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SUBJECT
Review PhD thesis Jan Kotál

Dear chairman of the PhD committee,

I hereby provide you with my review of the PhD dissertation of Jan Kotál on "*Functional analysis of tick salivary serine and cysteine protease inhibitors*" with supervisor Dr. Michail Kotsyfakis and co-supervisor Dr. Jindřich Chmelař.

The PhD thesis of Jan Kotál describes the results of original research which resulted in significant and valuable novel insights in the biology of hard ticks and their interaction with the mammalian host. The PhD thesis from Jan Kotál mainly reports on the biochemical, molecular and functional characterization of protease inhibitors present in saliva of the tick *Ixodes ricinus*, an important vector of human and animal diseases in Europe. Besides the fundamental scientific insights, the results presented in the thesis can contribute, in the near future, to improved control of *I. ricinus* in Europe.

The data about a cystatin in tick saliva have already been published in an excellent international peer-reviewed journal (Cellular and Molecular Life Sciences, IF ~ 7). In this paper (Manuscript 3), the authors characterize a cystatin, named Iristatin, a novel molecule contributing to successful modulation of host immunity. Iristatin has been shown to inhibit mammalian cathepsins C and L and modulate the response of various leukocytes and their proliferation and infiltration to sites of inflammation. Furthermore, the authors published the crystal structure of Iristatin, which is still quite a rare component of tick-related publications and illustrates the scientific rigor of the presented work.

The identification of a serpin from tick saliva is presented in Manuscript 4, which is already prepared for publication. The probability of publication in a good journal is high, since these data are innovative, being a significant contribution to the still incomplete knowledge of the role of protease inhibitors at the tick-host interface. The presented serpin IRS-8 plays a role in blocking of blood coagulation and host complement system, probably due to its universal specificity towards many host serine proteases of physiological importance.

Unquestionably, Jan Kotál had a fundamental participation in the elaboration of these two manuscripts since he is the first author of both. Besides his work on salivary protease inhibitors, it is evident from his CV that he familiarized also with molecular interactions occurring in the tick midgut. It is also obvious that the PhD candidate had the opportunity to work with several technical approaches in a multidisciplinary setting which undoubtedly gave him a very broad skill set for the enzymatic, cellular, biochemical and molecular studies, besides others.

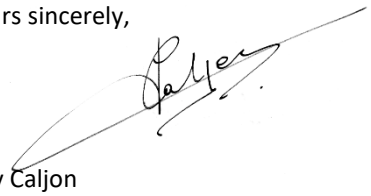
Taken the PhD thesis format into consideration, I would like to point out that the introduction is relatively concise but provides fundamental information to allow the readers to understand the important aspects of tick-host interaction with a focus on tick protease inhibitors. Two already published review papers (Manuscripts 1 and 2;

with Jan Kotál as first and shared first author respectively) provide an additional and more detailed information on the topic that readers might appreciate as a part of the introduction. MS1 summarizes the role of tick saliva as modulator of various host immune processes and cell populations. MS2 focuses specifically on tick salivary cystatins and serpins and it can therefore be assumed that it served as a guide in designing experiments described in MS3 and MS4.

I do consider that the PhD project work, developed by Jan Kotál in the hosting research group, has an excellent scientific merit and that the thesis was carefully redacted. Therefore, I highly recommend accepting the thesis of Jan Kotál for his PhD defense.

Please do not hesitate to contact me if additional information would be required,

Yours sincerely,



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