The Effect of PRRS Viral Level and Isolate on Tonsil Gene Expression

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Summary and Implications

Porcine Reproductive and Respiratory Syndrome virus (PRRSV) can persist in tonsil tissue for >150 days post infection (dpi) without clinical signs. This can occur even when PRRSV is cleared from serum and can result in secondary outbreaks. Tonsil tissue from commercial crossbred pigs that were experimentally infected with one of two PRRSV isolates, NVSL-97-7985 (NVSL) or KS-2006-72109 (KS06), was used to identify genes that were differentially expressed in pigs with extreme high or low tonsil PRRS viremia at 42 dpi. Results provide insight on the mechanisms of PRRSV persistence in tonsils and help to identify bio-markers for PRRSV persistence in tonsil tissue. This may lead to the development of more effective strategies to reduce the chance of PRRS re-breaks.

Introduction

PRRS is one of the most costly swine diseases worldwide. PRRSV can persist in tonsil tissue, where it avoids immune response and can mutate or just persist and then return to the blood, causing re-breaks. Different PRRSV isolates vary in their pathogenic paths. Of the two isolates that were used in this study, NVSL is more pathogenic than KS06 because NVSL causes faster, higher serum viremia and a greater impact on growth rate of pigs. NVSL may also avoid immune responses more effectively by hiding in tonsil tissue.

The goal of this research was to identify differences in gene expression in tonsil, depending on PRRS viral level and isolate, in order to obtain a greater understanding of the mechanism of PRRSV persistence.

Materials and Methods

At 28 days average age, 184 and 180 commercial nursery pigs (Duroc×Landrace/Yorkshire) were experimentally infected (intramuscularly and intranasally) with 10^5 (TCID50) of NVSL or KS06, respectively, in two separate PRRS Host Genetics Consortium infection trials. At 42 dpi, pigs were euthanized and tonsil and serum samples were collected. Tonsil viral level and serum viremia were evaluated by a semi-quantitative PCR assay for PRRSV RNA. Based on high or low levels of PRRSV in the tonsil, tonsil samples were chosen for RNA-seq to assess abundance of mRNA for each gene expressed in tonsil tissue. As a result, 15 NVSL-high, 13 NVSL-low, 12 KS06-high, and 10 KS06-low samples were selected for RNA-seq analyses. The statistical analysis software QuasiSeq was used to identify differentially expressed genes (DEGs) (q<0.1) between pigs infected with NVSL versus KS06, pigs with high versus low tonsil viral level, and their interaction. The Ingenuity Pathway Analysis (IPA) software was used to identify causal relationships and biological functions of the DEGs.

Results and Discussion

At 42 dpi, more pigs infected with NVSL had cleared serum viremia (70.6%) than pigs infected with KS06 (26.1%). Tonsil viral level and serum viremia were higher in NVSL infected pigs than in KS06 infected pigs (Fig. 1).

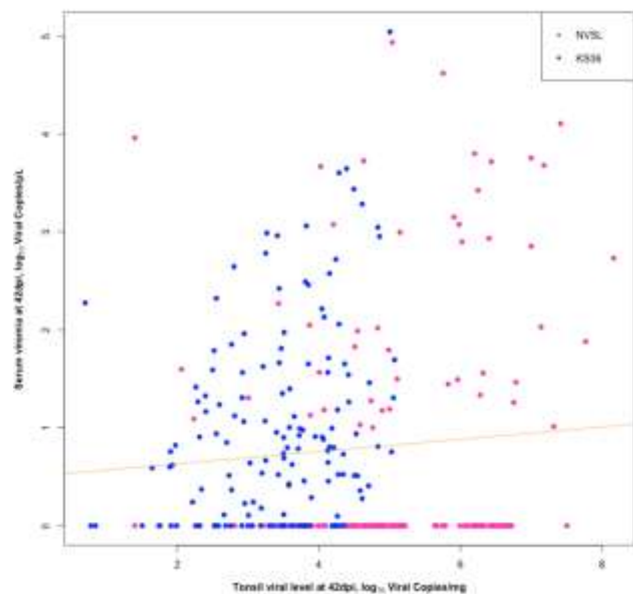

Figure 1. The scatter plot of tonsil viral level versus serum viremia at 42 days post infection with NVSL or KS06.

In total, 11,909 genes were determined to be expressed in the tonsil, of which 258 were DEGs between the two PRRSV isolates, 105 were DEG between pigs with high versus low tonsil virus level, and 7 DEGs showed a significant interaction between PRRSV isolate and tonsil viral level.

The 12 DEGs that were significantly up-regulated in tonsil from pigs infected with NVSL compared to KS06 are involved in inhibiting organismal death. Because KS06 is less pathogenic than NVSL, it may impair tonsil integrity less than NVSL. Genes involved in cell movement and quantity of cells had greater expression in pigs infected with KS06, which may increase interactions and quantity of immune cells that may be associated with immune modulation and viral clearance.

The 4 DEGs that were significantly up-regulated in tonsils from pigs from high versus low tonsil viral level were CXCL10, TBX21, CCL5 and CCL19. These genes likely activate the polarization of blood cell functions to trigger cellular immune response. Genes CCL5, RSAD2 and CXCL10, which were up-regulated in pigs with high tonsil viral level may inhibit virus replication in tonsil tissue.

Collectively, these results suggest that KS06 infection may result in less tonsil tissue damage by regulating genes related to cell and tissue morphology. NVSL infection stimulated genes that inhibited movement of immune cells and, thus, this isolate may be better at avoiding immune responses by hiding in tonsil tissue. High tonsil virus levels may activate the expression of genes that trigger cellular immune responses to clear virus that persists in tonsils and inhibits virus replication. These findings contribute to our understanding the mechanisms involved in tonsil pathology induced by PRRSV infection in pigs.

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