HHS Public Access

Author manuscript

Vaccine. Author manuscript; available in PMC 2016 March 24.

Published in final edited form as:

Vaccine. 2009 August 6; 27(36): 4953–4960. doi:10.1016/j.vaccine.2009.05.099.

Evaluation of two live attenuated cold-adapted H5N1 influenza virus vaccines in healthy adults

Ruth A. Karron[1], **Kawsar Talaat**[1], **Catherine Luke**[2], **Karen Callahan**[1], **Bhagvanji Thumar**[1], **Susan DiLorenzo**[1], **Josephine McAuliffe**[2], **Elizabeth Schappell**[1], **Amorsolo Suguitan Jr.**[2], **Kimberly Mills**[2], **Grace Chen**[2], **Elaine Lamirande**[2], **Kathleen Coelingh**[3], **Hong Jin**[3], **Brian R. Murphy**[2], **George Kemble**[3], and **Kanta Subbarao**[2]

[1]Center for Immunization Research, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 21205

[2]Laboratory of Infectious Diseases, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892

[3]MedImmune, One MedImmune Way, Gaithersburg, MD 20878

Abstract

Background—Development of live attenuated influenza vaccines (LAIV) against avian viruses with pandemic potential is an important public health strategy.

Methods and Findings—We performed open-label trials to evaluate the safety, infectivity, and immunogenicity of H5N1 VN 2004 AA *ca* and H5N1 HK 2003 AA *ca*. Each of these vaccines contains a modified H5 hemagglutinin and unmodified N1 neuraminidase from the respective wild-type (*wt*) parent virus and the six internal protein gene segments of the A/Ann Arbor/6/60 cold-adapted (*ca*) master donor virus. The H5N1 VN 2004 AA *ca* vaccine virus was evaluated at dosages of $10^{6.7}$ $TCID_{50}$ and $10^{7.5}$ $TCID_{50}$, and the H5N1 HK 2003 AA *ca* vaccine was evaluated at a dosage of $10^{7.5}$ $TCID_{50}$. Two doses were administered intranasally to healthy adults in isolation at 4 to 8 week intervals. Vaccine safety was assessed through daily examinations and infectivity was assessed by viral culture and by realtime reverse transcription-polymerase chain reaction testing of nasal wash (NW) specimens. Immunogenicity was assessed by measuring hemagglutination-inhibition (HI) antibodies, neutralizing antibodies, and IgG or IgA antibodies to recombinant (r)H5 VN 2004 hemagglutinin (HA) in serum or NW.

Fifty-nine participants were enrolled: 21 received $10^{6.7}$ $TCID_{50}$ and 21 received $10^{7.5}$ $TCID_{50}$ of H5N1 VN 2004 AA *ca* and 17 received H5N1 HK 2003 AA *ca*. Shedding of vaccine virus was minimal, as were HI and neutralizing antibody responses. Fifty-two percent of recipients of $10^{7.5}$ $TCID_{50}$ of H5N1 VN 2004 AA *ca* developed a serum IgA response to rH5 VN 2004 HA.

Correspondence: Dr. Ruth A. Karron, Center for Immunization Research, Hampton House 217, Johns Hopkins Bloomberg School of Public Health, 624 N. Broadway, Baltimore, MD 21205, rkarron@jhsph.edu.

Conflict of interest: Ruth Karron has been a consultant and a member of an advisory board for MedImmune. Kathleen Coelingh, Hong Jin, Josephine McAuliffe, and George Kemble are employees of MedImmune. No other authors have potential conflicts of interest to report.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

Conclusions—The live attenuated H5N1 VN 2004 and HK 2003 AA *ca* vaccines bearing avian H5 HA antigens were very restricted in replication and were more attenuated than seasonal LAIV bearing human H1, H3 or B HA antigens. The H5N1 AA *ca* LAIV elicited serum ELISA antibody but not HI or neutralizing antibody responses in healthy adults. (ClinicalTrials.gov Identifiers: NCT00347672 and NCT00488046).

Keywords

influenza; vaccine; pandemic; H5N1; clinical trial

The emergence of a number of avian influenza A subtypes (H5N1, H7N7, and H9N2) in human populations, and the intercontinental spread of H5N1 influenza (http://www.who.int/csr/disease/avian_influenza/country/cases_table_2008_03_18/en/index.html) have made the development of vaccines against these novel influenza viruses a global health priority. A number of strategies are currently being employed to produce these vaccines, including the development of killed whole virus vaccines, subunit vaccines, and recombinant viral proteins that are administered with standard or novel adjuvants, each of which has been evaluated in clinical trials [1–15]. Ideally, vaccines to be used in the event of an influenza pandemic will require a low dose, be able to be produced rapidly, and able to be self-administered or delivered by individuals with minimal training in health care delivery. Since stockpiled vaccines are unlikely to be a perfect antigenic match for a newly emerged pandemic strain or for pandemic strains that may evolve during the course of the pandemic, a vaccine that induces cross-protective immunity to antigenically drifted strains is also desirable. Live attenuated influenza vaccines (LAIV) for pandemic influenza viruses could potentially meet many of these requirements.

