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Efficacy of seasonal live attenuated influenza vaccine against virus replication and transmission of a pandemic 2009 H1N1 virus in ferrets

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ABSTRACT

In March 2009, a swine origin influenza A (2009 H1N1) virus was introduced into the human population and quickly spread from North America to multiple continents. Human serologic studies suggest that seasonal influenza virus vaccination or infection would provide little cross-reactive serologic immunity to the pandemic 2009 H1N1 virus. However, the efficacy of seasonal influenza infection or vaccination against 2009 H1N1 virus replication and transmission has not been adequately evaluated *in vivo*. Here, ferrets received one or two doses of the US licensed 2008–2009 live attenuated influenza vaccine (LAIV) intranasally. An additional group of ferrets were inoculated with the A/Brisbane/59/07 (H1N1) virus to model immunity induced by seasonal influenza virus infection. All vaccinated and infected animals possessed high titer homologous hemagglutination-inhibition (HI) and neutralizing antibodies, with no demonstrable cross-reactive antibodies against 2009 H1N1 virus. However, in comparison to non-immune controls, immunized ferrets challenged with pandemic A/Mexico/4482/09 virus displayed a significant reduction in body temperature and virus shedding. The impact of single-dose LAIV inoculation on 2009 H1N1 disease and virus transmission was also measured in vaccinated ferrets that were challenged with pandemic A/Netherlands/1132/09 virus. Although a single dose of LAIV reduced virus shedding and the frequency of transmission following homologous seasonal virus challenge, it failed to reduce respiratory droplet transmission of 2009 H1N1 virus. The results demonstrate that prior immunization with seasonal LAIV or H1N1 virus infection provides some cross-protection against the 2009 H1N1 virus, but had no significant effect on the transmission efficiency of the 2009 H1N1 virus.

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1. Introduction

The pandemic influenza A H1N1 virus was first identified in April 2009 and quickly spread from North America to multiple continents prompting the World Health Organization (WHO) to declare a pandemic on 11 June 2009 [1,2]. By the time WHO declared the pandemic over in August 2010, the 2009 H1N1 virus had spread globally, and the 18,000 deaths reported to WHO are almost certainly an underestimation of the true number that have occurred worldwide [3]. Because the pandemic 2009 virus is still circulating in some parts of the world, there continues to be a need to better understand the cross-protective immunity to 2009 H1N1 virus conferred by seasonal influenza vaccination or virus infection. Hancock et al. reported a human serologic study which measured the level of pre-existing immunity to the 2009 pandemic H1N1 virus resulting from seasonal infection or vaccination in different age groups [4]. Using stored-serum samples from trials of seasonal trivalent inactivated vaccine (TIV) predating the current pandemic, this study showed that vaccination with contemporary seasonal influenza vaccines provided little cross-reactive immunity to the 2009 H1N1 virus. Likewise, no seroconversion to the 2009 H1N1 virus was detected in adults or children who were vaccinated with the seasonal live attenuated influenza vaccine (LAIV). However, not all LAIV-elicited immune responses are measurable in serologic tests and therefore preclinical evaluations of seasonal LAIV are needed to fully determine the cross-protective efficacy against the 2009 H1N1 virus. Moreover, the effectiveness of 2008–2009 LAIV in restricting 2009 H1N1 virus spread and preventing illness caused by this pandemic virus has not been evaluated in humans.

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The seasonal trivalent LAIV used in the U.S. is administered in one or two doses by nasal spray. The vaccine viruses used in LAIV are reassortant viruses containing six internal genes originally derived from two stably attenuated cold-adapted influenza A and B master donor viruses and genes from the recommended contemporary wild-type influenza A and B viruses, encoding the surface proteins hemagglutinin (HA) and neuraminidase (NA). The use of LAIV is attractive because it more closely mimics natural infection compared with the injectable TIV. LAIV elicit local nasal wash IgA and cellular responses in a higher proportion of recipients [5,6]. Thus, LAIV are capable of inducing a broad range of immune responses and appear to offer cross-protection against antigenically distinct heterologous influenza viruses [7–9]. For example, the induction of cross-reactive nasal wash IgA antibody and T cell responses in LAIV (H2N2)-immunized mice was associated with protection from systemic H5N1 virus spread and death [7–9].

Ferrets are considered to be the most suitable animal model for influenza vaccine efficacy studies [10–13] and this animal model was used to demonstrate the inherent virulence of 2009 H1N1 viruses compared with seasonal H1N1 viruses, which was associated with more extensive pulmonary replication in this naïve host [14,15]. Using the ferret model, two recent studies addressed the impact of prior seasonal influenza vaccination on 2009 H1N1 influenza virus replication [16,17]. However, these studies did not test the efficacy of seasonal LAIV on both 2009 H1N1 replication and respiratory droplet transmission in ferrets. Here, we demonstrate that vaccination with LAIV or prior influenza H1N1 virus infection provides a detectable level of cross-protection against the pandemic 2009 H1N1 virus in the absence of detectable cross-reactive HI antibodies. Immune ferrets showed a significant reduction in viral shedding and body temperatures following 2009 H1N1 A/Mexico/4482/09 virus challenge. Despite alleviating some disease symptoms in vaccinated ferrets, seasonal LAIV administration did not block transmission of 2009 H1N1 A/Netherlands/1132/09 virus from vaccinated ferrets to naïve contact ferrets via respiratory droplets.

