the Regulations on the terms and conditions for acquiring scientific degrees and occupying academic positions at ImikB-BAS. The set includes all required documents.

Doctoral student Nikola Ralchev graduated with a bachelor's degree in "Molecular Biology" from Sofia University " St. Kliment Ohridski", 2018. After that, he promptly started his master's degree in "Cell Biology and Pathology" and defended his thesis on the topic "Molecular characterization of two Bcl-2 homologs encoded in the genome of the sea urchin Strongylocentrotus purpuratus" with an excellent grade. It shows a deep consistency and desire to develop in cell and molecular biology. His dedication is also evidenced by Nikola Ralchev's several internships during the bachelor's and master's programs. The first was in the Laboratory of Experimental Immunology, Institute of Microbiology, Bulgarian Academy of Sciences and is related to the development of therapy in animal models of house dust allergy, which he subsequently develops in detail in his dissertation. All this helped him study and master methods related to cultivation, characterization of different cell types, and upgrading methodological skills beyond cell cultivation and biochemical processes to perfection. His second internship was in the laboratory "Signaling, metabolism and tumour progression" at the Cancer Research Center of Lyon, CRC, France. It is on the topic: "Investigating the non-apoptotic functions of proteins from the Bcl-2 family". The study of the Bcl-2 family is part of

his thesis in his master's program and speaks of the determination and desire of the doctoral student to be as precise as possible in developing and presenting the scientific topic. In the same year, 2020, after obtaining a master's degree, Nikola Ralchev was enrolled as a full-time doctoral student at ImikB-BAS, and in 2021, he became an assistant professor at the Laboratory of Experimental Immunology ImikB-BAS. This detailed sequence is also visible in the dissertation, which is presented to me for opinion.

Nikola Ralchev is fluent in English (B2 level). CAE-Certificate in Advanced English), which allows him access to scientific sources in the global database on the scientific problem set in doctoral studies. As evidence of the scientific interest and desire to develop as a scientist, as well as the quality of the scientific production, are the numerous participations in scientific forums, scientific projects, publications not directly related to the topic of the dissertation, H index: Scopus – 3, as well as the awards received over the years.

During his doctoral studies, Nikola Ralchev participated in nine scientific forums and published two research papers in journals with an impact factor. He has also submitted certificates for completed courses and a report detailing the credits earned for evaluating and preparing doctoral students, reflecting his educational and research activities. Additionally, he meets the extra criteria for academic staff development at ImikB-BAS. Nikola not only meets but exceeds the required credit points. Supporting this assertion are the four awards he received between 2022 and 2024.

2. Relevance of the topic

Over the past 100 years, especially in the last 2-3 generations of the population of urbanized countries to which Bulgaria belongs, the cases of the so-called "diseases of civilization" with a chronic course have sharply increased. These are atherosclerosis, essential hypertension, metabolic disorders, autoimmune diseases and, last but not least, atopy and manifestations of allergy. Due to the so-called "hygiene hypothesis, " using xenobiotics, changes in eating habits, and the lack of appropriate genetic adaptation mechanisms lead to a significant change in the finely tuned human metabolism. There is evidence of increased production of reactive oxygen radicals and oxidative stress, development of hyperinsulinemia and insulin resistance, abnormal activation of the sympathetic nervous system, and the renin-angiotensin system in a "Western lifestyle diet". All this increases the tendency of the individual and/or family to develop sensitization and production of IgE class antibodies in response to common exposure to allergens. More and more data are accumulating to support the role of IgE in autoimmunity in patients with autoimmune thyroiditis and lupus erythematosus. Since 2016, people have been talking about histamine intolerance. Since 2011, the European Food Safety Authority (EFSA) published a scientific report warning that the histamine levels found in foods marketed in European Union countries may pose a health risk to consumers. All these facts make the mouse models presented in this dissertation of a chimeric antibody directed against inhibitory mouse FcγRIIb or human CR1 on allergen-specific Der p1 B cells extremely relevant. The results provide another possible alternative for modulating the allergen-specific B cell immune response and possible benefits for additional therapy in autoimmune patients positive for specific IgE or IgG1 antibodies against autoantigens.

3. Evaluation of the dissertation work and the contributions expressed therein

The dissertation is presented in 9 sections on 106 pages, clearly and concisely. Illustrated with 29 figures and 2 tables – excellently formatted. The literature used in the “Bibliography” section includes 211 literary sources, a significant portion of which are from the last 10 years. The dissertation submitted for consideration meets the requirements for sufficient volume and is appropriately structured.

The introduction is well formulated, outlining the main points discussed in the dissertation, and the transition to the literature review is smooth.

The literature review is structured correctly and built on the topic being developed and covers 33 pages. The scientific articles cited in it from recent years support the relevance of the chosen topic. The types of allergic reactions, the immunopathogenetic mechanisms, the types of allergens and the routes of their entry into the body are described sequentially. Very intriguing is section 2.4. “Evolution of the Th2 response and theories for the development of allergic reactions”. Gradually and smoothly, we move on to allergic reactions against house dust and mites and therapeutic strategies. I cannot fail to note the precision of the figures and the table in the scientific“review, as well as the original way of conclusion entitled: " Hypothesis and proposal for a new therapeutic approach aimed at the selective elimination of allergen-specific B cells through protein engineering molecules.” Here we can also see the role of the scientific supervisor in setting a topical topic with the results obtained duly evaluated by the international scientific community.

