Vaccine 29 (2011) 2712–2719

Contents lists available at ScienceDirect

Vaccine

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

Enhanced pneumonia and disease in pigs vaccinated with an inactivated human-like (δ-cluster) H1N2 vaccine and challenged with pandemic 2009 H1N1 influenza virus

Phillip C. Gauger[a,b], Amy L. Vincent[a,*], Crystal L. Loving[a], Kelly M. Lager[a], Bruce H. Janke[b], Marcus E. Kehrli Jr.[a], James A. Roth[c]

[a] Virus and Prion Research Unit, National Animal Disease Center, USDA, Agricultural Research Service, 1920 Dayton Avenue, PO Box 70, Ames, IA 50010, USA
[b] Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011, USA
[c] Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011, USA

ARTICLE INFO

Article history:
Received 3 November 2010
Received in revised form 21 January 2011
Accepted 25 January 2011
Available online 23 February 2011

Keywords:
Swine influenza virus
Enhanced pneumonia
Pandemic H1N1

ABSTRACT

Influenza is an economically important respiratory disease affecting swine world-wide with potential zoonotic implications. Genetic reassortment and drift has resulted in genetically and antigenically distinct swine influenza viruses (SIVs). Consequently, prevention of SIV infection is challenging due to the increased rate of genetic change and a potential lack of cross-protection between vaccine strains and circulating novel isolates. This report describes a vaccine-heterologous challenge model in which pigs were administered an inactivated H1N2 vaccine with a human-like (δ-cluster) H1 six and three weeks before challenge with H1 homosubtypic, heterologous 2009 pandemic H1N1. At necropsy, macroscopic and microscopic pneumonia scores were significantly higher in the vaccinated and challenged (Vx/Ch) group compared to non-vaccinated and challenged (NVx/Ch) pigs. The Vx/Ch group also demonstrated enhanced clinical disease and a significantly elevated pro-inflammatory cytokine profile in bronchoalveolar lavage fluid compared to the NVx/Ch group. In contrast, viral shedding and replication were significantly higher in NVx/Ch pigs although all challenged pigs, including Vx/Ch pigs, were shedding virus in nasal secretions. Hemagglutination inhibition (HI) and serum neutralizing (SN) antibodies were detected to the priming antigen in the Vx/Ch pigs but no measurable cross-reacting HI or SN antibodies were detected to pandemic H1N1 (pH1N1). Overall, these results suggest that inactivated SIV vaccines may potentiate clinical signs, inflammation and pneumonia following challenge with divergent homosubtypic viruses that do not share cross-reacting HI or SN antibodies.

Published by Elsevier Ltd.

1. Introduction

Swine influenza is caused by influenza A virus of the *Orthomyxoviridae* family and the cause of an acute respiratory disease in swine. Orthomyxoviruses have a negative-sense, segmented RNA genome that allows for genetic reassortment and generation of novel viruses. Currently, three major influenza subtypes, H1N1, H3N2 and H1N2, co-circulate in the major swine producing regions of the United States (US) and Canada [1–4]. However, two additional subtypes, H3N1 and H2N3, have been identified in North American swine, and drift variants of the predominant subtypes are increasingly more common [4–11]. The increased rate of genetic change in North American swine influenza virus (SIV) H1 subtypes is attributed to the introduction of the human–avian–swine triple reassortant H3N2 subtype in 1998 and more importantly, to the acquisition of the triple reassortant internal gene (TRIG) cassette [10,12]. SIV subtypes include different combinations of the HA and neuraminidase (NA) genes, however, the TRIG cassette, which includes the NP, M, and NS, genes of classical swine lineage, PB2 and PA genes of avian lineage, and PB1 of human lineage, have been consistently identified among contemporary isolates circulating in the North American swine population [2]. The TRIG appears to have an enhanced ability to acquire a variety of surface glycoprotein gene segments generating novel isolates such as the H2N3 subtype identified in 2006, when the TRIG was shown to have acquired an avian H2 and N3, producing a novel triple reassortant SIV [10,13]. Antigenic drift resulted in the evolution of three distinct H1 phylogenetic clusters (α, β, and γ) from the classical swine lineage. The δ-cluster emerged in 2005 in the US and includes SIVs with the HA gene of human seasonal virus origin [10]. The hu-like H1 (δ) influenza viruses introduced a fourth cluster currently endemic in US swine [10,14].

* Corresponding author. Tel.: +1 515 337 7557; fax: +1 515 337 7428.
E-mail address: amy.vincent@ars.usda.gov (A.L. Vincent).

0264-410X/$ – see front matter. Published by Elsevier Ltd.
doi:10.1016/j.vaccine.2011.01.082

Table 1
Experimental design.

Group	Vaccine	Challenge	*N*	Weeks of age		
				Priming vaccine	Booster vaccine	Challenge
Vx/Ch[a]	MN08	pH1N1	10	4	7	10
NVx/Ch[b]	None	pH1N1	10	4	7	10
NVx/NCh[c]	None	None	5	4	7	10

[a] Vx/Ch: vaccinated/challenged.
[b] NVx/Ch: non-vaccinated/challenged.
[c] NVx/NCh: non-vaccinated/non-challenged.

