Immunogenicity of Fowlpox Virus Expressing the Avian Influenza Virus H5 Gene (TROVAC AIV-H5) in Cats

Kemal Karaca, David E. Swayne, Deborah Grosenhaugh, Michel Bublot, Amy Robles, Erica Spackman, and Robert Nordgren

Merial Ltd., Athens, Georgia; Southeastern Poultry Research Laboratory, Athens, Georgia; and Merial SAS, Lyon, France

Received 2 June 2005/Returned for modification 11 July 2005/Accepted 5 August 2005

Vaccination of cats with fowlpox virus expressing the avian influenza (AI) virus H5 hemagglutinin gene (TROVAC AI) resulted in detectable hemagglutination inhibition (HI) antibody responses to the homologous A/Turkey/Ireland/1378/83 (H5N8) (A/tky/Ire/83) AI virus antigen. The HI antibody responses to heterologous A/Chicken/Indonesia/7/03 (H5N1) (A/ck/Indonesia/03) AI virus antigen were also detected in all vaccinated cats, but only after booster vaccinations. The vaccine described in this study and other poxvirus-vectored vaccines may be of value for the prophylaxis of AI virus-associated morbidity and mortality in mammals.

It has recently been reported that H5N1 avian influenza (AI) virus (AIV) can productively infect domestic cats. Feeding on infected material such as poultry or intratracheal inoculation with H5N1 AIV resulted in clinical disease and death (6). Horizontal transmission of the H5N1 AIV from infected cats to sentinel cats has also been demonstrated (6). In addition, AIV (H5N1) has been isolated from the lungs of a tiger and a leopard, both of which died unexpectedly after consuming chicken carcasses presumably infected with AIV (5). The probable horizontal transmission among tigers was also recently reported (17).

The current strategies for the control of human influenza outbreaks consist of vaccination and treatment with antiviral drugs (1, 3, 19). Although the potential value of the antiviral drugs was demonstrated during the recent H7N7 outbreak (3), their widespread use is limited due to concerns over the emergence of drug-resistant variants, side effects, and stockpiling requirements. While several experimental vaccine modalities (inactivated, live attenuated, replication-defective influenza viruses, virus and DNA vectored) are available, only inactivated and live attenuated influenza vaccines have been licensed for human use in the United States (1), whereas, in addition to inactivated and live attenuated vaccines, canarypox-vectored equine influenza and fowlpox-vectored avian influenza vaccines have been successfully used in horses (8, 18) and chickens (12), respectively.

The objectives of this study were to evaluate the ability of fowlpox virus expressing the AIV H5 hemagglutinin (HA) gene derived from A/tky/Ire/83 (TROVAC AIV-H5) to induce H5 HA-specific antibodies in cats and to further demonstrate the utility of avipox-vectored influenza vaccines in mammals.

Eight- to 10-week-old domestic short-hair cats were purchased from Liberty Research Inc. (Liberty, NY). These cats were previously vaccinated against feline leukemia virus (FeLV), challenged with FeLV, and shown to be free of persistent FeLV antigenemia. The cats were housed at two to four cats per pen, fed commercial feline diet, and provided with water ad libitum. The animals were handled in compliance with Merial Institutional Animal Care and Use Committee requirements.

Fowlpox expressing the avian influenza virus H5 HA gene derived from A/tky/Ire/83 has been described previously (15, 16). Lyophilized virus was rehydrated with 1 ml sterile distilled water immediately prior to vaccination.

Twenty cats approximately 6 months of age were placed into two groups of 10 cats each. The first group of 10 cats was administered 10^6.8 50% tissue culture infective doses/dose of TROVAC AIV-H5 subcutaneously on days 0 and 29. The second group of 10 cats served as a control. The presence of clinical signs (fever, lethargy, anaphylactic shock, and vomiting) and injection site reactions (swelling, tumefaction, ulceration, and pain) were assessed on days 1 to 4 and days 30 to 33. Blood was collected from all cats 3 days prior to the first vaccination and also on days 7, 14, 21, 29, 35, and 42 after the first vaccination.

The hemagglutination inhibition (HI) test was performed as described previously (14). Briefly, twofold serial dilutions of chicken red blood cell (cRBC)-treated cat serum was initially diluted 1:8 and was then incubated with 4 HA units of homologous (A/tky/Ire/83) or heterologous (A/ck/Indonesia/03) H5 AIV antigens (inactivated with 0.1% beta-propiolactone) and a 0.5% (vol/vol) suspension of cRBCs per well. Antibody titers corresponding to the reciprocal of the highest dilution that inhibited hemagglutination were expressed as geometric mean titers (GMTs).

