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Influence of maternally-derived antibodies on live attenuated influenza vaccine efficacy in pigs

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ABSTRACT

Vaccination during pregnancy is practiced in swine farms as one measure to control swine influenza virus (SIV) infection in piglets at an early age. Vaccine-induced maternal antibodies transfer to piglets through colostrum and stabilize the herd: however, maternally derived antibodies (MDA) interfere with immune response following influenza vaccination in piglets at the later stage of life. In addition, MDA is related to enhanced respiratory disease in SIV infection. Previously, we have developed a bivalent live attenuated influenza vaccine (LAIV) which harbors both H1 and H3 HAs. We demonstrated vaccination of this LAIV provided protection to homologous and heterologous SIV infection in pigs. In this study we aimed to investigate the influence of MDA on LAIV efficacy. To this end, SIV sero-negative sows were vaccinated with a commercial vaccine. After parturition, nursery piglets were vaccinated with LAIV intranasally or intramuscularly, and were then challenged with SIV. We report that MDA hampered serum antibody response induced by intramuscular vaccination but not by intranasal vaccination of the LAIV. Viral challenge in the presence of MDA caused exacerbated respiratory disease in unvaccinated piglets. In contrast, all LAIV vaccinated piglets were protected from homologous viral infection regardless of the route of vaccination and the presence of MDA. Our results demonstrated that LAIV conferred protection in the presence of MDA without inciting exacerbated respiratory disease.

1. Introduction

Swine influenza is an acute, highly contagious respiratory disease caused by swine influenza virus (SIV) [1]. Typical swine influenza infection is characterized as a sudden onset of fever, coughing, respiratory distress and nasal discharge. The disease can usually be resolved within 7 to 10 days. Although the mortality is low, morbidity can approach 100%, leading to a significant economic loss [2]. Swine plays a unique role in influenza A virus evolution as a mixing vessel to generate reassortant influenza viruses, which creates concerns for influenza pandemic [3,4].

Whole inactivated virus (WIV) based vaccines are commonly used in pregnant sows to prevent SIV infection in piglets [5]. Levels of maternally derived antibodies (MDA) may vary and protective levels of MDA were detected in piglets up to 14 weeks after birth [6]. Vaccination of piglets in the presence of MDA with WIV raises two major concerns. One is the suppression of antibody responses [6–8] and the other is vaccine associated enhanced respiratory disease (VAERD) [9], an aggravated pneumonia accompanied by severe pathology. The inhibitory effect of MDA has been perceived for a long time in both human and veterinary vaccines including influenza vaccines [10–12], but the exact mechanism of MDA interference is not clear [13]. The messages of these studies are clear, that vaccination regiment and the vaccine formula in neonates should be determined by taking MDA interference into account [14].

Another issue with MDA is they enhance respiratory diseases following influenza infection, this has also been reported in other respiratory viral infections [15]. A retrospective study of the 2009 pandemic found the pre-existing non-neutralizing and cross-reactive antibodies correlated with the severity of disease in patients [16,17]. This correlation was also demonstrated in an experimental setting where the vaccine and challenge viruses were homosubtypic [18].

We previously generated a live attenuated influenza vaccine (LAIV) that expresses both H1 and H3 HA antigens (SIV/606). We demonstrated that vaccination of SIV/606 protected pigs from both H1N1 and H3N2 SIV infections [19,20]. In this study, we explored whether vaccination of SIV/606 in piglets would overcome the MDA-associated obstacles.

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2. Materials and methods

2.1. Cells and viruses

Madin-Darby canine kidney (MDCK) cells were used in virus propagation and titration. A/swine/Saskatchewan/18789/02 (SIV/SK02, North American avian lineage), was propagated and purified as previously described [21]. SIV/SK02 was used for viral challenge, as a coating antigen for ELISA, as a standardized antigen for hemagglutination inhibition (HI) and for micro-neutralization assay. The LAIV, SIV/606, was originated from SIV/SK02 with part of its NA segment replaced by H3 HA derived from A/Swine/Texas/4199-2/98 [19].

2.2. Experimental design

An in vivo study was performed at VIDO-InterVac, University of Saskatchewan. All procedures complied with the ethical guidelines of the Canadian Council of Animal Care. Four pregnant sows were procured from an SIV free herd (a farrow to finish farm, Saskatchewan, Canada). Sows were sero-negative, as tested by commercial SIV ELISA kits (IDEXX, USA) and were negative in HI test to SIV/SK02. Two sows were vaccinated with FluSure XP (Pfizer Animal Health) and two were mock vaccinated at 5 and 3 weeks before parturition (day 0). FluSure XP contained A/swine/North Carolina/031/05 (H1N1), A/swine/Missouri/069/05 (H3N2), A/swine/Iowa/110600/00 (H1N1) and A/swine/Oklahoma 0726H/2008 (H1N2). Piglets were fed by the sows after birth, and were vaccinated either intramuscularly or intranasally with SIV/606 on day 16. Piglets were weaned on day 21 and were boosted on day 30 followed by virus challenge on day 37. Vaccine dose for SIV/606 was 2×10^6 PFU in 1 ml of MEM for both intramuscular and intranasal vaccination. Piglets in the control group were vaccinated with 1 ml of PBS intramuscularly. To ensure that all challenge virus was delivered to the lung, piglets were intratracheally inoculated with 8×10^5 PFU of SIV/SK02 in 4 ml of MEM. Group assignment and vaccination information are described in Table 1. A group of piglets were kept separately from other challenged groups and served as a non-vaccinated/non-challenged control. After virus challenge, all animals were observed daily for 5 days for body temperature and clinical signs.

