Title: Development of a broadly protective PRRS vaccine candidate: Application of non-toxic enterotoxin and E. coli as the adjuvant-delivery system - NPB #12-127

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Scientific Abstract:
Porcine reproductive and respiratory syndrome (PRRS) continues to be a threat to the swine industry. Current commercial available vaccines are not always efficacious in protection against infection from a wide array of heterologous PRRS virus (PRRSV) isolates in the field. Epitope-based vaccine represents a new approach to achieve protective immunity. The objective of this study was to construct and evaluate the immunogenicity of an epitope-based candidate vaccine for its potential application in future PRRS vaccine development. Initially, four multi-epitope antigens (chimera A-D) were constructed, which contain a set of consensus T-cell epitopes derived from PRRSV proteins of nsp9, nsp10, GP4, GP5, and N. To enhance immune responses of PRRSV T-cell epitopes, these multi-epitope antigens were genetically linked to a detoxified bacterial heat-labile enterotoxin (LT_{192}) as an adjuvant. These four epitope-toxin chimeras were transformed into a swine non-pathogenic E. coli strain to use as a potential live attenuated vaccine (designated as E. coli /epitope-toxin_mix). The in vitro expression of each multi-epitope antigen was detected in the supernatant of transformed E. coli culture. Immunogenicity of this candidate vaccine was evaluated in a PRRSV challenge pig model. The result demonstrated that specific T-cell immune responses were stimulated after immunization. At 28 days post vaccination, we observed the increased frequency of IFNγ+CD4+CD8+ cells and IFNγ+γδ T-cell populations in PBMCs stimulated by pooled synthetic peptides of T-cell epitopes. After challenge, strong IFNγ+γδ T cell response was observed. Peptide D2 (VRHHFTPSE) from N protein was identified as the epitope of γδ T cell lymphocytes; peptides A3 (CPGKNSFLDEAAYCNHL) and C3 (VRILAGWCGKNSFLD) from nsp10 were identified as epitopes of CD4+T lymphocytes; peptides B3 (VRGNPERVKGVLQNTFRF) from nsp2 and C2 (KGRLVWRSPVIEK) from GP5 were identified as epitopes of CD8+T lymphocytes. In comparison to the non-immunized pigs, pigs immunized with the E. coli /epitope-toxin_mix showed improved protection against virulent PRRSV challenge, with about 50% decrease of pneumonic lung lesions and 10-fold reduction of the viral load in serum and lung tissues at 14 days post challenge. This study establishes a platform for future construction of epitope-based vaccines against PRRSV infection.