Abstract

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Toll-like receptors in bacterial infections.

Diploma thesis

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Aim: The aim of this diploma thesis was to determine the expression and the activation of the toll-like receptors (TLRs) 2, 4, 5 and 9 in bacterial infections by reverse transcription by real time polymerase chain reaction (RT-qPCR) and by Western blotting (WB). Another aim was to introduce detection of TLRs in a pig by WB in the Institute of Microbiology of the ASCR, v.v.i. in Nový Hrádek.

Methods: 1) Optimization of WB – extraction of proteins, protein quantification, electrophoresis, Western blotting, immunodetection of proteins. 2) Four groups of one-week-old gnotobiotic piglets: germfree as a control (GF), piglets infected with *Salmonella enterica* serovar Typhimurium strain LT2 (LT2), piglets infected with *Escherichia coli* O55 (O55) and piglets colonized with *Escherichia coli* Nissle 1917 (EcN). 3) RT-qPCR - purification of a total RNA, spectrophotometric RNA quantification and estimation of purity, synthesis of cDNA, RT-qPCR, normalization and relativization of real-time PCR data.

Results: WB analysis of TLR5 in the piglet ileum and mesenteric lymph nodes was optimized. Antibodies against TLR2, 4 and 9 were tested. The mRNA expression of TLR5 and 9 was increased in the piglet ileum of EcN group. There were no significant differences in the TLRs expression between the group O55 and the group GF. The expression of TLR2, 4 and 5 was increased in mesenteric lymph nodes of group LT2.

Conclusion: Different ways of sample preparation for WB were evaluated. The expression of mRNA of TLR2, 4, 5 and 9 in piglet ileum and mesenteric lymph nodes after colonization/infection of germ-free piglets with probiotic bacteria or intestinal pathogens were described. The detection of gene message and protein expression of TLRs make possible good imagination dealing with a regulation and function of TLRs.