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A polyvalent influenza DNA vaccine applied by needle-free intradermal delivery induces cross-reactive humoral and cellular immune responses in pigs

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ABSTRACT

Background: Pigs are natural hosts for influenza A viruses, and the infection is widely prevalent in swine herds throughout the world. Current commercial influenza vaccines for pigs induce a narrow immune response and are not very effective against antigenically diverse viruses. To control influenza in pigs, the development of more effective swine influenza vaccines inducing broader cross-protective immune responses is needed. Previously, we have shown that a polyvalent influenza DNA vaccine using vectors containing antibiotic resistance genes induced a broadly protective immune response in pigs and ferrets using intradermal injection followed by electroporation. However, this vaccination approach is not practical in large swine herds, and DNA vaccine vectors containing antibiotic resistance genes are undesirable.
Objectives: To investigate the immunogenicity of an optimized version of our preceding polyvalent DNA vaccine, characterized by a next-generation expression vector without antibiotic resistance markers and delivered by a convenient needle-free intradermal application approach.
Methods: The humoral and cellular immune responses induced by three different doses of the optimized DNA vaccine were evaluated in groups of five to six pigs. The DNA vaccine consisted of six selected influenza genes of pandemic origin, including internally expressed matrix and nucleoprotein and externally expressed hemagglutinin and neuraminidase.
Results: Needle-free vaccination of growing pigs with the optimized DNA vaccine resulted in specific, dose-dependent immunity down to the lowest dose (200 μg DNA/vaccination). Both the antibody-mediated and the recall lymphocyte immune responses demonstrated high reactivity against vaccine-specific strains and cross-reactivity to vaccine-heterologous strains.
Conclusion: The results suggest that polyvalent DNA influenza vaccination may provide a strong tool for broad protection against swine influenza strains threatening animal as well as public health. In addition, the needle-free administration technique used for this DNA vaccine will provide an easy and practical approach for the large-scale vaccination of swine.

1. Introduction

Influenza virus is endemic in pigs and affects the majority of herds in modern swine production [1]. 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 for the swine industry [2]. It is well known that pigs and humans can exchange influenza viruses, and a recent example is the triple reassortant H1N1pdm09, composed of genes from three known swine viruses, which spread rapidly among humans during the pandemic in 2009 and later transmitted from humans to pigs [3]. Protection of pigs against influenza infection by effective vaccination would provide

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a crucial tool to benefit swine health and reduce risks to public health.

Current vaccines against influenza virus for pigs are based on inactivated virus and only induce immunity against the virus strains included in the vaccines, thus providing limited protection against the diverse spectrum of other circulating influenza strains [1]. Thus, an effective intervention strategy for the control of influenza in pigs requires improved vaccines. DNA technology enables vaccination with versatile combinations of antigens that can simply be substituted. The DNA platform was tested early on in the influenza field with variable results [4,5]. However, a direct comparison between early results [6–8] and more recent studies are complicated due to recent improvements in DNA vaccines as well as the improved techniques to evaluate cell-mediated immune responses. Thus, codon-optimization of genes [9–14], improved delivery [12,15–17] and DNA vector improvements [18] have enhanced the immunogenicity of DNA vaccines, and a number of DNA vaccine candidates have been successful in both animal and human studies [13–15,19–21]. DNA vaccines have the advantage of inducing both cellular and humoral immunity, both of which are believed to serve important roles in protection against influenza virus infections and shedding of virus [1,15,22].

Previously, we and others have tested DNA vaccines against influenza in pigs in different experimental settings [6–8,15,20,23,24]. Recently, we published the optimization of a polyvalent influenza DNA vaccine using next-generation antibiotic-free vectors together with a needle-free intradermal (i.d.) application in rabbits [25]. In the present study, we conducted a DNA dose titration study in pigs to investigate the immunogenicity of our optimized influenza DNA vaccine containing pandemic genes from the 1918 H1N1-, 1968 H3N2- and pdm09 H1N1-influenza viruses. Thus, we tested the induction of both cellular and humoral immune responses directed against antigens both homologous and heterologous to the vaccine.

2. Materials and methods

2.1. Construction of DNA vaccines

The six influenza DNA vaccine genes have been described previously [25]. The NTC9385R plasmid was used as an expression vector [18,25].

