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Vaccination of pigs with a codon-pair bias de-optimized live attenuated influenza vaccine protects from homologous challenge

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ABSTRACT

Influenza A virus (IAV) in swine constitutes a major economic burden for producers as well as a potential threat to public health. Whole inactivated virus vaccines (WIV) are the predominant countermeasure employed to control IAV in swine herds in the United States despite the superior protection, and diminished adverse effects, induced by live attenuated influenza vaccines (LAIV). A major hurdle for the development of LAIV exists in achieving the proper level of attenuation while maintaining immunogenicity. Using Synthetic Attenuated Virus Engineering (SAVE) to introduce codon-pair bias de-optimization (CPBD) into the hemagglutinin (HA) and neuraminidase (NA) gene segments of pandemic H1N1 IAV, a novel LAIV was produced and evaluated for attenuation, immunogenicity, and efficacy in pigs. The CPBD LAIV induced inappreciable pathology following intranasal administration yet induced robust serum and mucosal antibody titers. CPBD LAIV vaccinated pigs challenged with wild-type virus showed protection from disease and virus detection, highlighted by the absence of detectable virus titers in the nasal passages and lungs. These results demonstrate the efficacy of a LAIV designed by SAVE codon de-optimization in pigs, providing support for the continued development of CPBD LAIV for use in swine.

1. Introduction

Influenza A viruses are endemic respiratory pathogens in domestic swine populations [1] that constitute a significant economic burden through lost production, and a concern for public health via spill-over into human populations [2–4]. Despite its significance, control of IAV in domestic swine herds continues to be problematic. The paucity of effective control measures is due in part to the substantial genetic and antigenic diversity of swine IAV. Presently, three subtypes of IAV; H1N1, H1N2, and H3N2; are endemic in US swine populations that can be further divided into multiple genetic lineages and antigenic groups [5,6]. In the United States, the H3 subtype was classified into three major antigenic groups with multiple outliers [7] and the H1 subtype was classified into seven phylogenetic, antigenically distinct clades [8,9]. Additionally, neuraminidase (NA) inhibiting antibodies contribute to immunity against IAV, as has recently been shown for NA subtype N2, where vaccination with one of two antigenically distinct lineages failed to protect from challenge with the heterologous NA [10]. Further, continued introductions of human seasonal IAV to pig populations are contributing to the extensive genomic and antigenic diversity [11–13].

Traditionally, vaccination with adjuvanted, whole inactivated virus vaccines (WIV) formulated with oil-in-water adjuvant was the most highly utilized method of IAV control in the US swine industry. The immunity provided by WIV is strain specific, eliciting strong protection against homologous antigen but poor cross-protective responses across lineages and subtypes [14–16]. Vaccine efficacy in growing pigs is complicated by the negative interference of maternal antibodies present in piglets, as sows are the predominant recipients of WIV in an effort to protect the neonatal pig via passive antibody [17]. Further, infection with a heterologous strain of the same subtype following vaccination with WIV has been shown to cause enhanced respiratory disease [14,18–20]. Vaccine associated enhanced respiratory disease, or VAERD, is manifested in pigs following WIV administration and subsequent infection with a drifted, homosubtypic virus. The increased lung pathology observed in VAERD affected pigs is thought to be mediated by non-neutralizing IgG antibodies that

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target the conserved stalk, HA2 domain, of the IAV hemagglutinin, that are readily induced by WIV [21].

Live attenuated influenza viruses (LAIV) have been shown to offer superior protection to WIV under certain conditions. In pigs, LAIV have been shown to provide protection from a broader range of antigens without inducing VAERD [22–24]. Several LAIV platforms are currently being developed for veterinary use, though questions concerning efficacy against heterologous virus and increasing the IAV subtype content exist [25,26]. Codon-pair bias de-optimization (CPBD) introduces underrepresented codon pairs into the viral genome reducing translation on host-cell ribosomes [27,28]. A computational approach, termed Synthetic Attenuated Virus Engineering (SAVE), has been developed to aid in the design and increase the speed of genomic recoding [29]. Recently, a CPBD LAIV based on the 2009 pH1N1 A/California/07/2009 virus was shown to be clinically attenuated and protect ferrets against homologous challenge [30], demonstrating efficacy in an outbred animal model.

To investigate the utility of a CPBD LAIV in an agricultural setting, we assessed the attenuation, immunogenicity, and protection of a 2009 pH1N1 A/California/07/2009 attenuated virus in pigs. We found the 2009 pH1N1 CPBD LAIV to be significantly attenuated compared to wild-type virus and capable of preventing clinical disease and pathology. Additionally, we show vaccination induced robust mucosal and humoral immunity which correlated with protection from disease and virus detection following homologous challenge.

2. Materials and methods

2.1. Virus

Synthetic attenuated virus engineering (SAVE) was used to introduce codon-pair bias into the coding sequences of the hemagglutinin and neuraminidase gene segments of influenza as previously described, introducing 346 synonymous mutations in 1465 nucleotides of the HA, and 293 synonymous mutations in 1196 nucleotides of the NA [28,29]. CPBD attenuated H1N1 (AttH1N1) and wild-type A/California/04/2009 H1N1 (CA/09) were cultured on Madin-Darby canine kidney (MDCK) cells in Opti-MEM (Life Technologies) supplemented with fetal calf serum, L-glutamine, vitamins, and antibiotics/antimycotics.

