Title: Mechanism of PRRSV inhibition of interferon-mediated antiviral response – **NPB #10-118**

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Date submitted: November 3, 2012

Scientific Abstract:

Porcine reproductive and respiratory syndrome virus (PRRSV) interferes with interferon (IFN)-activated antiviral response. In this study, different PRRSV strains were compared for their effects on IFN signal transduction pathway. One strain of genotype 1 PRRSV (LeLystad) and six strains of genotype 2 (VR-2385, Ingelvac PRRS MLV, VR-2332, NVSL, A2MC2 and MN-184) were used in this experiment. Compared to uninfected MARC-145 cells, all of the strains tested except A2MC2 and MN-184 had much lower protein level of IFN-stimulated gene 56 (ISG56) and signal transducer and activator of transcription 2 (STAT2) post-IFN treatment. Infection of the cells with VR-2385, MLV, VR-2332 and NVSL led to significant reduction of the transcript level of ISG15 and ISG56. In primary porcine alveolar macrophages (PAMs), all the strains except MLV and NVSL inhibited IFN-induced elevation of STAT2 protein. To determine which PRRSV protein(s) are responsible for the inhibition of the IFN signaling, individual proteins of VR-2385 were cloned for overexpression. IFN signaling luciferase reporter assay showed that in addition to nsp1β, GP3, GP4 and N proteins inhibited the IFN-activated reporter expression. Nsp1β protein was selected for further analysis and comparison between the different PRRSV strains for their effects on the IFN-activated JAK/STAT signaling. Nsp1β from these strains were cloned into an expression vector and their effect on IFN signaling was compared. Overexpression of all the nsp1β plasmids except MLV led to inhibition of the IFN reporter expression. This indicates that nsp1β from all the strains except MLV has inhibitory effect on IFN signaling. Our findings delineate the different effects of these strains on IFN-activated signal transduction.

Type I IFNs induce the expression of IFN-stimulated genes by activating phosphorylation of both the STAT1 and STAT2, which form heterodimers, interact with IRF9 and translocate to the nucleus in heterotrimers (ISGF3). PRRSV nsp1β was found to block the nuclear translocation of ISGF3 complex. We further discovered that nsp1β induced degradation of karyopherin-α1 (KPNA1, also known as importin-α5). KPNA1 is known to mediate import of ISGF3 from cytoplasm to the nucleus. Overexpression of nsp1β led to a reduction of KPNA1 but had little effect on its transcript level. Treatment of the nsp1β-transfected cells with proteasome inhibitor MG132 restored KPNA1 protein level. Presence of nsp1β led to elevation of KPNA1 ubiquitination and a shortening of its half-life. Analysis of nsp1β deletion constructs showed that the N-terminal domain of nsp1β was involved in the ubiquitin-proteasomal degradation of KPNA1. Infection of MARC-145 cells by PRRSV strain VR-2332 and VR-2385 led to a reduction of KPNA1, while a low virulent strain MLV had little effect. These results indicate that nsp1β blocks ISGF3 nuclear translocation by way of stimulating KPNA1 degradation.

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.

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