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Review of PhD thesis by Mgr. Hana Tykalová

entitled

Interaction of tick-borne encephalitis virus with host and vector cells

PhD thesis by Mgr. Hana Tykalová was prepared in the University of South Bohemia, Faculty of Science, Biology Centre of the ASCR, Institute of Parasitology in České Budějovice under supervision of Prof. RNDr. Libor Grubhoffer CSc., Hon. D.Sc., dr. h. c., and co-supervision of Prof. Alain Kohl.

The thesis contributes to understanding of different levels of interaction of tick-borne encephalitis virus (TBEV) with host and vector cells. The thesis is introduced by very well written overview summarizing the state of art of the patho-immunobiology of host and vector infection by TBEV. The Result part is divided into three sections. The first two sections are focused on characterization of vector [manuscript 1 & 2] and of the host neural cell response [manuscript 3 & 4] to TBEV infection, while the third section is focused to adaptation of TBEV to the vector and host cell background [manuscript 5].

Methodologically the thesis reveals transcriptomic and proteomic responses in TBEV-infected cells, and it also shows the phenomenon of impairment of host protein and rRNA synthesis upon TBEV infection. In addition, Hana Tykalová shows in her thesis the importance of quasispecies in the virus adaptation to vector and host cells. The thesis is finished by excellent discussion, in which are summarized the results and conclusions of TBEV interaction with host and vector cells and given the future perspectives. Also is attached the list of abbreviations and 375 references.

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Hana Tykalová could clarify and discuss during defense of her thesis the following points.

1. *Introduction:* Is it possible to define based on presented results the categories of "intrinsic" (constitutionally present) and the PAMP-induced antiviral mechanism of the "innate immunity"?
2. *manuscript 2 & 3:* Translational shut-off occurring in neuronal cells following TBEV infection is of a special interest. This finding is the first description of transcriptional and translational shut-off in neuronal cells infected with flaviviruses. Hana Tykalová shows that it is accompanied by interference with the host cell transcription and a decrease of RNA polymerase 1-produced transcripts, 18S and 28S rRNA. It will be interesting to compare these changes in the host neuronal cells with differential expression analysis and protein levels in Ixodes ricinus-derived vector cell lines infected with TBEV.
3. What is known about a possible role of extracellular vesicles (ECV) in the TBEV transmission from arthropod to human cells and to neuronal cells especially? What could be an effect of ECV-mediated transmission on cellular tropism? What could be the effect of ECV on neuroinvasiveness, and neurovirulence, and blood-brain barrier permeability?
4. *manuscript 4*: IFNs-lambda (type III IFNs) confer initial antiviral protection at anatomical barriers without provoking unnecessary inflammation. Type I IFNs come up as a second line of defense, following escape from IFN-lambda control, to enhance antiviral and immunoinflammatory responses, at the expense of collateral damage. The onset of type III IFNs action is more gradual and less pungent yet less harmful (Andreakos et al. Curr Opin Immunol. 2019). Hana Tykalová et al [*manuscript 4*] showed that „neurons and astrocytes differed in the duration of IFN-lambda response. While neurons activated the IFN-lambda response in early intervals only, astrocytes were able to sustain the IFN-lambda response to the later time point." Different expression of type I and III IFNs in neurons and astrocytes result in different levels of ISGs. Is it possible to quantitate based on presented results what was the ratio of type I and type III IFNs, and that of the IFNAR and IFNLR receptors in TBEV-infected neurons and astrocytes? What could be the consequences for the observed differences in the virulence manifestation of TBEV in different neural cell lines.
5. *manuscript 5:* The study of TBEV quasispecies in different host environments imply that TBEV exists as a heterogeneous population that contains virus variants pre-adapted to reproduction in different environments, probably enabling virus survival

in ticks and mammals. How fast is established the distribution of TBEV variants characteristic for a given host environment in a serially passaged plaque isolate when the major pre-adapted variants are initially not present? What are the consequences for TBEV infectivity?

6. What is the advantage (if any) of a low TBEV virulence for a virus evolution?

In conclusion, the thesis of Mgr. Hana Tykalová perfectly fulfills requirements demanded for the level of dissertation work. Hana Tykalová is the shared first author of one excellent paper, and co-author of four other high-quality papers published in impacted journals. I heartily recommend submitted work for defense and depending on the outcome of defense procedure for approval of doctor degree.

Prague, October 13, 2022

Prof. Ivan Hirsch, PhD