RESEARCH ABSTRACT

SWINE HEALTH

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**

Investigator: Ying Fang, Kansas State University

Co-Investigator: Weiping Zhang, Kansas State University
Collaborators: Bhupinder Bawa, Kansas State University; Renukaradhya Gourapura, The Ohio State University; Raymond R. R. Rowland, Kansas State University

Date Submitted: May 27, 2014

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 (VRILAGGWCPGKNSFLD) from nsp10 were identified as epitopes of CD4+T lymphocytes; peptides B3 (VRGNPERVKGVLQNTRF) from nsp2 and C2 (KGRLYRWRSPVIIEK) 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.