

Report of the supervisor on Bc. thesis by Barbora Svobodova „LIPOPOLYSACCHARIDE CONTAMINATION OF RECOMBINANT PROTEINS AND ITS SIGNIFICANCE FOR IMMUNOLOGICAL STUDIES“

During the production of recombinant proteins in *Escherichia coli* expression system we often meet problems with contamination of produced proteins with LPS, the structural constituent of *E. coli* cell wall. This contamination can markedly affect the results of immunological experiments performed with recombinant proteins, because LPS in minute amounts stimulates macrophages and B lymphocytes through TLR4 receptor, which leads to cell proliferation and production of various cytokines.

Therefore the main aim of Barbara's thesis was the removal of LPS contamination, determination of residual LPS in the preparation of recombinant proteins and estimation of minimum "efficient concentration" of LPS which can induce the cytokine response in mouse splenocyte culture.

I found Barbora responsible, hard working student with sufficient amount of laboratory skill. She was capable of creative thinking, designing experiments, carrying them out, interpreting the results and discussing them critically. Understandably she suffered from the lack of immunology knowledge because she hasn't attended any lecture from immunology. Due to the lack of experience with writing of such kind of theses the discussion and comparison of her results with scientific literature are not deep enough.

Nevertheless, she learned methods of LPS determination, its removal from protein samples, work with immunocompetent cells in culture, determination of cytokines by ELISA. She was able to reduce LPS contamination below the level which is able to induce production of cytokines and this way to affect the results of immunological experiments.

I strongly recommend the bachelor thesis by Barbora Svobodová for the defence.

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Jan Kopecký