Trivalent LAIV (licensed in the United States for eligible persons aged 2 – 49 years) is comprised of a type B vaccine strain and two type A vaccine strains that contain internal protein gene segments derived from the cold-adapted, temperature-sensitive, attenuated A/Ann Arbor (AA)/6/60 (H2N2) master donor strain and hemagglutinin (HA) and neuraminidase (NA) gene segments from contemporary epidemic strains. Trivalent LAIV is delivered intranasally, can be produced rapidly and in high yield, and has been shown to provide cross-protection against drifted H1N1 and H3N2 influenza A strains in children and adults [16–21]. Development of LAIV with similar characteristics for influenza viruses of pandemic potential would be highly desirable.

A number of LAIV candidate vaccines containing avian HA and NA gene segments and the internal protein gene segments of the A/AA/6/60 master donor strain have been produced and evaluated in preclinical studies [22–24]. The H5N1, H7N3 and H9N2 AA *ca* LAIV have been shown to be highly attenuated in chickens, and attenuated and restricted in replication in the upper and lower respiratory tracts of mice and ferrets [22–24]. Additionally, these candidate vaccines protect mice and ferrets against challenge with homologous and heterologous wild-type (*wt*) H5, H7 and H9 viruses, respectively [22–24]. We have previously reported that a candidate H9N2 AA *ca* LAIV is attenuated and immunogenic in individuals who were previously seronegative to the H9 virus [25]. Here, we describe the clinical evaluation of two LAIV containing modified avian H5 HA and

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

unmodified N1 NA from clade 1 H5N1 viruses (A/VietNam/1203/2004 and A/Hong Kong/213/2003) and the six internal gene segments (PB1, PB2, PB, NP, M and NS) of the A/AA/6/60 master donor virus.

PARTICIPANTS, MATERIALS AND METHODS

Vaccine virus

H5N1 VN 2004/AA *ca* and H5N1 HK 2003/AA *ca* are live attenuated, cold-adapted, temperature-sensitive influenza viruses derived from A/AA/6/60 *ca* (H2N2) Master Donor Virus (MDV-A, MedImmune) and wild-type (*wt*) A/Viet Nam/1203/2004 (H5N1) or wild-type A/Hong Kong/213/2003 (H5N1), respectively. Each vaccine virus contains the six gene segments encoding the internal proteins from the MDV-A virus and the respective HA and NA from the *wt* donor virus. In each vaccine, the *wt* HA was modified to remove the multibasic cleavage site.

The pre-Master Virus Seeds (pre-MVS) were produced at the Laboratory of Infectious Diseases (LID), National Institute of Allergy and Infectious Disease (NIAID), NIH, in BSL-3 containment. Attenuation of the H5N1 VN 2004/AA *ca* pre-MVS virus and the H5N1 HK 2003/AA *ca* pre-MVS in mice and chickens was confirmed, so that the manufacture of the vaccines could be conducted in BSL-2 containment. The pre-MVS were then transferred to MedImmune, CA, for manufacture of the drug product under GMP conditions. The bulk drug substances were subjected to characterization and lot release testing. Vaccine virus was blended in SPG buffer at the expected potency and filled into AccuSpray™ sprayers (0.5 mL). Filled sprayers were stored frozen at −60°C or below and thawed just prior to administration.

The H5N1 VN 2004/AA *ca* vaccine was evaluated at doses of $10^{6.7}$ $TCID_{50}$ (low dose) and $10^{7.5}$ $TCID_{50}$ (high dose). The H5N1 HK 2003/AA *ca* vaccine was only evaluated at a dose of $10^{7.5}$ $TCID_{50}$.

Study Population

Clinical trials were conducted at the Center for Immunization Research (CIR) Isolation Unit at the Johns Hopkins Bayview Medical Center and at the CIR outpatient clinic. A total of 59 healthy adult subjects from the Baltimore metropolitan area were recruited and enrolled into three separate cohorts. Each cohort consisted of 16–22 subjects who were screened and selected to participate in the evaluation of two doses of an H5N1 virus vaccine candidate, administered 4 to 8 weeks apart. The first cohort received two $10^{6.7}$ $TCID_{50}$ doses of the H5N1 VN 2004 AA *ca* vaccine, the second cohort received two $10^{7.5}$ $TCID_{50}$ doses of the H5N1 VN 2004 AA *ca* vaccine, and the third cohort received two $10^{7.5}$ $TCID_{50}$ doses of the H5N1 HK 2003 AA *ca* vaccine. The evaluation of the first $10^{6.7}$ $TCID_{50}$ dose of the H5N1 VN 2004 AA *ca* vaccine was accomplished in stages: 9 subjects received a first dose in June, 2006, and when their safety and clinical assessment was complete, 12 additional subjects received a first dose of vaccine in July, 2006. Subjects from both cohorts were readmitted in August, 2006 to receive a second dose of vaccine.

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

These studies were sponsored by the Regulatory Control and Human Subjects Protection Branch of the National Institute of Allergy and Infectious Diseases and were conducted under investigational new drug applications (BB-IND #12588 and 13378). All clinical protocols were reviewed and approved by the Committee on Human Research Institutional Review Board (IRB) of the Johns Hopkins Bloomberg School of Public Health and the Institutional Biosafety Committee of Johns Hopkins University. Informed, witnessed, written consent was obtained from each participant. Healthy adult men and non-pregnant women, 18 to 49 years of age, were enrolled in the clinical trial if they met eligibility criteria and were willing to remain on the isolation unit for the duration of the inpatient portion of the trial.

