RESEARCH ABSTRACT

SWINE HEALTH

Title: Recombinant porcine interferon to control PRRSV and select other viral pathogens" – #19-217 IPPA

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Scientific Abstract:

Interferons (IFNs), particularly IFN-α and IFN-β, provide one of the first lines of defense against virus infections. Many viruses that affect swine, i.e. porcine reproductive and respiratory syndrome virus (PRRSV), Seneca Valley virus (SVV), porcine epidemic diarrhea virus (PEDV) and swine influenza virus (SIV), encode proteins that block IFN activity. These viruses have a devastating economic impact on the swine industry. Restoring the anti-viral state in pigs by IFN administration could greatly reduce the economic losses associated with viral disease outbreaks. The goals of this project were to produce inexpensive and industrially scalable porcine IFN using recombinant technology and to assess the ability of these IFNs to suppress the *in vitro* replication of PRRSV, SVV, PEDV and SIV in porcine cells. Recombinant IFN-α and IFN-β were produced using a *Bacillus megaterium* shuttle vector. First, however, it was necessary to modify the shuttle vector by cloning a cleavable self-aggregating (cSAT) module downstream of the xylose promoter (PxylA). The module consists of the coding sequence for a gyrA intein, a PT linker and a self-aggregating ELK16 tag. Next, the coding sequences of porcine IFN-α and IFN-β were codon optimized and cloned into the vector. The IFN coding sequences were introduced upstream of and in-frame to the cSAT module, resulting in the production of an IFN fusion protein. The IFN genes were be under the control of the inducible promoter PxylA, which could be activated by supplementation of the bacterial culture media with xylose. The shuttle vector was constructed in *E.coli* then transformed directly into *B. megaterium* by electroporation. Transformants were selected for tetracycline resistance. Expression of the fusion proteins was induced by providing xylose to the culture media. Aliquots of culture samples were collected at increasing optical densities until the optical density reached 1.5, at which point the bacteria were pelleted for protein purification. The aliquots were tested for the fusion protein by western blotting using tag-specific antibodies. These experiments revealed that the proteins were successfully purified. Bacterial pellets were sonicated and centrifuged to separate the soluble and insoluble fractions. Due to self-aggregating nature of the ELK16 tag in the cSAT module, the IFN fusion proteins accumulated in the insoluble fractions. IFN was released from the insoluble fractions by activating the self-cleaving ability of the GyrA intein using reducing buffer. The insoluble, cleaved cSAT module was removed from the soluble IFN by centrifugation followed by dialysis in a refolding buffer. The biological activity of purified porcine IFN-α and IFN-β was measured in Vero cells using the lytic vesicular stomatitis virus cytopathic assay. We compared the biological activity of our IFNs to commercially available recombinant human IFN-α and β (BPS BioSciences). Cells were incubated with serial twofold dilutions of each IFN starting with 100 pg/ml for 20 hr. Twenty-four hours after inoculation with VSV, cells were fixed and virus titers were

calculated. VSV titers were approximately 1 x 10^8 pfu/ml in the absence of IFN. Virus titers were greatly reduced in the presence of all four IFNs that were tested. These findings demonstrate that the porcine IFNs that we produced are biologically active, although their ability to suppress VSV replication was approximately tenfold less as that observed with the commercially available human IFNs. Next, we assessed the ability of recombinant porcine IFNs to suppress the *in vitro* replication of PRRSV, SVV, PEDV and SIV in swine alveolar macrophages (MARC-145 cells). The cells were cultured in six-well plates then inoculated with virus in the presence or absence of IFN. All four viruses (PRRSV, SVV, PEDV and SIV) were used at a multiplicity of infection of 1.0 and the IFNs (porcine IFN-α and IFN-β) were tested both separately and together. Plaque assay experiments revealed that the ability of each virus to replicate in MARC-145 did not significantly differ when comparisons were performed between cultures with no IFNs, 2 units of rPor-IFN-α or rPor-IFN-β or 2 units of both rPor-IFN-α and rPor-IFN-β.