MicroRNA expression in lung tissue and blood isolated from pigs suffering from bacterial pneumonia

MicroRNAs (miRNAs) are a highly evolutionarily conserved group of small non-coding RNA molecules, which regulate the activity of other genes at the post-transcriptional level. Recently it has become evident that miRNA plays an important role in modulating and fine tuning of the innate and adaptive immune responses. Still, little is known about the impact of miRNAs in the development and pathogenesis of lung infections. Expression of miRNA, known to be induced by bacterial (i.e., LPS) ligands and thus supposed to play a role in the regulation of antimicrobial defence, were studied in lung tissue from pigs experimentally infected with Actinobacillus pleuropneumoniae serotype 2 and 6. Circulating miRNAs were studied in blood from pigs infected with A. pleuropneumoniae serotype 2 using real time-qPCR (RT-qPCR). Expression profiles of miRNA in blood of seven animals before and after infection, were also studied using miRCURY™ LNA arrays (Exiqon, Denmark). Piglets were inoculated by dripping 1ml bacterial suspension, into each nostril during inhalation. Each time group is a different set of 4-6 pigs. Most of the inoculated pigs revealed characteristic, well demarcated, lung lesions. No pathological changes were seen in lungs from control animals. All AP infected animals had a significantly higher level of mRNA coding for the acute-phase protein SAA-2 in the liver compared to the control group. Whole Blood samples were collected in PAXgene Blood RNA Tubes (PrenalytiX) before (control) and after infection of piglets (6 h., 12 h., 24 h. and 48 h.). Total RNA was extracted from blood samples using PAXgene™ Blood RNA kit (Qiagen/ PrenalytiX). The quantity of extracted total RNA was determined using a Nanodrop ND-1000 and the quality of extracted RNA was estimated by on-chip electrophoresis (Nanochip 6000) on an Agilent 2100 Bioanalyzer, a RNA integrity number (RIN) was assigned to each sample. Expression levels of selected miRNA were further studied in lung tissue collected at two time points (6 h. and 24 h.) after A. pleuropneumoniae serotype 2 and 6 infection. 600 ng total RNA from blood samples before and after infection were labelled with Hy5™ and Hy3™ fluorescent label, respectively, using the miRCURY™ LNA Array power labelling kit (Exiqon, Denmark). Sample were mixed pair-wise and hybridized to miRCURY™ LNA array version 11.0 (Exiqon, Denmark), which contains capture probes targeting all miRNAs for human, mouse and rat. The miRCURY™ LNA array microarray slides were scanned, and image analysis was carried out using the ImaGene 8.0 software (BioDiscovery, Inc., USA). A two-tailed T-test calculated between infected and control identified 10 of 1263 miRNA to be differentially expressed (p-values lower than 0.05). MicroRNA expression in lung tissue over time in response to the two different serotypes were very similar. miR-223 was found to be highly up regulated, followed by miR-146a and to a lesser degree miR-233 in lung tissue of the AP serotype 2 infected animals. MiR-233 was also found to be up regulated in blood based on both microarray and RT-qPCR, mir-233 is a negative regulator of neutrophil proliferation and activation and might act to limit the potentially harmful consequences of the accumulation of infiltrating neutrophils in AP infected lungs. More data of microRNA expression in blood of pigs infected with A. pleuropneumonia serotype 2 will be presented.

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