

Review of the Ph.D. thesis by Tetyana Kobets: Methods for *Leishmania* parasite detection and quantification as a tool for study of pathogen-vector-host interactions

The thesis deals with the important subject, methods of *Leishmania* parasite load measurement and their applications in the immunoparasitology research. Leishmaniasis threatens millions of people in tropical and subtropical zone and understanding of the role of host immune mechanisms in defence against this infection, mechanisms, by which the parasite escapes the immunological control and immunomodulatory effects of sand fly saliva, facilitating transmission and dissemination of the parasite in the host, is very important for successful development of vaccines, for diagnostics and therapy of *Leishmania* infections.

Detection and mainly proper quantification of the parasite in the host organs and tissues represents the obligatory prerequisite for all studies dealing with the effect of vector saliva on parasite transmission, natural resistance and development of adaptive immunity against the parasite including the genetic control of parasite elimination, dissemination, and disease development. Tetyana Kobets developed the improved PCR-ELISA method for detection and quantification of *Leishmania* parasites, which was published in the highly impacted journal Nature Protocols. The improvement consists in the use of two labelled primers while the step with hybridization with labelled probes is omitted. This increases the reliability of the method enabling inclusion of additional controls. The method is very sensitive (threshold of 0.3 fg of DNA which corresponds to 0.004 parasites). The applicability of the method in the immunoparasitology research is documented in two additional papers also published in very good highly impacted journals.

The thesis begins with the detailed introduction focused on the *Leishmania* parasite including its life cycle, clinics, epidemiology and distribution. The second chapter comprises mouse models used for genetic studies and in the third one assays for parasite detection and quantification are discussed in detail. The introduction is comprehensive and well written.

The aims of the study are clearly formulated and in my opinion they were accomplished completely. Materials and methods rather repeat what is written in particular papers. Three papers published in Nature Protocols (IF = 8.362), Immunogenetics (IF = 2.942) and International Journal for Parasitology (IF = 3.822) ensue. The fourth manuscript deals with the genetic murine model for the analysis of susceptibility to *Leishmania tropica* infection. Each paper is introduced with the short summary of results and the individual

contribution of the Ph.D. student which I very appreciated. Sometimes the contribution of particular authors is difficult to distinguish.

In my opinion the well done experimental work led to publishing three excellent papers in highly impacted journals. Conclusion of the thesis represents very informed discussion of the topic and documents that Tetyana Kobets was really involved in the problem. Instead of description of particular parts of the thesis in detail, I would like to encourage the discussion by following questions and comments.

1. In the past, Nrpmp gene used to be connected with the susceptibility/resistance to *Leishmania* and other intracellular pathogens. What is the recent opinion of the role of this gene in natural resistance to leishmaniasis?
2. In the second paper (Immunogenetics), the level of IgE was observed in genetically different mice infected with *L. major*. What was the reason of involving IgE? Does this immunoglobulin play any role in the protection against leishmaniasis or it was considered just as a sign of Th2 polarization?
3. To which extent the murine genetic models are applicable to humans?
4. In the past, the pathogenesis of leishmaniasis was mostly connected with macrophages. Recently, it was shown that *Leishmania* parasites can infect other cells of immune system like dendritic cells and neutrophils. What is the role of neutrophils in the immune response to *Leishmania* infection?
5. Just a comment to the MS. You observed many cytokines, but omitted several very important. TNF is important in activation of macrophages to kill *Leishmania* parasites. IL-10 and TGF- β are important in the development of alternatively activated and regulatory macrophages permissive for *Leishmania* parasites.
6. In the manuscript, you have used the term “relative units” in the expression of parasite load measured by limiting dilution technique. But this term is not defined in the MS. In addition, numbers of parasites after three days of cultivation need not correspond to their number at the moment of lymphoid organ dissection. Can you comment on it?
7. In discussion to the paper devoted to the effect of immunization by sand fly bites on the *Leishmania* infection Tetyana Kobets speculates that the non-protective immunity achieved by long term immunization results from skewing the immune system towards

the Th2 response. This hypothesis could be easily confirmed by measuring IgG2a: IgG1 ratio in immunized mice. The high ratio indicates Th2 polarization.

Conclusion: The thesis meets requirements for PhD. thesis and provides valuable information about newly developed methods of *Leishmania* detection and quantification and their application in the immunoparasitology research. I strongly recommend accepting the candidate's thesis as the basis for obtaining the degree **Ph.D.**

In České Budějovice 11.8. 2011

Doc. RNDr. Jan Kopecký, CSc.