RESEARCHREPORT

SWINE HEALTH

Title: Long lasting vaccine-induced immunity for swine flu (H3N2).
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Abstract: Swine influenza virus (SIV) strain H3N2 emerged in North America in 1998 causing severe respiratory disease in pigs. In this study 2 recombinant adenoviruses were developed as potential vaccines against these H3N2 influenza strains. To construct the recombinants, the SIV hemagglutinin (H3) gene and the nucleocapsid protein (NP) gene were subcloned into a shuttle vector and by homologous recombination were inserted into the E1 region of replication-defective human adenovirus-5 vector vaccine. This vector virus has been shown to infect pigs but the course of the infection is limited to only one round of viral replication. Each recombinant virus expressed its respective protein as determined by immunoblotting, by immunoprecipiation, and by immunocytochemical staining. All 3 immunological procedure were carried out with specific monoclonal antibodies.

Three groups of pigs (10 pigs/group) were vaccinated intramuscularly with the recombinants; one group was vaccinated with the recombinant adenovirus expressing the H3 protein, one group was vaccinated with the recombinant adenovirus expressing the NP protein, and one group was vaccinated with both recombinants in a mix. Two additional control groups were included in the animal trial. One control group was challenged with a virulent H3N2 field strain and one control group remained unchallenged. The results showed that pigs in the groups given the recombinant adenovirus expressing the H3 protein developed high levels of hemagglutination inhibition (HI) antibody by 4 weeks post vaccination. Pigs in the group vaccinated with both recombinant viruses in a mixture were completely protected. Complete protection was shown by the lack nasal shedding of virus following challenge and by the lack of lung lesions at one week following the challenge infection.

Introduction: Since 1998, swine producers in North America have experienced severe respiratory disease in their herds caused by a new swine influenza virus (SIV). Significant influenza disease occurred even in vaccinated herds. The signs of acute influenza disease were animals with high fevers (104 - 107 F), coughing, labored breathing, abortions and a low percentage of deaths in sows and in boar studs. The cause of the more severe influenza disease was a H3N2 swine influenza strain that

recently emerged in North America. Previous to 1998, swine influenza in North America was caused by strain H1N1. The commercially available inactivated virus vaccine provided resistance to the homologous H1N1 strain but did not give significant protection from the emerging disease caused by H3N2.

In the past few years the H3N2 strain of swine influenza has become widespread in the United States. The National Veterinary Services Laboratory has reported 153 swine influenza accessions as H3N2. These accessions have come from the Midwest, Texas and North Carolina (Zhou et al., J. Virol. 73:8851-8856, 1999). There have also been 103 accessions reported as H1N1 so both strains are causing disease in North America. In fact, there have been some instances of mixed infections when both influenza strains were isolated from the same herd. Thus, there is a need for a vaccine that can induce umbrella immunity for both swine influenza strains. Vaccines that are capable of inducing specific neutralizing antibody plus cell mediated immunity will provide superior protection against the acute influenza diseases.

Recombinant adenovirus vaccines can provide the needed broad spectrum and longer lasting immunity because they replicate in cells, unlike the commercial inactivated viral vaccines. Adeno-vectored vaccines expressing the influenza (strain H3N2) hemagglutinin gene and the influenza nucleocapsid protein gene have been constructed. These vectored vaccines should be superior to killed vaccines because the expressed hemagglutinin protein will induce specific antibody to neutralize the H3N2 strain while the expressed nucleocapsid protein will stimulate cytotoxic T-cells for broad immunity to all swine influenza strains.

Objectives: Traditional swine flu (H1N1) has been partially controlled in North America by a killed virus vaccine. In the past few years a new influenza strain, H3N2, has appeared. It has been particularly severe for respiratory disease in nursery and in finishing pigs and has caused abortions and some deaths in gestating sows. Our goal is to develop an Adenovirus vectored vaccine that will stimulate both humoral and cell mediated immunity to the strain H3N2 hemagglutinin protein (H3) and the nucleocapsid protein (NP) for superior protection against the new swine influenza strain. The level of active immunity and the degree of protection for pigs will be determined by vaccination trials. Eventually, these vectored vaccines can be expanded to include protective immunity from H1N1 swine influenza srtrains and the vectored vaccines can be compared directly in terms of lasting immunity with the current killed vaccine.

