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Protective effect of a polyvalent influenza DNA vaccine in pigs

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ABSTRACT

Background: Influenza A virus in swine herds represents a major problem for the swine industry and poses a constant threat for the emergence of novel pandemic viruses and the development of more effective influenza vaccines for pigs is desired. By optimizing the vector backbone and using a needle-free delivery method, we have recently demonstrated a polyvalent influenza DNA vaccine that induces a broad immune response, including both humoral and cellular immunity.

Objectives: To investigate the protection of our polyvalent influenza DNA vaccine approach in a pig challenge study.

Methods: By intradermal needle-free delivery to the skin, we immunized pigs with two different doses (500 μg and 800 μg) of an influenza DNA vaccine based on six genes of pandemic origin, including internally expressed matrix and nucleoprotein and externally expressed hemagglutinin and neuraminidase as previously demonstrated. Two weeks following immunization, the pigs were challenged with the 2009 pandemic H1N1 virus.

Results: When challenged with 2009 pandemic H1N1, 0/5 vaccinated pigs (800 μg DNA) became infected whereas 5/5 unvaccinated control pigs were infected. The pigs vaccinated with the low dose (500 μg DNA) were only partially protected. The DNA vaccine elicited binding-, hemagglutination inhibitory (HI) − as well as cross-reactive neutralizing antibody activity and neuraminidase inhibiting antibodies in the immunized pigs, in a dose-dependent manner.

Conclusion: The present data, together with the previously demonstrated immunogenicity of our influenza DNA vaccine, indicate that naked DNA vaccine technology provides a strong approach for the development of improved pig vaccines, applying realistic low doses of DNA and a convenient delivery method for mass vaccination.

1. Introduction

Influenza A virus infections in swine herds constitute a well-known challenge to the swine industry. Reproductive problems together with weight loss and aggravation of secondary infections are characteristic of swine influenza and result in serious animal welfare problems and economic losses (Bennett et al., 1999; Olsen et al., 2006). The influenza infection in pigs resembles the infection in humans. The virus replicates in the epithelium of the entire respiratory tract but rarely infects other tissues (van der Laan et al., 2008). The disease lasts for 7–10 days seldom results in death of the animals (van der Laan et al., 2008). In addition, the tremendous genetic plasticity of the virus can result in transmission between animals as well as zoonotic transmission and adaptation to human hosts, resulting in novel pandemic influenza strains such as the pandemic 2009 H1N1 strain (Ito et al., 1998; Nelson and Vincent, 2015; Smith et al., 2009). A successful, more broadly

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protective vaccine for pigs against influenza A virus is very much desired, since it will improve the health in pig herds, limit the use of antibiotics and lower the risk of transmission to other species, such as humans. The current vaccines against influenza A virus for pigs are based on inactivated virus and only induce immunity against the virus strains included in the vaccines, thus providing only limited protection against the diverse spectrum of other circulating influenza strains (Sandbulte et al., 2015). DNA vaccine technology is already approved for use in pigs (Thacker et al., 2006) and has many advantages required for an effective influenza vaccine, such as rapid production, easy plasmid modification, a stable formulation and *in vivo* antigen expression leading to induction of both broad and long-lived cellular and humoral immunity (Kutzler and Weiner, 2008; Li and Petrovsky, 2015; Liu, 2011). The technique has previously been tested by us and others in pigs against influenza (Bragstad et al., 2013; Eriksson et al., 1998; Gorres et al., 2011; Heinen et al., 2002; Larsen et al., 2001; Macklin et al., 1998; Olsen, 2000). Several optimizations can be applied today to improve the production and immunogenicity of the vaccines. We and others have described improvements of influenza DNA vaccines, including optimizing the plasmid vector backbone (Borggren et al., 2015; Williams, 2013). and delivery of the vaccine intradermally with a convenient needle-free device developed for mass vaccination (Borggren et al., 2015; Martelli et al., 2007).

Recently, we reported a broad immune response induced in pigs by a DNA vaccine *in vivo* expressing six different genes of pandemic viral origin (Borggren et al., 2016, 2015). The pandemic nature of the DNA genes makes them the ancestor of all subsequent strains and are naturally less glycosylated when expressed *in vivo*, compared to circulating virus strains with more glycosylation acquired by antigenic drift (Sriwilaijaroen and Suzuki, 2012; Wang et al., 2009; Wei et al., 2010). Consequently, a broader range of epitopes can be recognized by the DNA vaccine-induced response, thus producing a more cross-reactive immunity. Thus, our DNA vaccine induced both humoral and cellular immunity against virus strains homologous and heterologous to the DNA vaccine genes (Borggren et al., 2016). In the present study, we have evaluated the immunogenicity, dose-response, and protective effect of the same polyvalent DNA vaccine in an influenza-virus challenge study in pigs.