2.3. Sample collection and pathological examination

Sow blood was collected at vaccinations (day-35 and -21), at farrowing (day 0), during the nursing period (day 16) and at weaning (day 21). Colostrum and milk were also collected on days 0, 16 and 21. Piglets were bled on day 0, 16, 30, 37 and 42. The blood samples on day 0 were collected after the piglets had consumed colostrum. On 5 day post infection (pi), all piglets were euthanized and were examined for SIV induced lesions in the lungs. The percentage of each lobe affected by SIV (dark red consolidation) was estimated visually. The total percentage affected, for the entire lung, was the sum of the percentages of areas affected for all lobes. The weighted value of each lung lobe was based on the weight of each lobe relative to the total lung weight [21]. A single experienced veterinarian scored all samples and was blinded for the experimental groups. [21]. Three biopsy pieces were collected from the right cranial, middle and caudal lobes using an 8 mm biopsy punch (Miltex, Inc., York, PA, USA) and were pooled in 1 ml MEM for virus titration.

Table 1
Group assignment of piglets.

Group (number of piglets)	Status of MDA	Vaccination (Route)		Virus challenge (day 37)
		1st(day 16)	2nd(day 30)	
A ($n=6$)	Positive	PBS (IM)	PBS (IM)	+
B ($n=7$)	Positive	LAIV(IM)	LAIV(IM)	+
C ($n=6$)	Positive	LAIV (IN)	LAIV(IN)	+
D ($n=7$)	Negative	PBS (IM)	PBS(IM)	+
E ($n=7$)	Negative	LAIV(IM)	LAIV(IM)	+
F ($n=6$)	Negative	LAIV (IN)	LAIV (IN)	+
G ($n=6$)	Negative	–	–	–

2.4. Antigen specific ELISA, virus isolation, HI and micro-neutralization assay

Antigen specific IgG and IgA ELISA were performed as described previously [22]. Colostrum and milk were treated with Rennet to separate the whey for ELISA [23]. Virus titration, HI and micro-neutralization assays were performed as described in "WHO manual on animal influenza diagnosis and surveillance" [24].

2.5. Statistics

One-way ANOVA followed by Tukey's multiple comparison was used to determine the statistical significance of the difference between groups using GraphPad Prism 6 (GraphPad Software Inc., USA). Virus titers were transformed by rank for statistical analysis. We did not perform the statistical analysis on ELISA results of sow samples because of the small sample size ($n=2$).

3. Results

3.1. Vaccination of commercial SIV vaccine induces cross reactive IgG in sows

Sows were screened for H1N1 and H3N2 SIVs and found to be sero-negative. The farm was closed to incoming animals for over 8 years and did not have a history of SIV outbreaks. The SIV vaccine had not been used in the herd. Four sows were chosen among the ones synchronized to farrow at a similar time. In order to induce serum IgG, the sows were vaccinated with a commercial vaccine twice, at 5 and 3 weeks before parturition. Serum samples were collected from the sows after each vaccination and the antibodies that are cross-reactive to SIV/SK02 were determined by ELISA. The IgG level peaked on farrowing day (day 0) and sustained at an elevated level until weaning. No IgG titer was detected in both PBS vaccinated sows during this period (Fig. 1A). Colostrum and milk were collected at parturition, during the nursing period and at weaning. Although there were individual variations, SIV/SK02 specific IgG and IgA levels in colostrum and milk exhibited the highest titer at parturition (Fig. 1B and C). Also, we noted that in WIV vaccinated sows, total SIV specific IgG titers in colostrum were higher than that in serum.

3.2. MDA interferes IgG induction of LAIV delivered through intramuscular but not intranasal route

Piglets were checked for SIV/SK02 specific serum IgG levels prior to vaccination with LAIV. All 19 piglets fed by vaccinated sows absorbed IgG quickly after birth and obtained notable amounts of SIV/SK02 specific IgG in the blood (Fig. 2A, mean titer: 1142 ± 222). The IgG levels remained high until the first vaccination of LAIV (mean titer: 484 ± 98) in this group. The 26 piglets fed by non-vaccinated sows did not show noticeable IgG titers. We evaluated the serum IgG titers in piglets after they were vaccinated with LAIV. While intramuscular vaccination of LAIV induced serum IgG after the first vaccination (mean titer: 473 ± 334), intranasal vaccination induced slightly lower IgG titers (mean titer: 192 ± 132) (Fig. 2B). Similarly, a boost of LAIV through the intramuscular route induced

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