Development of Novel Chimeric Vaccine and Delivery System for Classical Swine Fever Virus

Pengcheng Shang[1], Adriana Avila[2], Rui Guo[1], Susan K. Whitaker[3], Yanhua Li[1], John Tomich[3], Raymond R. R. Rowland[1], Ying Fang[1]

[1]Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, KS;

[2]Department of Chemistry and Biochemistry, Auburn University, Auburn, AL; [3]Department of Biochemistry and Molecular Biophysics, Kansas State University, Manhattan, KS.

Introduction

Historically, the greatest threats to the US swine production are from foreign animal diseases, such as CSF. CSF is caused by CSFV, which is highly contagious to pigs and wild boars. CSFV belongs to the family Flaviviridae, genus Pestivirus. The genome of pestiviruses is a positive-sense, single stranded RNA. It comprises a single open reading frame (ORF) that encodes a polyprotein of about 4000 amino acids, which is processed by viral and cellular proteases to generate four structural proteins (C, Erns, E1 and E2) and nine nonstructural proteins. Infections caused by CSFV can have a deleterious effect on swine production, causing excessive morbidity and mortality. Modified live virus (MLV) vaccines for CSF, such as the CSFV C-strain, are effective in controlling infection but are not routinely used, primarily due to the lack of diagnostic tools that can differentiate infected from vaccinated animals (DIVA), which hampers disease control measures relying on serology. Furthermore, MLV vaccines may pose concerns on biosecurity issues, since virulent revertant virus can be easily obtained (and modified) from the vaccinated animals in the field. There are restrictions on the international trade in pig products from countries using CSF MLV vaccines. To circumvent these problems, subunit vaccines have been developed. Previous studies demonstrated that the CSFV E2 protein can induce specific neutralizing antibodies. Subunit vaccines based on the E2 glycoprotein have been tested extensively for their efficacy, and the companion DIVA test has been developed based on ELISAs that detects E2 and another viral glycoprotein, the Erns. In comparison to MLV vaccines, the main limitation of these subunit vaccines is their higher cost for production of antigens; on the other hand, immunization with E2 protein alone is less efficacious and the early stimulation of innate immune responses is needed for initial protection of immunized animals. To overcome these limitations, this proposed study will use a novel approach for CSF vaccine development, in which a modified live PRRSV will be used as a vector to express CSFV E2 protein. To eliminate the need for a cold chain, DNA vaccine approach was used, in which nanoparticles composed of branched amphiphilic peptide capsules (BAPCs,) were employed as the DNA delivery agent. The candidate vaccine and the DNA delivery system were evaluated in *in vitro* expression system and a nursery pig model. This study developed a candidate viral vectored chimeric vaccine and established DNA vaccine delivery system, which could be applied to other emerging and transboundary swine pathogens in the future.

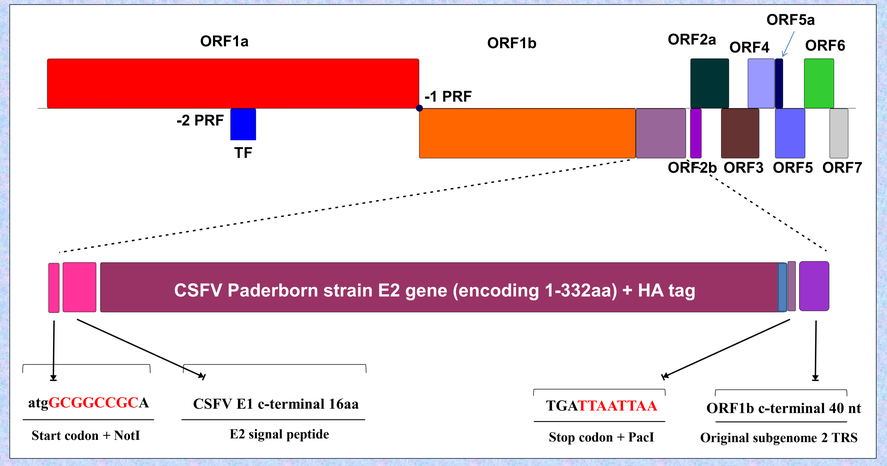

Figure 1. Construction of recombinant PRRSV expressing CSFV truncated E2 protein (CSFV E2-PRRSV chimera). Nucleotides encoding CSFV E2 protein (1-332aa, TM-deleted) and signal peptide were inserted between PRRSV ORF1b / ORF2a junction as an independent ORF. The E2 ORF was followed by the original transcription regulatory sequence (TRS) of subgenome 2 (ORF2a and ORF2b). PRF, programmed ribosomal frameshifting site.

