Prague, 30th August 2022

RE: Evaluation Report – Diploma Thesis by Sabína Vančíková

Dear Committee Members,

The diploma thesis authored by Sabína Vančíková aims to elucidate the immune responses to gut mycobiota in patients suffering from inflammatory bowel disease (IBD). I would like to highlight that the thesis is written in English and have standard chapter composition. The literature overview focuses mainly on gut barrier function and IBD, much less space is devoted to mycobiota and immune responses against it.

The literature review is sometimes lacking a deeper focus and synthesis of information from different sources so much required from an excellent diploma thesis or even lacks some crucial citations. To mention just some examples:

1. In the fungal disease susceptibility chapter only the *CX3CR1, Dectin-1/2, and DC-SIGN* mutation(s) are mentioned (p. 18-19). However, there is much more to be discussed e.g. *RORC*[1], *IL17F*[2], *STAT3*[3,4], *CLEC7A*[5], *CARD9*[6] or *STAT1*[7,8] mutations to mention just the main ones associated with candidiasis susceptibility in humans.

2. Following the same line, the interaction of mycobiota with the host´s immune system is limited in the literature overview to the description of receptors involved in sensing or endocytosis of fungi (p.17-18). There is not a single piece of information about the adaptive immune response to fungi. Given the fact, that Ig-response/B-cell response is in the central focus of the experimental part of the thesis I consider this as the major drawback of the literature overview.

3. Moreover, several recent papers dealing with the central topic of the thesis are not cited nor discussed at all[9-13]. All of the above-mentioned papers deal with the IBD/CD, mycobiome and/or Ig-response induction by mycobiome. These are crucial papers for the literature overview as well as for the discussion part. Yet, they are not mentioned at all.

Thus, the literature overview is rather incomplete from my point of view and I would suggest discussing the complexity of immune response to mycobiota in more detail during the thesis defense presentation.

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The abovementioned citations No. 9-13 should be discussed in the context of the obtained results during the thesis defense as well.

The Material and Methods section contains all the necessary information for replicating the experiments described in the thesis. This is a well-documented part with all details needed.

The main goals of the experimental part are clearly stated and they are represented by three interconnected, yet self-standing aims: 1) analyzing biomarkers associated with gut permeability in IBD, 2) determining levels of antifungal antibodies in IBD patients and 3) the effect of anti-fungal lysates on the reactivity PBMCs derived macrophages. The first Aim is solely devoted to the characterization of IBD patients with respect to their gut permeability. In order to achieve this, several biomarkers are measured in the serum of IBD patients and controls by ELISA. When the IBD patients were stratified according to their CD/UC or PSC some biomarkers were found to be associated with a particular type of IBD.

> 4. I am wondering if the stratification according to the treatment, sex or age was tested and if resulted in differences in these biomarkers distribution? The MBL marker shows in the case of PSC-IBD kind of bimodal distribution. Can you associate this bimodal distribution with some clinical parameters? Lastly, I think it would be more beneficial to include also the control subjects to Figure 7 (if this is going to be presented during thesis defense).

The second aim was focused on the detection of anti-fungal antibodies in the sera of IBD patients and controls. First, based on the cultivation assay from feces several fungal species were selected from obtained single colonies and individual sequencing of ITS region of rDNA was performed. It is of note, that this was done on 3 IBD patients and 2 controls.

> 5. It is not clear how were the participants of this part of the study (cultivation assay from feces) selected. What was the criterion? The same applies to the selection of the controls. A detailed description of these subjects concerning their age, sex, treatment, etc. is missing in the thesis. This should be clarified.

Next, the identified fungal strains were used to prepare cell lysates, that were individually incubated with serum-derived from IBD patients and controls and the IgM, IgG and IgA reactivity to fungi lysates was visualized using specific secondary antibodies in ELISA.

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6. Just of note, the presence of particular fungal species was not measured in the vast majority of the individuals. If done, this would aloud to determine if the IBD patients are preferentially colonized by (some) fungal species in comparison to healthy controls or if the response to particular fungal species is dysregulated in IBD patients.

7. Related to figure 8 and the discussion part: Is there any functional difference between IgA in the gut and IgA in the serum?

Subsequently, the presence of particular types of anti-fungal antibodies in all individuals was correlated to each other.

8. In my opinion, this mainly reflects the presence of shared epitopes between particular fungal strains (as mentioned briefly in the discussion part of the thesis).

9. It is not clear to me, why the presence of antibodies to particular fungal species was not correlated to various clinical parameters of IBD patients (same as point No. 4). Or this was tried and there is no significant outcome? If so, this should be reported and discussed as well… If not tried at all, this is a missed opportunity to shine.

The macrophages polarized from PBMCs from healthy volunteers were stimulated by particular fungal species identified previously. Several parameters were measured using flow cytometry and ELISA.

10. There is just a representative FACS gating strategy for PBMCs derived cells and various measured markers (no FMO controls shown). Original data highlighting the reported differences achieved by stimulation using fungal lysates or other factors are not reported at all. This should be presented during the thesis defense.

11. Figure 10 + 11 – the number of replicates per group and their nature (biological, technical) is not indicated. Dot plots should be used instead of bar graphs. Moreover, how was the ELISA in figure 11 performed? I guess the cultivation with different fungal lysates might impact the viability of PBMCs derived cells in the cultivation and secondly impact cytokine production. How this was controlled or normalized?

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Lastly, the discussion part is lacking several important papers relevant to the thesis topic and their impact on the result obtained by the author (at least the abovementioned citations No. 9-13). They should be discussed in the context of the obtained data during the thesis defense.

To summarize, I would like to highlight the positive aspects of the thesis – it is written in English, it is easy to follow and it contains schemes nicely illustrating some important aspects relevant to the literature overview. The material and methods section is in detail documenting all the experiments performed. On the other hand, the literature overview and discussion are sometimes a bit vague and lack necessary detail or important information from several new papers relevant to the topic of the thesis. Also at least the correlation between the clinical parameters of IBD patients and the presence of anti-fungal antibodies is lacking. There are some additional issues associated with the experimental part, but I guess they can be clarified or improved during the thesis presentation. I am leaving the overall classification of the thesis to the Committee Members based on the performance during the thesis defense.

With best regards

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