



REVIEWERS COMMENTS ON THE DOCTORAL THESIS

By: **Oksana Tsyklauri, M.Sc.**

MECHANISMS OF THE TOLERANCE AND HOMEOSTASIS OF IMMUNE CELLS

The submitted high-quality dissertation is written in a semi-abbreviated form on a very central biomedical topic, using the whole spectrum of relevant methodologies. Oksana Tsyklauri chosen optimal format and extent of the thesis - on the one hand she wrote the thesis in very good English with a minimum of formal inaccuracies (I am only curious what is "anti-idiotic activity" on page 12), at the same time she balanced appropriately in the literary overview between the necessary generalities and the need to explain the relevant molecular mechanisms in detail. The opening part is very well prepared and readily presents the recent published information thematised by the Treg function and diversity in context of autoimmunity, put in a general immunological context. I also appreciate the ability to interconnect the Treg story with the Bardet-Biedel syndrome research into one synthetic and synergic narrative about the self-tolerance. I would also like to appreciate the tightness of the text, that it does not go beyond the topic of the thesis and that "between the lines" proves the non-trivial fact - that the author clearly understands the studied issue in detail. The literary sources used are a representative and in many respects exhaustive overview of the relevant literature.

The strengths of the submitted dissertation are the methodologies and clearly and creatively chosen or created models. Individual experimental arrangements are very well thought out, allowing critical interpretation and then answering the questions asked. These are in the mainstream of immunological research, in many cases testing the hypotheses related to the "textbook" basis of immunological theory - including the mechanisms behind the activity of the Tregs in the controlling of autoimmune reactions (limiting the IL-2) mediated by cytotoxic T lymphocytes or characterizing the functional diversity of effector T-cells (including the novel specialized type ("super-effector" CD8 T cells). Of a very high quality are also pioneering contributions to the molecular and cellular mechanisms behind the symptoms of the Bardet-Biedel syndrome viewed from different experimental perspectives.

The understanding of the techniques used is convincingly documented in the work - which is another (along with the ability to critically think through literary contexts) unequivocal argument for granting the Ph.D. degree. The techniques used to fulfil the specific aims of the study are on the edge of current technical and model possibilities. It is also evident that Oksana Tsyklauri makes use of the excellent intellectual and methodological background of the training laboratory, where, thanks to the erudition of the supervisor she can perform research competitive in quality with the best institutions elsewhere.

The strongest aspect of the submitted dissertation is the quality of the presented scientific work. Oksana Tsyklauri can rely on 5 excellent publications (including one first author paper on bioRxiv) in exceptionally high quality biomedical journals. The dissertation is based on 4 already accepted publications (Oksana is on two shared first author - together with Veronika Niederlová – EMBO Rep. and Eur. J. Immunol.). Individual publications are presented and discussed in the sufficient extent enabling to understand the "big picture" behind the research performed. The author's contribution is clearly stated, making the solid base for the overall highly positive evaluation. Again, the conciseness and sophistication of this part of the work is to be appreciated.

In summary, the quality of the published papers, together with the quality of the respective journals (and also the quality of the dissertation itself, is quite sufficient for my highly positive evaluation.

Finally, I would like to ask a few very curious questions:

1. On page 14 is discussed the role of obesity in autoimmunity. The popular habit modulating the activity and even transdifferentiating of the white adipose tissue to beige adipose tissue is hardening. Could be such behavioural intervention used as an anti-autoimmunity treatment? What is the mechanistic explanation for the obese phenotype as one of the BBS symptoms? Why is BBS-linked obesity more manifested in females compared to males?
2. On page 20 you discuss the role of AIRE in regulation of the tissue restricted antigens and consequently in controlling the repertoire of the Tregs. Is anything known about the role of other TRAs controlling factors (DEAF1 in the periphery and in the thesis mentioned FEZF2 in the thymus) in the generation of the Tregs?
3. At page 25 you mention secretion of the extracellular vesicles by Tregs. Exosomes seems to play important roles in various contexts, modulation of their production could pleiotropically effect the overall immunological equilibrium – like seen in BBS. Could be there a link between the Bardet-Biedl syndrome proteins and modulation of the release of the bioactive extracellular vesicles?
4. The homology between the cilium and the immunological synapse is mentioned in the thesis without further explanation. Could you provide some details about this extremely interesting topic? Which other cell types, similarly to leukocytes, lack the primary cilium? What is your opinion about the publication: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5747971/> ?
5. At page 39 you discuss the previously unknown CD8+ T cell subset nicknamed as super-effector T cell. I am curious about the argumentation behind the superiority of the cells in their effector function.

Finally, I would like to state that the dissertation submitted by Oksana Tsyklauri is of high quality and demonstrates the author's scientific erudition as well as the ability to formulate complex scientific facts and hypotheses in an understandable way. The dissertation "Mechanisms of the tolerance and homeostasis of immune cells" meets the requirements given by Charles University, Faculty of Science for doctoral theses and I strongly recommend it for successful defence.

Prague, April 20, 2022



prof. RNDr. Jan Černý, Ph.D.