RESEARCH ABSTRACT

SWINE HEALTH

Title: Molecular Identification of Type I Interferon Antagonistic Components of PRRSV Proteins – **NPB# #09-239**

Investigation: Kay S. Faaberg, PhD

Institution: USDA/ARS/NADC

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Scientific Abstract

We set out to define the regions of PRRSV Type 2 strain MN184 responsible for inhibiting type 1 interferon using cloned PRRSV proteins expressed in MARC-145 cells, which supports virus replication. The individual genes for each nonstructural and structural proteins of MN184 proteins was best implemented by the use of the eukaryotic expression vector pCI, modified to include a Flag tag. The original bioassay used NDV modified to express green fluorescent protein that stimulates type 1 interferon in Marc-145 cells. Thus it was used an indicator for IFN inhibition by PRRSV. We found that this bioassay system was extremely difficult to optimize for reliable and consistent results. Our final bioassay eliminated the NDV-GFP indicator step, since the chosen ELISA system measured IFN-β protein when present in small amounts. A commercially prepared qRT-PCR test was implemented to assess the level of cellular mRNA for IFN-β. The NS1 gene of pandemic influenza was to be used as our positive control for inhibition. However, the pandemic flu NS1 gene was found not to inhibit type 1 interferon substantially using this assay, as recently confirmed by others. Additionally, our results suggested that MARC-145 cell IFN-β inhibition by MN184 proteins was not detected. Rather, we saw that strain MN184 nsps 3, 7-11 actually modestly induced IFN-β protein into the cell supernatant. We have yet to establish the level and protein product sizes of our expressed PRRSV proteins. The reasons for our failure to detect type 1 IFN inhibition may lie in the strain of PRRSV tested, the type of cells utilized, the methods used to detect interferon, and the IFN chosen for ELISA or mRNA detection. However, the MN184 protein expression system developed through this funding will be used to further define the host response to PRRSV infection.