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Short communication

Induction of a cross-reactive antibody response to influenza virus M2 antigen in pigs by using a Sendai virus vector

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ABSTRACT

Protecting pigs from simultaneous infection with avian, swine, and human influenza viruses would be an effective strategy to prevent the emergence of reassortants with pandemic potential. M2 protein is a candidate antigen for so-called 'universal vaccines,' which confer cross-protection to different influenza viruses in a strain- and subtype-independent manner. We tested whether a recombinant F gene-deleted Sendai virus vector that contained an M2 gene derived from an H5N1 avian influenza virus (SeV/ΔF/H5N1M2) could induce a cross-reactive antibody response to the extracellular domain of M2 protein (M2e) in pigs. SeV/ΔF/H5N1M2 induced an antibody response to M2e when the vector was inoculated intramuscularly. The antibodies induced by SeV/ΔF/H5N1M2 cross-reacted with M2e derived from different avian, swine, and human influenza viruses. In mice, however, SeV/ΔF/H5N1M2 did not confer cross-protection to challenge with a heterologous H3N2 influenza virus. Our results confirm those of other groups indicating that antibodies to M2e do not mediate protection to influenza viruses in pigs.

1. Introduction

Most pandemic influenza viruses to date, including recent 2009 H1N1 viruses, are reassortants of avian, swine, and human influenza viruses (Neumann et al., 2009). Pigs support replication of these different influenza viruses in their respiratory tracts and serve as 'mixing vessels' for the generation of reassortants (Kida et al., 1994). Therefore, protecting pigs from simultaneous infection with avian, swine, and human influenza viruses would be an effective strategy to prevent the emergence of reassortants with pandemic potential.

Conventional inactivated influenza vaccines induce an antibody response to hemagglutinin (HA) on the virus surface. For an inactivated influenza vaccine to be effective, the antigenicity of the HA of the vaccine strain has to match that of field strains. However, the antigenicity of HA is highly variable among different influenza viruses. In particular, conventional inactivated swine influenza vaccines likely do not provide protection against avian and human influenza viruses in pigs (Ma and Richt, 2010). Therefore, so-called 'universal vaccines,' which confer cross-protection to different influenza viruses in a strain- and subtype-independent manner (Epstein and Price, 2010), should be developed for pigs.

M2 protein is a candidate antigen for universal vaccines (Fiers et al., 2009). The N-terminal amino acid sequences of the extracellular domain of M2 protein (M2e) are highly conserved among different influenza viruses (Liu et al., 2005). Antibodies to M2e do not have virus-neutralizing activity, but they bind M2e on virus-infected cells and interfere with virus budding (Zebedee and Lamb, 1988; Fu et al., 2009). Antibodies to M2e mediate cross-protection to different influenza viruses in mouse models (Neirynck

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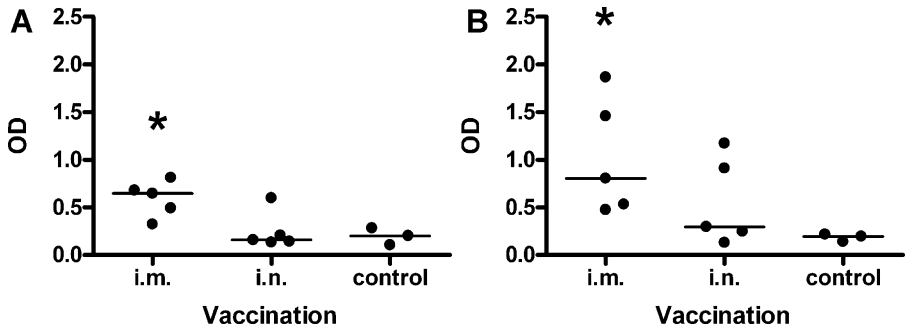

Fig. 1. Antibody response to M2e in pigs vaccinated with SeV/ΔF/H5N1M2. Serum antibody titers were measured by ELISA using an M2e peptide derived from an H5N1 avian influenza virus (A/chicken/Yamaguchi/7/2004). (A) Antibody titers at 4 wk after the first vaccination. (B) Antibody titers at 4 wk after the second vaccination. Data are given as OD_{450} values of serum samples diluted at 1:400. Horizontal bars indicate median. Asterisks indicate statistically significant differences ($P < 0.05$, Mann–Whitney U test) compared with values for unvaccinated control pigs. i.m., intramuscular; i.n., intranasal.

et al., 1999; Fan et al., 2004; Thompkins et al., 2007; Price et al., 2009). So far, however, only a few studies have investigated the efficacy of M2 antigen-based vaccines in pigs (Heinen et al., 2002; Kitikoon et al., 2009).