The nucleotide and protein identities between the HA1 regions (nucleotides 1 to 1050 of the coding region) of the HA gene of Turkey/Ireland/1378/83 and the HA1 gene regions of Chicken/Indonesia/7/03 were determined by multiple-sequence alignment of the nucleotide and amino acid sequences with ClustalW (Lasergene99, version 5; DNAStar, Madison, WI).

Vaccination did not induce systemic or local adverse reactions. Figure 1 shows the HI antibody responses of the TROVAC AIV-H5-vaccinated cats to homologous and hetero-
87% amino acid identity, although protection was related to among heterologous strains of the H5 subtypes with as little as (TROVAC AIV-H5) in poultry resulted in cross-protection for protection. Although it has not been defined for cats, the greater than what is antigenically important.

acids has not been well defined for H5, and also, when viruses observed, the role of antigenically important specific amino acids was not related, amino acid sequences may diverge at levels of the HA1 proteins of these two viruses revealed 85.9% nucleotide sequence identity and also, when viruses are not related, amino acid sequences may diverge at levels greater than what is antigenically important.

HI titers are probably the best indicators of the potential for protection. Although it has not been defined for cats, the use of fowlpox virus expressing the AI virus H5 HA gene (TROVAC AIV-H5) in poultry resulted in cross-protection among heterologous strains of the H5 subtypes with as little as 87% amino acid identity, although protection was related to the similarity of the vaccine strain to the challenge strain (13).

Booster vaccination, as shown here, can also increase protection against nonhomologous strains.

Vaccination has been an important tool in the prevention of influenza in humans since the 1940s (1). Vaccination against swine and equine influenza is routinely used in animals in the United States because these viruses are endemic and disease is common in the respective populations that they infect. By contrast, vaccination against AI has not been widely practiced in the United States because infections are rare and are usually eradicated by biosecurity measures. In other parts of the world where AI is common in poultry, vaccination either has been routine or has been used as a means of emergency response during epizootics of highly pathogenic AI.

The current Asian H5N1 AI viruses have the potential to be the origin of the next human pandemic virus or to establish endemic infections in various domesticated mammals and birds. Recently, several vaccine development strategies have been proposed for the prevention of the next human influenza pandemic (9, 19). These vaccines include inactivated influenza A viruses derived from recent isolates, inactivated influenza A viruses generated by reverse genetics, and live attenuated influenza A viruses (9, 19). Little attention has been given to the potential use of canarypox- and/or fowlpox-vectored influenza vaccines against current H5N1 AIV, despite their significant safety and efficacy profiles in animals and humans (2, 4, 7, 9, 10, 11, 12, 16, 18).

For example, the fowlpox-vectored AI vaccine used in the current study was shown to be effective in reducing the morbidity and mortality induced by H5 AIV isolates in chickens (12). Vaccination with this fowlpox-vectored influenza vaccine was also shown to be effective in reducing oral and cloacal viral shedding 30- to 1,000-fold in comparison to the level of viral shedding by unvaccinated chickens (12). In addition, it has been shown that the canarypox vectored equine influenza virus vaccine is effective in reducing morbidity caused by recently emerging equine influenza virus (H3N8) isolates (18). Furthermore, several other established commercial vaccines for cats (canarypox virus-vectored rabies and canarypox virus-vectored canarypox feline leukemia virus), dogs (canarypox virus-vectored canine distemper virus), horses (canarypox virus-vectored West Nile virus and canarypox virus-vectored equine influenza virus), chickens (fowlpox virus-vectored Newcastle disease virus and fowlpox virus-vectored avian influenza) are available for animals and are used widely without significant adverse effects, supporting the concept that avipox-vectored vaccines are safe and efficacious.

The current study showed that the TROVAC AIV-H5 is capable of inducing high levels of antibodies to H5 AI virus. More importantly, the antibody responses were detected as early as 1 week after the first vaccination. Furthermore, cats mounted a booster response to the second vaccination that cross-reacted with a recent highly pathogenic Asian H5N1 isolate. These results suggest that poxvirus-vectored influenza vaccines should be considered as an alternative in the development of specific influenza vaccines for mammalian species.

We thank J. Beck and Debra Walls for technical assistance.

REFERENCES

1. Centers for Disease Control and Prevention, National Immunization Program. 2004. Influenza. In Epidemiology and prevention of vaccine-prevent-
able diseases ("the pink book"), 8th ed. Centers for Disease Control and Prevention, Atlanta, Ga.

2. de Bruyn, G., A. J. Rossini, Y. L. Chiu, D. Holman, M. L. Elizaga, S. E. Frey, D. Burke, T. G. Evans, L. Corey, and M. C. Keef. 2004. Safety profile of recombinant canarypox HIV vaccines. Vaccine 26:704–713.