2.2. Experimental design

Forty-seven three-week-old pigs obtained from a herd free of IAV and porcine reproductive and respiratory syndrome virus (PRRSV) were housed in a biosafety level 2 containment facility in compliance with USDA NADC Animal Care and Use Committee protocols. Prior to study onset, all pigs were confirmed to be seronegative for IAV by commercial nucleoprotein ELISA kit (IDEXX, Westbrook, ME). Pigs were implanted with a subcutaneous temperature transponder to measure body temperature (Destron Fearing, Dallas, TX).

For the vaccine safety evaluation in study 1, pigs were divided into five groups (Table 1). Pigs were inoculated intranasally with 1 ml (0.5 ml/nostril) of low dose (10^3 $TCID_{50}$) or high dose (10^5 $TCID_{50}$) AttH1N1 virus, wild-type virus (10^5 $TCID_{50}$) by slowly dripping the inoculum by syringe into each nostril. Intranasal inoculations were performed under gentle physical restraint without anesthesia. To compare attenuation of codon-pair bias de-optimized virus with wild-type A/California/04/2009, nasal swabs were collected from groups 1–4 at 0, 3, and 5 days post vaccination (dpv). Further, 5 pigs from groups 1, 3 and 4 were euthanized on 5 dpv for pathological examination of the lungs and trachea and collection of bronchoalveolar lavage fluid (BALF), collected with 50 ml minimal essential media (MEM), to assess virus titer.

Table 1
Design of study 1 evaluating attenuation of codon-pair bias de-optimized live attenuated H1N1 vaccine.

Group	Treatment	n
1	None	5[a,b]
2	AttH1N1 low (10^3)[c]	5[b]
3	AttH1N1 high (10^5)[c]	5[a,b]
4	A/CA/04/2009 (10^5)[c,d]	5[a,b]

[a] Number of pigs euthanized at 5 days post-vaccination.
[b] Number of nasal swabs collected at 1, 3, 5 dpv.
[c] Vaccine dose in $TCID_{50}$, administered via intranasal route.
[d] Wild-type A/California/04/2009.

In study 2, pigs were challenged at 28 dpv with 10^5 $TCID_{50}$ per mL wild type CA/09 via intranasal and intra-tracheal routes to evaluate protection efficacy (Table 2). Pigs received 2 ml virus inoculum intratracheally and 1 ml intranasally under anesthesia by intramuscular injection with ketamine (8 mg/kg of body weight), xylazine (4 mg/kg), and Telazol (6 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA), or remained unchallenged as negative controls (NV/NC). Prior to challenge, nasal swabs and serum were collected to confirm IAV status appropriate for each treatment group. Nasal swabs were collected at 3 and 5 days post challenge (dpc) and all pigs were euthanized for pathological examination of lungs and trachea and collection of BALF on 5 dpi.

2.3. Pathological examination

The lungs of pigs euthanized at 5 dpv and 5 dpi were examined for macroscopic and histologic signs of lung pathology as described previously [31]. Macroscopic lung lesions were scored as the percentage of the lung observed with areas of purple-red consolidation, which is a hallmark of IAV infection. Tissue samples from the right middle or affected lung lobe and trachea were fixed in 10% buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin (H&E) for histopathologic examination.

2.4. Viral replication and shedding

The presence of IAV in nasal swab samples was assessed by virus isolation on MDCK cells as previously described [24]. Briefly, samples in approximately 2 ml MEM, were filtered through a 0.45 mm syringe filter prior to inoculation of PBS washed, confluent MDCK monolayers in 24 well plates. Following an incubation period of 1 h at 37 °C, 200 µl serum-free MEM supplemented with 1 µg/ml tosylsulfonyl phenylalanyl chloromethyl ketone (TPCK)-trypsin and antibiotics/antimycotics was added to all wells. Cell monolayers were observed for cytopathic effect (CPE) at 48 and 72 h. At 72 h, plates were fixed with 10% buffered formaldehyde in PBS-0.05% Tween-20 and stained using an anti-influenza A virus

Table 2
Design of study 2 for evaluating protection of codon-pair bias de-optimized live attenuated H1N1 vaccine against homologous challenge.

Group	Treatment	Challenge	5 dpc[a,b]
1	None	None	8
2	AttH1N1 low (10^3)[c]	A/CA/04/2009 (10^5)[d]	8
3	AttH1N1 high (10^5)[c]	A/CA/04/2009 (10^5)[d]	8
4	None	A/CA/04/2009 (10^5)[d]	8

[a] Number of pigs euthanized at 5 days post-challenge.
[b] Number of nasal swabs collected at 1, 3, 5 dpc.
[c] Challenge dose in $\log_{10}$ $TCID_{50}$, administered via intranasal and intratracheal routes.
[d] Wild-type A/California/04/2009.