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Report on the Ph.D. Thesis of MSc. Zhengyu Du named:

"Role of bacteria and mucosal immune system and their interaction in the pathogenesis of inflammatory bowel disease"

Msc. Zhengyu Du submitted a Ph.D. thesis that deals with the highly actual problematic of the development of inflammatory bowel disease and its causative factors. In particular, the work studies the interplay between microbiota and host immune system and aims to provide understanding of microbial entity and its molecular basis for induction of inflammatory bowel disease.

The Ph.D. thesis itself consists of 139 pages and is composed of 6 chapters. The obligatory introductory chapter has 29 pages, 5 figures, 1 table and 174 references and represents up-to-date review of the literature. This chapter is followed by a chapter defining the dissertation goal, and main body of the dissertation consisting of 3 previously peer-reviewed and published articles. The last chapter of the Ph.D. thesis is a discussion of 16 pages and 63 references. The Ph.D. thesis is concluded with a list of abbreviations, candidate CV and list of publications.

The submitted Ph.D. thesis is coherent, well-organized and well-written. The number of typos and discrepancies is minimal. Msc. Zhengyu Du demonstrated critical knowledge and judgement of the literature while being able to evaluate her own results. Indeed, the key aspects of the submitted Ph.D. thesis are 3 previously peer-reviewed and impacted publications. Msc. Zhengyu Du is either first author or first co-author of two of these publications while being the 9^{th} co-author of 13 authors on the third one.

Questions for general discussion:

1) I would like the candidate to define her specific contribution for the listed publications and list the publication impact factor during her Ph.D. thesis defense.

2) Question related to the work: “Development of gut inflammation in mice colonized with mucosa-associated bacteria from patients with ulcerative colitis.” It seems that preserving the microbiome diversity and species representation is a major limiting factor in studies defining the microbiome role in induction of the disease by transferring the microbiota from human to mice. Would candidate discuss this topic and think of any suggestions how to approach this issue in the future?

3) Question related to the work: “Secretion of alfa-hemolysin by *E. coli* disrupts tight junctions in ulcerative colitis patients.” The work demonstrates that knocking-out both alfa-hemolysin genes in *E. coli* p19a prevents the decrease of the transepithelial electric resistance in Caco-2 cells that serves as a read out for tight junction integrity. Would candidate speculate on signaling induced by alfa-hemolysin that could lead to the tight junction dissolution? Further, why the transepithelial electric resistance in Caco-2 increases even above the level of control once alfa-hemolysin genes were deleted (Fig.4.4)? Was the phenotype reassessed by alfa-hemolysin complementation on plasmid in a knock-out strain?

4) Question related to the work: “Protective effect of *Clostridium tyrobutyricum* in acute dextran sodium sulphate-induced colitis.” Would candidate think of any experiments how to prove that it is the butyrate production by the *Clostridium tyrobutyricum* that is responsible for the observed protective effects?

5) General question: Would candidate speculate on the possible causes of the increase of incidence of inflammatory bowel disease observed in the recent years?

14.11. 2016 in Prague,

Jana Kamanova