RESEARCH ABSTRACT

SWINE HEALTH

Title: Gene expression in lymph nodes of PRRSV-infected pigs – **NPB #08-247**

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Scientific Abstract

Porcine reproductive and respiratory syndrome virus (PRRSV) is a major pathogen of swine worldwide and causes considerable economic loss. Previous in vitro PRRSV infection studies of the primary target cells, porcine alveolar macrophages (PAMs) in our lab have identified,through Serial Analysis of Gene Expression (SAGE) data, specific pathways that associate with variation in PRRSV replication and macrophage function. The goal of the current study to identify significant changes in gene expression in homogenized tracheobronchial lymph nodes (TBLN) during the acute phase of a PRRSV infection in vivo using Digital Gene Expression Tag Profiling. Comparative functional genomics of the acute response against PRRSV, porcine circovirus type 2 (PCV2), and swine influenza virus (SIV) will more clearly define the negative effect of PRRSV on the pig immune system. 12 sows purchased from a source that can provide PCV2, SIV, and PRRSV negative animals were farrowed at NADC to provide 80 early-weaned pigs for the animal experiment at 5 weeks of age. Pigs were allotted to one of 4 equal sized treatment groups according to litter PCV2 maternal antibody status: Group 1 – sham inoculated control, Group 2 – PRRSV challenge, Group 3 - PCV2 challenge, or Group 4 SIV-challenge. On 0 dpi pigs received an intranasal challenge with 2 ml of either sham or virus inoculum. Challenge viruses were PRRSV SDSU73, PCV2 Group 2 European-like, and SIV H1N1 OH07 given at 1 x 10^5 cell culture infectious dose 50% ($CCID_{50}$) per pig. Sham inoculum was prepared from the 3 cell cultures (MARC-145, PK-15, and MDCK cells) used to propagate the viruses. Five pigs from each group were euthanized and necropsied on 1, 3, 6, and 14 dpi. Temperatures of pigs intended for necropsy on 14 dpi were recorded daily. As expected, each virus had its own unique febrile response signature. Pigs in the PRRSV group exhibited dyspnea and lethargy beginning at 8 dpi. At necropsy, lungs were scored for gross lesions. Bronchioalveolar lavage fluid (BALF) was cultured for presence of bacterial pathogens. Sections of tracheal-bronchial lymph nodes (TBLN) were homogenized and sent for flow cytometry analysis, cytokine analysis or RNA extraction. Total RNA were collected from TBLN, pooled for each group and timepoint to make 16 libraries, for analysis with Digital Gene Expression Tag Profiling whole-genome expression analysis platform (Illumina Technologies). The data generated underwent image analysis, base calling, and standard filtering to generate a list of sequence tags and counts. Multidimensional statistical tests and clustering analysis were applied to determine which changes in tag abundance are significant. Tags were annotated with available transcript information. The updated analysis pipeline contains 7804 swine RefSeq sequences and 240420 HarvardGI Accessions (SSGI release 14) allowing us to associate tags with transcripts and genes. Virus sequences from Refseq and GenBank allowed us to determine viral tag counts in the libraries. The experimental results have been integrated with previous studies to develop a robust model of swine respiratory virus infection. For select genes of interest, significant changes were validated by real-time RT-PCR, and changes in transcript abundance mapped to known metabolic, signaling and other pathways/networks. Gaining insight into how the virus causes disease may aid development of more cross-protective vaccines that would certainly lead to the production of healthier swine. If more efficacious vaccines were available, then they may lead to strategies to eliminate PRRSV from U.S. swine, a feat that would provide long-term economic impact.

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.