Inactivated influenza A vaccines are approved for use in US swine in pigs 3 weeks of age or older and have played a significant role in preventing clinical disease [15,16]. However, inactivated vaccines have shown limited efficacy or cross-protective immunity against heterologous homosubtypic or heterosubtypic viruses [15,17–19]. Consequently, use of autogenous vaccines specific to the farm of origin has also increased in an attempt to control disease in the face of the escalating diversity within North American influenza A viruses [3]. In contrast, exposure to live H1N1 and H3N2 virus has demonstrated complete protection against an SIV with an unrelated HA protein in some studies, suggesting live exposure results in improved cross-protection between heterologous SIVs [20]. Collectively, genetically and antigenically diverse SIVs have made prevention more challenging due to the increasing lack of cross-protection among heterologous viruses and the inability to update vaccines as rapidly as viruses change [3].

A potential problem with vaccination was demonstrated when enhanced pneumonia in a subset of pigs administered an inactivated H1N1 vaccine followed by challenge with a heterologous virus was reported [19]. The viruses in that study were shown to have no cross-reactivity either as anti-sera or antigen in the hemagglutination inhibition (HI) assay. The inactivated α-cluster swine H1N1 vaccine failed to protect against challenge with a heterologous γ-cluster H1N2 SIV and resulted in enhanced pneumonic lesions in one-third of the pigs [19], and in all similarly treated pigs in a subsequent study [21]. In the study described here, pigs were administered an inactivated 2008 H1N2 hu-like δ-cluster SIV vaccine followed by challenge with 2009 pandemic H1N1 (pH1N1). We report the inactivated vaccine did not protect against challenge with pH1N1 virus. Furthermore, pigs in the vaccinated and challenged group (Vx/Ch) demonstrated enhanced macroscopic and microscopic pneumonia as well as an elevated inflammatory cytokine profile suggesting vaccination potentiated the clinical disease and pneumonia in the Vx/Ch group followed by heterologous challenge with pH1N1.

2. Materials and methods

2.1. Experimental design

Twenty-five, three-week-old cross-bred pigs were obtained from a herd free of SIV and porcine reproductive and respiratory syndrome virus (PRRSV) and treated with ceftiofur crystalline free acid (Pfizer Animal Health, New York, NY) and enrofloxacin injectable solution (Bayer Animal Health, Shawnee Mission, KS) according to label directions to reduce bacterial contaminants prior to the start of the study. Pigs were housed in biosafety level 2 (BSL2) containment during the vaccine phase of the study. Pigs were transferred to ABSL3 containment on the day of challenge for the remainder of the experiment. Pigs were cared for in compliance with the Institutional Animal Care and Use Committee of the National Animal Disease Center. The experimental design is described in Table 1.

SIV vaccine was prepared with A/Sw/MN/02011/08 H1N2 (MN08) at approximately 32 HA units and inactivated by ultraviolet irradiation with the addition of a commercial oil-in-water adjuvant (Emulsigen D, MVP Labs) at a v:v ratio of 4:1 virus to adjuvant. Pigs were vaccinated with 2 ml by the intramuscular route at approximately 4 weeks of age and boosted at 7 weeks of age (Table 1). Pigs challenged at 10 weeks of age were inoculated intratracheally with 2 ml of 1×10^5 50% tissue culture infectious dose ($TCID_{50}$) of A/CA/04/09 pH1N1 (pH1N1) propagated in Madin–Darby canine kidney (MDCK) cells, as previously described [14]. Pigs were observed daily for signs of clinical disease. Rectal temperatures were taken on −1, 0, 1, 2, 3, 4, and 5 days post-infection (dpi). Nasal swabs (Fisherbrand Dacron swabs, Fisher Scientific, Pittsburg, PA) were taken on 0, 3, and 5 dpi to evaluate nasal virus shedding by dipping the swab in minimal essential medium (MEM) and inserting the swab approximately 2.5 cm into each nares. Swabs were then placed into 2 ml MEM and stored at −80 °C until study completion. Pigs were humanely euthanized with a lethal dose of pentobarbital (Sleepaway, Fort Dodge Animal Health, Fort Dodge, IA) on 5 dpi to evaluate lung lesions and viral load in the lungs. Postmortem samples included serum, bronchoalveolar lavage, lung and trachea.

2.2. Pathologic examination of lungs

At necropsy, lungs were removed and evaluated for the percentage of the lung affected with purple-red consolidation typical of SIV. The percent of the surface affected with pneumonia was visually estimated for each lung lobe, and then a total percentage for the entire lung was calculated based on weighted proportions of each lobe to the total lung volume [22]. Tissue samples from the trachea and right cardiac lung lobe were taken and fixed in 10% buffered formalin for histopathologic examination. Tissues were routinely processed and stained with hematoxylin and eosin. Lung sections were given a score from 0 to 3 and tracheal sections were given a score from 0 to 2.5 to reflect the severity of bronchial and tracheal epithelial changes based on previously described methods [9]. The lung sections were scored according to the following criteria: 0.0: no significant lesions; 1.0: a few airways affected with bronchiolar epithelial damage and light peribronchiolar lymphocytic cuffing often accompanied by mild focal interstitial pneumonia; 1.5: more than a few airways affected (up to 25%) often with mild focal interstitial pneumonia; 2.0: 26–50% airways affected often with moderate interstitial pneumonia; 2.5: approximately 51–75% airways affected, usually with significant interstitial pneumonia; 3.0: greater than 75% airways affected, usually with significant interstitial pneumonia. Trachea sections were scored according to the following criteria: 0.0: normal; 1.0: focal squamous metaplasia of the epithelial layer; 2.0: diffuse squamous metaplasia of much of the epithelial layer, cilia are focally evident; 2.5: diffuse squamous metaplasia with an absence of cilia. A single pathologist scored all slides and was blinded to the treatment groups.