2. Methods

2.1. Viruses

Virus stocks for seasonal A/Brisbane/59/2007 (Brisbane/59) and A/South Dakota/6/2007 (SD/2007), a Brisbane/59-like virus, were propagated in the allantoic cavities of embryonating hen's eggs. Stocks were titered in a standard plaque assay and expressed as plaque forming units (pfu) [12,18]. A/Mexico/4482/2009 (Mex/4482) virus was isolated from a 29-year-old female patient with severe respiratory disease [19]. A/Netherlands/1132/2009 (Neth/1132) virus was isolated from a 17-year-old male patient with non-fatal respiratory symptoms. Mex/4482 and Neth/1132 viruses were propagated and titrated by plaque assay. All experiments were performed in negative pressure biosafety level 3 laboratories with enhancements as outlined in the Biomedical Microbiological and Biomedical Laboratory [20]. Animal research was conducted under the guidance of the Centers for Disease Control and Prevention's Institutional Animal Care and Use Committee in an Association for Assessment and Accreditation of Laboratory Animal Care International-accredited animal facility.

2.2. Vaccination and challenge

Male Fitch ferrets, 7–12 months of age (Triple F Farms, Sayre, PA), serologically negative by hemagglutination inhibition (HI) assay for currently circulating influenza viruses, were used in this study. For vaccinations, 10 ferrets were vaccinated twice, 4 weeks apart, with a human dose (0.2 ml) of the 2008–2009 seasonal LAIV (FluMist®) (referred to as LAIV throughout text) delivered by the intranasal route. The seasonal LAIV was kindly provided by Drs. Kathleen Coelingh and Hong Jin at MedImmune (Mountain View, CA). Each 0.2 ml dose of FluMist contains $10^{6.5–5.75}$ FFU (fluorescent focus units) of each of the three influenza virus reassortants: SD/2007, A/Uruguay/716/2007 (H3N2) and B/Florida/4/2006. Nine and seven animals served as PBS controls for homologous and heterologous challenge, respectively. A second group of 10 ferrets were intranasally inoculated with 10^6 pfu of Brisbane/59 virus in a total volume of 1 ml. For uniform and consistent infections, all ferrets were immobilized with ketamine/xylazine/atropine cocktail (25 mg/kg ketamine, 2 mg/kg xylazine, and 0.05 mg/kg of atropine) prior to inoculation [12].

Challenge of immune ferrets and controls occurred 4.5 weeks after vaccine boost (8.5 weeks after initial vaccination); challenge of Brisbane/59-infected ferrets occurred 8 weeks after infection. Prior to vaccination boost and challenge, serum was collected to assess antibody response to vaccination or infection. Baseline weights and temperatures for ferrets were obtained prior to challenge. Ferrets were challenged intranasally with 10^6 pfu of virus diluted in PBS for both challenge viruses; SD/2007 (homologous virus) and Mex/4482 (heterologous virus). Following challenge, ferrets were monitored for changes in body weight and temperature as well as clinical signs of illness such as sneezing, lethargy, nasal or ocular discharge, diarrhea and neurological dysfunction [12]. Body temperatures were measured on days 1–8 post-challenge (p.c.) using an implantable subcutaneous temperature transponder (BioMedic Data Systems, Inc., Seaford, DE). Any ferret that lost greater than 25% of its baseline weight was euthanized. Virus shedding was measured in nasal washes collected from anesthetized ferrets on days 2, 4, 6, and 8 post-challenge. Samples were immediately frozen on dry ice and stored at −70 °C until analysis. Viruses were titrated in eggs for virus infectivity from initial dilutions of 1:10 and the limit of virus detection was $10^{1.5}$ EID_{50}/ml [18]. Statistical significance of virus titers in nasal washes was determined by Student's *t*-test.

2.3. Transmission experiments

To assess airborne transmissibility (referred to as respiratory droplet transmission), ferrets were housed in adjacent transmission cages as described [15]. The adjacent walls of the cages were replaced with a stainless-steel wall containing perforations 1–5 mm in diameter and spaced 3 mm apart to allow transfer of respiratory droplets through the air while preventing direct contact between ferrets. Nine ferrets were vaccinated once with the 2008–2009 seasonal LAIV as above; six additional ferrets were mock vaccinated with PBS for controls. Vaccinated ferrets were intranasally inoculated with either the 2009 H1N1 Neth/1132 virus or, as a control, the seasonal SD/2007 virus at a dose of 10^6 pfu in 1 ml PBS. Twenty-four hours after inoculation, a naïve ferret was placed in each cage adjacent to an inoculated ferret. Contact ferrets were always handled before inoculated ferrets and all equipment was decontaminated after handling of each contact ferret to avoid inadvertent transmission by investigators. Ferrets were monitored for clinical signs of disease as detailed above. Nasal washes were collected every other day post-infection for at least 11 days; samples were stored and analyzed as described above. Post-exposure sera were collected 21 days after inoculation and subjected to HI analysis.

2.4. Antibody assays

All sera was treated with receptor-destroying enzyme (RDE, Denka Seiken, Tokyo, Japan) and tested for reactivity to relevant combinations of SD/2007, Mex/4482, Neth/1132 and Brisbane/59