EVALUATING IMMUNOGENICITY OF AFRICAN SWINE FEVER VIRUS 9GL AND 151R ANTIGENS

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Evaluating Immunogenicity of African Swine Fever Virus 9GL and CD2v Antigens

(May 2013)

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Swine and wild boars in Africa, and parts of Europe and Russia continue being decimated by a severe form of hemorrhagic fever known as African Swine Fever (ASF). This viral disease has been responsible for the deaths of millions of swine and poses a significant risk to the United States pork industry since it is easily transmitted across continents. However, there is no vaccine or treatment available and thus, it is important to develop a vaccine that can prevent future infections, and reduce the economic and social impacts resulting from this disease. My research will focus on generating recombinant lentivirus expressing 9GL and CD2v ASFV antigens which will be used to evaluate antigen-specific cytotoxic T cell responses in pigs immunized with recombinant adenoviruses expressing these two antigens.

ACKNOWLEDGEMENTS

I would like to thank Dr. Waithaka Mwangi for his willingness to allow me to join this project and work in his laboratory. I appreciate the invaluable help and experience that you have provided me. Thank you for providing me with the skills and experience needed in order for me to be successful in my future academic pursuits.

CHAPTER I

INTRODUCTION

African Swine Fever Virus (ASFV) is a foreign animal pathogen that produces severe hemorrhagic fever and death in affected swine. With a mortality rate of ~100%, this virus has had devastating effects on the food security and economy of the nations in which the virus is endemic (1). Although first reported in Kenya, ASFV has expanded its range to various countries including Europe, Russia, Brazil, Cuba, and parts of the Middle East (2-4). With no vaccine available, disease management has been limited to the mass slaughter of affected swine, leading to substantial economic losses. Furthermore, with past outbreaks occurring in neighboring countries such as Cuba, there is significant concern due to the possibility of ASFV spreading to the US mainland (5, 6). As a result, it is imperative that research be dedicated towards the development of a safe and effective vaccine in preparation for potential future outbreaks in U.S.A.

Domestic pigs are most commonly infected by ASFV through virus inhalation, or ingestion of products or wastes contaminated with the virus (7). Onset of the disease begins with fever (>105^0F) which persists for the first 2-5 days of infection and dips slightly prior to death. In addition, infected pigs are anorexic, dull and huddle together, exhibiting arched backs when moved. Disease progress is associated with bloody diarrhea, and eventually hemorrhages of the skin, snout, abdomen, and legs. The majority of pigs affected die within the first 5 days of infection, and necropsies performed show enlarged spleen, hemorrhages in multiple body organs including lymph nodes, and button ulcers in the caecum and colon. The few pigs that survive infection remain emaciated and are lifelong carriers of the disease. The presence of survivors and

the variety of symptoms suggests that disease presentation and severity could be dependent on the ASFV isolate contracted (8).

The overarching goal of this project in Dr. Mwangi's laboratory is to develop a safe vaccine that will provide immunity against ASFV infections in pigs. In order to accomplish this, my research will focus on evaluating immunogenicity of two proteins, 9GL and 151R, which are known to contribute to ASFV pathogenicity (Figure 1). The first protein of interest, 9GL has been shown to target host cell macrophages and induce plaque formation (9). The second protein of interest, 151R, is a chaperon protein for 9GL and is essential for virus replication. Furthermore it is involved in the expression of the capsid protein p72 (10). My project will involve sub-cloning the genes encoding these two proteins into a lentivirus vector and thereafter use the DNA constructs to generate recombinant lentivirus expressing the respective antigens. The recombinant lentiviruses will be used for *in vitro* readouts to evaluate antigen-specific T cell responses in pigs immunized with recombinant adenoviruses expressing these antigens.

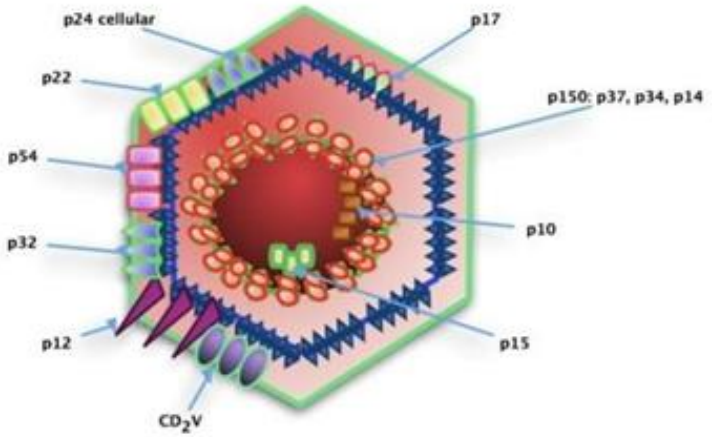

Figure 1: Structure of ASFV virus protein including all surface and internal proteins.

