Veterinary Microbiology 191 (2016) 35–43

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Particle and subunit-based hemagglutinin vaccines provide protective efficacy against H1N1 influenza in pigs

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ARTICLE INFO

Article history:
Received 26 October 2015
Received in revised form 9 May 2016
Accepted 23 May 2016

Keywords:
Influenza
Swine
Vaccine
Hemagglutinin
Baculovirus
Subunit
VLP

ABSTRACT

The increasing diversity of influenza strains circulating in swine herds escalates the potential for the emergence of novel pandemic viruses and highlights the need for swift development of new vaccines. Baculovirus has proven to be a flexible platform for the generation of recombinant forms of hemagglutinin (HA) including subunit, VLP-displayed, and baculovirus-displayed antigens. These presentations have been shown to be efficacious in mouse, chicken, and ferret models but little is known about their immunogenicity in pigs. To assess the utility of these HA presentations in swine, Baculovirus constructs expressing HA fused to swine IgG2a Fc, displayed in a FeLV gag VLP, or displayed in the baculoviral envelope were generated. Vaccines formulated with these antigens wer The e administered to groups of pigs who were subsequently challenged with H1α cluster H1N1 swine influenza virus (SIV) A/Swine/Indiana/1726/88. Our results demonstrate that vaccination with any of these three vaccines elicits robust hemagglutinin inhibition titers in the serum and decreased the severity of SIV-associated lung lesions after challenge when compared to placebo-vaccinated controls. In addition, the number of pigs with virus detected in the lungs and nasal passages was reduced. Taken together, the results demonstrate that these recombinant approaches expressed with the baculovirus expression vector system may be viable options for development of SIV vaccines for swine.

1. Introduction

Influenza A viruses (IAV) are enveloped, segmented, negative-sense RNA viruses that infect birds and a variety of mammals including humans and swine. A significant problem for swine producers, swine influenza virus (SIV) infection ranks as one of the top three respiratory challenges in breeding, nursery, and finishing operations where estimated additional costs associated with the disease can reach $10.31 per pig to market (Holtkamp et al., 2007; Donovan, 2008; USDA, 2008). In addition to its economic impact on the swine industry, SIV poses a significant threat to human health due to the susceptibility of pigs to both avian and human influenza virus strains. As demonstrated by the 2009 outbreak of pandemic influenza strain A/H1N1/09, pigs can serve as "mixing vessels" with the capacity to generate novel, potentially pandemic, influenza stains via reassortment (Ma et al., 2008).

Although a single SIV subtype, "classical" H1N1, predominated for decades in North American swine populations, the current landscape of swine influenza viruses is much more heterogeneous. With the emergence of triple reassortant H3N2 SIV at the turn of the century, reassortant viruses containing human, swine and avian gene segments have become common in US swine herds (Thacker and Janke, 2008). The resulting antigenic variation has led to minimal cross protection among currently circulating strains, presenting a significant challenge in controlling SIV infection by vaccination. Although vaccines are available to combat SIV infection, these products contain only the major circulating strains as they are mostly based on inactivated formulations of field isolates that have been adapted to *in vitro* cultivation (Chen et al., 2012). The adaptation of field strains to *in vitro* cultivation carries two inherent disadvantages. First, some field strains may not propagate to acceptable titers by standard *in vitro* culture methods, including embryonated chicken eggs and cell culture. Second, the *in vitro* adaptation process can introduce mutations that may negatively impact vaccine efficacy of the final product (Krammer and Grabherr, 2010). These disadvantages coupled with the strict USDA licensing requirements for influenza vaccines can lead to extended development timelines of up to 5 years, hampering the response time to emerging strains (Thacker and Janke, 2008). In order to more quickly respond to outbreaks of new SIV strains not

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http://dx.doi.org/10.1016/j.vetmic.2016.05.011
0378-1135/

controlled by the currently licensed vaccines, a more rapid and flexible solution is needed.

Recombinant vaccines based on the major influenza antigens present a favorable alternative to traditional inactivated virus vaccines. By tapping into established expression platforms, recombinant antigens can be produced quickly with the flexibility for exchange of antigens as new strains emerge. The baculovirus expression vector system (BEVS) is an established expression platform that is currently in use for human and veterinary vaccines (Cox et al., 2008; Beach and Meng, 2012). A large number of studies have been published detailing the efficacy of experimental recombinant influenza vaccines prepared using the BEVS. Subunit approaches, involving expression of hemagglutinin (HA) and subsequent purification by anion exchange and/or lentil lectin chromatography have been reported (Powers et al., 1997; Lin et al., 2008; Loureiro et al., 2011). Enveloped virus-like particle (VLP)-based approaches displaying HA and/or neuraminidase (NA) via particles comprised of influenza or other virus structural proteins have also been reported (Bright et al., 2007; Haynes et al., 2009; Loureiro et al., 2011; Choi et al., 2013). In addition, published reports demonstrate that the baculovirus itself can be utilized in envelope-display and/or transduction based approaches (Lu et al., 2007; Chen et al., 2013; Prabakaran et al., 2014). Although the published data suggest that these approaches are promising candidates for new influenza vaccines, the majority of the efficacy data has been generated in mice or birds with minimal efficacy data available in pigs (Pyo et al., 2012).

In order to address the scarcity of swine-based efficacy data and determine the utility of these recombinant approaches for the development of influenza vaccines for swine, several baculovirus constructs expressing various iterations of recombinant HA were generated in this study. Recombinant HA was designed as a fusion protein with a swine immunoglobulin Fc domain (H1-2aFc), modified for insertion into the baculovirus envelope (H1-BD), or expressed in tandem with feline leukemia virus (FeLV) Gag (H1-Fgag). The recombinant HA antigens expressed from these constructs were evaluated for efficacy against a heterologous, classical SIV H1N1 challenge in pigs.

