



MEDICAL UNIVERSITY
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MEDICAL UNIVERSITY OF VIENNA
Center of Pathophysiology, Infectiology and Immunology
Institute of Specific Prophylaxis and Tropical Medicine

Head: Univ. Prof. Dr. Ursula Wiedermann-Schmidt

Kinderspitalgasse 15, A-1090 Vienna Office: Tel.: +43-1-40160-38291 Fax: +43-1-40160-938293 www.meduniwien.ac.at/tropenmedizin

Vienna, 27th August 2020

Dear Prof. Horak,
Dear Univ. Doc. Krulova,
Dear Colleagues,

Please, find enclosed my evaluation of the PhD dissertation of Mgr. Tomas Machacek with the title: "The immune response of naïve mice infected with the neuropathogenic schistosome *Trichobilharzia regenti*" that was performed under the supervision of Prof. Petr Horak and Univ. Doc. Magdalena Krulova at the Faculty of Science, Charles University in Prague.

The cumulative paper thesis comprises five publications. Mag. Machacek is a first and corresponding author of two papers published/accepted in the journal *Parasite & Vectors* (IF 2.824 according to the Web of Science 2019; ranking 9/39 in the category Parasitology) and co-author of three papers, published in the journals: *Micron*, *Frontiers in Cellular and Infection Microbiology*, and *Parasite Immunology*, all scientific journals with a rigorous peer review.

The thesis concentrates on the cross-talk between helminth parasite and the host, with a specific focus on the host immune responses and the central nervous system (CNS). The ability of parasites to enter the CNS of its host and to manipulate its immune responses or behaviour has always been a source of fascination for parasitologists. Although the induction of cellular and humoral immune responses by helminth parasites affecting e.g. the gut, liver, or lung have been extensively investigated in clinical trials and experimental models, the immune responses to helminth neuroinfections remain neglected. Some of the possible explanations might be i) obvious obstacles associated with the collection of clinical samples as well as ii) the lack of suitable experimental animal models.

The thesis focuses on the interactions between the neuropathogenic avian schistosome *T. regenti* and its accidental mammalian host, the mice. *T. regenti* is a trigger of cercarial dermatitis in humans and a suitable comparative model for clinically relevant schistosomes. The major findings are i) the characterization of the host immune responses triggered by *T. regenti*-derived molecules in the periphery or within the CNS; ii) description of the role of nitric oxide on *T. regenti*; and iii) the visualization of migration of *T. regenti* in the spinal cord of mice by fluorescent 3D-imaging techniques based on newly established labelling method. The results of this thesis are valuable not only in the field of parasitic neuroinfections, such as neurotoxocarosis or neurocysticercosis, but also for research on the mechanism of immunopathological processes associated with autoimmune neurodegenerative diseases.

Generally, the thesis has a clear scientific focus; it is very well structured, written in very good English, and presented in a superb design. The thesis has five chapters. The rationale and background for the thesis

are explained in detail and are straightforward. The aims are clearly outlined and are indicative that the candidate precisely planned the thesis. Mgr. Machacek provided an extensive and informative introduction into the two main topics of his thesis, touching all the relevant aspects. Particularly, I appreciated the self-designed figures and tables in the introduction which helped to understand the presented information. Together with a large number of citations to previous work I had the impression that the student conducted the thesis based on profound and excellent knowledge.

The technical quality of this thesis is impressive. The candidate established and employed a broad array of techniques (e.g. assessment of schistosomula viability, intravital fluorescent staining of cercariae, mice handling, cell culture, ELISA, or testing of peptidase activity) to address the aims of the thesis. The candidate contributions to the publications are listed in Chapter 3. Appropriate methods were performed at a high level of quality and sophistication and are described in detail in the publications. Results in published papers are presented in a clear and easy-to-follow fashion. Chapter 4 summarizes the main findings of all thesis papers back-to-back and these findings are further discussed in the light of the relevant literature.

