

The effect of tick salivary proteins on innate immunity cells

PhD thesis by RNDr. Jana Páleníková

Supervised by Prof. Jan Kopecky Csc. and Mgr. Jaroslava Lieskovská PhD

Assessment by Juan Anguita, PhD. Ikerbasque Professor. CIC bioGUNE. Derio, Spain.

This thesis explores activities associated with tick saliva that influence immune responses by directly affecting cells of the innate immune system. The candidate presents three publications, one as first author in *Infection and Immunity*, and two as second author in *Parasites and Vectors* and *Parasite Immunology*. There is a big effort worldwide to understand the complex array of pharmacological activities present in the saliva of *Ixodes* ticks, vectors of pathogens of great importance in both human and veterinary medicine. The work carried out by the candidate has shed light into some of the activities carried out by some of the proteins present in tick saliva that are injected into the host: serpins and sialostatins. Therefore, the work published by the candidate is of high significance not only to understand the interaction between the ticks and their mammalian hosts, but also to identify potential therapeutic targets against these arthropods.

The candidate, who has passed the state exam in Infection Biology, has done a superb job reviewing the state of the art in our knowledge of the interaction between tick salivary antigens and immune cells. This is presented in a well-designed and coherent introduction that leads to clear objectives for the thesis and finalizes with clear conclusions and aspects to consider for future studies.

As mentioned, the thesis is built on three published manuscripts in journals that are highly relevant for the field. The first manuscript, in particular, is noteworthy because not only dwelves into the effect of the serpin IRS-2 on dendritic cell responses to the causative agent of Lyme borreliosis, but also demonstrates the effect of this modulation on the differentiation of CD4 T cells. This aspect has long-term consequences during infection with *Borrelia spirochetes*

and the pathological manifestations during the infection. The other two manuscripts address the effect of sialostatins on dendritic cell responses.

Overall, this is an excellent thesis that highlights the efforts in clarifying the interaction of tick salivary antigens and dendritic cells with excellent and relevant results. I fully support its acceptance for defense.

These are some of the questions for the candidate:

1. The candidate makes an excellent review of the signaling pathways initiated by innate immune cells mediated by PRRs, mentioning the importance of where those signals are initiated (page 9) in the case of TLRs. Since phagocytosis is a key factor that determines the quality and quantity of Borrelia-induced signals, is there any evidence of the ability of tick saliva to modulate the internalization of the pathogen at the site of deposition that could affect the overall output of cells such as dendritic cells and modulate downstream acquired immune responses?
2. In the first manuscript, it is clearly demonstrated that IRS-2 inhibits IL-6 production in response to Borrelia antigens, resulting in lower downstream Th17 responses (Fig. 4). In these experiments, there was any observation that the restimulated T cells were skewed towards a different phenotype (e.g. Th1) or the conditions of the assay resulted in lower general activation?
3. Does the candidate think that the activities associated with sialostatins could be related to the inability of mice to develop tick immunity?

Sincerely,



Juan Anguita, PhD



Páleníková, J., 2016: **The effect of tick salivary proteins on innate immunity cells**. PhD. thesis. University of South Bohemia, Faculty of Science, School of Doctoral Studies in Biological Sciences, České Budějovice, Czech republic. p.80

Thesis review

Dr. Páleníková is an author of four published papers, out of which three are presented in her PhD thesis, including the one where she is a first author. All three papers were published last year, two of them have already been cited. Dr. Páleníková performed the experiments, analysed the data, and prepared figures. In one paper, she also contributed to the study design. All papers are published in peer-reviewed journals with impact factors ranging between 2.1 and 4.2, confirming high quality of the published studies.

Question 1: Dr. Páleníková is not a corresponding author of any of the papers. Does she have an experience with the submission process itself?

The thesis came into being under the supervision of Prof. Kopecký and co-supervision of Dr. Lieskovská in a well-established and internationally-recognized laboratory. It is focused on the effect of tick salivary proteins, serpins and cystatins, on dendritic cells that serve as an important bridge between innate and adaptive immunity. Tick saliva and their effect on host haemostatic mechanisms and on immune system is an exciting research area with potential application in human and veterinary medicine. Dr. Páleníková substantially contributed to this research field; she performed a deep analysis of three tick salivary proteins – serpin IRS-2 and two sialostatins (L and L2) – on signalling pathways as well as on effector functions using murine dendritic cells.

Question 2: In future, do you plan to include also dendritic cells of human origin? Would you expect some differences between those two host species (mice versus humans) in terms of the effect of tick salivary proteins on dendritic cells?

Question 3: In all published studies you used recombinant proteins expressed in *Escherichia coli*. Are there any putative or proven posttranslational modifications in the native proteins? Could they contribute to the protein immunomodulatory function? How would you design the experiment to test this?

Question 4: Your current research interest includes the effect of tick cystatins and serpins on neutrophil functions. Do you already have some interesting findings?

In a well-written Introduction, Dr. Páleníková gives a comprehensive background of ticks as disease vectors, followed by detailed review of tick-host interactions, including the phenomenon of saliva assisted transmission. Last chapter of the introduction is devoted to cysteine and serine proteases and their inhibitors. The papers are summarized and briefly discussed on 5 pages. Dr. Páleníková refers here to several unpublished data using them to show working hypothesis that were proven wrong. One page long conclusion includes future perspectives. This part of the thesis show Dr. Páleníková ability to review relevant literature (the thesis is based on more than 170 references), to indicate possible errors and to suggest improvements and next step research.

Question 5: It is mentioned that the tick proteins could hypothetically be used in medicine as a new therapeutical agents, e.g. for asthma. Are there any drugs in use or in the phase of clinical testing that are based on tick salivary proteins or salivary proteins of bloodfeeding insects? What would be the advantages and disadvantages of such an approach?

Question 6: How far is the current research of vaccine from tick salivary proteins that would block transmission of tick-borne pathogens? Are tick's cystatins and serpins suitable candidate proteins for such transmission-blocking vaccine or not?

Question 7: As an expert in tick-host interactions, you might receive questions from public or media regarding this topic. What would be your answer to this email: "Dear Dr. Páleníková, I have recently read an article on the internet that freak me out. Several people got an allergy to red meat after being bitten by a tick. Is it possible? Is this alpha-gal also in Czech ticks? I have found ticks feeding on me many times. How can I protect myself from developing such allergy? Thank you in advance. Yours sincerely, Ms. XY"

In conclusion, the work presented by Dr. Páleníková is well written and brings new high quality results about the importance of tick salivary cystatins and serpins as immunomodulators and factors of saliva assisted transmission. Therefore, I certify that Dr. Páleníková thesis fulfils all the requirements necessary to be awarded with a PhD title.

In Prague, December 8, 2016

Iva Kolářová

RNDr. Iva Kolářová, Ph.D.
Department of Parasitology
Faculty of Science
Charles University
Vinicna 7, 128 44 Prague 2
Czech Republic
Tel.: +420 604 862 742
E-mail: kolarova2011@gmail.com
iva.rohousova@natur.cuni.cz