2. Materials and methods

2.1. Cells and virus

Semi-adherent Sf9 insect cells were maintained in TNM-FH medium (BD Biosciences, San Jose, CA) at 28 °C in tissue culture flasks. Suspension SF+ insect cells were maintained in SF900III SFM (Life Technologies, Grand Island, NY) at 28 °C in spinner flasks. MDCK cells were maintained in EMEM (Sigma Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum at 37 °C with 5% CO_2. H1N1 SIV A/Swine/Indiana/1726/88 was maintained in specific pathogen-free (SPF) chicken eggs.

2.2. Plasmids

Baculovirus constructs were prepared using H1 hemagglutinin (H1DB) from swine influenza virus strain NVSL 96–37181 (H1α cluster) in plasmid pVL1393-H1DB. For H1-2aFc, the H1DB coding sequence was amplified by PCR with primers designed to remove the signal sequence, transmembrane domain and C-terminal tail. The truncated H1DB coding sequence was cloned into pVAX1#4-IgG2aFc in frame with a mouse κ-light chain signal sequence on the 5′ end and a swine IgG2a Fc domain, including a 6X GGS linker, on the 3′ end. The entire coding sequence was then excised via BamHI/NotI restriction sites and cloned into pVL1393 resulting in pVL1393-H1-2aFc. For H1-BD, a truncated H1DB coding sequence was amplified similar to that of H1-2aFc but without BamHI restriction sites. Synthetic ssDNA fragments encoding baculovirus gp64 signal peptide and C-terminal tail were prepared (Integrated DNA Technologies, Iowa City, IA) and attached to the truncated H1 coding sequence on the 5′ and 3′ ends respectively by overlap-extension PCR (OE-PCR). The fused coding sequence was cloned into pVL1393 via BamHI and NotI restriction sites resulting in pVL1393-H1-BD. For H1-Fgag, full length H1DB was excised from pVL1393-H1DB via BamHI and NotI restriction sites and cloned into the multiple cloning site 1 (MCS1) of plasmid pORB-MCS1-sIRES-FeLV gag resulting in pORB-H1DB-sIRES-FeLV gag.

2.3. Generation of recombinant baculovirus

pVL1393-H1-2aFc, pVL1393-H1-BD, and pORB-H1DB-sIRES-FeLV gag were used to generate recombinant baculovirus BacDB-H1-2aFc, BacDB-H1-BD, and BacFBU-H1-Fgag respectively by co-transfection of Sf9 cells with linearized Diamond Bac (BacDB) or FlashBAC ULTRA (BacFBU) baculovirus DNA. Recombinant baculoviruses were amplified on Sf9 cells and harvested by centrifugation at 1000*g* for 5 min. Harvest supernatants were further processed by 0.2 μm filtration and stored at 4 °C. Amplified baculoviral stocks were titered on Sf9 cells by a fluorescent antibody infectious dose 50 ($FAID_{50}$) method. Briefly, baculoviral stocks were 10-fold serially diluted in cell medium and Sf9 cell layers in 96-well plates were infected with each dilution set, 10 wells per dilution, on duplicate plates. The plates were incubated at 28 °C for 5 days, after which, the media was discarded and the cell layers were fixed with 50:50 acetone/methanol. Baculovirus-infected wells were detected by an indirect fluorescent antibody (IFA) test using anti-baculovirus gp64 monoclonal antibody AcVI (eBioscience, San Diego, CA). Infected/non-infected wells were determined for each dilution and the viral titer in $FAID_{50}$/mL was calculated using the Reed-Muench method.

2.4. Evaluation of recombinant HA constructs

Recombinant baculovirus was used to infect SF+ cell cultures in spinner flasks at 28 °C with agitation at 100 rpm. BacDB-H1-2aFc cultures were incubated for 3 days while BacDB-H1-BD and BacFBU-H1-Fgag cultures were incubated for 5 days. At harvest, culture supernatants were centrifuged at 1000*g* for 5 min to pellet the remaining cells. The clarified supernatants were further processed by 0.2 μm filtration to remove any remaining cellular debris. Final harvests were used to evaluate H1 expression from each construct.

For BacDB-H1-2aFc, harvests were directly assessed by SDS-PAGE and Western blot using rabbit anti-H1 polyclonal serum with peroxidase-conjugated goat anti-rabbit IgG as a secondary antibody or goat anti-swine IgG conjugated with peroxidase alone (Jackson ImmunoResearch, West Grove, PA). In addition, harvests were processed via protein A affinity chromatography and SDS-PAGE to confirm fusion of H1 with the IgG2a Fc domain.

For BacDB-H1-BD and BacFBU-H1-Fgag, harvest samples were pelleted by ultracentrifugation at 100,000*g* for 2 h at 4 °C to pellet large molecular weight species. The resulting supernatants were removed and pellets were resuspended in Tris-buffered saline (TBS, 20 mM Tris, 150 mM NCl, pH 7.5). Resuspended pellets were separated on discontinuous sucrose gradients (10%–60% sucrose in 10% steps) by ultracentrifugation at 100,000*g* for 2 h at 4 °C. Gradients were fractionated from the top down and analyzed by SDS-PAGE and Western blot utilizing the previously described anti-H1 serum and mouse monoclonal antibody against FeLV gag p27 (BaculoFBU-H1-Fgag only) coupled with peroxidase conjugated goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA). Fractions containing H1 (BacDB-H1-BD) or H1 and FeLV gag (BacFBU-H1-Fgag) were pooled and dialyzed against TBS, pH 7.5 to