

Reviewer's comments to the Ph.D. Thesis of Mgr. Jana Plchová „Differential expression of tick *Ixodes ricinus* genes induced by blood feeding of infection: genetic analysis of ML domain containing proteins“

Knowledge of genes differentially expressed in pathogen organisms under different conditions is extremely important for the development of efficient treatments. The localization of the expression proteins is equally important. Mgr. Plchova substantially contributed to the explanation of the role of two different proteins in the lifecycle of the tick *Ixodes ricinus* which contain the MD-2-related lipid-recognition (ML) domain in their molecule. She identified two new genes in this tick species activated by blood feeding: for Der f 2 allergen like protein (D-A1 protein) and for ML-domain containing protein (IrML protein), studied their expression, prepared the recombinant proteins, compared their structure with similar proteins and tested several questions concerning their function. These proteins were suggested to form a beta structure containing multiple strands and to mediate several biological functions (pathogen recognition, transport) in tick organism because of the interaction with pathogen related products. Mgr. Plchová published her original results in two papers in impacted journals and which thus underwent the review process. Parts of her work were also presented as posters or lectures on several international meetings. The objectives of her research formulated on p. 29 were successfully fulfilled.

The Thesis is a result of extensive experimental work in the course of which Mgr Plchova mastered a remarkable number of experimental methods as well as methods of bioinformatics. She also showed very good orientation in literature (the work is documented by more than 200 references) and the databases of gene and protein structures. She successfully aligned the new proteins with several other proteins isolated from related organisms, especially mites.

I have the following questions to her:


- 1) p. 32, p.49 – specific polyclonal antibodies were prepared; how did the author evaluate the presence of antibodies, their specificity and affinity?
- 2) On several places the author is mentioning „expression in hemolymph“. I would suggest to write: „concentration of the gene product in hemolymph“. It seems difficult to imagine expression of a gene in the hemolymph. Please comment.
- 3) Personal communications of Jacot S. are cited several times. Is there any progress in this matter? Were the data published in the meantime?
- 4) On p. 48. it is written: „Analysis of gene encoding D-A1 protein expression in tissues revealed that it is mainly gut-specific, but it is also detected in hemolymph. *In situ* hybridization technique did not reveal localization of mRNA in the gut.“ Please comment.

As far as the formal arrangement of the Ph.D. Thesis is concerned, I miss several parts that are usually constituents of theses, specifically summary and list of abbreviations. The list of abbreviations would help the reader to better orientate him/herself in the text and would also make it easier for the doctorand to write the Theses. I also wonder why the two papers included in the Thesis were re-written and not scanned. The re-writing gives rise to errors, as e.g. on p. 39, Fig. 2. Other point that is not contributing to easy readability of the Mgr. Plchova's Thesis are the Figures. Often Figs. are mentioned in the text, but the reader has difficulty to find them. In case that the Figures are not at the same page as the reference to them, there should be given reference to the page where the reader can find them, e.g. Fig. 15, p. 100 of the Appendix etc. Also, the legends to several Figs. are not fully satisfactorily explaining them (e.g. Fig. 13). As far as the "Summary of Ph.D. Thesis" is concerned I consider its form as rather unfortunate though Mgr. Plchova followed strictly the recommendations of the University. There should be a clear one page summary (and that not only in English but also in one other language) followed by a concise descriptions of objectives and results and a critical discussion of results. However it is the matter of the University to formulate the demands.

Conclusion: Mgr. Jana Plchová showed without doubt her ability to perform independent research activity. The work contains original results published in two international journals with impact factor (the doctorand in both cases as a first author).

Based on these facts (though having reservation to the formal aspect of the Thesis given above), I recommend to accept the given Ph.D. Thesis to the defense according to the Study and Examination Rules of University of South Bohemia.