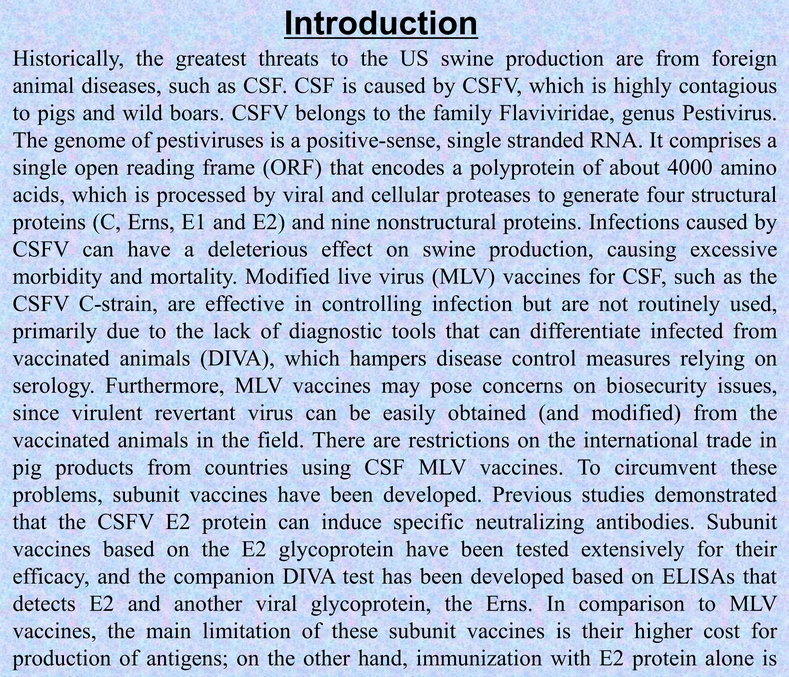

Figure 3. Transmission electron microscopy images of the BACP:DNA nanoparticles at N:P = 20.8. (**A**) Single BAPCs interacting with pDNA. Scale bar = 10 nm. (**B**) Cluster of BAPCs interacting with DNA. Scale bar = 100 nm. (**C**) Schematic representation of potential BAPC-DNA interactions.

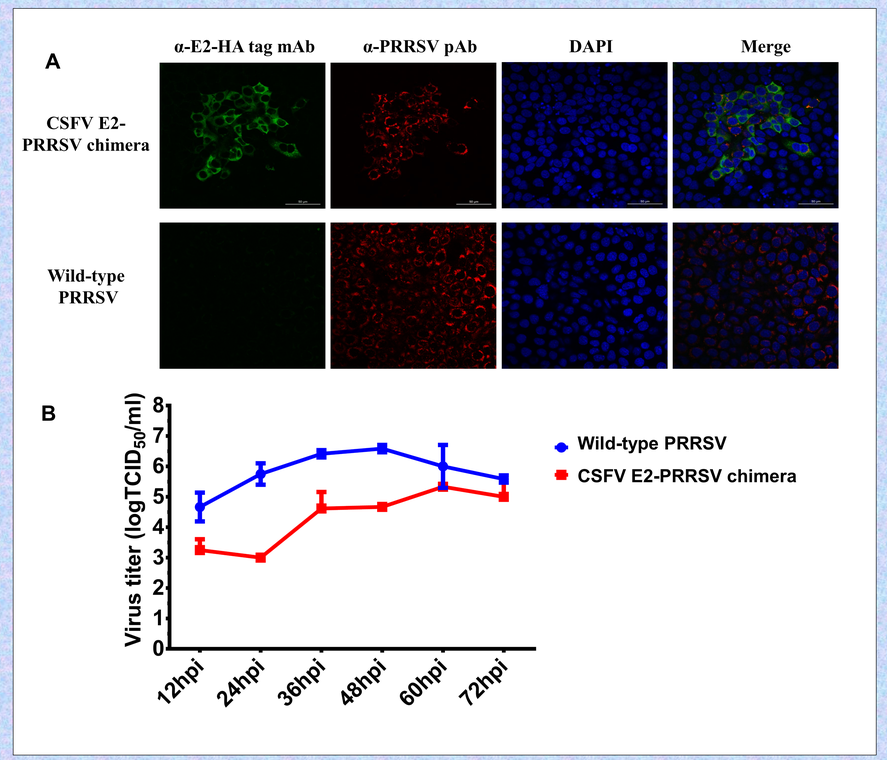

Figure 2. *In vitro* characterization of CSFV E2-PRRSV recombinant virus. (A) E2 expression in CSFV E2-PRRSV chimeric virus. E2 expression was detected by α-HA mAb in chimeric and wild-type virus-infected cells using indirect immunofluorescence assay (IFA). **(B)** Growth kinetics of the CSFV E2-PRRSV chimeric virus in comparison to that of wild-type PRRSV. MARC-145 cells were infected in parallel with passage 2 recombinant or wild-type viruses (MOI of 0.01). At 12, 24, 36, 48, 60, and 72 hours post infection, cells were harvested and the virus titers were determined by virus titration on MARC-145 cells and the result was interpreted as TCID50/ml.