3. Galama, J. M. 2003. Avian influenza and oseltamivir; a retrospective view. Ned. Tijdschr. Geneeskd. 147:1100–1102.

4. Jourdier, T. M., C. Moste, M. C. Bonnet, F. Delisle, J. P. Tafani, P. Devauchelle, J. Tarraglia, and P. Moingeon. 2003. Local immunotherapy of spontaneous feline fibrosarcomas using recombinant poxviruses expressing interleukin 2 (IL2). Gene Ther. 10:2126–2132.

5. Keawcharoen, J., K. Oraveerakul, T. Kuiken, R. A. M. Fouchier, A. Amonsin, S. Payungporn, S. Noppornpanth, S. Wattandorn, T. Apiradee, R. Tantilercharoen, R. Pattanarangsang, N. Arya, P. Ratana, A. D. M. E. Osterhaus, and Y. Poovorawan. 2004. Avian influenza H5N1 in tigers and leopards. Emerg. Infect. Dis. 10:2189–2191.

6. Kuiken, T., G. Rimmelzwaan, G. van Amerongen, M. Baars, R. Fouchier, and A. Osterhaus. 2004. Avian H5N1 influenza in cats. Science 306:241.

7. Marshall, J. L., J. L. Gulley, P. M. Arten, P. K. Beetham, K. Y. Tsang, R. Slack, J. W. Hodge, S. Doren, D. W. Grosenbach, J. Hwang, E. Fox, L. Odugwu, S. Park, D. Puni, and J. Schlom. 2005. Phase I study of sequential vaccinations with fowlpox-CEA(6D)-TRICOM alone and sequentially with vaccinia-CEA(6D)-TRICOM, with and without granulocyte-macrophage colony-stimulating factor, in patients with carcinomaembryonic antigen-expressing carcinomas. J. Clin. Oncol. 23:720–731.

8. Minke, J. M., J.-C. Audonnet, and L. Fischer. 2004. Equine viral vaccines: the past, present and future. Vet. Res. 35:425–443.

9. Palese, P., and A. Garcia-Sastre. 2002. Influenza vaccines: present and future. J. Clin. Investig. 110:8–13.

10. Paoletti, E. 1996. Application of poxvirus vectors to vaccination: an update. Proc. Natl. Acad. Sci. USA 93:11349–11353.

11. Pastoret, P. P., and A. Vanderplaschen. 2003. Poxviruses as vaccine vectors. Comp. Immunol. Microbiol. Infect. Dis. 26:343–355.

12. Swayne, D. E. 2004. Application of new vaccine technologies for the control of transboundary diseases. Dev. Biol. 119:219–228.

13. Swayne, D. E., J. R. Beck, M. Garcia, and H. D. Stone. 1999. Influence of virus strain and antigen mass on efficacy of H5 avian influenza inactivated vaccines. Avian Pathol. 28:245–255.

14. Swayne, D. E., D. A. Senne, and C. W. Reard. 1997. Avian influenza, p. 150–155. In D. E. Swayne, J. R. Glisson, M. W. Jackwood, J. E. Pearson, and W. M. Reed (ed.), A laboratory manual for the isolation and identification of avian pathogens, 4th ed. American Association of Avian Pathologists, New Bolton Center, Pa.

15. Taylor, J., R. Weinberg, Y. Kawaoka, R. G. Webster, and E. Paoletti. 1988. Protective immunity against avian influenza induced by a fowlpox virus recombinant. Vaccine 6:504–508.

16. Taylor, J., R. Weinberg, B. Languet, P. Desmettre, and E. Paoletti. 1988. Recombinant fowlpox virus inducing protective immunity in non-avian species. Vaccine 6:497–503.

17. Thanawongnuwech, R., A. Amonsin, R. Tantilercharoen, S. Damrongwatanapokin, S. Payungporn, K. Nanthapornphiphat, K., S. Ratana and Kunlaphloth, E. Tunak, T. Songer, V. Vivaaththanavichan, T. Leekumrongiosk, S. Kesdangakunw, S. Tunhikorn, and Y. Poovorawan. 2005. Probable tiger-to-tiger transmission of avian influenza H5N1. Emerg. Infect. Dis. 11:699–701.

18. Toulemendo, C. E., J. Daly, T. Sindle, P. M. Guigal, J. C. Audonnet, and J. M. Minke. 2005. Efficacy of a recombinant equine influenza virus vaccine against challenge with an American lineage H3N8 influenza virus responsible for 2003 outbreak in the United Kingdom. Vet. Rec. 156:367–371.

19. Webby, R., and R. G. Webster. 2003. Are we ready for pandemic influenza? Science 302:1519–1522.