CHAPTER II

MATERIALS AND METHODS

Synthetic genes encoding the 9 GL and 151R proteins were available in the puc57 cloning vector. These genes were amplified by PCR using primers that introduced EcoR V and EcoR I restriction sites at the the 5' and 3'ends respectively. These primers also introduced a FLAG and HA tag at the 5' and 3' ends, respectively, which allowed the proteins to be easily tracked in later experiments using tag-specific antibodies. Once the correct PCR products were obtained, they were cloned into the PCR-TOPO Blunt vector, transformed into TOP 10 chemically competent cells and subsequently plated on LB agar plates containing carbenicillin antibiotic. The bacterial colonies produced on these plates were screened by PCR in order to identify recombinant plasmid clones which contained the 9GL and 151R genes. The positive clones identified were later sequenced for confirmation of the presence of genes encoding the 9GL and 151R proteins. Following sequencing the positive clones were subjected to an EcoR V (5' end) and EcoR I (3' end) restriction digest and gene cleaned, resulting in the production of the purified genes. These genes were then subcloned into a lentivirus vector cut with at the 5' end with EcoR V and at the 3' end with EcoR I restriction enzymes and two constructs designated as pLenti-9GL and pLenti-151R were generated. These constructs were transformed into Stbl3 chemically competent cells and plated onto LB agar plates. Positive clones were identified by EcoR V and EcoR I restriction digestion of miniprep DNA. Clones expressing the 9GL and 151R proteins were identified by immunocytometric analysis of 293A cells transfected with the miniprep DNA and then probed with monoclonal antibodies against the FLAG and HA tags. In addition, protein expression was validated using ASFV-specific serum. Clones that successfully expressed the 9GL and 151R

proteins were retransformed into Stbl3 chemically competent cells and plated on LB Broth containing carbenicillin antibiotic. Midiprep DNA was prepared from these cultures and used to transfect 293 FT cells. Protein expression was determined once again through immunocytometric analysis using monoclonal antibodies against the FLAG and HA tags as above. Based on transfection efficiency, one clone per construct was selected and used to generated the recombinant lentivirus designated as Lenti-9GL and Lenti-151R, respectively.

Once the Lenti-9GL and Lenti-151R constructs are produced, they will be amplified, validated for protein expression, and virus titers will be determined ready for *in vitro* evaluation of T cell response.

CHAPTER III

RESULTS

In the course of this experiment we have been successful in isolating the 9GL and 151R inserts from the puc57 vector. Insertion into the TOPO-Blunt vector and subsequent transformation into TOP 10 cells produced colonies which showed the presence of these inserts when screened by PCR. These colonies were then successfully cut by EcoR V and EcoR I restriction sites and subcloned into the lentivirus vector. Transformation of the pLenti 9GL and 151R inserts into Stbl3 cells produced a moderate number of colonies which showed the presence of these genes when screened by PCR and sequenced.

Upon generating the miniprep DNA of these positive clones, we were able to transfect 293A cells with both the pLenti 9GL and 151R constructs. When transfected with 1μg of miniprep DNA, the cells demonstrated 9GL and 151R protein expression, however, the transfection efficiency was only ~10-20% (Figure 2-4). However, when 293A cells were transfected with 2μg of the 9GL and 151R miniprep DNA the transfection efficiency increased to ~50%. This suggests that the 293A cells are not suitable candidates for use in determining transfection efficiency and protein expression of the pLenti constructs.

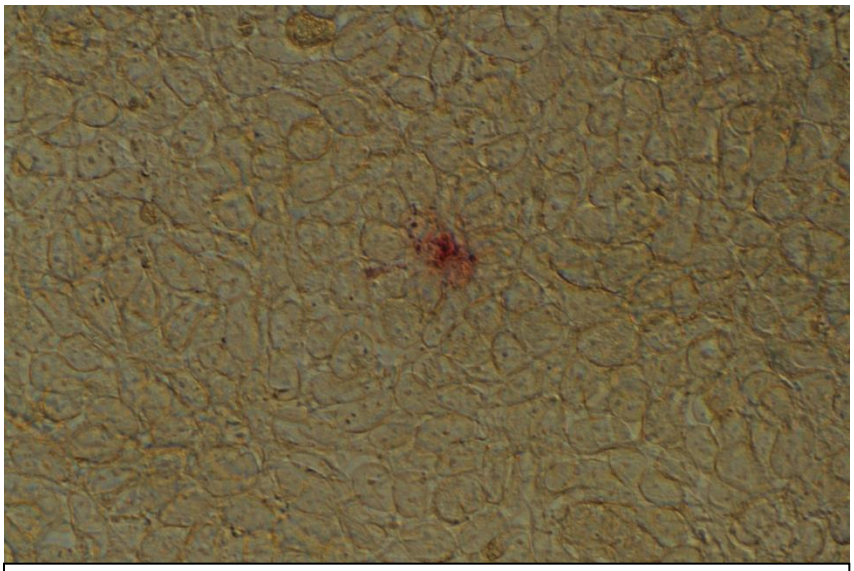

Figure 2: 293A cells transfected with miniprep DNA showing 9GL protein expression

