Title: Evaluation of envelope proteins for rapid induction of protective immune response against classical swine fever - **NPB#11-045**

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Scientific Abstract:

We have analyzed the individual effect of each of CSFV structural proteins (E0, E1, and E2) on the induction of humoral response and protection against the infection. Native version of each of these proteins was produced in baculovirus. Briefly, His-tagged CSFV E0, E1, or E2 genes were synthesized using polymerase chain reaction (PCR) followed by cloning of amplified genes into the pENTR/D-TOPO entry vector (Invitrogen, Carlsbad, CA). Expression of envelope proteins was achieved by cloning those genes into entry vectors suitable for recombination with baculovirus (Invitrogen). Entry vectors were verified for the presence and fidelity of cloned genes by sequencing. Recombinant baculoviruses were generated after recombination of entry clones with linearized baculovirus DNA and transfection of suitable insect cells. Insect cell culture medium containing recombinant viruses were harvested and used to infect fresh insect cells to produce high-titer viral stocks. Infected insect cells were used to analyze the expression of CSFV envelope proteins by Western blot. Expressed envelope proteins were purified using metal ion affinity chromatography on cobalt resin columns. The fractions collected during purification were analyzed by Western blot. Details in the production of recombinant baculovirus, protein expression and their purification are described in Gavrilov *et al*., (2011). Thirty to forty lbs pigs allotted into groups (n=5) were vaccinated intramuscularly with 50 micrograms of purified proteins in water-in-oil emulsions followed by an intranasal challenge with 10^5 TCID50 of highly virulent strain Brescia (BICv) 7 days after the last inoculation. In each experiment, a control group was mock vaccinated and received the challenge as previously described. Blood, nasal swabs, and tonsil scrapings were collected from vaccinated and mock-vaccinated control pigs after challenge. Viral loads in those clinical samples were determined by virus titration in SK6 cells or real time RT-PCR. Serum samples were collected throughout the experiment and the presence of CSFV E0 and E2 antibodies were assessed using commercial ELISA test kits. Neutralizing antibody response was assessed using virus neutralization assays. Detailed information about these experiments could be found in the attached paper (Gavrilov *et al*., 2011). Results demonstrated that as expected, E2 is able to induce a neutralizing antibody response as well as to protect swine against the virulent challenge. Interestingly, purified E0 was also efficient in raising a neutralizing antibody response and protection against the challenge.

In order to increase antigenicity and immunogenicity over wild-type proteins, E0 and E2 were then modified by fusing encoding genes to either an immunostimulatory molecule, flagellin; or to a protein moiety that delivers molecules to professional antigen presenting cells: a single chain anti-class II antibodies. Flagellin is the major structural component of the bacterial flagella. Cells of the innate immune system recognize conserved pathogen-associated molecular patterns (PAMPs) through Toll-like receptors (TLRs). Flagellin is recognized by TLR5 that signals through TLR5/TLR4, likely

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activating the interferon pathway. Therefore, flagellin is considered a potent immune activator that rapidly induces expression of proinflammatory cytokines, chemokines, and costimulatory molecules. Flagellin gene was kindly provided by Dr. M.A. Martinez (INIA, Spain) and was synthetically fused to the His-tagged E0 and E2. In addition the efficacy of subunit vaccines might be also improved by using an immunotargeting approach. This system is based on the hypothesis that antigens coupled to monoclonal antibodies specific for class II MHC could be targeted into antigen presenting cells. Here we used a recombinant single chain antibody directed to an invariant epitope of the porcine MHC II DR molecule attempting to target E0 and E2 to cells harboring this invariant MHC Class II epitope. After expression, the antigenicity (i.e.: induction of antibody response) of E0 and E2 was assessed in swine. Pigs, 30-40 lbs (2 animals/group), received 1 dose of E0sc, E0flag, E2sc, or E2flag via IM. Sera were collected from inoculated animals at 7, 14, and 21 days post incoculation and tested by ELISA (Idexx CSFV Ab test). All animals tested negative for CSFV antibodies suggesting that the fusion proteins as designed and synthesized were not antigenic. Further alternatives, including the production of new constructs and changes in the synthesis process, are being pursued at this time.