Procedures:

Viruses: Two swine influenza viruses (H3N2) from different farms in Iowa were used. The transgenes were prepared from the RNA of a H3N2 influenza virus isolated in the fall of 1999 from pigs on a farm in northwest Iowa that was experiencing a severe outbreak of respiratory disease. The challenge H3N2 virus was provided by the ISU Veterinary Diagnostic Laboratory and was isolated by Dr. Mengeling's group from tissues submitted from a farm with severe respiratory disease.

Construction of recombinant vaccines: The replication-defective Adenovirus-5 recombinants were constructed as described in the preliminary report. The expression of the transgenes in recombinant adenovirus infected 293 cells was carried out by immunoblotting, by immunoprecipitation, and by immunocytochemical staining as described. Stock recombinant viruses were purified by CsCl density gradient centrifugation following no more than 4 passages on 293 cells. The final titers were 10^{11} $TCID_{50}$/ml for the recombinant adenovirus expressing the H3 protein (Ad-H3-14.2)

and 2 x 10^{11} $TCID_{50}$/ml for the recombinant adenovirus expressing the NP protein (Ad-NP-13.4).

Recombinant vaccine trials in pigs: The experimental design comprised 50 SPF pigs randomly assigned to 5 groups (10 pigs/group). These pigs were weaned at 2 weeks of age, delivered to National Animal Disease Center and allowed to acclimate to their new environment and new feed for one week. At 3 weeks of age they were vaccinated and 5 weeks later they were challenged as indicated by the Table below.

Group	Vaccination	Challenge
I	No	No
II	No	Yes
III	Yes (hemagglutinin)	Yes
IV	Yes (bivalent)	Yes
V	Yes (nucleocapsid)	Yes

For vaccination 2 x 10^{10} $TCID_{50}$ of recombinant virus was given to each pig intramuscularly in 0.5 ml. For Group IV both viruses were given at 2 x 10^{10} $TCID_{50}$ in a mixture. The challenge virus was serially passed only in pigs. Lung lavage fliuds from lungs of infected pigs showing the most extensive lesions were pooled. The challenge virus titer from the pooled lung lavage fluids was 7 x 10^5 $TCID_{50}$ /ml. For challenge, the pigs were anesthetized with a telazol/ketamine/xylazine solution. While the anesthetized pigs were breathing deeply the challenge virus was given as 1.5 ml per nostril with a syringe adapted with a tight fitting nasal tip. To reduce the possibility of secondary bacterial infections, oxytetracycline (9 mg/lb) was given IM at the time of challenge and once again at 2 days post challenge.

The antibody response was measured by the hemagglutination-inhibition (HI) test before vaccination and at 2, 4, 5 and 6 weeks post vaccination. The last serum sample was taken one week following the challenge infection. Clinical signs post challenge were monitored by observing the animals twice per day and daily body temperatures were determined for 5 days post challenge. For virus shedding, nasal swabs from each pig were collected daily on day 0 - day 5 and virus amounts were estimated by inoculating MDCK cells in 24 well plates and monitoring virus-induced CPE. At 7 days post challenge the control pigs and principals were euthanized, lungs were examined for gross lesions and lung tissues were saved for histopathology.

Results: The results of this vaccination study are summarized by the enclosed 3 figures and 2 tables.

Antibody response to vaccination and to challenge: Blood was collected and serum prepared from each pig prior to vaccination and at 2, 4 and 5 weeks post vaccination and at necropsy which was at 6 weeks post vaccination. Challenge virus inoculation of pigs in Groups II through V occurred after the 5th week post vaccination bleeding followed one week later by necropsy and collection of the final blood samples at 6 weeks post vaccination. The antibody response for influenza virus was tested by the HI test and the results are shown in Figure 1. Pigs vaccinated with recombinant adenovirus expressing the H3 protein (Groups III and IV) showed some HI antibody response at 2 weeks post vaccination and significant HI titers by the 4^{th} and 5^{th} week post vaccination. The pigs in these 2 groups that had already developed high levels of antibody to influenza virus did not show a marked increase in HI titer one week following the challenge infection. The control pigs of group II and the pigs vaccinated with recombinant adenovirus expressing NP alone (Group V) developed no HI antibody through 5 weeks post vaccination and both groups showed a small increase in HI titer

one week after challenge. The environmental group (Group I) remained negative for HI antibody throughout the duration of the experiment.

These results show that pigs vaccinated with recombinant adenovirus-5 expressing the H3 influenza protein either given alone (Group III) or in combination with recombinants expressing the NP protein (Group IV) induce high levels of serum antibody by the 4^{th} and 5^{th} weeks after IM vaccination. In the case of influenza infections, significant levels of serum HI antibody correlate with solid protective immunity.