Study design

These studies were conducted between April 1st and December 20th of 2006 and 2007, when *wt* human influenza viruses would be unlikely to be circulating in the local community. To further reduce the risk of reassortment between a naturally occurring epidemic *wt* human influenza virus and the vaccine virus, the presence of *wt* influenza viruses in the community was monitored between April 1st–May 1st and October 1st–December 20th by obtaining information regarding influenza virus detection from the Diagnostic Virology Laboratory and Department of Hospital Epidemiology and Infection Control at Johns Hopkins Hospital. Participants would not have been enrolled if there were 3 influenza hospitalizations in the week preceding the planned vaccination.

These studies were conducted as open-label inpatient trials with all participants receiving vaccine. Participants were screened to establish health status and, if eligible, received 0.5 mL of vaccine via nasal spray. Participants were then examined daily while on the inpatient unit by a health care provider (study physician, physician assistant, or nurse practitioner).

Several IRB-approved modifications to the study protocol were made during these studies. First, participants in the evaluation of the low dose of the H5N1 VN 2004 AA *ca* vaccine were not screened for pre-existing H5 hemagglutination inhibiting (HI) antibody. However, based upon reports of H5 seropositivity in 1–3% of populations not known to be exposed to H5 influenza viruses, we pre-screened volunteers for H5 HI antibody in the evaluations of the high dose of the H5N1 VN 2004 AA *ca* vaccine and of the HK 2003 AA *ca* vaccine and only included seronegative participants (those with H5 HI antibody titers 1:8) in these studies. Second, both nasal washes and throat swabs were obtained during the evaluation of the low dose of the H5N1 VN 2004/AA *ca* vaccine based upon reports that *wt* H5N1 virus was recovered more efficiently from throat swabs of infected patients than from nasal washes [26]. However, since vaccine virus was not detected by culture or rRT-PCR in any throat swab, collection of throat swabs was discontinued for studies in the other two cohorts. Finally, based upon the pattern of vaccine virus shedding observed in the H5N1 VN 2004 AA *ca* vaccine low dose study, the duration of the inpatient stay was shortened from a total of 14 days (3 days before vaccination and 11 days following vaccination) to 12 days (3 days before vaccination and 9 days following vaccination) for the other two studies, providing that vaccine virus was not detected by rRT-PCR from nasal washes obtained for 3

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

consecutive days prior to discharge day. None of the participants was required to stay on the isolation unit longer than anticipated.

Isolation Unit and Staffing

The CIR Isolation Unit is a non-smoking, dormitory-like setting designed to house up to 40 adult men and women. The isolation unit has a self-contained ventilation and hot water system as well as an externally vented HEPA filtered exhaust. Study-specific requirements for individuals staffing the isolation unit were as previously described [25].

Study procedures

To establish the health status of potential participants, CIR staff elicited medical histories, including menstrual and contraceptive history and/or history of surgical sterility for female participants, and performed physical examinations. Potential participants were screened for tuberculosis using PPD tests, and for viral hepatitis, HIV infection, and antibody to H5N1 influenza A viruses (for studies of $10^{7.5}$ $TCID_{50}$ of the H5N1 VN2004/AA *ca* vaccine and H5N1 HK 2003/AA *ca* vaccine) using serologic assays. Blood specimens were obtained for hematology and serum biochemistries, and urinalyses were performed via dipstick. Potential participants who were female were tested for pregnancy using urine β-HCG assays and were counseled to avoid becoming pregnant during the study.

Procedures for admission to the isolation unit, for vaccination, for daily clinical evaluation, and for use of protective clothing (gown, gloves, and mask) by study staff were performed as previously described [25]. Vaccine was administered on study day 0 [25]. Nasal washes were collected daily during the inpatient portion of the study and were tested for vaccine virus (see below). In the event of a respiratory or febrile illness, nasal wash specimens were also cultured for adventitious respiratory viruses, including *wt* influenza viruses, parainfluenza viruses types 1, 2, and 3, respiratory syncytial virus, and adenovirus. Because *wt* H5N1 infection can be associated with abnormalities of liver function and with neutropenia [26], serum alanine aminotransferase (ALT) levels and complete blood counts (CBCs) were determined before vaccination and on day 7 following vaccination. Any abnormal results were followed until resolution. For the study of high dose ($10^{7.5}$ $TCID_{50}$) of the H5N1 VN2004/AA *ca* vaccine and the study of the H5N1 HK 2003/AA *ca* vaccine, serum ALT levels were also measured on day 3 following vaccination.

All adverse events were graded for severity using a 1–4 scale. Clinical signs or symptoms were graded as follows: grade 1, no interference with daily activity; grade 2, some interference with daily activity; grade 3, significant, prevents daily activity; grade 4, requires emergency room visit or hospitalization. Specific grading scales were also devised for each laboratory abnormality; grading scales for ALT elevations, neutropenia, and thrombocytopenia are included here. ALT abnormalities were graded as follows: grade 1=1.5 to 3 times the upper limit of laboratory normal values (ULN); grade 2= 3.1–6 times the ULN; grade 3= 6.1–10 times the ULN; grade 4= >10 times the ULN. Neutropenia was graded as follows: grade 1=2001–2499/mm^3, grade 2=1501–2000/mm^3, grade 3=1000–1500/mm^3, grade 4= <1000/mm^3. Thrombocytopenia was graded as follows: grade

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

1=100,000–124,999/mm^3, grade 2=50,000–99.999/mm^3, grade 3=25,000–49,999/mm^3, grade 4= <25,000/mm^3.