2. Materials and methods

2.1. Construction of DNA vaccines

The six influenza DNA vaccine genes have been described previously (Borggren et al., 2016, 2015). Briefly, the 6 influenza DNA vaccine genes were designed from nucleotide sequences published in GenBank derived from only pandemic influenza strains; 1918 NP: A/Brevig Mission/1/18(H1N1), 1918 M: A/Brevig Mission/1/18(H1N1), 2009 HA: A/California/04/2009(H1N1)pdm09, 2009 NA: A/California/04/2009(H1N1)pdm09, 1968 HA: A/Aichi/2/1968(H3N2), 1968 NA: A/Aichi/2/1968(H3N2). The genes were synthesized and designed to include the appropriate restriction enzymes and the Kozak-sequence (GCCACC) upstream from the start codon, for efficient cloning and transcription into the expression vector. All genes were synthesized using only codons from highly expressed human or mammalian genes (codon optimized), except the M gene that was not codon optimized. The minimal NTC9385R plasmid, free of antibiotic resistance genes, was used as the expression vector backbone (Williams, 2013).

2.2. Animals and experimental design

Fifteen 5-to-6-week-old, recently weaned male pigs (Yorkshire x Landrace breed), tested influenza-negative by ID Screen® Influenza A Antibody Competition Multi-species ELISA (ID.VET, France), were procured from a commercial Spanish high-health herd free from Porcine Respiratory and Reproductive Syndrome (PRRS), Aujeszky's disease, *Pasteurella multocida* and *Brachyspira* spp., but positive for *Haemophilus parasuis*, *Mycoplasma hyopneumoniae* and *Actinobacillus pleuropneumoniae* serotype 2. Prior to weaning, the pigs had been vaccinated against porcine circovirus type 2 (PCV2) and *Mycoplasma hyopneumoniae*. The pigs were randomly assigned to three groups of five animals (two vaccinated groups allocated in one box and one non-vaccinated group in another one). Boxes were subjected to negative pressure at the biosafety level 3 isolation facilities of the Centre de Recerca en Sanitat Animal (CReSA), Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Spain. Pigs were allowed to acclimatize for one week before the initiation of the experiment.

With an interval of three weeks, two groups of pigs were vaccinated twice on the dorsal side of the back using the needle-free Intra-Dermal Application of Liquids (IDAL® MSD Animal Health) device (Ferrari et al., 2011; Visser et al., 1994). For use of the IDAL® device, the vaccine constructs were premixed at a 1:1 vol ratio with an α-tocopherol-based aqueous solution (Diluvac Forte®, MSD Animal Health) (Borggren et al., 2016, 2015). Five pigs were immunized with 500 μg of DNA (83 μg per gene/plasmid) each (one shot of 200 μl (2.5 mg/ml Diluvac)) on the back of individual pigs). Five pigs received 800 μg of DNA (133 μg per gene/plasmid) each, distributed into four shot sites à 200 μl (1 mg/ml of Diluvac) on the back of individual pigs. Five pigs remained unvaccinated and constituted a non-immunized control group. Two weeks after the second vaccination, all pigs were challenged intranasally (i.n.) with 10^6 ($TCID_{50}$)/pig of pandemic A/California/7/09 (H1N1)pdm09 applied in 1.5 ml into each nostril. All pigs were monitored daily for clinical signs of disease or any adverse vaccination-related effects. Rectal body temperatures were recorded daily starting from two days before challenge until the end of the experiment. Whole-blood samples were collected from the anterior vena cava of all pigs on days −36, −28, −21, −15, −7, 0, 7 and 13 post challenge (pc). Serum was isolated and stored at −20 °C for subsequent examination. On days 0, 3, 5, 7, 9 and 13 pc, nasal swab samples were collected in virus transport medium (phosphate-buffered saline (PBS) containing antimicrobial drugs (100 U/mL penicillin and 0.1 mg/ml streptomycin)) from all pigs to evaluate nasal virus shedding. Samples were stored at −80 °C until testing. Upon termination of the experiment, on day 13 pc, the pigs were euthanized by intravenous injection of a lethal dose of pentobarbital followed by exsanguination. Post mortem, gross-pathological evaluation was carried out and lung tissues (apical and middle lobes as well as other potential lobes if evidence of gross lesions) were taken and fixed by immersion in 10% buffered formalin. Lung tissues were subsequently embedded in paraffin, cut in 4 μm sections, stained with hematoxylin-eosin stain, and slides were observed under an optical microscope. Potential swine influenza-like lesions (broncho-interstitial pneumonia) were scored using a previously published work (Detmer et al., 2013).

The present study was approved by IRTA's Ethics Committee for Animal Experimentation and the Animal Experimentation Commission from the Autonomous Community of Catalonia Government in compliance with the Directive, UE 63/2010 and the Spanish Legislation, RD 53/2013 and the Catalan Law 5/1995 and Decree 214/1997

2.3. Influenza virus detection

A quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assay was utilized to monitor viral loads in nasal swab samples (day 0, 3, 5, 7, 9 and 13 pc). RNA was extracted with a MagNA Pure LC Instrument applying the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche diagnostics). Primers and probes for the neuraminidase (NA) gene of challenge virus A/California/7/09 (H1N1) pdm09 were used to detect the challenge virus. The beta-actin housekeeping cellular gene was used as a control for correct sampling that should contain pig derived cell material in the swaps. Quantification of virus was performed by using a standard curve developed by serial dilutions of H1N1pdm09 virus with known $TCID_{50}$/ml concentration,