I have a few comments on this section and a question:

1. I recommend replacing the term "sensitization" used in points 2.3.1 and 2.3.2 with the clinically accepted term sensitization.
2. Commenting on the role of H2 blockers in item 4, "Therapeutic approaches to allergy," is also helpful.
3. It is good to describe, and not just cite, the alternative function of mouse CR1 in B lymphocytes (p. 37). It is undoubtedly fascinating to readers that mouse CR1 is not associated with an inhibitory signal of BCR-dependent activation.
4. Occludin and claudin-1 are molecules associated with intestinal permeability and diseases such as Crohn's disease and ulcerative colitis. What would be the likely immunopathogenetic mechanism when Der p1 enters the intestinal mucosa, especially in atopic dermatitis or asthma?

The goal is well formulated and follows the dissertation topic. The task set (four in number) is clearly defined and meets the goal. They are sufficient, excellently implemented, and commented on in the dissertation.

The Materials and Methods section shows the Doctoral Student's competence and contribution to the dissertation work. The section is written in a highly systematic and, at the same time, detailed manner. The variety of methods used and their affiliation to different areas of cellular, molecular biology, cellular engineering and immunology show the value of my proposed dissertation work and Nikola Ralchev's capacity to handle technical matters and summarize data from the results obtained. Here, one can also see the continuity in the use of well-developed and internationally recognized methods and chimeric molecules, a "trademark" of the team led by the scientific supervisor of the Doctoral Student.

I have one remark about this section:

In item 1.8, " Phenotyping of pulmonary infiltrates," viable cells were counted using a Burker chamber after obtaining a single-cell suspension. It would be good to specify how this vitality was established by staining with a 0.4% solution of the Trypan blue dye.

The results of the experimental studies are presented on 22 pages, meet the set tasks and follow the sequence in the described methods. They are very well illustrated with 17 precisely made figures. They are described in detail and with the necessary criticality, both in terms of the direct and indirect influence of the Dp52-71 chimaeras on lymphocyte subpopulations, cells of the non-specific immune response, molecules part of humoral immunity, analysis of pro-inflammatory proteins in BALF, and also in situ analysis of lung tissue from the experimental models.

Nikola Ralchev demonstrates exceptional skill and a deep understanding of experimental immunology, particularly concerning mouse models, a complex and specialized field. The numerous results he presents are compact, competent, and well-organized. These findings are intriguing, hold significant scientific and practical value, and align well with the objectives outlined in the dissertation.

In the **Discussion section**, the doctoral student successfully defends the idea of experimental productions and discusses in depth and critically the obtained results in comparison with the known data in the world literature. For example, "epitope spreading" is skillfully and logically commented on as a probable possibility for the allergen-specific IgE immune response change. The comprehensiveness of the constructed experimental models, "the humanized mouse model of allergy to HDM, and "the chronic mouse model of allergy" is impressive, leaving readers with the idea of a well-conceived and perfectly executed scientific project. The doctoral student Nikola Ralchev has done an excellent job with this section.

10 Conclusions have been made, clearly divided into two sub-points, depending on the mouse model and chimeric antibody used. They logically follow the results and discussion, synthesizing the large-scale and heterogeneous methodology dissertation work. The contributions are united in 2 categories of fundamental importance and a scientific-applied nature. They clearly show the significance and innovation of the obtained scientific results and the undeniable relevance of the dissertation work, not only in the scientific sphere but also in practical medicine.

4. Evaluation of the publications and personal contribution of the Doctoral Student

Two publications have been presented concerning the dissertation work. The first was published in the Scandinavian Journal of Immunology, which has a Journal Rank of Q2 and an Impact Factor of 3.7 (2022). The second publication appeared in the International Journal of Molecular Sciences, with a Journal Rank of Q1 and an Impact Factor of 4.9 (2023). These publications reflect the significance of the results obtained in the dissertation and highlight the high regard held by the international scientific community, as evidenced by the journals' quartile rankings and Impact Factors. In both publications, Nikola Ralchev is listed as the first author, clearly demonstrating the PhD student's substantial contribution to developing and analyzing the findings presented in the dissertation work.

The findings were presented at nine national and international scientific forums. I must highlight the four awards that underscore the impressive qualities of the doctoral student. Nikola Ralchev's dissertation received an excellent evaluation.

5. Abstract

This document is 58 pages long and includes the key components of the entire dissertation. It adheres to the regulations governing the conditions and procedures for obtaining scientific degrees and holding academic positions at ImikB-BAS.

6. Conclusion

The dissertation submitted for my review represents a complete, well-executed, and thoroughly justified scientific work. The relevance of the findings, the variety of methods employed, and the effective combination of these methods to yield publishable results are commendable. The doctoral student, Nikola Ralchev, is a qualified young scientist with diverse skills, firm capabilities, and the creative enthusiasm necessary to achieve these outcomes. The results presented in the dissertation could serve as a foundational step toward a new therapeutic approach to modulate the specific B-cell immune response in various allergic, autoimmune, and chronic inflammatory conditions.

I believe that Nikola Ralchev's dissertation meets the requirements outlined in the Act on the Development of the Academic Staff in the Republic of Bulgaria and the regulations set by ImikB-BAS for awarding the educational and scientific degree of "Doctor." Based on this assessment, I confidently support the dissertation, the abstract, and the results and contributions presented. I therefore propose to the esteemed members of the scientific jury that they award the educational and scientific degree of "Doctor" to Nikola Ralchev Ralchev in the doctoral program "Immunology."

25.02.2025

Prepared the opinion:……
Assoc. Dr. Ekaterina Ivanova-Todorova, MD