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Safety, immunogenicity, and efficacy of an alphavirus replicon-based swine influenza virus hemagglutinin vaccine

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ABSTRACT

A single-cycle, propagation-defective replicon particle (RP) vaccine expressing a swine influenza virus hemagglutinin (HA) gene was constructed and evaluated in several different animal studies. Studies done in both the intended host (pigs) and non-host (mice) species demonstrated that the RP vaccine is not shed or spread by vaccinated animals to comingled cohorts, nor does it revert to virulence following vaccination. In addition, vaccinated pigs develop both specific humoral and IFN-γ immune responses, and young pigs are protected against homologous influenza virus challenge.

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1. Introduction

Swine influenza virus (SIV) continues to be problematic in the swine industry. SIV is characterized by a sudden onset of respiratory illness, and is usually accompanied by anorexia, lethargy, and fever. In addition to the clinical complications associated with SIV in production animals, there have been several published reports implicating swine in the transfer of influenza viruses to humans [1–3]. Most recently, the 2009 pandemic H1N1 influenza virus was shown to have components of swine origin [4]. Our group has reported the rapid development of a swine vaccine against the pandemic H1N1 virus based on the alphavirus replicon system [5]. In addition to the possibility of zoonotic transfer, swine influenza viruses within the swine population continue to evolve at a rapid pace. Until 1998, swine influenza in the United States was caused almost exclusively by classical H1N1 [6], originally isolated in 1930 [7]. However, in 1998 both double and triple reassortant H3N2 viruses emerged [8–10]. Since then, there have been many influenza reassortment events that have led to the emergence of new subtypes and clusters [11–17]. Commercially available SIV vaccines often do not protect against new and emerging virus subtypes/clusters and must be periodically updated to match currently circulating strains. As such, novel swine influenza vaccines that are safe, effective, and can be rapidly altered to antigenically match an emerging strain should be considered as alternatives to traditional swine influenza vaccines. The United States Department of Agriculture Center for Veterinary Biologics (USDA CVB) has guidelines on the design of such safety studies for modified live vaccines (Veterinary Services Memorandum 800.201), but to date, has no specific guidance on shed spread or reversion to virulence studies for recombinant replication-incompetent vaccines. Thus, the studies included in this paper represent novel study designs and results that have been approved by the USDA CVB specifically for this replication-incompetent alphavirus-based replicon particle (RP) SIV vaccine.

An alphavirus replicon vector system has been derived from the attenuated TC-83 strain of Venezuelan equine encephalitis virus (VEEV) [18]. The ~11 kb VEEV positive-sense genome contains two open reading frames (ORFs). The 5′ ORF encodes four nonstructural

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proteins (nsp1–4) and the 3′ ORF encodes the virus structural proteins (capsid and glycoproteins (E3, E2, 6K and E1)) (reviewed in [19,20]). The nonstructural proteins are translated from the positive-sense genomic RNA and function to transcribe full-length negative-sense RNA. This negative-sense RNA is a template for both additional genomic RNA as well as 26S subgenomic mRNA. The 26S promoter is located between the two ORFs on the negative-sense RNA and is recognized by the nonstructural proteins for transcription of a subgenomic mRNA, from which the structural proteins are translated. This 26S mRNA is produced in 10-fold molar excess when compared to genomic RNA [19]. Foreign genes of interest can be inserted in the place of VEEV structural genes in a cDNA clone generating a self-replicating RNA (replicon) capable of expressing the foreign gene when introduced into cells. The self-amplifying replicon RNA directs the translation of large amounts of protein in transfected cells, reaching levels as high as 15–20% of total cell protein [21]. This replicon RNA does not contain any of the VEEV structural genes, so the RNA is propagation-defective. The replicon RNA can also be packaged into RP by supplying the structural genes *in trans* in the form of capsid and glycoprotein helper RNAs [21,22]. When the helper and replicon RNAs are combined and cotransfected into cells, the replicon RNA is efficiently packaged into single-cycle, propagation-defective RP.

Early production of RP was hampered by recombination events that resulted in the generation of replication-competent virus (RCV) (reviewed in [20]); first generation helper RNAs encoded the capsid and glycoprotein genes on the same RNA molecule, and thus only required one recombination event to create RCV [21,23,24]. The probability of this event occurring was greatly reduced by separating the helpers onto two separate RNAs ("split helper" system). This bipartite or split conformation greatly reduced the occurrence of RCV, as separation of the helper RNAs requires two independent recombination events to occur for generation of RCV [21,25]. These initial versions of the helper RNAs were designed to contain a 26S promoter downstream of the 5′ untranslated region. However, recent studies have demonstrated that the 26S promoter is not required for functional helper RNAs [26]. Removal of the 26S promoter results in helper RNAs that are not independent transcriptional units, and further reduces the possibility of functional recombinations between the replicon and helper RNAs. In addition to the removal of the 26S promoter, a stop codon has been introduced at the 3′ end of the capsid gene in place of the chymotrypsin-like cleavage site [26]. This mutation negates the cleavage activity of the capsid protein, adding another safeguard against functional recombination. Thus, helper RNAs lacking 26S promoters and containing an engineered capsid stop codon further reduce the probability of functional recombination than the standard split helper RNA system [26].