After discharge from the isolation unit, participants were asked to return to the CIR clinic for outpatient visits on study day 28 (+7 days) following each dose of vaccine. At each visit, staff obtained vital signs, reviewed interim histories, and obtained blood and nasal wash samples for antibody testing.

Isolation, quantitation and identification of the H5N1 vaccine viruses

Nasal washes were obtained prior to vaccination and then daily from the day of vaccination until the day of discharge. As noted above, daily throat swabs were also obtained in studies of the low dose of the H5N1 VN2004/AA *ca* vaccine. Specimens were tested for the presence of vaccine virus by quantitative viral culture on Madin Darby Canine Kidney (MDCK) cells and using an rRT-PCR assay that amplified a portion of the influenza A M2 gene, both as previously described [25]. The sensitivity of the rRT-PCR assay [25] for vaccine virus was approximately $10^{0.5}$ $TCID_{50}$/mL of nasal wash.

Immunologic Assays

Sera were tested for HI antibodies to H5N1 virus using horse red blood cells (RBC) for the VN 2004 study [27] and turkey RBC for the HK 2003 study as previously described [28] and for neutralizing antibodies using a modified version of a previously described microneutralization assay, in which we assessed titers of neutralizing antibody to vaccine virus [25].

Sera were also tested for IgG and IgA antibody to the H5 VN 2004 HA by ELISA. Immulon 2 plates were coated with 30ng/well of recombinant baculovirus-expressed H5 VN2004 HA (Protein Sciences, Meriden, CT), and the ELISA was performed using endpoint titration [28]. As described below, a subset of sera were also tested for IgA antibody to recombinant baculovirus-expressed H1 A/New Caledonia/20/99 and H7 A/Netherlands/219/03 HA (Protein Sciences, Meriden, CT). Nasal wash specimens were concentrated [28] and were tested using the same antigen to measure vaccine-specific IgA, expressed as a percent of total IgA [28].

Total and influenza vaccine-specific IgG and IgA antibody secreting cells (ASC) were measured in recipients of $10^{7.5}$ $TCID_{50}$ of H5N1 VN 2004/AA *ca* and of H5N1 HK 2003/AA *ca* using an enzyme-linked immunospot (ELISPOT) assay based on an assay described by Sasaki et al. [29]. Briefly, our assay differed from the published assay in that the wells were coated with one of the following: 1) rH5 VN2004 HA protein diluted to 10μg/mL in Dulbecco’s phosphate-buffered saline (Invitrogen); 2) beta-propiolactone treated H5N1/AA *ca* vaccine virus; 3) beta-propiolactone treated cold-adapted A/Ann Arbor/6/60 (H2N2) virus diluted to 5000 HAU/mL; or 4) purified goat anti-human IgA + IgG + IgM (Kirkegaard & Perry Laboratories, Gaithersburg, MD) at a concentration of 5μg/mL in D-PBS. PBS alone and human CCRF-CEM cells (human T-lymphoblasts; ATCC, Manassas, VA) cells were used as negative controls; human IM9 cells (human IgG+ lymphoblasts; ATCC) were used as a positive control. Plate images were recorded and

Author Manuscript

counted using ImmunoSpot 4 software (Cellular Technologies Ltd., Shaker Heights, OH). Human IgA ASC were visualized as red spots and IgG ASC were visualized as blue spots. The number of ASC was expressed per 10^6 total IgG or IgA ASC.

Data Analysis

Infection after immunization with either vaccine was defined as: 1) shedding of vaccine virus detected by culture and/or 2) shedding of vaccine virus detected by rRT/PCR any time after study day 1 and/or 3) a 4-fold rise in serum HI, neutralizing, or H5 IgG or IgA serum antibodies. Participants whose nasal washes were rRT/PCR positive on study day 1 only but who were without other evidence of infection were not considered infected because this phenomenon occurred frequently and we could not exclude the possibility that input virus, rather than replicating virus, was being detected. Antibody titers were expressed as reciprocal $\log_2$ for calculation of mean values. Student's *t*-test was used to compare mean ages and antibody titers. Fisher's exact test was used to compare proportions.

RESULTS

Study participants

One hundred ninety four potential participants were screened for the H5N1 virus vaccine trials and 59 participants were enrolled: 21 in the cohort that received $10^{6.7}$ $TCID_{50}$ of H5N1 VN 2004 AA *ca* (18 received a second dose), 21 in the cohort that received $10^{7.5}$ $TCID_{50}$ of H5N1 VN 2004 AA *ca* (19 received a second dose), and 17 in the cohort that received $10^{7.5}$ $TCID_{50}$ of H5N1 HK2003 AA *ca* vaccine (16 received a second dose). Participants ranged in age from 18 to 49 years. Of the 59 participants, 19 (32%) were female, 53 (90%) black, 5 (8%) white, 3 (6%) Asian, and 1 described herself as other. For studies of $10^{7.5}$ $TCID_{50}$ of H5N1 VN 2004 AA *ca* and of H5N1 HK2003 AA *ca*, 122 potential participants were screened for H5 HI antibody. Eight of these individuals had titers 1:8 and were excluded from participation.

