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Research Note—

Efficacy of a Fowlpox-Vectored Avian Influenza H5 Vaccine Against Asian H5N1 Highly Pathogenic Avian Influenza Virus Challenge

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SUMMARY. A recombinant fowlpox-avian influenza (AI) H5 vaccine (rFP-AIV-H5) expressing the hemagglutinin of the A/turkey/Ireland/1378/83 H5N8 AI isolate has been used in Central America since 1998 to control H5N2 low pathogenicity AI. Previously, this vaccine was shown to induce full protection against a panel of H5 highly pathogenic (HP) AI isolates, including HPAI H5N1. Here, we evaluate the efficacy of rFP-AIV-H5 against escalating doses of HPAI H5N1 A/chicken/SouthKorea/ES/03 isolate and against the HPAI H5N1 A/chicken/Vietnam/0008/2004 isolate. In both studies, 1-day-old specific pathogen-free (SPF) chickens were vaccinated by subcutaneous route with rFP-AIV-H5 and challenged 3 wk later by the oronasal route. In the first study, full protection was observed up to a challenge dose of 6.5 $\log_{10}$ embryo infectious dose (EID_{50}), and the 50% chicken infectious dose was estimated to be 3.1 and 8.5 $\log_{10}$ EID_{50} in the control and the rFP-AIV-H5-vaccinated group, respectively. A 2–4 $\log_{10}$ and >4 $\log_{10}$ reduction of oral and cloacal shedding was observed in rFP-AIV-H5 vaccinated birds, respectively. The rFP-AIV-H5 vaccine induced hemagglutination inhibition antibodies (5.2 $\log_2$) detectable with homologous H5N8 antigen. In the second study, rFP-AIV-H5-vaccinated chicks were fully protected against morbidity and mortality after challenge with the 2004 Vietnam isolate, whereas unvaccinated chickens died within 2 days of challenge. Shedding in cloacal swabs was detected in all unvaccinated controls but in none of the rFP-AIV-H5-vaccinated chickens. Together, these results confirm the excellent level of protection induced by rFP-AIV-H5 in SPF chickens against two recent Asian HPAI H5N1 isolates.

RESUMEN. *Nota de Investigación*—Eficacia de una vacuna recombinante H5 de influenza aviar con viruela aviar como vector, contra un desafío con un virus Asiático H5N1 de influenza aviar de alta patogenicidad.

Una vacuna recombinante H5 de influenza aviar con viruela aviar como vector (por sus siglas en Inglés rFP-AIV-H5) expresando la hemaglutinina del aislamiento de influenza aviar A/turkey/Ireland/1378/83 H5N8, ha sido utilizada en América Central desde el año 1998 para controlar la influenza aviar de baja patogenicidad H5N2. Con anterioridad, esta vacuna ha demostrado inducir protección plena contra un grupo de aislamientos H5 de influenza aviar de alta patogenicidad, incluyendo aislamientos de influenza aviar de alta patogenicidad H5N1. En el presente trabajo se evalúa la eficacia de la vacuna rFP-AIV-H5 contra dosis crecientes de los aislamientos de influenza aviar de alta patogenicidad H5N1 A/chicken/South Korea/ES/03 y A/chicken/Vietnam/0008/2004. En ambos estudios se vacunaron pollos libres de patógenos específicos de un día de edad con la vacuna rFP-AIV-H5 por vía subcutánea y fueron desafiados tres semanas después por la ruta oro-nasal. En el primer estudio se observó protección plena ante una dosis de desafío de 6.5 $\log_{10}$ dosis infecciosa para embrión de pollo. La dosis infecciosa 50% para pollos se estimó en 3.1 y 8.5 $\log_{10}$ dosis infecciosa para embrión de pollo para los controles y el grupo vacunado con rFP-AIV-H5, respectivamente. En el grupo vacunado con rFP-AIV-H5 se observó una reducción de 2–4 $\log_{10}$ en la diseminación oral del virus y de más de 4 $\log_{10}$ en la diseminación cloacal. Utilizando el antígeno homólogo H5N8, la vacuna rFP-AIV-H5 indujo anticuerpos inhibidores de la hemoaglutinación (5.2 $\log_2$). En el segundo estudio, luego del desafío con el aislamiento Vietnamita del año 2004, las aves vacunadas con rFP-AIV-H5 resultaron completamente protegidas contra morbilidad y mortalidad, mientras que las aves no vacunadas murieron dentro de los dos primeros días posteriores al desafío. Se detectó diseminación viral en hisopos cloacales en todos los controles no vacunados, pero no en las aves vacunadas con rFP-AIV-H5. Estos resultados confirman el excelente nivel de protección contra dos aislamientos recientes de influenza aviar de alta patogenicidad H5N1 inducido por la vacuna rFP-AIV-H5 en aves libres de patógenos.

