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Evaluation of a Virus-like Replicon Particle Vaccine Expressing Proteins of Swine Influenza Virus in Pigs With and Without Maternally Derived Antibodies

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Summary and Implications

A major hurdle to swine influenza vaccination of young piglets is maternal antibody interference. This interference is transient as it disappears when pigs reach about 3 months of age. We vaccinated piglets without and with interfering maternal antibody using a recombinant vector vaccine. In the absence of interfering maternal antibody, the vaccine was effective in inducing a strong immune response and greatly reduced the amount of virus. However, this same recombinant vaccine was not effective when interfering maternal antibodies were present. We are currently trying a higher dose of vaccine and different genes from SIV in hopes we can overcome this maternal antibody. Preliminary data from these new studies are promising.

Introduction

Swine influenza virus (SIV) causes serious disease in the swine industry due to clinical disease, delayed time to market, and zoonotic potential. A major hurdle for SIV vaccination in young piglets is maternally derived antibodies. This passive immunity is beneficial for protecting newborn piglets from clinical disease but detrimental to the induction of active immunity. Because antibodies to SIV in colostrum can cause current vaccines to fail, delayed vaccination following antibody decay is routinely practiced. This causes a period of time in which piglets are susceptible to influenza due to declining maternal immunity and prior to vaccine induced immunity. The use of recombinant vectors which mimic natural infection by producing high levels of antigen *in vivo* is one possible way to avoid such interference. The ability of hemagglutinin/ nucleoprotein (HA/NP) vaccine in a recombinant vector has been shown to overcome maternal antibody to SIV. The objectives of this study were to determine if 1) virus-like replicon particle (RP) vaccine expressing the HA gene induced an active immune response in pigs lacking maternal antibodies and 2) RP vaccine expressing HA could overcome maternal antibodies to SIV. The objectives were determined via a vaccination-challenge study.

Materials and Methods

Pigs came from two sources and their incorporation in the study is shown in Table 1. One source was SIV antibody negative and did not use SIV vaccine; the other source routinely used SIV vaccine in gilts and sows. Neither source had experienced recent clinical outbreaks of swine influenza. Piglets for the vaccination-challenge study that are naïve or had maternal antibody came from pregnant swine that received either a sham or a commercially available inactivated SIV H3N2 vaccine. Piglets were weaned at 2 ½ weeks, transported to the ISU livestock infectious disease isolation facility and divided into 6 groups of 10 pigs each. At 3 and 5 weeks of age (Day 0 and 14) pigs either received the RP vaccine or a placebo intramuscularly (IM). All pigs were challenged intratracheally (IT) with 10 ml of 1×10^7 TCID₅₀ (50% tissue culture infectious dose) of virulent cluster 4 H3N2 SIV (homologous to HA used in RP vaccine) at Day 53. Pigs were monitored for increased temperature and euthanized 4 days post-inoculation (PI, Day 57). Tissues were collected for quantitative gross lung lesion scoring, homologous hemagglutination inhibition (HI) assay, and virus titration.

To construct the RP vaccine, replicon plasmids containing the HA gene and helper plasmids containing Venezuelan Equine Encephalitis Virus (VEEV) capsid or glycoprotein genes were prepared. To prepare a replicon with the homologous HA gene, the gene was PCR amplified using specific primers. The PCR products encoded for unique EcoRV and AscI restriction sites at the 5' and 3' ends, respectively. The PCR products were then cloned into a transfer vector, pCRII-Blunt (Invitrogen) and sequenced to ensure no errors were introduced into the gene during PCR amplification. Each gene was then cloned into a replicon vector using the unique EcoRV and AscI restriction sites. RNA transcripts were produced *in vitro* (RiboMAX T7 Express, Promega) from the replicon and helper plasmids and purified, followed by agarose gel analysis to assess integrity, and quantification by ultraviolet (UV) absorbance. The replicon and helper RNAs were mixed with Vero cells in electroporation chambers and pulsed. Replicon electroporated cells were incubated at 37°C for 18 hours. Cell lysates were prepared from electroporated cells for Western blot analysis. RP was harvested from culture fluids and the infectious titer of the RP preparation was measured by antigen-specific IFA. RP were tested in a

cytopathic effect (CPE) assay to assure the absence of detectable replication-competent virus.