Response to $10^{6.7}$ $TCID_{50}$ of H5N1 VN 2004 AA ca vaccine—As noted above, 21 participants received $10^{6.7}$ $TCID_{50}$ of the VN 2004 AA *ca* vaccine, 18 of whom received a second dose. The vaccine was generally well-tolerated (Table 1). One participant had transient asymptomatic wheezing noted by auscultation on days 2 and 7 following vaccination. Further questioning revealed that she had a previous history of asymptomatic wheezing noted by a health care provider that was not included in the subject's self-reported history. This subject did not shed vaccine virus or develop an antibody response. Other solicited adverse events noted after the first dose of vaccine (Table 1) included headache (3 participants), diarrhea (1 participant), and pharyngitis (1 participant). All of these illnesses were of mild (grade 1) or moderate (grade 2) severity, and none occurred in subjects who shed vaccine virus. A number of unsolicited adverse events were also reported, including toothache/dental abscess, heat rash, tinea corporis, knee pain, and heartburn. In addition, ALT elevations were noted in 3 participants (2 of grade 1 and 1 of grade 2 severity). None of these was associated with vaccine virus shedding. Following the second dose of vaccine, solicited adverse events in 18 participants included headache (2 participants) and cough (1 participant). Vaccine virus was not recovered from these individuals. ALT elevations were

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

observed in 5 individuals on study day 7; 3 of grade 1 severity and 2 of grade 2 severity. One of the individuals with a grade 2 elevation in ALT had vaccine virus detected by rRT-PCR on study day 1 only; vaccine virus was not detected by culture.

The $10^{6.7}$ $TCID_{50}$ dose of the VN2004 AA *ca* vaccine was minimally infectious and immunogenic. Vaccine virus was not detected by culture in any participant and was detected by rRT-PCR in 2 participants following dose 1 (days 2 and 3 and day 1, respectively; Table 2) and in 3 participants following dose 2 (all on day 1 only). Antibody responses to this dose of vaccine were also minimal. When considering any 4-fold rise in antibody titer among the 21 recipients of 1 or 2 doses of vaccine, serum HI and serum IgA responses were each detected in 2 recipients (10%), and nasal wash IgA responses were detected in 5 recipients (24%; Table 2). Neutralizing antibody and serum IgG antibody responses were not detected in any recipient.

Response to $10^{7.5}$ $TCID_{50}$ of H5N1 VN 2004/AA ca vaccine—Twenty-one participants received $10^{7.5}$ $TCID_{50}$ of H5N1 VN 2004/AA *ca* vaccine, 19 of whom received a second dose. This dosage of the VN 2004 vaccine was also generally well-tolerated. Following the first dose, headache was the most frequently reported solicited adverse event (5 participants), followed by vomiting, myalgia, diarrhea, and epistaxis (1 participant each; Table 1). Transient ALT elevations were also observed in 3 participants. All signs, symptoms, and laboratory abnormalities were of grade 1 severity. Following the second dose, solicited adverse events included headache (3 participants), rhinorrhea (3 participants, one of whom was culture positive for human parainfluenza type 3) and fever (1 participant; Table 1). Each of these symptoms was of grade 1 severity. The febrile participant had a temperature of 38.0°C on the evening of vaccination (Study Day 0) which was not rechecked. The subject was afebrile on Study Day 1 and on all days thereafter. Additionally, 3 subjects experienced transient asymptomatic neutropenia (2 of grade 1 and 1 of grade 4 severity) on Study Day 7 following the second dose of vaccine, which had resolved at the time of repeat determination on Study Day 9. In particular, the subject with grade 4 neutropenia had a normal absolute neutrophil count (ANC) prior to vaccination, an ANC of 443 on study day 7 and of 2036 on study day 9. Shedding of vaccine virus was not detected at the time of neutropenia in any participant, although the individual with grade 4 neutropenia did have vaccine vírus detected by rRT-PCR on study days 1 and 4 and by culture on Day 4 only following the second dose of vaccine, with a titer of $10^{1.3}$ $TCID_{50}$/mL of nasal wash.

The $10^{7.5}$ $TCID_{50}$ dose of H5N1 VN 2004/AA *ca* vaccine was more infectious than the lower dose of vaccine. Vaccine virus was detected by rRT-PCR in 3 participants following the first dose (2 on study day 1 only; 1 on study day 2 only), and in 15 participants following the second dose of vaccine (13 on study day 1 only; 1 on study days 1 and 4, and 1 on study days 1, 2, and 5; Tables 1 and 3). Vaccine vírus was also detected by culture in 2 participants on a single day each following the second dose of vaccine, at titers of $10^{0.75}$ $TCID_{50}$ (study day 1) and $10^{1.3}$ $TCID_{50}$ (study day 4).

Serum HI responses and neutralizing antibody responses to the higher dose of VN 2004 AA *ca* were minimal and comparable to those measured with the lower dose of vaccine (Tables

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

2 and 3). Nasal wash IgA responses were detected in 4/21 (19%) vaccinees (Table 2). However, serum IgA responses were detected more frequently in these vaccinees than in the recipients of the lower dose of H5N1 VN 2004/AA *ca* vaccine: 11/21 of these vaccinees had a fourfold or greater rise in serum IgA antibody titer to rH5 antigen (Tables 2 and 3), as compared with 2 of 21 recipients of the lower dose of vaccine ($p=.006$).

