Institute of Microbiology
Academy of Sciences of the Czech Republic, v.v.i.

Division of Immunology and Gnotobiology
Head: prof. RNDr. B. Říhová, DSc.
Vídeňská 1083
142 20 Prague 4

Reviewer: Milada Šírová, PhD.
Laboratory of Tumor Immunology
Institute of Microbiology ASCR, v.v.i.

Review of doctoral thesis
Igor Grekov: Experimental murine leishmaniasis and its application for drug discovery and study of host-pathogen interactions

The thesis is organized in a commonly used format, i.e. it contains an introductory part and a set of published papers. Introduction is followed by a chapter on methods, and a concise formulation of aims. The core of the thesis consists of four papers. In two of them the applicant is a co-author, in one he is the first author, the last paper was included as a submitted manuscript - again, the applicant is the first author. Each paper is introduced by a short text summarizing the aim of the study and principal results. A statement confirming how the applicant participated in the experiments and creation of the paper is given. In a final part of the thesis, a short discussion, conclusions, and a list of references are included.
The most significant results are the following:
1. Refinement of the protocol for *Leishmania* cultivation using biphasic medium
2. Improvement of the PCR-ELISA protocol for quantitative detection of *Leishmania* parasites in tissue samples
3. Mapping of five genetic loci that are involved in control of *Leishmania major* dissemination and pathology
4. Demonstration of cytostatic and cytotoxic effect of calcimycin on *Leishmania* promastigotes and amastigotes *in vitro*
5. Identification of constitutive NO synthase of *Leishmania* as a potential therapeutic target

Tel.: -420 241 062 345 Fax.: -420 241 721 143
E-mail: immuno@biomed.cas.cz

A substantial part of these results (points 4-5) forms a content of the last paper included in the thesis. However, the paper was submitted but has not yet been accepted for publication; therefore it should not be accepted as a subject of evaluation.

I have the following comments:

The Introduction is well written and enables the reader to gain a basic insight into all the studied issues. My concern would be that in some parts the chapter does not respect proportions. For instance, there is a detailed description of treatment protocols using lipidic formulations of Amphotericin B (pp. 32-33). On the other hand, disulfiram as an emerging alternative treatment is not mentioned in the Introduction, although it was used as a kind of standard in the study of cytostatic and cytotoxic effects of calcimycin (Grekov et al "Calcium Ionophore Calcimycin…").

Chapter on methods are usually no regular component of theses written in this form. The chapter provides an overview of all experimental procedures and methods used, but in fact all of them are described in the papers.

The aims are formulated clearly and concisely. Discussion briefly reviews the results in all particular projects and compares the obtained data with the published results of others. Conclusion is brief and specific, documenting that the aims were accomplished. The whole text is easy to read. There are some (but infrequent) typing errors or mistakes (e.g. Fig. 1 cited on p. 48 is missing; a paragraph on p. 128 is repeated).

Questions:

Could you explain what the reasons were for choosing calcimycin as a candidate anti-leishmanial agent? Why did you compare calcimycin with disulfiram and did not use established drugs such as miltefosin or paromomycin?

Obviously, calcimycin was a model compound that made it possible to establish constitutive NO synthase as a potential target for treatment, but could it be used as an *in vivo* drug?

Do you have data on the potential effect of combination of calcimycin and disulfiram?

Conclusion: The thesis meets the requirements for PhD. thesis. I recommend it as the basis for obtaining the PhD. degree.

Prague, September 14th, 2011-09-14

Milada Šírová, PhD.