

Supervisor's report on Zdenka Hanusova's Diploma thesis:

Physiological role of SIGIRR in an early embryonic development.

The study describes an investigation of the role of SIGIRR in regulating Toll-like receptor (TLR) signaling and the possible mechanism of its function. SIGIRR has been characterized as a negative regulator of TLR signaling in some epithelial cells as well as dendritic cells, however, how this effect is achieved mechanistically is not precisely known. Interestingly, our previous data showed that SIGIRR is abundantly expressed during very early stages of embryonic development (E6.5-8.5). To better understand the physiological role of SIGIRR, Zdenka explored several molecular, genetic and microscopic approaches to take a more comprehensive look at the SIGIRR mechanism of action and its specific role in the early mammalian development.

The work of Zdenka Hanusova provided the very first evidence that at least in some hematopoietic cells, notably macrophages and B cells, SIGIRR has little or no effect on TLR4 mediated activation of cytokine and IgM expression, respectively. That strongly suggests that SIGIRR functions in a cell content-dependent manner. Moreover, she showed that SIGIRR function can be affected by its posttranslational mechanism, likely glycosylation, which seems to be also cell type-dependent. Importantly, Zdenka's discovery that the SIGIRR function can be predicated by its association with membrane lipid rafts is in my view the most intriguing finding of her thesis and it is worth of further exploration. Zdenka was also able to prepare a new reporter system for identification of *bona-fide* SIGIRR ligand which will be used in future experiments. The availability of SIGIRR deficient mice made it possible to assess the effect of SIGIRR absence on the expression of other TIR-domain containing receptors and adaptor proteins as well as IRAK kinase during embryonic development. Indeed, Zdenka found that the expression of all of these molecules is significantly affected in SIGIRR^{-/-} mice. Moreover, the basal level and LPS-mediated upregulation of IL-1 β cytokine were significantly enhanced in SIGIRR KO mice, suggesting an important physiological role of SIGIRR in the maintenance of homeostasis in developing embryo. In my opinion, the completion of these experiments will likely result in papers published in relatively well recognized international journals.

The thesis is written up in English, in a standard format. Chapters are well balanced and transitions between them are smooth. The Introduction, Literature review and Materials and Methods sections highlight the rationale of the study, describe its design and the procedures and methods required for its analysis. The result section documents important observations in a clear way. Figures are labelled properly and easy to understand. While Zdenka's data contradicted the current understanding of SIGIRR signaling function, they, on the other hand, opened a whole new area of research for future exploration.

Zdenka Hanusova joined my research group in the fall of 2010. She quickly adapted to laboratory life and routine. In several months she was able to conduct relatively complex experiments independently. She is very mature intellectually and technically skillful and thus both sides benefited from mutual collaboration. Unfortunately, several additional experiments designed to elucidate the role of SIGIRR that has been

already initiated remained unfinished or incomplete. Also, few experiments reported in the thesis were performed only once and could not be repeated before the completion of this thesis. This was largely due to the fact that Zdenka for medical and personal reasons could not fully commit herself to these experiments. Thus, while there is some experimental and formal limitation of this thesis, in general, all major objectives of this investigation has been achieved and many interesting insights into the function of SIGIRR were gained.

Conclusions and recommendation

In my opinion, Zdenka Hanusova is highly efficient, accurate and well organized student. Her work represents an important step towards the understanding of fundamental events underpinning the physiological role of SIGIRR in normal physiology. Richness of experimental approaches, decent presentation of results and a thorough discussion fully attest that Zdenka is well prepared for the scientific carrier. Moreover, the fact that the thesis is written up in English further strengthens the potential of Zdenka Hanusova as a successful PhD candidate.

Based on the above review, I endorse Zdenka Hanusova thesis to be accepted as a fulfilment of requirements for the degree “Master of Science”.

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