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A review of Ph.D. Thesis

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

*by MSc. Zhengyu Du*

The submitted work deals with the topical issue of inflammatory bowel diseases (IBD), an illness with constantly increasing incidence and not yet fully elucidated pathogenesis. The experimental work was carried out in the Institute of Microbiology (Laboratory of Immunology and Gnotobiology), Academy of Sciences of the Czech Republic and in Wageningen University, The Netherlands and realized under the Marie Curie ITN Project. Working in the laboratory of Immunology and Gnotobiology enabled the use of unique germ-free animal models.

The thesis is based on three original articles published in impacted international journals, the candidate is the first author in one of them. The thesis is composed of Abstract, General introduction, Aims, full text of above mentioned articles comprising a detailed discussion and summarizing General discussion. The Introduction brings a current knowledge concerning IBD pathogenesis with special emphasis on immune reactions and intestinal microbiota. All the parts of the thesis are accompanied with large number of references which proves the deep knowledge of the issue studied. The work is formally well edited with the exception of wrong figure numbering in the text of Chapter 1 and some small printing errors. Fig. 1.3 concerns probably European population and it would be given in the figure description because of large differences among various ethnics.

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Methods used in experimental work are suitable, modern and often sophisticated and comprise work with experimental animals, bacteriological methods, immunochemical methods, methods of molecular biology and genetics, immunofluorescence, histological methods and gas chromatography. It is evident that the candidate has gained a number of valuable methodological experience.

The first publication follows the effect of bacteria originated from biopsies of patients with ulcerative colitis transferred to GF mice. These bacteria does not induce colitis in mice but in some cases increase sensitivity to DSS induced experimental colitis. Very interesting is the increased sensitivity of F4 mice which raises the question of possible disappearance of some protective bacteria. I wonder what is the qualitative difference between mouse and human microbiom? Is it possible to draw conclusions from the results obtained in mice applicable to human pathology?

The second publication brings the important and original results concerning the possible effect of *E. coli* hemolysin on intestinal permeability. It is possible to regard hemolysin of *E.coli* as one of pathogenetic factors in ulcerative colitis.

A possible probiotic candidate *Clostridium tyrobutyricum* is evaluated in the third paper. It was shown to be very efficient in the prevention of experimental DSS colitis in mice. Mechanism of its function was studied. Immunocompetent BALB/c mice and deficient SCID mice were used as animal models. I see certain discrepancy between very similar character of DSS colitis in the both mouse strains used on one hand and in described importance of T cells in the pathogenesis of colitis on the other hand. What is the real role of specific immunity in the pathogenesis of DSS colitis? The differences between BALB/c and SCID mice were found in the production of inflammatory cytokines TNF and IL-18. Why the production of TNF was followed in culture supernatants by ELISA method and that of IL-18 in tissue sections by immunofluorescence? Was tested the total IL-18 or only its active form? Are there some efforts to use *Clostridium tyrobutyricum* in clinical trials?

Concluding General discussion is well structured underlining the principal results and future perspectives of further research. The main not fully answered question remains – it is the role of intestinal microbiota: are their changes in microbiota described in IBD the cause or the consequence of IBD?

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## **Conclusion**

The thesis submitted brings interesting and original results published in international journals. The work shows the deep theoretical knowledge and large methodological experience of the author. The work meets the requirements for the Ph.D. thesis and can be used as a basis for awarding Ph.D. degree.

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