RESEARCH ABSTRACT

SWINE HEALTH

Title: Development of PRRS virus-like-particles containing nanoparticle vaccine and its evaluation in pigs - **NPB # 12-166**

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Scientific Abstract:
Porcine Reproductive and Respiratory syndrome (PRRS) is an economically devastating problem plaguing the global swine industry. Since early 1990s both live attenuated and inactivated vaccines are in use, but still control and transmission of PRRS is a major problem. Most of the inactivated virus and subunit vaccines are poorly immunogenic due to their soluble nature and rapid degradation *in vivo*. Alternatively, virus like particles (VLPs) generated using viral surface proteins mimics the morphology of the native virus, and it is non-infectious as it lacks the viral genetic material. Until now only two reports on PRRS-VLPs are available and they are GP5 and M containing VLPs and influenza nucleocapsid protein and PRRSV GP5 chimeric (NA/GP5) VLPs, but their immunogenicity was not evaluated in pigs. Since putative PRRSV neutralizing epitopes are not limited to GP5 and M, we hypothesized that PRRS-VLPs comprising of all (5 or 6) viral membrane proteins (GP2a, E, GP3, GP4, GP5 and M) serve as a potent candidate vaccine. We cloned full-length genes of the six surface proteins of a type 2 PRRSV (strain SD09-28) in to a baculovirus transfer vector, transformed in to competent *E. coli*, and transfected into Sf9 cells to generate recombinant baculoviruses (rBVs). High titered rBVs stocks were used to co-infect Sf9 cells in different combinations to generate PRRS-VLPs. Our in vitro results revealed that VLPs were formed from co-infection of GP5-M, GP2a-GP3-GP4-GP5-M, GP2a-E-GP3-GP4, GP2a-GP3-GP4, as measured by transmission electron microscopy the size of the particles was in the range of 30-80nm. Nanoparticles made of biodegradable and biocompatible polymers [e.g. PLGA [poly(lactide co-glycolide)] are approved by FDA to use in vaccine delivery systems. In a pilot vaccine trial in nursery pigs, BEI inactivated PRRS-VLPs containing GP5-M and GP2a-GP3-GP4-GP5-M entrapped in PLGA nanoparticles or unentrapped were coadministered intranasally twice with a potent mucosal adjuvant, *Mycobacterium tuberculosis* whole cell lysate, and challenged with a virulent heterologous PRRSV strain 1-4-4. Analysis of viremia suggested the reduced challenged viral RNA load and infectious virus in pigs received PRRS-VLPs irrespective of entrapment in NPs, while in the lungs of pigs vaccinated with PRRS-VLPs without encasing in NPs had helped to significantly reduce the viral load. In conclusion, we generated PRRS-VLPs containing all the six PRRSV membrane glycoproteins, and that could be a potential candidate vaccine when delivered with a potent adjuvant.

Further studies are required to confirm the dose-dependent PRRS-VLPs vaccine efficacy and the degree of cross-protection against other heterologous PRRSV strains. In conclusion, our results suggested that PRRSV-VLPs containing all the viral membrane proteins are beneficial in inducing the cross-protective immune response in pigs, when the candidate vaccine is used twice through intranasal route and co-administered with a potent adjuvant.