2.2. Animals and experimental design

Twenty-two five-week-old, recently weaned pigs obtained from a Danish specific pathogen free (SPF) herd were randomly assigned to four groups of five or six animals. The pigs were housed without contact to other animals in separate isolation facilities at the Department of Animal Science, Aarhus University. The pigs were allowed to acclimatize for 1 week before the initiation of the experiment. With an interval of 3 weeks, three groups of pigs were vaccinated twice on the dorsal site of the back using the needle-free IntraDermal Application of Liquids (IDAL®) device (Henke Sass Wolf). Six pigs were vaccinated with 200 μg of DNA each (one injection site on the back), another six pigs received 800 μg of DNA each (distributed into four injection sites) and five pigs received 1972 μg of DNA (distributed into 10 injection sites). For use of the IDAL® device, the vaccine constructs were premixed at a 1:1 volume ratio with an α-tocopherol-based aqueous solution (Diluvac Forte®, MSD Animal Health). Two pigs remained unvaccinated, and three additional pigs received the Diluvac Forte® solution without any DNA vaccine. The latter five pigs displayed similar immune profiles in the analyses and were thus combined into the non-treated control group. All pigs were monitored daily for clinical signs of disease or any adverse vaccination-related effects. Rectal body temperatures were recorded 2 days before and 2 days after each vaccination. Whole-blood samples were collected from the anterior vena cava of all pigs on days 0, 7, 14, 21, 28 and 35 post-first vaccination (pv1). Serum was isolated and stored at -20 °C for subsequent examination. On day 35pv1, peripheral blood mononuclear cells (PBMC) were isolated from freshly collected heparinized blood samples by density gradient centrifugation and cryopreserved until use. On a weekly basis starting from day 0pv1, nasal swab (MicroRheologics) samples were collected in virus transport medium from all pigs to test for potential accidental influenza infection during the experiment. Upon termination of the experiment, on day 35pv1, the pigs were euthanized by i.v. injection of a lethal dose of pentobarbital. All animal handling and experimentation procedures were approved by the Danish Animal Experiments Inspectorate (2014-15-0201-00251).

2.3. Influenza detection

Nasal swab samples (day 0, 7, 14, 21, 28 and 35pv1) were examined for influenza A virus RNA using an in-house real-time reverse transcription (RT)-PCR assay. Primers and probes for the matrix gene of influenza A virus, the NA gene of H1N1pdm09 and the HA gene of human seasonal H3N2 were used.

2.4. Enzyme-linked immunosorbent assay (ELISA)

ELISA was conducted to measure influenza-specific IgG responses in the sera as previously described [25]. The influenza virus proteins used for coating were HA from A/California/04/09(H1N1)pdm09, A/Aichi/2/1968(H3N2), A/swine/Guangxi/13/2006(H1N2) or A/Brisbane/59/07(H1N1); NA from A/Aichi/2/1968(H3N2); NP from A/California/07/09(H1N1)pdm09; M1 protein from A/Brevig Mission/1/1918(H1N1) (all from Sino Biological Inc.); or M2e polypeptide (GenScript). A horseradish peroxidase-conjugated anti-pig-IgG antibody (AbD Serotec) was used for detection.

2.5. Hemagglutination inhibition (HI) assay

The HI assay was performed according to the protocols of the WHO [26] as previously described [25]. The virus isolates tested were two swine strains, A/swine/Denmark (DK)/10409/2013(H1N1pdm) and A/swine/DK/10525/2008(H1N2).

2.6. Microneutralization assay (MN)

Development of neutralizing antibodies was determined according to the protocols of the WHO [27]. Viruses used were A/California/07/09(H1N1pdm09), A/NewCaledonia/20/99(H1N1), and A/swine/DK/10409/2013(H1N1pdm), with 100 $TCID_{50}$ as the inoculum.

2.7. PBMC stimulation and cell-mediated immune assays

Prior to stimulation, the cryopreserved PBMC were thawed and rested overnight in R10 (RPMI, Gibco) supplemented with 10% heat-inactivated FBS (Gibco) and 1% penicillin–streptomycin (Gibco) (culture medium) at 37 °C with 5% CO_2. During stimulation, the R10 was supplemented with 50 ng/ml porcine IL-18 (R&D). The PBMC were stimulated with 5 μg/ml recombinant influenza proteins, including NP from A/California/07/09(H1N1)pdm09 and A/Brevig Mission/1/1918(H1N1), HA from A/California/04/09(H1N1)pdm09 or matrix 1 (M1) from A/Brevig Mission/1/1918(H1N1) (all from Sino Biological Inc.). One microgram per milliliter *Staphylococcus* Enterotoxin B (SEB, Sigma) served as a positive control and media alone served as a negative control. After 18 h of

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