To assess the specificity of the serum IgA response to rH5 antigen, we measured serum IgA antibody titers to rH1 and rH7 antigens in pre and post sera from recipients of the low and high dosages of VN 2004 AA *ca*. We chose H1 because of previous reports of cross-reactivity between H5 and H1 antibodies [30], and H7 because we expected no cross-reactivity to this antigen. As expected, mean reciprocal $\log_2$ prevaccination titers to H1 were significantly higher than to H7 (for the low dose group, 11.2 vs. 5.7, $p<.001$ and for the high dose group, 10.4 vs 4.9, $p<.001$; data not shown). Among the 21 recipients of the low dose of vaccine, fourfold or greater rises in serum IgA titer were not detected to either the H1 or the H7 antigen. Among the 21 recipients of the high dose of vaccine, we observed a fourfold or greater rise in serum IgA titer to H1 antigen in 1 individual and to the H7 antigen in a second individual (data not shown). These findings confirm the specificity of the serum IgA response to rH5 HA antigen that was observed in recipients of $10^{7.5}$ $TCID_{50}$ of VN 2004 AA *ca*.

When the numbers of vaccine specific IgG and IgA ASCs were determined in the peripheral blood of recipients of the high dose of vaccine, 1 individual had an increase over background in vaccine-specific IgG ASC and a second individual had an increase over background in vaccine-specific IgA ASC (data not shown).

Response to $10^{7.5}$ $TCID_{50}$ of H5N1 HK 2003/AA ca vaccine—Seventeen participants received $10^{7.5}$ $TCID_{50}$ of H5N1 HK 2003/AA *ca* vaccine, 16 of whom received a second dose (Table 1). This vaccine was also generally well-tolerated. Following the first dose, headache was the most frequently reported solicited adverse event (3 participants; Table 1). A single participant reported an episode of chills on study day 1 (Table 1). On study day 7, an additional participant had a grade 1 decrease in platelelet count (resolved by study day 9) and a grade 1 ALT elevation (resolved by study day 15). Following the second dose, solicited adverse events included headache (4 participants), and rhinorrhea (2 participants, Table 1).

Replication of the H5N1 HK 2003/AA *ca* vaccine appeared to be comparable to that observed with the VN 2004/AA *ca* vaccine. Vaccine vírus was detected by rRT-PCR in 8 participants following the first dose (6 on study day 1 only; 1 on study day 2 only, 1 on study days 1 and 2), and in 9 participants following the second dose of vaccine (7 on study day 1 only, 1 on study day 2 only, 1 on study days 1 and 2; Table 1). Vaccine vírus was also detected by culture in 1 participant on a single day following the first dose of vaccine, at a titer of $10^{1.0}$ $TCID_{50}$ on study day 1 (Table 1).

None of the recipients of the H5N1 HK 2003/AA *ca* vaccine developed serum HI responses or neutralizing antibody responses to the vaccine virus (Table 2). Only 1 vaccinee developed a serum IgG response to rH5 antigen (Table 2). In contrast to recipients of $10^{7.5}$ $TCID_{50}$ of

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

H5N1 VN 2004/AA *ca* vaccine, only 3 of 17 recipients of the H5N1 HK 2003/AA *ca* vaccine developed serum IgA responses to rH5 antigen (Table 2). Following the first and second dose of vaccine, 1 individual had an increase over background in vaccine-specific IgG ASC, but none had an increase over background in vaccine-specific IgA ASC (data not shown).

DISCUSSION

In this study, we evaluated two LAIVs containing H5 and N1 antigens: H5N1 VN 2004/AA *ca* and H5N1 HK 2003/AA *ca*. Each of these vaccines was generally well-tolerated in healthy young adults: reported signs and symptoms were mild to moderate in severity, with headache being the most frequently reported symptom. However, these vaccines were highly restricted in replication and did not induce immune responses in the majority of participants.

There are a number of potential explanations for the low infectivity of the H5N1 vaccine viruses. First, the H5 HA may have had limited capacity to bind to cells of the upper airway: H5N1 VN04 virus binds preferentially to sialic acid receptors terminating in N-acetylneuraminic acid α2,3 galactose (α2,3Gal), whereas α2,6Gal receptors predominate in human upper airway epithelium [31]. Secondly, it is likely that most of the study participants had been previously infected with H1N1 influenza A viruses: testing of pre-immunization sera from recipients of the H5N1 VN2004 AA *ca* virus indicated that all individuals had detectable antibody to rH1 antigen derived from A/New Caledonia/20/99. It is possible that pre-existing antibody to the human H1 hemagglutinin (HA) and/or the human N1 neuraminidase (NA) cross-reacted with the avian H5 HA and/or the N1 NA and decreased vaccine virus replication. Some monoclonal antibodies derived from Turkish survivors of H5N1 influenza infection bind to H5 HA and are able to neutralize both H5N1 and H1N1 viruses [30], indicating that shared neutralizing epitope(s) exist on some H5 and H1 HAs. Although we were not able to detect cross-reacting antibodies to H5 in HI or neutralization assays, it is possible that such antibodies were present at a level below the limit of detection of our assays, but were able to limit viral replication of a highly attenuated virus having an avian-like HA. Antibodies directed at the NA will not prevent infection with influenza virus [32, 33], but can reduce viral replication [33–35]. Cross-reactive antibodies to avian N1 NA have been detected in mice immunized with DNA encoding the human N1 NA from A/New Caledonia/20/99 (H1N1) and in approximately 20% of a small number of adult human sera [33, 36]. Finally, it is possible that the combination of the avian influenza HA and NA genes with internal protein genes from a human influenza virus resulted in a virus with a host-range restricted phenotype for humans. A previous study showed that a reassortant virus containing H5N1 HA and NA and internal protein genes from a human H3N2 influenza virus was less infectious in MDCK cells and in ferrets than the *wt* H5N1 virus [37]. In addition, H5N1 reassortant viruses containing human influenza virus internal protein genes have not been detected, despite coincident epidemics of H5N1 influenza in birds and of H3N2 or H1N1 influenza in humans for more than 10 years. These observations suggest that reassortment between human influenza viruses and avian H5N1 influenza viruses may produce progeny viruses that are restricted in replication in humans.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Two factors may have contributed to the poor antibody responses observed in recipients of these H5N1 LAIVs. First, the highly restricted replication of the H5N1 LAIVs is likely responsible for the antibody responses observed in these clinical trials because generally a virus that does not replicate efficiently presents a low antigen load. Second, the H5 HA is intrinsically less immunogenic in humans than human influenza HAs [1]. The induction of moderate to high levels of HI or neutralizing antibody by inactivated H5N1 virus vaccines has required either large amounts of antigen [1], or the use of adjuvants [3–15].

