Recombinant Paramyxovirus Type 1-Avian Influenza-H7 Virus as a Vaccine for Protection of Chickens Against Influenza and Newcastle Disease

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Recombinant Paramyxovirus Type 1-Avian Influenza-H7 Virus as a Vaccine for Protection of Chickens Against Influenza and Newcastle Disease


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SUMMARY. Current vaccines to prevent avian influenza rely upon labor-intensive parenteral injection. A more advantageous vaccine would be capable of administration by mass immunization methods such as spray or water vaccination. A recombinant vaccine (rNDV-AIV-H7) was constructed by using a lentogenic paramyxovirus type 1 vector (Newcastle disease virus [NDV] B1 strain) with insertion of the hemagglutinin (HA) gene from avian influenza virus (AIV) A/chicken/NY/13142-5/94 (H7N2). The recombinant virus had stable insertion and expression of the H7 AIV HA gene as evident by detection of HA expression via immunofluorescence in infected Vero cells. The rNDV-AIV-H7 replicated in 9–10 day embryonating chicken eggs and exhibited hemagglutinating activity from both NDV and AI proteins that was inhibited by antisera against both NDV and AIV H7. Groups of 2-week-old white Leghorn chickens were vaccinated with transfectant NDV vector (tNDV), rNDV-AIV-H7, or sterile allantoic fluid and were challenged 2 weeks later with viscerotropic velogenic NDV (vvNDV) or highly pathogenic (HP) AIV. The sham-vaccinated birds were not protected from vvNDV or HP AIV challenge. The transfectant NDV vaccine provided 70% protection for NDV challenge but did not protect against AIV challenge. The rNDV-AIV-H7 vaccine provided partial protection (40%) from vvNDV and HP AIV challenge. The serologic response was examined in chickens that received one or two immunizations of the rNDV-AIV-H7 vaccine. Based on hemagglutination inhibition and enzyme-linked immunosorbent assay (ELISA) tests, chickens that received a vaccine boost seroconverted to AIV H7, but the serologic response was weak in birds that received only one vaccination. This demonstrates the potential for NDV for use as a vaccine vector in expressing AIV proteins.

RESUMEN. Recombinante con paramixovirus tipo 1 e influenza aviar H7, como vacuna para la protección de pollos contra la influenza y la enfermedad de Newcastle.

La vacunas actuales para prevenir la influenza aviar dependen de la inyección parenteral, representando una aumento en la mano de obra empleada. Una vacuna con mayores ventajas sería aquella que pudiera administrarse por aplicación masiva, como la vacunación por aerosol o en el agua de bebida. Se desarrolló una vacuna recombinante (rNDV-AIV-H7) usando un vector paramixovirus tipo 1 (copia B1 del virus de la enfermedad de Newcastle) con inserción del gen de la hemaglutinina (HA) del virus de influenza aviar A/pollo/NY/13142-5/94 (H7N2). El virus recombinante presentaba una inserción y expresión estable del gen de la hemaglutinina H7 como se hizo evidente por la detección de la expresión de la hemaglutinina por medio de la inmunofluorescencia en células Vero infectadas. El rNDV-AIV-H7 se replicó en huevos embrionados de pollo de 9 a 10 días y mostró actividad hemaglutinante para ambas proteínas virales, que fueron inhibidas por antisueros contra el virus de Newcastle y contra influenza aviar subtipo H7, respectivamente. Grupos de aves Leghorn de dos semanas de edad fueron vacunadas con el vector transfectante del virus de la enfermedad de Newcastle, con el virus recombinante rNDV-AIV-H7, o con fluido alantoideo estéril. Las aves fueron desafiadas dos semanas después

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con una cepa velogénica viscerotrópica del virus de Newcastle o con el virus de influenza aviar de alta patogenicidad. Las aves que recibieron el líquido alantoideo no fueron protegidas contra el desafío con el virus velogénico viscerotrópico de Newcastle o con el virus de influenza aviar de alta patogenicidad. La vacuna transfectante de Newcastle confirió 70% de protección contra el desafío de Newcastle pero no contra el desafío contra influenza aviar. La vacuna recombinante rNDV-AIV-H7 confirió protección parcial (40%) contra el desafío de ambos virus. La respuesta serológica fue examinada en pollos que recibieron una o dos inmunizaciones de la vacuna rNDV-AIV-H7. Con base en las pruebas de inhibición de la hemaglutinación y de ELISA, los pollos que recibieron una vacuna de refuerzo presentaron seroconversión para el virus de influenza H7, pero la respuesta serológica fue débil en las aves que recibieron una sola vacunación. Esto demuestra el potencial del virus de Newcastle para su uso como vector y para expresar proteínas del virus de influenza.