Prague, March 12, 2012


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Minor comments

- i) p.30 The description of amino acid changes occurring in different isoforms is confused; Lys is not a hydrophobic amino acid, if Lys is changed, thus according to the Table for Arg.
- ii) Fig 9 is confusing, the arrows are in opposite directions; the source of the Fig is not given, the Npc2-like protein is not specified
- iii) p.48, line 5 from the top: there should be Figure 11 not 12
- iv) Fig. 8 is not at all referred in the text
- v) in the legend to Fig. 7, p.61, it should be stated whether the data are averages of several experiments or from one typical experiment performed in duplicates or triplicates.

Report on the on the PhD thesis (Biological Sciences) submitted by Ms Mgr. Jana Plchová with the title:

“Differential expression of tick *Ixodes ricinus* genes induced by blood feeding or infection: genetic analysis of ML domain containing proteins”.

Ms Plchová has submitted a thesis on the subject of ML domain proteins expressed by the castor bean tick (*Ixodes ricinus*) with a focus on the identification, isolation and characterization of these proteins. ML proteins expressed by *I. ricinus* are postulated to be involved in the tick immune response to infection and/or to be mediators of a tick defensive reaction. The aim of the thesis was to identify ML domain containing proteins in the genome of *I. ricinus* using bioinformatic tools, to analyze the expression patterns and localization of the proteins in tick tissue and to test whether recombinantly expressed ML proteins play a role in tick innate immunity by assessing the possible anti-microbial activity of the proteins. A further focus was on the ML-domain containing protein and Der-p2 allergen like protein of *I. ricinus* regarding its possible IgE binding ability.

The submitted dissertation on this subject has been divided into different chapters beginning in Chapter 1 with an introduction and literature review on the subject of ticks and tick immunity as well as with a general introduction on the ML protein family. The introduction represents an extensive literature review, whereby the subject relevant literature has been critically evaluated and includes well compiled specific up to date information.

The objective of the thesis is clearly stated and outlined in Chapter 2, which is followed by the results section in Chapter 3. The data have been divided into two categories of findings, one pertaining to the characterization of an identified ML domain containing protein, IrML, the second related to findings concerning the Der-p2 allergen-like protein.

The ML domain containing protein IrML was extensively characterized regarding the observation of polymorphic forms, the prediction of putative lipid binding sites potentially important in anti-microbial activity of the protein, and prediction of secretion and tertiary structure. Expression of IrML was found in the gut, hemolymph and salivary glands of *I. ricinus*, and was shown to be influenced by blood feeding of the tick. It was investigated whether IrML could be involved in the tick immune response to infection, indicating that mRNA expression is upregulated in tick cell lines exposed to the gram-negative bacteria *E. coli* and *B. burgdorferi*, however the protein did not show an impact on bacterial cell survival.

In the second part of the results, the Der p2 allergen-like protein of *I. ricinus* was also extensively characterized with respect to sequence, structure, expression and putative lipid binding capacity. The recombinantly expressed Der p2 allergen-like protein of *I. ricinus* showed some binding activity to *E. coli* and *M. luteus* but in analogy to the findings with IrML did not show bactericidal activity. The protein was also investigated with respect to its capacity to bind IgE, with positive results.

The findings of the experimental work are discussed in Chapter 4. The discussion is performed in context with findings published in the literature, rounded up by conclusions drawn up in Chapter 5. In Chapter 6 a detailed description of the methods employed is given.

In summary, the research data and findings are presented and discussed in a very good way. Ms Plchová has used a wide range of methods in a very proficient manner to generate the findings of the studies and has provided ample evidence that she is conversant with the methods of scientific investigation. This is underlined by the fact that both sets of results generated within the thesis have been published in respected peer-reviewed journals.

The presentation of the results, the interpretation of the data and the discussion of the relevance of the findings is exemplary and makes for a very comprehensive reading of the work. The findings of the PhD study can be regarded as novel, constituting an original contribution to science in the field.

I thus recommend to accept the thesis.

A handwritten signature in black ink, appearing to read 'Ulrike Seitzer', written twice side-by-side.

