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Research paper

Safety and immunogenicity of mammalian cell derived and Modified Vaccinia Ankara vectored African swine fever subunit antigens in swine

Jaime Lopera-Madrid[a], Jorge E. Osorio[a], Yongqun He[b], Zuoshuang Xiang[b], L. Garry Adams[c], Richard C. Laughlin[c], Waithaka Mwangi[c], Sandesh Subramanya[d], John Neilan[e], David Brake[e], Thomas G. Burrage[e], William Clay Brown[f], Alfonso Clavijo[g], Mangkey A. Bounpheng[h,*]

[a] Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, 53706, United States
[b] Unit for Laboratory Animal Medicine, Department of Microbiology and Immunology, Center for Computational Medicine and Bioinformatics, and Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, MI, 48109, United States
[c] Department of Veterinary Pathobiology, College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, College Station, TX, 77843-4467, United States
[d] Bioo Scientific Corporation, 7050 Burleson Rd., Austin, TX, 78744, United States
[e] Plum Island Animal Disease Center, U. S. Department of Homeland Security Science and Technology, Greenport, New York, United States
[f] Life Sciences Institute, University of Michigan, Ann Arbor, MI, 48109, United States
[g] Institute for Infectious Animal Disease, 2501 Earl Rudder Hwy, Suite 701, College Station, TX, 77845, United States
[h] Texas A&M Veterinary Medical Diagnostic Laboratory,1 Sippel Rd., College Station, TX, 77843, United States

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ABSTRACT

A reverse vaccinology system, Vaxign, was used to identify and select a subset of five African Swine Fever (ASF) antigens that were successfully purified from human embryonic kidney 293 (HEK) cells and produced in Modified vaccinia virus Ankara (MVA) viral vectors. Three HEK-purified antigens [B646L (p72), E183L (p54), and O61R (p12)], and three MVA-vectored antigens [B646L, EP153R, and EP402R (CD2v)] were evaluated using a prime-boost immunization regimen swine safety and immunogenicity study. Antibody responses were detected in pigs following prime-boost immunization four weeks apart with the HEK-293-purified p72, p54, and p12 antigens. Notably, sera from the vaccinees were positive by immunofluorescence on ASFV (Georgia 2007/1)-infected primary macrophages. Although MVA-vectored p72, CD2v, and EP153R failed to induce antibody responses, interferon-gamma (IFN-γ^+) spot forming cell responses against all three antigens were detected one week post-boost. The highest IFN-γ^+ spot forming cell responses were detected against p72 in pigs primed with MVA-p72 and boosted with the recombinant p72. Antigen-specific (p12, p72, CD2v, and EP153R) T-cell proliferative responses were also detected post-boost. Collectively, these results are the first demonstration that ASFV subunit antigens purified from mammalian cells or expressed in MVA vectors are safe and can induce ASFV-specific antibody and T-cell responses following a prime-boost immunization regimen in swine.

1. Introduction

African swine fever (ASF) is one of the most important disease of domestic pigs (Sánchez-Vizcaíno et al., 2015). The etiology agent, ASF virus (ASFV), is a large, complex DNA arbovirus and only member of the *Asfarviridae* family (Tulman et al., 2009). ASFV shares some features with poxviruses, including cytoplasmic genome organization and gene expression (Tulman et al., 2009). Although ASFV infection is generally asymptomatic in African wild suids, ASFV infection of domestic pigs usually results in a highly contagious hemorrhagic disease (Costard et al., 2013). Pigs that survive and recover initial infection may become persistently infected and

* Corresponding author.
E-mail addresses: loperamadrid@wisc.edu (J. Lopera-Madrid), osorio@svm.vetmed.wisc.edu (J.E. Osorio), yongqunh@med.umich.edu (Y. He), xiangzsh@gmail.com (Z. Xiang), GADAMS@cvm.tamu.edu (L.G. Adams), richard.laughlin@tamuk.edu (R.C. Laughlin), wmwangi@vet.k-state.edu (W. Mwangi), sandeshs@biooscientific.com (S. Subramanya), john.neilan@HQ.DHS.GOV (J. Neilan), David.Brake@ST.DHS.GOV (D. Brake), thomas.burrage@HQ.DHS.GOV (T.G. Burrage), wclayb@umich.edu (W.C. Brown), Alfonso.Clavijo@inspection.gc.ca (A. Clavijo), mbounpheng@gmail.com (M.A. Bounpheng).