Sendai virus (SeV) is a paramyxovirus that causes pneumonia in laboratory mice. The viral envelope contains two spike proteins: hemagglutinin-neuraminidase, which mediates the attachment of virions to host cells, and fusion protein (F), which facilitates the penetration of viral ribonucleoprotein into infected cells. A transmission-incompetent SeV vector (SeV/ΔF) was developed by deleting the F gene (Li et al., 2000). SeV vectors are now used as efficient and safe viral vectors for gene transfer into airway epithelial cells (Griesenbach et al., 2005).

Here, we tested whether a recombinant SeV/ΔF containing an M2 gene derived from an H5N1 avian influenza virus could induce a cross-reactive antibody response to M2e in pigs. We further evaluated the protective efficacy of this M2-based vaccine in a mouse model after challenge with a heterologous H3N2 influenza virus.

2. Materials and methods

2.1. Vaccination and infection

An M2 gene was cloned from an H5N1 avian influenza virus (A/chicken/Yamaguchi/7/2004) and inserted into SeV/ΔF (designated SeV/ΔF/H5N1M2) as previously described (Li et al., 2000). Five-week-old pigs were inoculated with SeV/ΔF/H5N1M2 (10^8 cell culture infectious units; CIU) twice at a 4-wk interval via the intramuscular or intranasal route by using nasal atomizers for humans (Fujifilm, Japan). Eight-week-old female C57BL/6 mice were anesthetized with 2,2,2-tribromoethanol and inoculated with SeV/ΔF/H5N1M2 (5×10^7 CIU) twice at a 4-wk interval via the intramuscular, intranasal, subcutaneous, intraocular, or sublingual route. At 4 wk after the second vaccination, inoculated mice were challenged with 1 LD_{50} ($10^{5.5}$ EID_{50}) of an H3N2 influenza virus (A/HKx31).

2.2. ELISA

ELISA plates (Nunc, Denmark) were coated with M2e peptide (1 μg) in 0.05 M carbonate-bicarbonate buffer (pH 9.6) overnight at 4 °C. Wells were blocked for 30 min at room temperature with 50 mM Tris-buffered saline (pH 8.0) containing 1% BSA (TBS + BSA). Serum samples were serially diluted in TBS + BSA containing 0.05% Tween 20 and incubated in the wells for 1 h at room temperature. To detect antibodies bound to the wells, horseradish peroxidase-conjugated anti-pig or anti-mouse IgG (Bethyl Laboratory, USA) and TMB enzyme substrate (KPL, USA) were added to the wells. Optical density (OD) values were read at a wavelength of 450 nm after TMB Stop Buffer (KPL) was added. The amino acid sequences of M2e peptides used in ELISA were: MSLLTEVETPTRNEWECRCSDSSD (A/chicken/Ymaguchi/7/2004, H5N1); MSLLTEVETPIRNGWECKCSDSND (A/chicken/Ibaraki/1/2005, H5N2); MSLLTEVETPIKSEWGCRCNDSSD (A/swine/Okinawa/2/2005, H1N1); MSLLTEVETPTRSEWECRCDDSND (A/swine/Miyazaki/1/2006, H1N2); MSLLTEVETPIRNEWGCRCNDSSD (A/Niigata/1150/2010, H3N2); and MSLLTEVETPTRSEWECRCSDSSD (A/Narita/1/2009, H1N1).

3. Results and discussion

First we tested whether SeV/ΔF/H5N1M2 could induce an antibody response to M2e in pigs. Pigs were vaccinated twice with SeV/ΔF/H5N1M2 via the intramuscular or intranasal route. At 4 wk after each vaccination, serum antibody titers were measured by ELISA using an M2e peptide derived from a homologous H5N1 influenza virus (A/chicken/Yamaguchi/7/2004). The results showed that SeV/ΔF/H5N1M2 induced significant ($P < 0.05$) antibody titers to M2e in pigs that were vaccinated by the intramuscular route (Fig. 1). Antibody titers after the second intramuscular vaccination (Fig. 1B) did not differ significantly from those after the first (Fig. 1A), indicating that the additional SeV inoculation had, at best, only a moderate boosting effect. Two-dose vaccination by the intranasal route induced moderate (but not statistically significant) antibody titers (Fig. 1B).

Next we tested whether the antibodies induced by SeV/ΔF/H5N1M2 could cross-react with M2e derived from various avian, swine, and human influenza viruses. For this analysis, we chose the four serum samples that showed the highest antibody titers to an M2e