The HI assay was performed on sera stored at -20C. Geometric means at 5 time points (Day 0, 14, 28, 53 and 57) were determined for each group.

Nasal swabs and lung lavage fluid were collected and viral amount determined by qPCR performed. Bronchial alveolar lavage (BAL) fluid was collected by pipetting 50 ml of sterile DMEM with antibiotics into the lungs and collected into sterile 15 ml tubes and stored at -80C until titration.

Analysis of variance (ANOVA) was used to analyze results from gross lung lesion scoring, log 2 transformed serum HI titers, and log 10 transformed virus titers from nasal swabs and BAL fluid. Rectal temperature data was also analyzed via ANOVA.

Results and Discussion

Rectal temperatures in all sham treated groups (1 and 3) and group 4 increased day 1 PI when compared to day 0 PI (Figure 1). This demonstrates the ability of SIV challenge to cause a febrile response that persisted for a day. The group with no maternal antibody given HA RP vaccine (group 2) did not have a significant temperature increase at day 1 PI when compared to day 0 PI (Figure 1). This demonstrates the ability of the RP vaccine to significantly reduce the febrile affect of SIV challenge.

The HA RP vaccine induced a strong antibody response when given to piglets with no maternal antibody (Group 2). The geometric mean HI 14 days after the last vaccine was administered (day 28) and day 0 PI was 368 and 92 respectively. The sham group (group 1) was negative on the HI test at these time points. This demonstrates that the source is serologically naïve and sham vaccinated pigs remained so during the study. The HA RP vaccine induced a robust immune response that was detected by HI. The maternal antibody positive groups 3 and 4 had geometric mean titers of 260 and 422 respectively at day 0. All pigs in these two groups had negative titers (<40) at the day of challenge, demonstrating that maternal antibody had waned but the HA RP vaccine given at day 0 and 14 did not overcome maternal interference

SIV was not detected via qPCR (<10²/ml) in nasal swabs day 2 PI in groups 1 and 2 and therefore, the amount of virus at this time point in nasal swabs from groups 3 and 4 was not determined. We speculate the lack of detectable virus in nasal swabs day 2 PI was because high amounts of virus were not being replicated and excreted at this time. There was significantly more virus detected in nasal swabs and BAL fluid at day 4 PI in group 1, 4.1 log 10 and 4.6 log 10/ml respectively, than in group 2, 0.5 log 10 and 2.8 log 10/ml respectively. This demonstrated that HA RP vaccine reduced virus amounts when pigs were from a serologically naïve source. The amount of virus in lungs 4 days PI in pigs with maternal

antibody, groups 3 and 4, were the same statistically and this shows the HA RP vaccine did not overcome maternal antibody.

The gross lung lesions were more severe in group 1 versus group 2, 20.8 percent and 7.4 percent respectively, as was neutrophilic exudate evident in histologic examination of lungs. There were no differences in gross and microscopic lung lesions in group 3 and 4. Taken together, this demonstrates that lung lesions due to SIV are reduced when HA RP vaccine is used in seronegative pigs, but not in pigs with maternal antibody.

In conclusion, HA RP vaccine reduced the febrile response, lung lesions and amount of virus when serologically negative pigs are challenged with SIV. However, RP vaccine containing HA alone did not overcome the inhibitory affects of maternal antibody.

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Table 1. Groups for vaccination-challenge pig trial.

Group No.	No. of Pigs	Dam Treatment	Piglet Treatment	Piglet Challenge
1	10	Sham	Sham	Homologous SIV
2	10	Sham	H3 VRP	Homologous SIV
3	10	Inactivated SIV	Sham	Homologous SIV
4	10	Inactivated SIV	H3 VRP	Homologous SIV

Figure 1. Average Rectal Temperatures.