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serve as virus reservoirs in areas where the disease is endemic (Oie, 2012). Outside the African continent, the disease is endemic to Madagascar and Sardinia, and intensive eradication programs following ASFV outbreaks in Portugal, Spain, South America, and the Caribbean were successful. Since its introduction into Georgia in 2007, ASFV has spread rapidly into vast areas of Western and Southern Russia, Ukraine, and the Republic of Belarus (Costard et al., 2009). There is no effective treatment or vaccine against ASF, thus current control measures rely mainly on detection and elimination of infected animals (Sánchez-Vizcaíno et al., 2013). Studies in domestic pigs using conventional vaccine approaches such as inactivated, avirulent or live attenuated ASF viruses (tissue culture adapted, rational gene deleted) have reported varying levels of homologous protection, however, some surviving animals have been shown to develop subclinical disease, thus raising the possibility of vaccinated animals becoming carriers (Sanchez-Vizcaino et al., 2009). Although protective immune mechanisms are poorly understood, cellular immune responses (particularly CD8+ T-cells) (Oura et al., 2005; Takamatsu et al., 2013), and humoral responses (Escribano et al., 2013; Onisk et al., 1994; Wardley et al., 1985) are thought to have important roles in host protection. Early vaccination studies that tested several, B-cell immunodominant ASFV subunit, recombinant proteins (i.e., p30, p54 and p72) produced using either baculovirus or DNA-based vaccines yielded variable success (Argilaguet et al., 2012, 2011; Barderas et al., 2001; Gómez-Puertas et al., 1998). Interestingly, immune responses elicited by DNA vaccines were variable and dependent on the fusion tag (i.e., soluble HA or ubiquitin) selected for ASFV recombinant antigen expression (Argilaguet et al., 2012). A recent study using an ASFV E75 expression library containing approximately 4000 individual plasmid clones (excluding p30, p54, and CD2v) demonstrated a correlation between protection and CD8+ T-cell response (Lacasta et al., 2014). Results from this study showed that the ASFV genome (~170–190 kb) contains additional antigens with protective potential, and implied that identification of such determinants would enable advances in the development of protective subunit vaccine candidates. To this end, we applied an *in silico* bioinformatic tool to identify and rank ASFV open reading frames (ORFs) that possess attributes desirable in selecting vaccine targets. Vaxign is the first web-based vaccine design program that predicts vaccine targets based on genomic sequences utilizing the reverse vaccinology (RV) strategy (He et al., 2010b). Using the entire annotated protein sequences from genome(s), Vaxign identifies open reading frames that possess a high probability of being good vaccine candidates based on (i) protein subcellular location, (ii) transmembrane helices, (iii) adhesin probability, (iv) sequence ortholog analysis among pathogenic strains, (v) sequence exclusion from genome(s) of nonpathogenic strain(s), and (vi) epitope binding to MHC (major histocompatibility complex) class I and class II. Vaxign has been used for rational design of experimental vaccines against several intracellular pathogens including Brucella and *Rickettsia prowazekii* (Caro-Gomez et al., 2014; Gomez et al., 2013), and has been successfully used for prediction of potential vaccine targets in uropathogenic *Escherichia coli* (He et al., 2010b), *Streptococcus agalactiae* (Pereira et al., 2013), and human herpes simplex viruses (Xiang and He, 2013).

