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## **Review of the PhD thesis: Toll-like receptors and myeloid cells in development and disease**

Proposed PhD thesis submitted by Jana Balounova summarizes the experimental data from three published studies and one manuscript in revision. Jana Balounova is the first author of the paper in the European Journal of Immunology and manuscript in revision in Genes and Development and co-author on the papers in the Journal of Proteome Research and in Cellular Immunology. Besides papers directly linked to the thesis, she coauthored two other manuscripts.

In her thesis, Jana first provides an overview of the biology of Toll-like receptors and summary of experimental methods. Results part of the thesis is based on three published papers and one submitted manuscript.

Paper in the European Journal of Immunology and manuscript submitted to the Genes and Development reports a very interesting and extensive study of the expression and function of Toll-like receptors during the embryonic development. Authors describe the kinetics of TLRs expression not only on the cells of myeloid origin but also on early hematopoietic progenitors and characterize not only the temporo-spatial aspect of TLRs expression but they also tackle the functional characteristics. This part of the thesis is novel and provocative and provides primary data on the role of TLRs in the development as TLRs have almost exclusively been studied on mature differentiated cells of innate immunity. This study also describes a generation of mouse TLR2-Cre transgenic strain allowing for mapping TLR2 expression during embryonic development.

In the remaining two papers, Jana describes the induction of autophagy during the course of *Francisella tularensis* infection and high expression of alpha defensins by eosinophils in type I diabetes patients.

Structure of the thesis is logical, written in a very good English and is easy to follow. I would appreciate slightly more detailed introduction into the biology of TLRs. Especially the chapters about the TLR mediated autophagy and TLRs in disease are very brief and do not provide sufficient summary of the current knowledge.

I have the following questions complementing the published reports:

- Could you please provide an update on the status of the submitted manuscripts?
- Most of the data on the role of TLRs during the embryonic development deals with TLR-2. I assume that it's caused by the limited availability of the suitable reagents for other TLRs. Could you comment on the

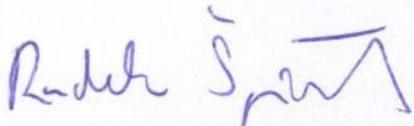
potential role of other members of TLR family? Would you expect the other TLRs to behave similarly to TLR-2?

- Regarding the finding in patients with type I diabetes. Have you had a chance to analyze alpha defensins expression in relatives of the patients at high risk of type I diabetes or in patients at the prediabetic stage? Would you expect to see similar profile early in the course of the disease, or even in the pre-disease stage?
- Have you checked for alpha defensins expression in other autoimmune diseases or in patients with infection related inflammation?

Taken together, I highly appreciate the quality of the submitted thesis. Especially the part about the role of TLRs during the embryonic development is novel and extends our current understanding of the role of TLRs. I hope that this effort will be rewarded by the acceptance of the remaining manuscript into a prestigious journal.

During her PhD studies Jana Balounova showed a proficiency in the vast array of experimental methods as documented by very high quality of her research papers. In my opinion, the results of her scientific projects clearly exceed the average level of PhD candidates and she meets the requirements set by the Immunology board of PhD studies. I fully recommend that Jana Balounova is awarded the PhD degree in the field of immunology.

Radek Špíšek

A handwritten signature in blue ink, reading "Radek Špíšek". The signature is written in a cursive style with a prominent flourish at the end.