Key words: avian influenza, fowlpox recombinant, vaccine, H5N1 HPAI challenge, protection, shedding

Abbreviations: AGP = agar gel precipitation; AI = avian influenza; AIV = avian influenza virus; BPL = β-propiolactone; CID_{50} = 50% chicken infectious dose; ck/SK/03 = A/chicken/SouthKorea/ES/03; ck/Vtn/04 = A/chicken/Vietnam/0008/2004; DIVA = differentiating infected from vaccinated animals; EID_{50} = 50% egg infectious dose; HA = hemagglutinin; HI = hemagglutination inhibition; HP = highly pathogenic; i.n. = intranasally; s.c. = subcutaneous; SPF = specific pathogen free; $TCID_{50}$ = 50% tissue culture infective dose

A fowlpox virus vaccine containing the H5 hemagglutinin (HA) gene of A/turkey/Ireland/1378/83 (H5N8) (rFP-AIV-H5), when administered to 1-day-old chicks, protected chickens against a panel of different highly pathogenic (HP) avian influenza (AI) H5 strains (3,4,5), including a recent HPAI H5N1 isolate (A/chicken/SouthKorea/ES/03; ck/SK/03) (2). This vaccine received a U.S. Department of Agriculture (USDA) license for emergency use in 1998. More than 2 billion doses of rFP-AIV-H5 vaccine have been used,

Table 1. Mortality data after challenge with escalating doses of ck/SK/03 in unvaccinated controls (diluent) and in rFP-AIV-H5-vaccinated chickens.

Challenge dose (log_{10} EID_{50})	Control	rFP-AIV-H5
0.5	0/10[A]	0/10
2.0	0/10	0/10
3.5	8/10 (2.8)[B]	0/10
5.0	10/10 (2.4)	0/10
6.5	10/10 (2.0)	0/10
8.0	10/10 (2.0)	2/10 (4.5)

[A]Number of dead birds/total inoculated.
[B]Mean time to death in days.

mainly in Mexico, Guatemala, and El Salvador. This vaccine recently received a temporary authorization for use in Vietnam and in France. Here, we evaluated the efficacy of rFP-AIV-H5 against escalating doses of ck/SK/03 and against a 2004 Vietnam HPAI H5N1 isolate.

MATERIALS AND METHODS

Study 1. ck/SK/03 50% chicken infectious dose (CID_{50}). Sixty specific-pathogen-free (SPF) chickens (1 day old) were vaccinated by the subcutaneous (s.c.) route with a field dose ($10^{3.5}$ 50% tissue culture infective dose [$TCID_{50}$] in 200 μl) of rFP-AIV-H5 (TROVAC™ AIV H5; Merial Select, Inc., Gainesville, GA), and 60 SPF (1 day old) chickens were s.c. injected with the diluent (unvaccinated controls). Twelve groups (six vaccinated and six controls) of 10 chickens each were challenged intranasally (i.n.) 3 wk later with six escalating doses (0.5, 2.0, 3.5, 5.0, 6.5, and 8.0 log_{10} mean embryo infectious doses [EID_{50}]) of the ck/SK/03. Morbidity and mortality were recorded daily for 14 days postchallenge. The CID_{50} was evaluated using a statistical exponential model. Shedding was evaluated by egg titration in cloacal and oral swabs taken 48 hr postchallenge, as described previously (3). Hemagglutination inhibition (HI) titers were evaluated using β-propiolactone (BPL)-inactivated homologous A/turkey/Ireland/1378/83 H5N8 antigen and sera obtained before (day 21) and after challenge (day 35), as described previously (3).

