PROTEOMIC ANALYSIS OF THE RESPONSE OF PORCINE NEUTROPHILS TO STIMULATION WITH LPS.

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Neutrophils are the most abundant leukocytes in peripheral blood and constitute an essential cellular component of innate host response against microbial invasion. Studies of the immunomodulatory effects of LPS are of historical importance for description of the role these cells play in the infection and inflammation. Many questions concerning the neutrophils role in the immune repose remains to be answered. The aims of this work were to test the differential proteins expression in porcine neutrophils stimulated with LPS, in order to gain complete understanding of the nature of LPS signalling in host cell and identifying the proteins implicated in the innate immune response, using two-dimensional gel electrophoresis (2-DE), mass spectrometry technology and systems biology analysis.

To identify the protein expression changes induced by LPS stimulation, porcine neutrophils were isolated from the blood of health pigs and were incubated for 6, 9 and 18 h in the presence or absence of 100 ng/ml of LPS. The quantitative analysis of proteins extracts from neutrophils (control and LPS-treated cells) were performed by 2-DE. The number of proteins differentially expressed in neutrophils after LPS treatment varies through the time-course. We sought to investigate whether the detected proteins belonged to specific pathways and tried to integrate our protein data set into functional networks, using the IPA application. The molecular and cellular functions most significantly perturbed were cell death at 6 h post-stimulation and cell movement at 9h and 18 h. The association of the deregulated proteins with canonical pathways highlighted two major pathways: acute phase response signalling and NRF-2 mediate oxidative stress response at all times studied. The infectious and inflammatory diseases, cell-to-cell signalling and interaction and cell death were the most affected networks, reinforcing the idea that these processes are implicated in the response to LPS.