27th June 2014

It is my pleasure to provide an opinion on the PhD thesis **"*Leishmania tropica*: immunopathology and genetic control" by Yahya Sohrabi MS.c,** student of the Third Faculty of Medicine, Charles University in Prague.

The thesis is focused on *Leishmania* species which - although medically important, is one of the least studied. *Leishmania tropica* causes both cutaneous and visceral leishmaniasis in the Old Word with recent outbreaks in several urban foci, but the immune response to this parasite has never been studied thoroughly. The main reason was a lack of appropriate inbred mammal model.

The thesis consists of four main chapters – (1) Introduction, (2) Material and methods, (3) Results and (4) Discussion connected with Conclusions. Together with the extensive list of References the text comprises a considerable extend - 203 pages. However, some information is presented repeatedly; for example, the whole chapter Materials and methods in my opinion is redundant as it repeats information given in further text.

First chapter is a comprehensive review concerned on leishmaniasis - beginning with the history of the disease, biology of the genus *Leishmania*, diversity in the course and clinical manifestations of the diseases and overview of leishmaniases present in Europe with emphasis on *L. tropica*. Following text is focused on methods allowing research of immunology and genetic control of complex diseases including leishmaniasis using animal models and presents summary of results obtained with murine models in this field. I appreciate the Tables 4 and 5 which summarize genes identified to be involved in immune response to leishmaniasis and co-localization of these genes/loci in mice and human, respectively.

Results chapter consists of four studies, three of which were already published in impacted journals; the fourth is prepared for publication. Yahya Sohrabi is a first author of two of them. In the first work published in 2012 (PLOS Neglected Trop. Dis.) author and his colleagues analyzed disease outcome in a set of recombinant congenic Ccs/Dem mice strains infected with *L. tropica*. Subject of the following study is the recombinant strain CcS-9 which was more susceptible to *L. tropica* than parental strains, developed large skin lesions, hepatomegaly and splenomegaly. In the manuscript prepared for publication authors showed that the pathology in this strain was not caused by higher number of parasites in comparison with other strains, but it was connected with more severe inflammatory response. The other recombinant susceptible strain CcS-16 was characterized genetically and identification of 8 gene-loci controlling susceptibility to *L. tropica* was published in 2013 (PLOS Neglected Trop. Dis.). The fourth publication (2012, Microbes and Infections) revealed that memory CD8+ T cells are the main source of IFN- γ in people infected with cutaneous leishmaniasis and might be responsible for maintenance of the protective immune response against leishmaniasis.

In my opinion **the main aims of the thesis were fully accomplished -** author and his colleagues established the first reliable mouse model for genetic studies of *L. tropica* infection, described in detail pathology connected with the disease and genes involved in its control. They proved that multiple gene interactions control symptoms during *L. tropica* infection. Use of the same recombinant mice strains which were previously tested for *L. major* infection allowed evaluation of species specificity of immune response and diversity in susceptibility genes involved. Surprisingly large sex effect on infection and pathology caused by *L. tropica* was revealed. The results of the study confirmed the role of host genotype in development of leishmaniasis. In addition, recognition of important molecules and cells involved in the outcome of infection can contribute to novel strategies of the disease treatment.

CONCLUSION

I find this PhD project of high scientific quality. It brings unique, novel and valuable findings which fill the gap in recognition of the disease caused by *L. tropica*. Yahya Sohrabi proved to manage immunological and histological methods as well as methods of genetics and molecular biology. He is able to correctly evaluate and properly interpret the acquired results. It is my pleasure to fully recommend Yahya Sohrabi to obtain the PhD degree.

QUESTIONS/COMMENTS

I am experienced in vector – parasite interactions, so my questions and comments cover rather general biological aspects of the study:

1) The term phlebotomine sand flies should not be written in italics, use of italics in biological texts is restricted for genus and species names (pages 12, 13).

2) The Introduction is quite long and broad; however, I am missing more information about epidemiology of leishmaniases, especially *L. tropica* which is the crucial organism studied. For example, there is no information about important zoonotic foci of *L. tropica* in Israel. In contrast to statement in the text the role of hyraxes as reservoir hosts *of L. tropica* were confirmed by different authors, even experimentally. Other interesting but missing points are (1) the high genetic diversity of *L. tropica* strains from different geographical areas or (2) the question of species status of *L. killicki* and its recently described reservoir in Maghreb area.

3) Mice were infected with stationary phase promastigotes with added promastigote secretory gel. Did you also try to increase percentage of metacyclic forms in the inoculums or co-inoculate sand fly salivary glands to mimic natural mode of host infection?

4) For quantification of parasites in mice tissues you have used analysis of PCR product by ELISA. What is the advantage of PCR-ELISA against a classical quantitative real-time PCR analysis?

5) I did not find the strain CcS-9 among strains tested in the study published in 2012 in PLOS Neglected Trop. Dis., although according to the text on page 71 and Table 8 it was also tested. Could you explain this difference?

6) Could you estimate what is the reason of different epidemiology of *L. tropica* between localities, where this parasite circulate in a zoonotical cycle and localities with antroponotic transmission? Do you think that there are some genetically fixed differences among different strains of the species or is it a reversible ecological strategy of the same parasite? Is there some geographical or genetic connection between type of circulation (antroponotic vs. zoonotic) and tendency of *L. tropica* to visceralize?

RNDr. Jovana Sádlová, Ph.D.

Jovana Sádlová

Departmet of Parasitology, Faculty of Sciences, Charles University in Prague