Study 2. A/chicken/Vietnam/0008/2004 challenge. Twenty-two SPF chickens (1 day old) were vaccinated by s.c. route with a field dose ($10^{3.5}$ $TCID_{50}$ in 200 μl) of rFP-AIV-H5, and 12 birds were kept as unvaccinated controls. All chickens were i.n. challenged 3 wk later with 30 chicken lethal dose 50% (corresponding to 4.5 log_{10} EID_{50}/bird) per bird of the HPAI H5N1 A/chicken/Vietnam/0008/2004 (ck/Vtn/04) challenge strain. Morbidity and mortality were recorded daily for 10 days postchallenge. Shedding was evaluated at 2 days postchallenge from five birds of both groups in cloacal swabs by virus titration in embryonated chicken eggs. HI titers were evaluated using the gamma-irradiated (5 kGy) challenge ck/Vtn/04 isolate as the antigen.

RESULTS

Study 1. CK/SK/03 CID_{50}. All birds showing clinical signs of AI after challenge died soon after challenge. Mortality in the control groups reached 0% (0/10), 80% (8/10), and 100% (10/10) after challenge with $\leq$2.0, 3.5, and $\geq$5.0 log_{10} EID_{50} of ck/SK/03, respectively (Table 1). The surviving birds did not seroconvert, indicating that the challenge dose in these birds was not sufficient to initiate infection. The CID_{50} in the unvaccinated control group was 3.1 log_{10} EID_{50}. At the highest challenge doses (6.5 and 8.0 log_{10} EID_{50}), all control birds died within 2 days postchallenge. Full (10/10) and 80% (8/10) protection levels against mortality/morbidity were induced by rFP-AIV-H5 after challenge with $\leq$6.5 and 8.0 log_{10} EID_{50} of ck/SK/03, respectively. The CID_{50} in vaccinated groups was 8.5 log_{10} EID_{50}. The mean time to death of the two unprotected vaccinated chickens was longer (4.5 days) than in the unvaccinated group (2 days).

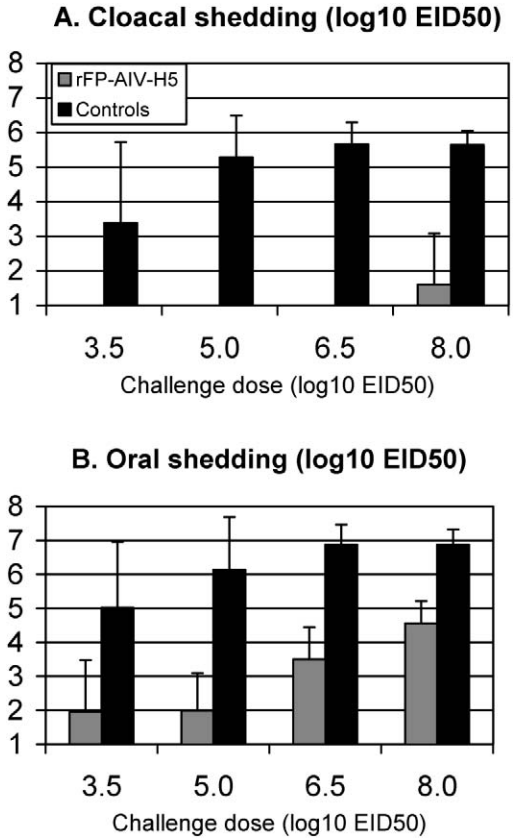

Fig. 1. Shedding of ck/SK/03 challenge virus (log_{10} EID_{50}/ml) in cloacal (A) and oral (B) swabs 48 hr postchallenge.