Borstel, March 13, 2012

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Referee's comments to PhD. thesis of Mgr. Jana Plchová, entitled "Differential expression of tick *Ixodes ricinus* genes induced by blood feeding or infection: genetic analysis of ML domain containing proteins"

Ticks are ecto-parasitic blood-feeding arthropods which, due to their ability to transmit a wide range of pathogenic microorganisms causing diseases of humans and animals, have great medical and veterinary impact. Nowadays, there is increasing knowledge on different aspects of the intimate relationships between ticks and their vertebrate hosts, as well as the various mechanisms of acquiring, survival, amplification and transmission of tick-borne pathogens. However, the biological processes on the host – tick – pathogen interface have yet to be understood and explored in more details, which could lead to the development of new and effective measures to control ticks and tick-borne diseases. Thus, the topic of the thesis is very actual.

The submitted thesis of Jana Plchová, a PhD. student of the University of South Bohemia, Faculty of Science, was elaborated in the Laboratory of Molecular Ecology of Vectors and Pathogens at the Institute of Parasitology, Biology Centre of the ASCR and supervised by Prof. Libor Grubhoffer and co-supervised by Natasha Rudenko. The Laboratory has achieved outstanding results on molecular mechanisms of pathogen transmission by ticks and gained international reputation in this field.

The main part of the PhD. thesis is based on two original first-author papers with important contributions of the candidate, published in recognized international journals – *Parasitology* (IF 2.522) and *Journal of Vector Ecology* (IF 1.256).

The thesis, inclusive copies of the two first-author papers and Appendix, is written on 110 pages and comprises introduction and literature review, objectives, results, discussion, conclusions, material and methods, a list of up-to-date referred sources and Appendix with alignments of known members within the different groups of the ML protein family.

The Introduction is an overview of general characteristics of ticks focused on tick immunity and contains a detailed characterization of the ML protein family inclusive ML proteins in ticks.

Members of the ML protein family are present in a wide range of organisms and exert various functions, however, the knowledge of their occurrence and functions in ticks is rather limited. The ML proteins in ticks identified so far belong to group II, their expression, depending on the tick species, was detected in different tissues and is induced by feeding/ The current results indicate their involvement in tick immunity. Thus, the investigation of ML proteins in *Ixodes ricinus*, the vector of a number of various pathogens, represents an important area for future studies.

The first paper by Horáčková (Plchová) et al. deals with *IrML* gene encoding a new member of the ML protein family in *I. ricinus* and reveals its differential expression in tick organs and its changes during the blood meal. Most important, this gene is similar to another gene encoding a ML domain protein which was found to be differentially expressed in uninfected and *Borrelia*-infected *I. ricinus* ticks and was assigned to a group of genes connected with pathogen recognition and transmission.

The latter was supported by unpublished results showing up-regulation of *IrML* expression in *I. ricinus* cell lines by the presence of *B. burgdorferi*.

The second paper is a valuable contribution to the knowledge on allergen-like proteins belonging to the ML domain protein family. For the first time, a gene encoding Der-p2 allergen-like protein was identified in *I. ricinus* and a complete analysis of the gene inclusive its tissue- and stage specific patterns of expression are given. It was revealed that the expression of the gene is induced by blood meal in the tick gut and to a lower extent in the haemolymph. The recombinant protein probably participates in tick immunity and neutralisation of host IgE ingested by blood meal.

I have no critical comments to the published papers as they underwent rigorous peer-reviews. Overall, the thesis is elaborated precisely, written concisely and only with few misspellings that do not decrease the value of the work and can be easily corrected in eventual future work and publications. The thesis fulfils international standards.

I have only one general question for discussion:

The author states that *IrML* is most probably involved in pathogen recognition, but not in their elimination (p. 32). Could the author explain in more details what could be the mechanism(s) of this recognition?

In conclusion, I would like to state that I highly appreciate the way the applicant coped with a very difficult task. I enjoyed reading and evaluating this thesis and suggest, if the authors find it reasonable, publishing the theoretical part (review of literature) of the thesis as a mini-review.

The thesis of Jana Plchová fulfils all requirements set for PhD. theses and I can fully recommend it for defense and fulfillment of the academic degree "***Philosophiae doctor***" (PhD).

Bratislava, 14.3.2012


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RNDr. Mária Kazimírová, CSc.