Key words: avian influenza, chicken, H7, Newcastle disease, vaccine

Abbreviations: AGP = agar gel precipitin; AI = avian influenza; AIV = avian influenza virus; HA = hemagglutinin; HI = hemagglutinin inhibition; HP = highly pathogenic; LP = low pathogenicity; ND = Newcastle disease; NDV = Newcastle disease virus; PBS = phosphate-buffered saline; rNDV-AIV-H7 = recombinant NDV vaccine; tNDV = transfectant NDV vaccine; vvNDV = velogenic viscerotropic NDV

Sporadic outbreaks of avian influenza (AI) in poultry have occurred throughout the world with occasional large epizootics (8). Control methods for AI outbreaks have varied, but vaccines have been used in the control of some low-pathogenicity (LP) AI outbreaks and recently with highly pathogenic (HP) AI outbreaks in Mexico (1995) and Pakistan (1994–95). Over the past 2 decades, the majority of AI vaccines have been inactivated whole virus vaccines selected for use based on protection elicited from humoral response against the homologous subtype of the hemagglutinin surface glycoprotein. Recently, a recombinant fowlpox vaccine with cDNA insert of the H5 hemagglutinin subtype was used to help control LPAI in central Mexico. However, both inactivated whole AI and recombinant fowlpox-AI H5 vaccines require administration by labor-intensive and expensive parenteral injection.

Improved delivery and lower cost of administration are needed for AI vaccines. Development of vaccines for mass administration such as via drinking water or spray would reduce the expense of parenteral administration. Live AI vaccines would achieve this goal, but their use creates additional problems including creating the potential for recombination with field avian influenza virus (AIV) or mutation to create viruses that could be pathogenic and cause disease.

Newcastle disease (ND) commonly occurs throughout the world, and vaccination is widely practiced in North and South America and other parts of the world. In the United States, eight billion broiler chickens and 300 million turkey poults are vaccinated yearly with live lentogenic vaccine strains—B1 and/or LaSota—through spray or water vaccination. Recently, infectious clones of ND viruses (NDV) have been developed as vaccine vectors for delivery of various viral antigens (6). Infectious laryngotracheitis has also been investigated as a mass immunizing vaccine vector for AI proteins (5).

This study was conducted to determine whether AIV H7 hemagglutinin cDNA could be stably inserted into the NDV genome; expressed in cell culture, embryonating chicken eggs, and chickens; and protect chickens from lethal NDV and H7 AIV challenge in chickens.

**MATERIALS AND METHODS**

**Vaccine viruses.** A previously developed infectious clone of the lentogenic paramyxovirus type 1 B1 vaccine strain was used as the virus vector (6). A recombinant NDV vaccine (rNDV-AIV-H7) was constructed by insertion of the hemagglutinin (HA) gene from LP AIV A/chicken/NY/13142-5/94 (H7N2) using methods described (6). The transfectant virus derived from the NDV B1 wild-type infectious clone was used as a NDV control vaccine (tNDV) and sterile allantoic fluid was used for the sham control group (Sham).

**Challenge viruses.** Vaccinated birds were either challenged with 10^4.7 mean embryo infectious dose (EID<sub>50</sub>) of velogenic viscerotropic NDV (Fontana strain—vvNDV) or 10^4.1 EID<sub>50</sub> of HP AIV (A/Steele/ACC-10/59 [H7N7]) at 4 weeks of age. The NDV virus was isolated from a poultry outbreak of NDV in California during 1972–73. The HPAIV was isolated from a man with hepatitis in 1959 and was later found to be a North American avian lineage of H7 HPAIV (1). The 100 μl of vvNDV challenge was given split
between nares and conjunctival sac (eye drop), while the 100 μl HPAIV challenge was administered through the choanal slit.

**Hemagglutination (HA) and hemagglutinin inhibition (HI) assays for vaccine and challenge virus determination.** The HA and HI tests were completed using conventional microtiter methods (3,7). All HA and HI assays were conducted with chicken erythrocytes, except for HA assays done in titrations of the tNDV and rNDV-AIV-H7 vaccines, which were conducted with turkey erythrocytes. For HI tests, serial twofold dilutions of chicken serum were made in phosphate-buffered saline, pH 7.2 (PBS); 4 HA units of test antigen were added to each dilution and incubated at room temperature for 30 min. An equal volume of 0.5% chicken erythrocytes in PBS was added as a test indicator. The HI endpoint was determined as the last dilution with complete inhibition of HA activity. The inactivated A/turkey/OR/71(H7N3) AIV strain and SEPRL B1 strain of NDV were used as antigen in HI assays (3).