Results of shedding are shown in Fig. 1. Cloacal shedding was fully prevented by rFP-AIV-H5 vaccination up to the 6.5 log_{10} EID_{50} challenge dose, and a 4 log_{10} EID_{50} reduction was detected at the highest challenge dose (8.0 log_{10} EID_{50}) (Fig. 1A). A challenge dose-dependent 2–4 log_{10} reduction of oral shedding was observed in rFP-AIV-H5-vaccinated birds (Fig. 1B).

The rFP-AIV-H5 vaccination induced HI antibodies (mean of 5.2 ± 1.3 log_2) detectable with the BPL-inactivated homologous turkey/Ireland/1378/83 H5N8 antigen. These titers were boosted postchallenge in a challenge dose-dependent manner (Fig. 2).

Study 2. ck/Vtn/04 challenge study. All 22 rFP-AIV-H5-vaccinated chickens were fully protected against morbidity and mortality after challenge with ck/Vtn/04, whereas all 12 unvaccinated chickens died within 2 days after challenge. Cloacal swabs collected at 2 days postchallenge were all positive for AI virus (mean titers of 5.7 ± 0.5 log_{10} EID_{50}/ml) in the control group and all negative in the rFP-AIV-H5 group. The mean HI titers by using the ck/Vtn/04 antigen were 2.5 ± 1.4 and 7.6 ± 1.3 log_2 before and after challenge, respectively.

DISCUSSION

Full clinical protection was provided by rFP-AIV-H5 against high doses of two Asian H5N1 HPAI viruses. The CID_{50} for the ck/SK/03 isolate was much higher (8.5 log_{10} EID_{50}) in the rFP-AIV-H5 vaccinates than in the control (3.1 log_{10} EID_{50}) group, indicating that >100,000 times more virus was necessary to lethally infect rFP-AIV-H5-vaccinated chickens than unvaccinated birds. The increased

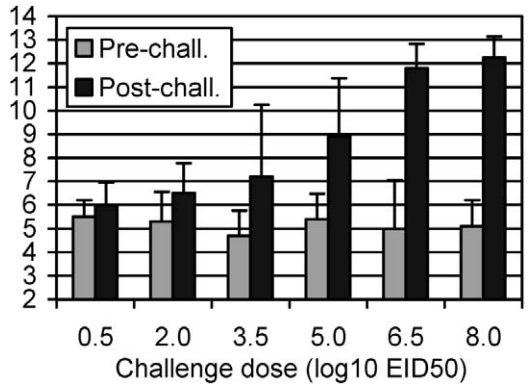

Fig. 2. HI titers (log_2) 3 wk after vaccination (Pre-chall.) and 2 wk after challenge (Post-chall.) by using the homologous BPL-inactivated A/turkey/Ireland/1378/83 antigen in rFP-AIV-H5-vaccinated chickens.

resistance of vaccinated birds to experimental infection was reported previously in a low pathogenicity AI challenge model in turkeys (1).

In study 1, prevention of cloacal shedding and reduction of oropharyngeal shedding in vaccinated birds were significant and challenge dose-dependent. Absence of detectable challenge virus in cloacal swabs of vaccinated chickens in the second study also indicated that challenge virus replication was impaired by vaccination. It should be mentioned that shedding was evaluated at only one time point (e.g., 2 days) after challenge. Although high levels of shedding are observed in the nonvaccinated control group at that time point, the kinetic of shedding in the vaccinated group may be delayed and would need to be further investigated.

In study 1, the increase in HI antibody titers after challenge correlated with the challenge dose and the level of oral shedding, strongly suggesting that it was dependent on the level of challenge virus replication. Anti-nucleoprotein and matrix protein antibodies as detected by agar gel precipitation (AGP) test were detected in the serum of chickens showing a significant increase (≥ 3 log_2) in HI titers after challenge (data not shown). The presence of AGP antibodies can be used as a differentiating infected from vaccinated animals (DIVA) test in rFP-AIV-H5-vaccinated flock because vaccinated chickens lack AGP antibodies unless infected by a field AI virus. Altogether, these results extend the high and broad level of protection induced by the rFP-AIV-H5 vaccine in SPF chickens to two recent Asian H5N1 HPAI viruses, making it an ideal DIVA vaccine for 1-day-old chicks administered at the hatchery.

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