Acknowledgements

This project is supported by the Center of Excellence for Emerging and Zoonotic Animal Diseases (CEEZAD) and the Terry Johnson Cancer Center at Kansas State University, and the Fundacão de Amparo à Pesquisa do Estado de São Paulo - FAPESP (2013/15360-2).

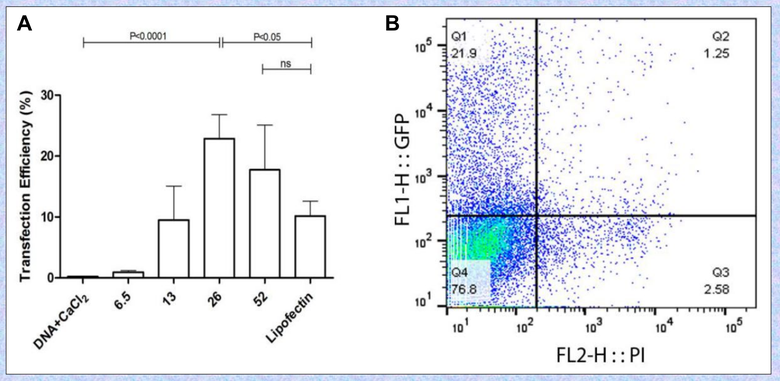

Figure 4. In vitro transfection efficiency of BAPCs-DNA nanoparticles. (**A**) HEK-293 cells transfected with nanoparticles prepared at different peptide:DNA charge ratios (N:P) with plasmid DNA of PRRSV infectious clone expressing green fluorescent protein (GFP). BAPCs-DNA mixture was incubated for 4 hours in reduced serum media and 1 mM $CaCl_2$. (**B**) Flow cytometry analysis of GFP-expressing HEK-293 cells after 48 h post transfection with BAPCs nanoparticles at N:P ratio of 26 with plasmid DNA of PRRSV infectious clone. Data represent mean values +SD of four experiments combined. Differences between values were compared by ANOVA using Bonferroni as post-test. Non-statistical significance (ns) was considered when P>0.05.

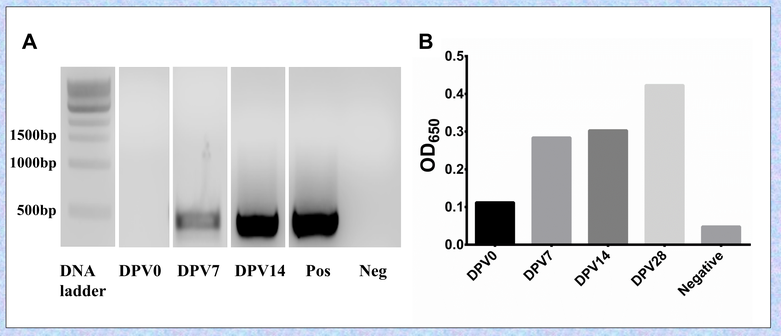

Figure 5. Detection of viral RNA and anti-E2 antibody response in serum samples from DNA vaccine immunized pig. (A) RT-PCR detection of chimeric virus RNA. Viral RNA was amplified by specific primers from the pig sera at 7 and 14 days post vaccination (DPV). **(B)** ELISA test of anti-E2 antibodies in serum samples. E2 specific IgG titers were measured by ELISA using serum samples from immunized pig at DPV 7, 14, and 28.

Results and Discussion

- CSFV E2 gene (without C-terminal 41aa) was inserted into the ORF1b/ORF2a site in PRRSV cDNA infection clone (**Fig. 1**). Viable recombinant virus, CSFV E2-PRRSV chimera, was recover in cell culture (**Fig. 2**).
- nanoparticles composed of branched amphiphilic peptide capsules (BAPCs) were employed as the DNA delivery agent (**Fig. 3**). Transfection of DNA-BAPC nanoparticles in cultured cells recovered infectious viruses, in which the transfection efficiency of BAPCs is comparable or superior to lipofectin (**Fig. 4**).
- DNA vaccination and BAPCs delivery approach were evaluated using a nursery pig model. Pigs were immunized by PRRSV-E2 chimera in a form of DNA-BAPC nanoparticles (group 1), DNA without nanoparticles (group 2), live recombinant chimeric virus (group 3), or mock-inoculated as a control (group 4). Viral RNA was detected by nested RT-PCR from group 1 and group 3 pigs at 7 days post inoculation, while virus specific antibody response was detected by 3 weeks post-infection.
- This study developed a chimeric dual user candidate vaccine for both PRRSV and CSFV. The chimeric vaccine platform and DNA delivery system established in this study could be applied to other emerging and transboundary swine pathogens in the future.