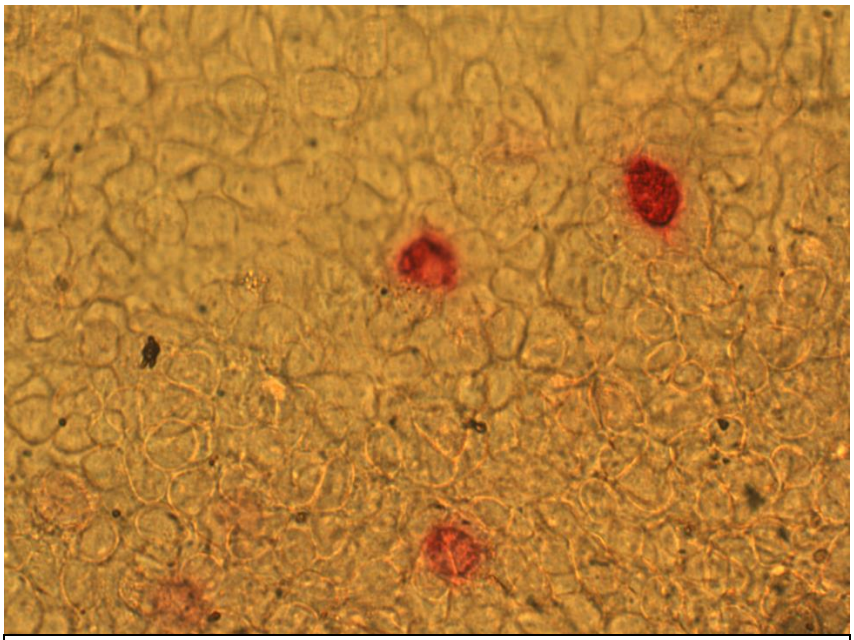

Figure 3: 293A cells transfected with miniprep DNA showing 9GL protein expression

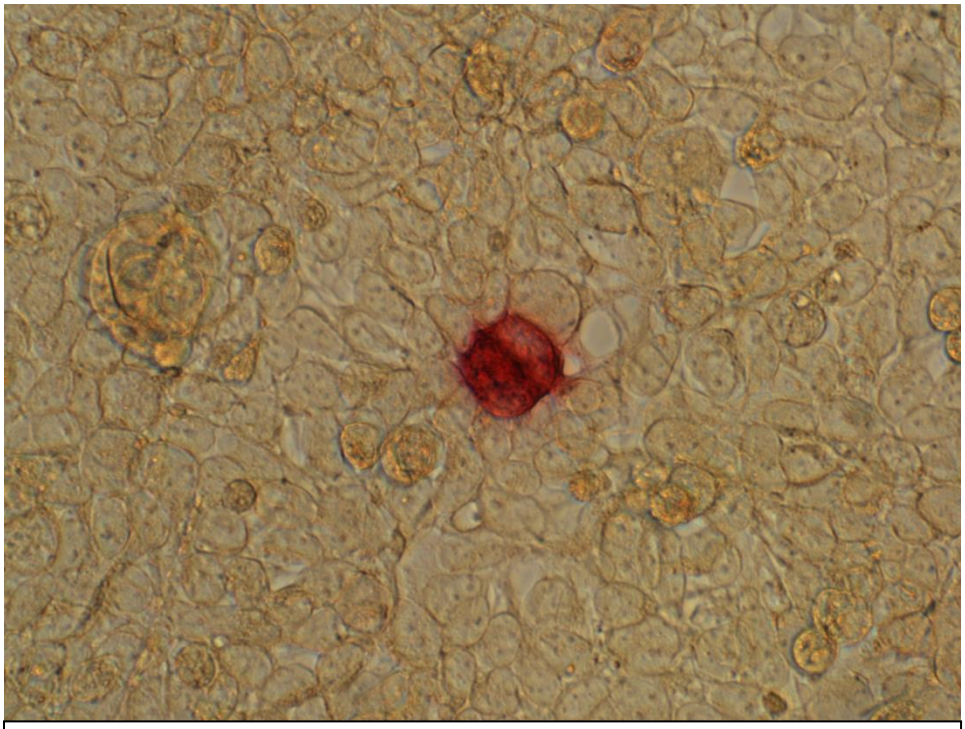

Figure 4: 293A cells transfected with miniprep DNA showing 151R protein expression.

Although we have shown through expression staining of 293A cells that we can successfully produce the 9GL and 151R proteins, we have been unable to show protein expression of these same genes in the 293FT cells. New 293 FT have been ordered: the previous batch had been contaminated with adenovirus based on assays conducted to determine poor performance. The new cells will be used to generate lentivirus.

CHAPTER IV

CONCLUSIONS

African Swine Fever is a devastating disease that has had a tremendous economic and social impact on countries in which it is endemic. With a mortality rate of ~100% it has destroyed the pork industry in countries in Africa and Europe and has the potential of becoming a threat to the U.S. pork industry. With the increase in concern of the impacts this disease can have socially and economically, much research has been devoted into developing some method for controlling the effects and spread of this disease. It is our hope that with the successful generation of the lentivirus for 9GL, 151R and a multitude of other ASF proteins, that we will be able to successfully generate a vaccine that can be safely used on swine in these affected areas.

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