Safety and tolerability of African Swine Fever Virus subunit vaccine candidates in commercial pigs

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Abstract

African Swine Fever Virus (ASFV) is currently the greatest threat to the global swine population. It has decimated the pork industry in much of Asia and developing a vaccine against it is of the upmost importance. While previous studies have shown that protection can be conferred using attenuated ASFV, this method is not safe for the animals. Since attenuated ASFV can induce immunity, this study aims to identify the antigens responsible for conferring protective immunity to swine. Doing so will greatly aid the process of developing a safe and effective vaccine for protection of domestic and feral pigs. To do this, all the ASFV open reading frames have been used to generate multicistronic expression cassettes. This novel approach allows for a more manageable and realistic expression of the whole ASFV proteome in the adenovirus vaccine vector as well as clearer selection of antigens that reactive lymphocytes are binding to in the proteome. The genes encoding the multicistronic expression cassettes have then been cloned into a shuttle vector that allows transfer of the cassettes into a plasmid backbone that is then used to generate a weakened recombinant human adenovirus expressing the ASFV antigens. Once the viruses are assembled, they will be used to formulate an experimental vaccine for immunization of pigs. Following two boosts, three weeks apart, the pigs will be challenged with wildtype ASFV. Antibodies and T cells from survivors will be used to identify the ASFV antigens involved in stimulating protective immunity. Identification of the protective antigens will allow development of a safe and efficacious subunit vaccine for protection of domestic and feral pigs.

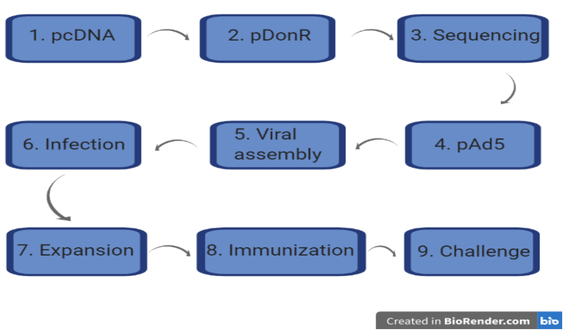
Introduction

ASFV, originally confined primarily to the warthog population in Africa, has now spread into parts of Europe and Asia and quickly become endemic in the feral pig populations of these regions. These feral pigs spread it quickly among domestic populations and with a mortality rate reaching close to 100% in some cases, it can quickly wipe out entire swine farms (1). While live attenuated viruses have shown the ability to confer protective immunity to immunized pigs, there are many associated safety risks that remain. Subunit vaccines offer the potential to confer protective immunity while also being safer than live virus vaccines (2). However, to do so requires the antigens that induce protection to be determined. This study seeks to identify these antigens to support the successful development of these subunit vaccines in the future.

Objective

- Identify antigens that confer protective immunity for ASFV in swine

Methods



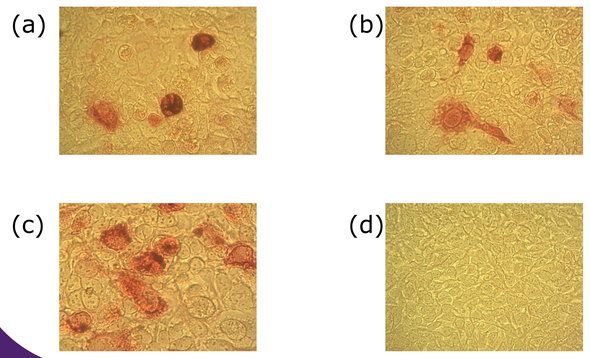
Methods (cont.)

Briefly, ASFV genes are PCR amplified from pcDNA constructs, restriction enzyme-digested, and then ligated into pDonR shuttle plasmid. The genes are validated by sequencing, shuttled into Adenovirus plasmid backbone (pAd5), and protein expression is evaluated using anti-FLAG antibody and confirmed by immunocytometric analyses using ASFV convalescent antibodies. The pAd5 constructs are transfected into 293A human embryonic kidney cells for virus assembly. Successfully assembled virus constructs are tested as above to confirm expression of ASFV genes. The recombinant viruses are expanded to produce virus stock for immunization of swine to induce immune responses.

Results

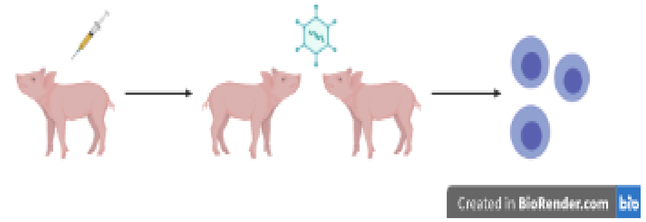
Protein expression was evaluated by immunocytometric analysis using an anti-FLAG antibody, staining red for recombinant viruses expressing ASFV genes tagged with the FLAG sequence.

(a) and (b) show constructs 50-2 and 50-11 respectively which both stained red, indicating the virus assembled with the correct genes expressed. (c) is a positive control and (d) is a negative control.



Next Steps

Once all ASFV genes are assembled in adenoviruses, they will be used to immunize swine in a prime-boost regimen three weeks apart. The pigs will be challenged with wildtype ASFV Georgia 2007/1 and lymphocytes will be harvested from the survivors to analyze which antigens stimulate protective immunity. These will then be selected and combined to make a more effective subunit vaccine.



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Acknowledgments

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