The candidate has presented his work at several national and international conferences (oral and poster presentations) and performed a research stay at two host institutions in Germany, demonstrating his mobility. The candidate is additionally a first author and co-author of further scientific publications, the first one dealing with cercarial dermatitis and the second one with immune responses to *Toxocara canis*. Finally, the candidate is author of seven popular publications and is associated with two societies for Parasitology.

I have the following questions and comments:

Theoretical part

Abstract. “Our *in vitro* experiments revealed that *T. regenti* cathepsin B2, a cysteine peptidase used for the skin penetration, activates bone marrow-derived dendritic cells much stronger than the parasite homogenate, suggesting its role in initiating the mixed type1/2 host immune response”. Firstly, I found it tricky to claim that TrCB2 activates BMDC “stronger” in comparison to homogenate, as the level of activation might be as well the question of the dose. Did you measure which concentration of the recombinant TrCB2 corresponds to the amount found in the homogenate? If not, how would you do it? The same counts for the claim: “We showed that living schistosomula induces production of interleukin 6 in astrocyte cultures, but their homogenate or active isoforms of *T. regenti* cysteine peptidases trigger even stronger re-action”. Secondly, the “activation” status per se is not indicative for the “mixed type 1/2” host response. Please, comment on this.

Page 12. “The cytokine milieu in the CSF of NT patients indicates the Th2 polarization. Specially, IL-1b, IL-4, IL-6, and IL-13 were elevated.....” Do you suggest that IL-1b and IL-6 are associated with Th2 polarization? In my point of view, IL-1b and IL-6 are not associated with Th2 responses, but I could be mistaken. Please, comment.

Page 13. “The concentration of IL-4 and IL-6 was even higher in the CSF than serum which suggests intrathecal production of the cytokines and favouring type 2 immunity.” Please, see my comment above.

Page 39. The role of parasite-derived extracellular vesicles (EV) in the parasite-host communication represents a very exciting topic. You correctly discussed the role of antigens present in EV in the initiation of the host immune response in the case of human schistosomes. Is there anything known about the role of parasite-derived EV in the neurotropic behaviour of parasites? How would you test the effect of EV on e.g. astrocytes or microglia?

Page 39. “The effects of rTrCB2 might be caused either by its direct immunogenicity or by its enzymatic activity which could be sensed by the protease-activated receptors.” Please, discuss which receptors might be involved.

Papers

Paper 1. Janecek *at al.* (2014) have shown that the distribution of larvae of *T. canis*, a parasite known to migrate to the CNS, depends on the mice strain used. Can you speculate, which factors might be involved in this phenomenon? In your study, you used mice on both BALB/c and C57Bl/6 background, similarly, as Janecek *at al.* Did you see any difference in the i) immune responses of the host or ii) *T. regenti* migration pattern between these two mice strains?

Scanning of fluorescent signals by confocal laser scanning microscopy (CLSM) allows non-invasive cell imaging *in vivo* and enables to obtain high-resolution images and 3D reconstructions. CLSM has been used previously in parasitology. Did you have a chance to analyse the migration of fluorescently labelled *T. regenti* by this method? If not, what were the reasons against?

Paper 2. You characterized the responses of murine astrocytes and microglia to *T. regenti* live parasite and *T. regenti*-derived molecules by measuring the levels of NO and cytokines. Did you evaluate the viability of these cells after *T. regenti* exposure? Along these lines, Chou *et al.* (2020) have shown that *T. canis* E/S products induce apoptosis in astrocytes. How would you test if *T. regenti* induces apoptosis or autophagy in astrocytes?

Altogether, the submitted thesis well documents that MSc. Tomas Machacek has fulfilled all criteria to be awarded the “PhD” degree.

I confirm that I have no conflict of interest regarding the reviewing of this doctoral thesis.

Yours sincerely,



Assoc. Prof. Univ. Doc. Irma Schabussova, PhD

T: +43 (0)1 40 160-38250

F: +43 (0)1 40 160-938293

Email: Irma.schabussova@meduniwien.ac.at

