REVIEWER´s REPORT

Ph.D. THESIS

Mgr. Adéla Chlastáková

Anti-inflammatory and immunomodulatory activities of *Ixodes ricinus* salivary proteins

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REPORT

The thesis of Mgr. Adéla Chlastáková is a cumulative paper thesis that consists of four papers accompanied by other mandatory parts, such as the Introduction, Summary of Results, Conclusion and Future Perspectives, References, and Curriculum vitae. It is written in English in a clear and concise manner, is well-structured and quotes an appropriate number of bibliography sources (176 in total).

Introduction

The introductory part is 24 pages long, accompanied with 5 figures and 1 table. I really enjoyed reading it since the text flows smoothly, all possible reader's questions are answered in the next sentence, figures were carefully selected to illustrate important parts of the text, and the text is without typos (I was able to found only one typo - page 3, chapter 1.2.1.1, "… even stops blow flow through the wound …"). Chapter 1.2 Reaction of a Host to Tick Feeding contains rather textbook information and I missed a more in-depth literature review connected to the effect of tick feeding. This specialized part is present as a one-page long chapter complemented by a table. The part devoted to serpins is better balanced between textbook and tick-oriented information.

Question (pg 8): Later in the text, you described different subsets of T cells. Are there different subsets also among monocytes and macrophages? In your experimental work, you used cells from peritoneal lavage - are there any differences between peritoneal macrophages and monocytes/macrophages that defend the skin (function, markers)?

Question (pg 14): In the paragraph dealing with "saliva-assisted transmission", it is not clear if this phenomenon describes the facilitated transmission from the tick to the host or vice versa, from the host to the tick (citation 94 versus 95). If both, the sentence citing papers no. 96, 97, and 98 should clearly state the direction of transmission. Is the SAT phenomenon known also in some other blood feeding vectors?

Question (pg 19): Chapter 1.6.1 Serpins of the Tick *I. ricinus* … In this chapter the author describes detailed features of only 3 out of the six serpins, since the other three are characterized in the thesis´ s papers. Nevertheless, it would be beneficial to add here or - even better - in the conclusion a table that will compare-contrast all six *I. ricinus* serpins and highlight the research gaps. Can you, please present such a summary table during the defense?

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Comment (pg 12, Table 1): “Many of the proteins have been shown to have anti-haemostatic, anti-inflammatory, anti-complement, and immunomodulatory activities (see Table 1).” I miss in the table particular proteins and peptides names. The table could be improved by adding other important information like the origin of the protein/peptide (native or recombinant), tick species, developmental stage, gender, and phase of blood feeding.

Comment (pg 20-22): It is important to mention, if the characteristics of described serpins have been obtained with native or recombinant protein, for later one mentioning also the type of the expression system.

Papers

The PhD thesis consists of two published research papers, one published review, and one unpublished research paper in the form of ready-to-be-submitted manuscript. However, author´s publication record consists in total of six published peer-reviewed papers, all of them in journals exceeding impact factor 6. According to WoS, her papers have been cited 31-times in 25 articles, reaching H index 3.

Question: Regarding the author´s contribution, it is not clear what kind of experiments the author conducted. What methods the author used and mastered during the PhD studies (bench-based and in silico)? Did she also participate in X-ray crystallography?

Question: In the presented research papers, the author used recombinant serpins for the experiments. Each protein has been expressed using a slightly different method. Can you compare and contrast them? If the author would have such a possibility, would she choose a different expression system? How can she justify this selection?

Question (Paper I): Could the lack of glycosylation somehow affect the activities of the recombinant iripin-3 compared to native protein?

Questions (Paper II):

* Based on the author's contribution statement, she participated in designing experiments and data analysis. Did she also perform the experiments?

* (pg 27) “A statistically significant inhibitory effect on the intrinsic pathway was already achieved with Iripin-8 used at 375 nM concentration …” Is it known (or could be calculated) what is the natural concentration of Iripin-8 in *I. ricinus* saliva?

* (pg 28) “... serpins with Iripin-8-like RCL … represent candidates for the development of a universal anti-tick vaccine …” How universal are iripin-8-like serpins among all ticks (including soft ticks) and is there any in humans?

Question (Paper III): Paper three is a review without experimental data. For what parts of this review was Adela in charge of? What figures and tables did she prepare?

Questions (Paper IV):

* This paper is presented in a manuscript form. What is the current status of this paper?

* In Figure 8 (pg 137), the refolding buffer for iripin-1 itself significantly affected the expression of surface molecules of LPS treated neutrophils (CD11a, CD11b, CD18). How can you explain this? In addition, taking this effect into account, it seems that the iripin-1 only neutralizes the effect of the refolding buffer since there is no difference between LPS and LPS+iripin-1 treated neutrophils for these neutrophil surface molecules. Thus Mac-1 expression is probably not affected by iripin-1. What is your opinion?

Conclusion and Future Perspectives

This part is three pages long, putting all four papers into the context of published literature, showing limitations of the study as well as directions for future experimental work. As already mentioned, this chapter could have been enhanced by a table comparing all six *I. ricinus* serpins.

Curriculum vitae

The readiness of Adela Chlastáková to be an independent researcher is further supported by the fact that she was a holder of a research grant, she has a 3-month experience from a foreign renowned laboratory at NIAID, NIH, USA, and she already presented her work on two international conferences abroad.

Question: Both presentations were in the form of posters. Besides this thesis defense, do you have any other experience presenting your work in English as a talk? Did you provide, e.g., a seminar talk during your research stay?

Question: What are your plans for the near future regarding your research career?

In my opinion, the thesis by Mgr. Adéla Chlastáková fulfils all requirements for obtaining the PhD degree and I recommend it for the defense.

In Prague, September 26, 2022

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