Alphavirus RP vaccines have been tested in multiple animal studies using multiple species (including humans) for more than 20 years [20,27–29]. However, formal safety studies have not been conducted in swine previously. We have used the alphavirus replicon system to produce an H3 SIV RP vaccine and we report studies performed evaluating the potential for this vaccine to shed, spread, and revert to virulence in both the intended host (pigs) and non-host (mice) species. Immunogenicity and efficacy were also evaluated in pigs of different ages.

2. Materials and methods

2.1. Replicon particle vaccine

The HA gene was PCR amplified from a cluster 4 H3N2 SIV isolate (H3) and cloned into the VEEV RP vector system using previously published methods [30].

2.2. Pig shed spread and reversion to virulence study

Twenty six-week-old cesarean-derived, colostrum-deprived (CDCD) pigs (12 gilts and 8 barrows) were obtained from Struve Labs (Manning, IA). All pigs were confirmed negative for antibodies to porcine reproductive and respiratory syndrome virus (PRRSV), SIV H1N1 and SIV H3N2 using commercial ELISA assays. The pigs were randomly assigned to H3 RP vaccinated or placebo groups. Upon arrival at the study facility, pigs were separated into five different pens located within the same room. Each pen contained two H3 RP vaccinated pigs and two placebo pigs. These two groups were comingled for the duration of the study, except for the 24 h immediately following vaccination to prevent physical transmission of the H3 RP vaccine to placebo pigs. The H3 RP vaccine was administered both intravenously (IV) in the right jugular vein and intramuscularly (IM) on the right side of the neck, both in 3 ml doses containing 1×10^{10} H3 RP, for a total of 2×10^{10} H3 RP. The placebo vaccine containing only the vaccine diluent was administered in identical dose volumes and injection sites. Both vaccines were administered by personnel blinded to vaccine composition to avoid potential bias regarding vaccine reactions. Pigs were observed daily for 14 days post-vaccination for any vaccine-related adverse effects. Serum, nasal swabs and rectal swabs were collected on study days −1, 0, 3, 7, 10, and 14. Nasal and rectal swabs were placed into 15 ml conical tubes containing 1 ml minimum essential media (MEM) (Invitrogen Life Technologies) + 1% antibiotic/antimycotic (Invitrogen Life Technologies). Samples were held at −80 °C until removal for further processing. Tissues collected at necropsy included injection site (right neck musculature), tonsil, spleen, heart, lung, right retropharyngeal lymph node, liver, intestine, brain, and kidney. Tissues were placed in whirl-pak bags at necropsy and held at −80 °C until removal for further processing. Samples collected throughout the study were assayed by the RT-PCR and CPE assays. All pigs were housed and treated in accordance with IACUC approved guidelines.

2.3. Mouse shed spread and reversion to virulence study

Twenty six-week-old BALB/c female mice were obtained from Charles River Laboratories (Portage, MI). The twenty mice were divided equally among five cages, with each cage containing two mice injected with H3 RP vaccine and two mice injected with placebo vaccine. Mice were identified within a cage by unique individual ear notches. The two groups were comingled for the duration of the study, except for the 24 h immediately following vaccination on study days 0 and 14 to prevent physical transmission of the H3 RP vaccine to the placebo mice. The H3 RP vaccine was delivered intraperitoneally in 200 μl doses containing 3×10^7 SIV RP. The placebo vaccine was administered in identical dose volumes and injection sites. Both vaccines were administered by personnel blinded to vaccine composition to avoid potential bias regarding vaccine reactions. Fecal pellets were collected from each cage at multiple time points throughout the study. Blood was collected post-euthanasia via cardiac puncture. Tissues collected at necropsy included brain, liver, heart, kidney, spleen, lungs, and intestine. A portion of each tissue was placed into individual microcentrifuge tubes, and a portion of the tissue samples were also fixed in 10% buffered formalin for histopathological analysis. All samples collected throughout the study and at necropsy were held at −80 °C until removal for further processing. Samples collected throughout the study were assayed by the RT-PCR and CPE assays. All mice were housed and treated in accordance with IACUC approved guidelines.

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