There were two unexpected findings in this study. The first of these was the inability to predict infectivity and immunogenicity of H5N1 LAIV in humans based upon replication in mice and ferrets. In these small animal models, the H5N1 VN 2004/AA *ca* and H5N1 HK 2003/AA *ca* viruses were each restricted in replication compared to the parent *wt* virus, but were readily recovered by culture: for example, the mean peak viral titer achieved in the nasal turbinates of ferrets was $10^{4.1}$ EID_{50}/gm for the H5N1 VN 2004/AA *ca* virus and $10^{4.5}$ EID_{50}/gm for the H5N1 HK 2003/AA *ca* virus [23]; unpublished data]. In addition, two doses of each vaccine induced HI and neutralizing antibody in these animal models: in ferrets, the mean reciprocal $\log_2$ titers of HI and neutralizing antibodies against the homologous virus were 5.3 and 3.6, respectively for the H5N1 VN 2004/AA *ca* virus, and 10.2 and 10.6, respectively for the H5N1 HK 2003/AA *ca* virus [23]; unpublished data]. In contrast, each of these vaccines replicated minimally in humans, and was detected primarily by rRT-PCR on study day 1, which might represent the presence of input virus. Antibody responses were rarely detected by HI and neutralization assays in these individuals. Taken together, these data suggest that replication of H5N1 LAIV in mice and ferrets were not predictive of replication of these viruses in humans, and that novel models of infectivity should be developed.

The observation that serum IgA was the most sensitive measure of infection with the H5N1 VN 2004/AA *ca* virus was also unexpected. The specificity of the serum IgA response is suggested by: 1) dose response, in that 2 of 21 recipients of $10^{6.7}$ $TCID_{50}$ and 11 of 21 recipients of $10^{7.5}$ $TCID_{50}$ of H5N1 VN 2004/AA *ca* developed serum IgA antibodies to the rH5 antigen and 2) very infrequent serum IgA responses to H1 or H7 antigens (1 to H1 and 1 to H7 in the 42 vaccinees whose sera were tested). The absence of a serum IgA response in recipients of the HK 2003/AA *ca* vaccine might be explained by antigenic differences, since the rH5 used to coat the plates was derived from the VN 2004 H5N1 virus. Serum IgA antibody responses to live attenuated respiratory virus vaccines can be measured in very young infants who may have circulating maternal IgG [38, 39], and has recently been detected in recipients of an H7N3 LAIV vaccine (K. Talaat, Vaccine, in press). The biological significance of this response remains to be determined, but results from this study and the H7N3 vaccine study suggest that the serum IgA assay should be included in future studies of LAIV developed for influenza viruses with pandemic potential.

In summary, two doses of the H5N1 VN 2004/AA *ca* and of the H5N1 HK 2003/AA *ca* virus vaccines were generally well-tolerated and minimally infectious. Serum HI and neutralizing antibody responses to these vaccines were minimal, but serum IgA responses to rH5 HA were detected in approximately 50% of subjects who received the higher dose of H5N1 VN 2004/AA *ca*. The correlates of protection for LAIV against seasonal influenza are

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

unknown, and it therefore remains to be determined whether immunity induced by these H5N1 LAIVs would protect against infection by H5N1 viruses. However, strategies to enhance the infectivity and immunogenicity of the H5N1 LAIV should be pursued.

Acknowledgments

Financial support: National Institutes of Health N01-AI-15444; MedImmune; NIAID Intramural Program.

We wish to thank Philana Liang, Julie McArthur, Barbara DeNearing, Arlene Bloom, Paula Williams-Soro, Nicole Yoder, and Kathy Wang for excellent clinical and technical assistance, Romeo Paredes for data management, Alfred Pan, Lynne Fitch, Silas Nwagwu, Steve Thompson, Chin-Fen Yang, Weidong Cui, Qiao BoBo and Edith Matsuyama of MedImmune for vaccine manufacture and testing, and Jamell Davis and Jason Morsell for assistance with manuscript preparation. This research was supported by the Intramural Research Program of the NIAID, NIH and was performed as part of a Cooperative Research and Development Agreement (CRADA No: AI-0155) between the Laboratory of Infectious Diseases, NIAID and MedImmune.

