



UNIVERSITA KARLOVA

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Dissertation review

Name: **Mechanisms of the tolerance and homeostasis of immune cells**

Ph.D. candidate: Oksana Tsyklauri, M.Sc.

Supervisor: Mgr. Ondřej Štěpánek, Ph.D.

The dissertation is focused on the research of immune cell tolerance in two areas. The first is the area of regulatory T cells, the second is immune dysregulation in Bardet-Biedl syndrome (BBS). The work is based on 4 publications in quality impact journals (3 original articles, 1 review) and one publication in the preprint stage. In one publication, the candidate is the first author.

The **formal** and linguistic level of the work is at a high level, out of inaccuracies I found only a typo in the numerical designation Scheme 2 (p. 42).

The **theoretical introduction** is relatively extensive, with a large number of relevant references to the literature (312 in total) and very well, up-to-date summarizes current knowledge about regulatory T cells (Tregs), their development and disorders. The section on BBS is a bit thematically distant from the unifying line, but this is due to the final results of studies that have found another basis for immune dysregulation in BBS.

The methods are summarized briefly and are mentioned in detail in separate publications. They include work with mouse models and their analysis using flow cytometry, Western blot, RT-qPCR and further analysis of publicly available human data from scRNAseq. The author mainly performed optimization and analysis of mouse models, data analysis and participated in writing of publications.

The results are presented in the form of comments on individual publications and summary diagrams. Because the individual parts are slightly thematically different, the results are discussed separately for each topic. The discussion is relevant and puts the obtained data in a broader context. The candidate's first-author publication on the topic of immune dysregulation in the BBS was published in the prestigious Embo Reports magazine (IF = 8.8), which is in itself a guarantee of quality. Another first-author publication is still in the preprint stage, but it presents very interesting findings about a presumed new subpopulation of effector CD8 T cells and their regulation by Tregs. I expect the paper will be also accepted in a prestigious journal.

I have the following questions:



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To the theoretical part:

Mice lacking CTLA-4 show signs of autoimmunity, while mice with heterozygous impairment of this gene are normal. In humans, a heterozygous mutation is sufficient to manifest autoimmunity. What can make the difference?

Recently, congenital PD-1 deficiency in humans has been described. Can some phenotype data help elucidate the role of PD-1 in Tregs?

To the controversy about the origin of peripheral Tregs and their role in microbial tolerance on the mucosal surface: Are there studies examining the emergence of this tolerance in old age and the associated decreased thymic function?

To the results:

Why did you remove the MAIT cells from the analysis of scRNA-seq data from the analysis of human super-effector-like cells?

According to your mouse model data, the "CD8 super-effector" cells you define are increased in the absence of Tregs. Do you anticipate that your predicted counterpart of this subpopulation in humans should be increased in patients with monogenic Tregs deficiency, or in genetic disorders or treatments leading to decreased Tregs function?

Conclusion:

This is a very well-processed work that met the goals and brought a number of scientifically and clinically significant results. The candidate's publications are in major international journals, whose strict review process is in itself an indicator of the quality of the submitted results. The work is at a very high level compared to the range of dissertations I have evaluated and is also a reflection of the quality scientific background of the team and the guidance of the supervisor.

The candidate has demonstrated her creative abilities and the ability of independent scientific work, I recommend her work for defense without reservations.

Eva Froňková, M.D.,Ph.D.

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