Clinical signs post vaccination and post challenge: Pigs vaccinated with the recombinant adenoviruses showed no untoward effects and no problems occurred at the IM vaccination sites.

The challenge virus (H3N2) given deep intranasally to anesthetized pigs produced no marked clinical signs. During the one week post challenge the pigs were normal –no coughing, no rapid breathing and no inappetence at least under the barn isolation room conditions. Body temperatures did increase during the 5 days immediately post challenge and the results are shown in Figure 2. The body temperature profiles also indicate that the challenge infection with H3N2 was relatively mild. For Group II, the unvaccinated control pigs that were challenged, the average temperature for the 10 pigs in the group peaked on day 2 post challenge but remained below 104 F – the threshold level for a fever. For these challenged control pigs only 4 of 10 pigs had body temperatures above 104 F on day 2 post challenge. In contrast, pigs from the 3 recombinant adenovirus vaccinated groups (III – V) had group average body temperatures just above 104 F by day 1 post challenge and on day 2 had average body temperatures similar to the challenge control pigs in Group II.

The reason that the vaccinated pigs responded in terms of body temperatures earlier and more vigorously to the challenge virus than did the control pigs is not entirely clear. It could be that the vaccinated pigs were primed with either SIV antigen and this pre-exposure accelerated the mildly febrile response following challenge.

Gross lung lesions at necropsy: Four examples of the reddish consolidation typically seen in the apical and cardiac lobes of the lungs are shown in Figure 3. Two sets of lungs are shown from the challenged control pigs (Group II) and 2 sets of lungs from pigs vaccinated with NP alone (Group V). Table 1 summarizes the gross lesion results for all the pigs in the experiment. Group II pigs were the most severely affected with the lungs of 6 pigs showing moderate lesions, 3 pigs with mild lesions and the lungs of 1 pig appeared to be normal. Amongst the vaccinated groups, the levels of protection from the least protected to completely normal were Group V to Group III to Group IV. Only 2 pigs in Group V had lungs that appeared normal. For Group III pigs, 8 of the lungs were normal with one of these having caudal diaphragmatic lobe lesions that did not appear to be caused by the SIV challenge. And for Group IV, all the pigs had normal lungs, just like the lungs of pigs from the environmental control pigs (Group I).

Nasal shedding post challenge: The data for nasal shedding is summarized in Table 2. Here again the Group IV pigs were protected the best. No pigs from Group IV shed virus post challenge. The next best group was Group III with recombinant expressing the H3 protein alone. Very little virus was shed from Group III vaccinated pigs; only 2 pigs shed a small amount of virus on day 2 post challenge and 2 other pigs from that group shed a small amount of virus on day 4 post challenge. The nasal shedding results confirm that there was a positive effect by vaccinating with the recombinant adenovirus expressing NP protein alone (Group V). All of these pigs were shedding

virus at different levels but the duration and extent of the shedding was less than the shedding of the challenged control pigs in Group II. This is best exemplified on the last day of sampling, day 5 post challenge, when only 3 of the 10 pigs in Group V were shedding only a small amounts of virus.

These results taken in total show clearly that the recombinant adenovirus vaccines induce high levels of protective antibody. Also shown is that vaccination of pigs with 2 recombinants expressing both SIV proteins completely prevented nasal shedding of the challenge virus and completely eliminated gross lung lesions. Therefore the combined vaccination with recombinants expressing both SIV proteins provides the highest degree of protection.

Advantages and benefits: The recombinant adenovirus vaccine was given IM in only a single dose that provided solid protective immunity. This investigator believes that the immunity can be enhanced further by including adjuvants or cytokines in the vaccine mixture. The adenovirus vaccines infect cells and present the foreign antigens to the host's immune system in a manner similar to an actual infecting virus. Thus, these vectored vaccines stimulate long-term memory, unlike killed vaccines, and provide for a long-lasting immune response. Last, but most significantly, the recombinant adenovirus vaccine can stimulate the neonatal pig's immune system even in the presence of maternally-derived antibodies that normally would block any immunity provided by an inactivated vaccine (Monteil et al., J. Gen. Virol. 78:3303-3310, 1997). This is a tremendous advantage the "primed" nursery-age pig that can be boosted at 8-10 wks of age for any number of respiratory pathogens.

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Figure 3. Montage of lungs from Group II (Panels B & D) and Group V (Panels A & C) pigs showing SIV-induced lesions (strain H3N2).