In the current study, the Vaxign tool was used to analyze 12 ASFV annotated genomes (using Georgia 2007/1 strain as the reference genome), and identify and rank open reading frames (antigens) for subsequent recombinant expression. Five ASFV genes were successfully purified from mammalian cell HEK 293 (Human embryonic kidney cells 293) and/or MVA (Modified Vaccinia virus Ankara) recombinant antigen expression systems and were subsequently evaluated for safety and immunogenicity in swine using different prime-boost immunization regimens.

2. Materials and methods

2.1. Ethics statement

Yorkshire barrows, weighing 18–20 kg, were used in accordance with USDA policies under the supervision of the Texas A&M University Institutional Animal Care and Use Committee (IACUC). The pigs were obtained from a local, commercial source (State of Texas Department of Criminal Justice, Wynne Unit, 810 FM Road West, Huntsville, TX 77349). All experimental animal work was approved under Animal Use Protocol 2013-009, and reviewed and approved by the Texas A&M University IACUC Permit 2009067. The experiments were performed under BSL-2 conditions for pigs receiving MVA-vectored ASFV antigens and under BLS-1 conditions for pigs receiving HEK 293-purified recombinant ASFV antigens through the termination of the experiment on Day 42 post-immunization. Pigs were monitored at least twice daily for any clinical signs and to document any localized and or systemic adverse effects throughout the post-immunization phase. Animal care to alleviate any animal suffering was provided by the attending veterinarian. The pigs were fed an antibiotic-free commercial pig ration twice daily and water *ad libitum*. At the end of the study, the pigs were euthanatized with an intravenous overdose of a commercial euthanasia solution (Beuthanasia-D Special, sodium pentobarbital 390 mg/ml and sodium phenytoin 50 mg/ml, Intervet/Merck Animal Health, Madison, NJ) and osculated with a stethoscope to confirm lack of heartbeat.

2.2. Vaxign ranking and recombinant antigen selection

A total of 12 ASFV genomes were used for Vaxign analysis. Five genomes were downloaded from the National Center for Biotechnology Information RefSeq database: BA71 V (Accession #: NC_001659.1), Benin 97/1 (AM712239.1), E75 (FN557520.1), Georgia 2007/1 (FR682468.1), and OURT 88/3 (avirulent field isolate) (AM712240.1). Seven genome sequences were kindly provided by Dr. John Neilan: Kenya 1950 (AY261360.1), Malawi Lil-20/1(1983) (AY261361.1), Mkuzi 1979 (AY261362.1), Pretoriuskop/96/4 (AY261363.1), Tengani 62 (AY261364.1), Warmbaths (AY261365.1), and Warthog (AY261366.1). Since annotations of individual genes from these seven genomes were unavailable at the time of the study, the gene coding sequences of these genomes were determined by using Glimmer software (Delcher et al., 2007). Annotated protein sequences were used as input for the Vaxign pipeline analysis using the Georgia 2007/1 strain genome (Chapman et al., 2011) as the seed genome. For each protein sequence of the Georgia 2007/1 genome, Vaxign calculated and predicted the following five features: i) transmembrane domains, ii) adhesin probability, iii) sequence conservation among other ASFV strains, and epitopes binding to iv) MHC class I and v) MHC class II molecules (He et al., 2010b; Xiang and He, 2013). These five features were selected for analysis based on the following rationale: 1) the presence of transmembrane domain(s) suggested a possible virion surface location; 2) adhesin proteins suggested to be critical for virus attachment and entry; 3) expansive protection against diverse strains requires the use of conserved genes among different pathogenic ASFV strains as vaccine candidates; and 4) MHC class I/II epitope predictions for immunity determinants.

The testing of Vaxign Vaxitope method in epitope prediction has been described in previous studies (He et al., 2010b; Xiang and He, 2013). Since limited swine leukocyte antigens (SLA) specific epitopes were available for training of the epitope prediction function of Vaxitope, the human leukocyte antigen (HLA) epitope prediction method in Vaxitope was used to predict ASFV epitopes for binding to swine MHC. For the epitope screening for HLA alleles, we used six “Supertype” HLA Class I alleles and eight “supertype”