RESEARCH REPORT
SWINE HEALTH

Project Title: A novel PRRS vaccine in a bacterial vector known to stimulate strong cell-mediated and humoral immunity - **NPB #03-086**

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Abstract: In this study, we have explored the usefulness of a newly developed bacterial vaccine vector, *Brucella abortus* RB51, for generating an efficacious vaccine for PRRS. The gene coding for GP5, a known protective protein, of PRRS virus was amplified via RT-PCR and cloned in plasmid pBBGroE. *B. abortus* RB51 transformed with the recombinant plasmid stably maintained the plasmid and expressed the GP5 antigen. The recombinant RB51 strain was used to immunize twelve 3-week old piglets by a single subcutaneous inoculation of 10^{10} colony forming units of the bacteria. The vaccinated pigs developed GP5-specific antibodies by 4 weeks post-vaccination as determined by the Western blot analysis of their serum samples. The presence of low titers of virus neutralization antibodies were also detected in these serum samples. However, the blood lymphocytes collected from the vaccinated pigs did not secrete significant amounts of interferon-gamma upon in vitro stimulation with either recombinant GP5 protein or whole PRRS virus antigen, suggesting a poor T cell-mediated immune response to the vaccine. In order to determine the protective efficacy of the induced immune responses, the vaccinated pigs were challenged by intranasal inoculation of highly virulent PRRSV strain P129. Another group of 12 unvaccinated pigs similarly challenged with the virus served as positive controls for the infection. The clinical signs of the challenged pigs were scored on daily basis, and the viral shedding in their nasal secretions and the viral load in their blood were determined twice a week for 3 weeks. At 1, 2, and 3 weeks post-challenge a minimum of 3 pigs each from the vaccinated and unvaccinated groups were euthanized to assess the gross and microscopic lesions in their lungs. Based on the RT-PCR detection, pigs in both vaccinated and unvaccinated groups equally shed the virus up to 2 weeks post-challenge. No difference was also detected between the two groups with regard to the clinical signs, blood viral load, and gross and microscopic lesions in the lungs. Taken together, these findings indicate that the level of GP5-specific antibody response induced by the recombinant RB51 strain vaccination does not confer any protection against intranasal challenge with virulent PRRS virus.

Introduction: The porcine reproductive and respiratory syndrome (PRRS) continues to be one of the major causes of economic loss to the swine industry in North America, Europe and Asia. In the US, at present, only a single modified live vaccine (RespPRRS/Repro, Boehringer Ingelheim) is commercially available for use in 3-18 week-old pigs and in non-pregnant females. However, this vaccine has several shortcomings. Efficacy is lacking against the wide variety of field PRRSV strains evidenced by frequent reports of PRRS outbreaks in vaccinated herds. In addition, safety is suboptimal due to suspected incomplete attenuation and the possibility of reversion to a virulent phenotype. Killed vaccines are not effective against PRRS mainly because of their inability to induce good cell-mediated immunity. Therefore, novel approaches are necessary for developing effective recombinant PRRS vaccines. In this regard, we have explored the potential applicability of a new bacterial vector-based expression and delivery system for generating PRRS vaccine. The bacterial vector, *Brucella abortus* strain RB51 is currently being used as the official live vaccine to control bovine brucellosis in the US. Animals vaccinated with this bacterial vaccine develop strong cell-mediated and antibody responses. In this study, we tested if a recombinant RB51 strain expressing a known protective antigen of PRRS virus could induce appropriate immune responses that would lead to resistance against PRRS virus infection.

Objectives:

Objective 1. Construct a recombinant *Brucella abortus* RB51 strain expressing a fusion protein consisting of antigenic fragments of GP4, GP5 and M proteins of virulent PRRSV strain P129.

Objective 2. Immunize 3 weeks-old pigs with the vaccine and characterize the humoral and CMI responses.

Objective 3. Challenge infection of the vaccinated pigs and confirm protective efficacy of the recombinant vaccine.

Materials & Methods:

Expression of PRRS viral proteins in *B. abortus* RB51: The ORFs encoding GP4, GP5 and M proteins were amplified via RT-PCR using the genomic RNA of virulent strain P129 as template and specifically designed primer-pairs based on the sequences determined in our laboratory. Unique restriction endonuclease recognition sites were engineered into the primers to facilitate a directional cloning into pBBgroE plasmid. The viral genes were amplified using One Step RT-PCR kit (Invitrogen, Inc.). The amplified fragments were cloned into pCR 2.1-TOPO vector, and then sequenced to confirm the gene sequences. From the pCR 2.1-TOPO vector, the PRRSV genes were excised using the engineered restriction sites and subcloned into pBBgroE. The recombinant pBBgroE plasmids were electroporated into *B. abortus* RB51 and the colonies harboring the plasmids were isolated on agar plates. Expression of the viral proteins in RB51 was confirmed by Western blot analysis. A recombinant RB51 strain expressing GP5, RB51/GP5, was used to perform vaccination studies in pigs.

Study design and vaccination: Twenty eight two-week-old piglets were purchased from a PRRSV-negative commercial swine herd, transported to Purdue University, randomly assigned to 1 of 3 groups (12 pigs each in groups 1 and 2, and 4 pigs in group 3), placed on decks in 3 separate environmentally regulated rooms in accordance with guidelines in “Guide for the Care and use of Laboratory Animals” and fed age-appropriate commercial diets and water ad libatum. The PRRS negative status of the source herd was established by negative PRRS clinical history and negative PRRSV

serologic profiling (Idexx HerdCheck™ ELISA test). Dams of purchased pigs were confirmed PRRSV negative by Idexx ELISA serology test (S:P ≤ 0.1) and nested RT-PCR for PRRSV RNA tests of serum samples. At 3-weeks of age, pigs in group 1 were immunized by subcutaneous inoculation with 5×10^{10} CFU of strain RB51/GP5. Pigs in groups 2 and 3 were sham-vaccinated with saline as non-vaccinated controls. At 21 and 28 days post-vaccination, blood was collected from all the animals in groups 1 and 2 for analysis of CMI and antibody responses.

