GENERATION AND EFFICACY ASSESSMENT OF A CHIMERIC ANTIGEN E2-CD154 AS A MARKER CLASSICAL SWINE FEVER VIRUS SUBUNIT VACCINE PRODUCED IN HEK 293 AND CHO K1 MAMMALIAN CELLS

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The E2 glycoprotein is the major antigen that induces neutralizing and protective antibodies in CSFV infected pigs, thus a marker vaccine based on this antigen appears to be the most promising alternative to induce a protective immune response against CSFV. However, the structural characteristics of this protein state the necessity to produce glycoprotein E2 in more complex expression systems such as mammalian cells. In this study, we use a lentivirus-based gene delivery system to establish a stable recombinant HEK 293 and CHO K1 cell line for the expression of E2 fused to porcine CD154 as immunostimulatory molecule. In a first experiment, E2his and E2-CD154 were compared in an immunization trial. The average antibody titers in E2his immunized pigs was in the range of 30-40% of blocking and the average antibody titers for E2-CD154 are above 40% at day 14, meaning that the chimeric antigen is able to raise antibodies at positive levels in a shorter time. Additionally, the blocking rate of E2his vaccinated group in ELISA ranged between 66-88% and in the E2-CD154- vaccinated groups ranged between 86-92%, one week after booster immunization. The NPLA antibody titers also increased greatly. Later on, the protective capacity of purified E2-CD154 glycoprotein was demonstrated in a challenge experiment in pigs using a biphasic immunization schedule with 25 and 50 µg. The immunized animals developed neutralizing antibodies that were protective when the animals were faced to a challenge with 10^5 LD50 of ‘‘Margarita’’ CSFV highly pathogenic strain. No clinical signs of the disease were detected in the vaccinated pigs. Unvaccinated pigs in the control group exhibited symptoms of CSF at 3–4 days after challenge and were euthanized from 7–9 days when the pigs became moribund. These results indicate that E2-CD154 produced in recombinant HEK 293 and CHOK1cell line is a high quality candidate for the development of a safe and effective CSFV subunit vaccine. In the next steps, pilot and production scale, E2-CD154 expression levels should be increased in 10 to 50 fold, arriving to a very attractive productive platform for an implementation of a commercial subunit vaccine against CSF.