**Experimental design.** Two-week-old specific pathogen free white Leghorn chicks were immunized by eye drop method with 100 μl of one of three vaccines: 1) rNDV-AIV-H7, 2) tNDV, and 3) Sham. At 4 weeks of age, 10 birds from each group were challenged with vvNDV and 10 chickens with the HPAIV. The birds were observed for clinical signs and lesions of vvNDV and HPAIV infections. Mortality was recorded. All survivors were euthanatized with sodium pentobarbital (100 mg/kg) at 6 weeks of age. Two additional groups of chickens that were not challenged were immunized once (2 weeks of age) or twice (2 and 4 weeks of age) with rNDV-AIV-H7 and bled for serology at 6 weeks of age.

Sera from birds were tested at 2, 4, and 6 weeks of age for antibodies against influenza A nucleoprotein and matrix protein (agar gel precipitin [AGP] test), H7 AIV hemagglutinin (hemagglutination inhibition [HI] test), and NDV hemagglutinin (HI test) as described previously (3,7).

**RESULTS AND DISCUSSION**

The rNDV-AIV-H7 and tNDV infected and replicated in both Vero cell cultures and 9–10 day embryonating chicken eggs as determined by indirect immunofluorescence assay and HA assays for expression of viral proteins, respectively. For the rNDV-AIV-H7, the specific expression of H7 HA was confirmed in infected Vero cell cultures by immunofluorescence using primary anti-H7N3 polyclonal chicken sera and secondary FITC-conjugated antichicken IgY. Mock and tNDV infected Vero cell cultures were negative for expression of AIV H7 HA. Both NDV and AIV HA's were present on rNDV-AIV-H7 as evident by inhibition of HA activity by both anti-H7 AIV and NDV antisera. These findings indicate stable insertion and expression of the AIV H7 HA gene in the recombinant NDV virus and its potential for use as a vaccine vector. Previous recombinant NDV constructs resulted in stable insertion and expression of chloramphenicol acetyltransferase reporter gene and influenza H1 HA (2,4,6).

The goal for a recombinant NDV containing an AIV HA gene insert would be to prevent mortality from challenge with both vvNDV and HP AIV. Groups of 2-week-old chickens were inoculated with tNDV, rNDV-AIV-H7, or sterile allantoic fluid (sham). No antibodies against NDV or AIV were detected on the day of vaccination (2 weeks of age) in any of the groups. In addition, no antibodies to AIV (HI and AGP tests) were detected 2 weeks after vaccination (4 weeks of age) in any of the groups, but antibodies against NDV were present in the majority of rNDV-AIV-H7 and in a few tNDV vaccinated chickens. Two weeks following challenge (6 weeks of age), all survivors lacked antibodies to AIV, but vvNDV challenged birds had antibodies against NDV. In a second group of chickens, we determined whether a boost with rNDV-AIV-H7 vaccine would induce a greater antibody response to AIV. Although the serologic response, as determined by hemagglutination inhibition (HI), and ELISA tests was weak in the once-vaccinated group, seroconversion to AIV H7 was detected in the twice-vaccinated group.

When evaluating for protection from mortality in once-vaccinated chickens, sham-vaccinated birds were not protected from vvNDV or HP AIV challenge as evident by 0% and 10% survivors in the respective groups. However, the tNDV vaccine provided 70% protection for vvNDV challenge. By contrast, the rNDV-AIV-H7 vaccine provided partial protection from vvNDV (40% survival) and HP AIV (40% survival) challenge.

In previous studies with commercial B1 NDV vaccine, single immunization with the recommended vaccine dose of 10^5 EID₅₀ produced 100% protection against vvNDV challenge. Likewise, single inoculation of chickens with live LP AIV provided protection from HP AIV of a homologous HA subtype. In the current study, the lack of an analogous level of protection by the tNDV and rNDV-AIV-H7 vaccines against vvNDV and HP AIV may have resulted from lower level of replication of the recombinant vaccine in the respiratory tracts with subsequent reduced levels of
protective immunity. Previously, mice vaccinated twice with recombinant NDV containing AIV H1 HA developed antibodies against NDV and AIV and were 100% protected against virulent H1N1 influenza virus challenge (6). In the current experiment, the lack of 100% protection against vvNDV and HP AIV in rNDV-AIV-H7 vaccinated chickens may be the result of the single immunization protocol.

This demonstrates the potential for NDV for use as a vaccine vector in expressing AIV proteins; however, improvements in the vaccine are still needed to improve efficacy. Future studies will examine the potential for two immunizations with rNDV-AIV-H7 to provide better protection from AIV and NDV.

REFERENCES


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