Immune response analysis: – Humoral immune response was analyzed by detecting PRRSV-specific antibodies using ELISA and Western blot analysis using recombinant PRRSV GP5 protein, and in vitro virus neutralization assay; Idexx ELISA test was used for the antibody response detection and fluorescence focus neutralization assay was used for detecting the virus neutralizing antibodies. CMI response was assessed by determining the amount of interferon-γ (IFN-γ) secreted by the lymphocytes in blood mononuclear cells upon in vitro stimulation with recombinant GP5 antigen and whole PRRS virus antigen; a commercially available antigen-capture ELISA kit (BioSource International) was used for IFN-γ quantification.

Production of recombinant GP5 antigen: Recombinant PRRSV GP5 protein was obtained by overexpressing in *E. coli* as a fusion protein with N-terminal His-tag using a commercially available expression system (pRSET A, B,C system, Invitrogen, Inc.). The recombinant proteins was purified on metal (Ni^{2+}) affinity chromatography and used for Western blot analysis and for in vitro antigen-specific stimulation of blood lymphocytes.

Challenge infection: At 4 weeks after vaccination, all the pigs in groups 1 and 2 were challenged by intranasal inoculation of 10^5 $TCID_{50}$ of virulent PRRSV strain P129 in 2 ml of tissue culture fluid. The challenge inoculum was prepared from a low cell culture passaged stock with proven virulence in this pig model. The 4 pigs in the third group were sham-inoculated intranasally with 2ml of sterile cell culture media and were held as non-vaccinated non-challenged PRRSV-negative controls.

Determination of protective efficacy: After challenge, the pigs were monitored daily for 21 days for clinical signs and were assigned a daily clinical score as follows: **score 1** = no clinical signs; **score 2** = mild dyspnea and/or mild erythema and/or occasional coughing; **score 3** = severe dyspnea and/or marked erythema and/or regular coughing; **score 4** = score 3 plus cyanosis and/or inactivity or segregating. Blood and nasal secretions were collected twice weekly for detection of PRRSV by RT-PCR. On days 7 and 14 after challenge, 3 pigs each from groups 1 and 2 as well as 1 pig from group 3 were euthanized. All the remaining pigs were euthanized on day 21 after challenge. Gross lesions in lungs and lymph nodes and weights of lungs and ileal lymph nodes were recorded. Samples of lung and inguinal lymph nodes were fixed in 10% neutral buffered formalin, processed by routine methods, stained, examined microscopically to record the lesions. Serum collected from the pigs was analyzed by PRRSV Idexx ELISA.

Results:

Objective 1.

The genes coding for the selected antigenic fragments of GP4, GP5 and M proteins of PRRSV strain p129 were amplified by RT-PCR and cloned individually and as a fusion construct in *B. abortus* RB51. Expression of the pertinent proteins in RB51 was analyzed by Western blot. Initial analysis revealed that the level of expression of the fusion protein was very low. Since optimal level of protein expression is required for the induction of immune response in pigs, several strategies were employed to first

enhance the level of expression of individual proteins in RB51. We were successful only in achieving optimal expression of GP5 in RB51; the expression of GP4 and M proteins remained very low. The recombinant RB51/GP5 strain was used as the vaccine for immunization of pigs.

Objective 2.

Antibodies specific to GP5 were detected by Western blot analysis in the vaccinated pigs at 4, but not 3, weeks post-vaccination. Serum collected at 4 weeks after vaccination also contained low titers of virus neutralizing antibodies; the titers ranged 2 to 4 in the vaccinated pigs, in comparison the positive control convalescent sera had a virus neutralizing titer of >64. As expected, serology by Idexx ELISA showed no positive reactivity with all the pigs, including the vaccinated ones. Blood monocnuclear cells collected at 3 and 4 weeks after vaccination did not secrete IFN-γ upon specific stimulation with recombinant GP5 antigen or with the whole virus antigen; however, secretion of large amounts of IFN-γ was detected when the cells were stimulated with a mitogen (Con A).

Objective 3.

After challenge with virulent PRRS virus strain P129, pigs in the vaccinated and unvaccinated groups showed similar extent of clinical signs. Also, no significant differences were observed between both the groups with regard to virus shedding in the nasal secretions and viral load in the blood. Necropsy findings in pigs from the both groups were similar and consistent with the PRRS virus infection. Serology by Idexx ELISA showed positive reactivity in all the challenged pigs starting at day 7 after vaccination. As expected, pigs in the unvaccinated and unchallenged group remained healthy and negative for PRRS virus infection throughout the study.

Discussion: Our research results suggest that *B. abortus* RB51 is not an appropriate vector for the development of PRRS vaccine. Though the vaccinated pigs developed low titers of virus neutralizing antibodies, no antigen-specific T cell responses were detected in these animals. A major problem with this bacterial vector system appears to be its inability to express significant levels of PRRS viral proteins. In the absence of an optimal amount of antigen, it is difficult to induce robust immune responses even with a bacterial vector system possessing unique adjuvant properties. All of our efforts to improve the level of expression of PRRS viral proteins in *B. abortus* RB51 were unsuccessful. Therefore, in our opinion, *B. abortus* RB51 is not very useful for developing effective PRRS vaccines.

Lay Interpretation: The results of this research project suggest that, despite several unique, advantageous properties, *Brucella abortus* RB51 is not very useful for developing effective recombinant PRRS vaccines.

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