References

1. Treanor JJ, Campbell JD, Zangwill KM, Rowe T, Wolff M. Safety and immunogenicity of an inactivated subvirion influenza A (H5N1) vaccine. N Engl J Med. 2006 Mar 30; 354(13):1343–51. [PubMed: 16571878]
2. Treanor JJ, Wilkinson BE, Masseoud F, Hu-Primmer J, Battaglia R, O'Brien D, et al. Safety and immunogenicity of a recombinant hemagglutinin vaccine for H5 influenza in humans. Vaccine. 2001 Feb 8; 19(13–14):1732–7. [PubMed: 11166898]
3. Bresson JL, Perronne C, Launay O, Gerdil C, Saville M, Wood J, et al. Safety and immunogenicity of an inactivated split-virion influenza A/Vietnam/1194/2004 (H5N1) vaccine: phase I randomised trial. Lancet. 2006 May 20; 367(9523):1657–64. [PubMed: 16714186]
4. Nicholson KG, Colegate AE, Podda A, Stephenson I, Wood J, Ypma E, et al. Safety and antigenicity of non-adjuvanted and MF59-adjuvanted influenza A/Duck/Singapore/97 (H5N3) vaccine: a randomised trial of two potential vaccines against H5N1 influenza. Lancet. 2001 Jun 16; 357(9272):1937–43. [PubMed: 11425416]
5. Stephenson I, Das RG, Wood JM, Katz JM. Comparison of neutralising antibody assays for detection of antibody to influenza A/H3N2 viruses: an international collaborative study. Vaccine. 2007 May 16; 25(20):4056–63. [PubMed: 17412461]
6. Stephenson I, Bugarini R, Nicholson KG, Podda A, Wood JM, Zambon MC, et al. Cross-reactivity to highly pathogenic avian influenza H5N1 viruses after vaccination with nonadjuvanted and MF59-adjuvanted influenza A/Duck/Singapore/97 (H5N3) vaccine: a potential priming strategy. J Infect Dis. 2005 Apr 15; 191(8):1210–5. [PubMed: 15776364]
7. Leroux-Roels I, Borkowski A, Vanwolleghem T, Drame M, Clement F, Hons E, et al. Antigen sparing and cross-reactive immunity with an adjuvanted rH5N1 prototype pandemic influenza vaccine: a randomised controlled trial. Lancet. 2007 Aug 18; 370(9587):580–9. [PubMed: 17707753]
8. Keitel WA, Atmar RL. Preparing for a possible pandemic: influenza A/H5N1 vaccine development. Curr Opin Pharmacol. 2007 Oct; 7(5):484–90. [PubMed: 17644429]
9. Ehrlich HJ, Muller M, Oh HM, Tambyah PA, Joukhadar C, Montomoli E, et al. A clinical trial of a whole-virus H5N1 vaccine derived from cell culture. N Engl J Med. 2008 Jun 12; 358(24):2573–84. [PubMed: 18550874]
10. Levie K, Leroux-Roels I, Hoppenbrouwers K, Kervyn AD, Vandermeulen C, Forgus S, et al. An adjuvanted, low-dose, pandemic influenza A (H5N1) vaccine candidate is safe, immunogenic, and induces cross-reactive immune responses in healthy adults. J Infect Dis. 2008 Sep 1; 198(5):642–9. [PubMed: 18576945]
11. Nolan T, Richmond PC, Formica NT, Hoschler K, Skeljo MV, Stoney T, et al. Safety and immunogenicity of a prototype adjuvanted inactivated split-virus influenza A (H5N1) vaccine in infants and children. Vaccine. 2008 Nov 25; 26(50):6383–91. [PubMed: 18801398]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

12. Nolan TM, Richmond PC, Skeljo MV, Pearce G, Hartel G, Formica NT, et al. Phase I and II randomised trials of the safety and immunogenicity of a prototype adjuvanted inactivated split-virus influenza A (H5N1) vaccine in healthy adults. Vaccine. 2008 Aug 5; 26(33):4160–7. [PubMed: 18599164]
13. Bernstein DI, Edwards KM, Dekker CL, Belshe R, Talbot HK, Graham IL, et al. Effects of adjuvants on the safety and immunogenicity of an avian influenza H5N1 vaccine in adults. J Infect Dis. 2008 Mar 1; 197(5):667–75. [PubMed: 18260764]
14. Fazekas G, Martosne-Mendi R, Jankovics I, Szilvasy I, Vajo Z. Cross-reactive immunity in adult and elderly patients against clade 2 influenza A H5N1 virus strains induced by Fluval, a reverse genetic-derived adjuvanted H5N1 clade 1 prototype pandemic influenza vaccine. Clin Vaccine Immunol. 2008 Nov 19.19:19.
15. Vajo Z, Kosa L, Szilvasy I, Pauliny Z, Bartha K, Visontay I, et al. Safety and immunogenicity of a prepandemic influenza A (H5N1) vaccine in children. Pediatr Infect Dis J. 2008 Dec; 27(12): 1052–6. [PubMed: 18978514]
16. Belshe RB, Edwards KM, Vesikari T, Black SV, Walker RE, Hultquist M, et al. Live attenuated versus inactivated influenza vaccine in infants and young children. N Engl J Med. 2007 Feb 15; 356(7):685–96. [PubMed: 17301299]
17. Belshe RB, Gruber WC, Mendelman PM, Cho I, Reisinger K, Block SL, et al. Efficacy of vaccination with live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine against a variant (A/Sydney) not contained in the vaccine. J Pediatr. 2000; 136:168–75. [PubMed: 10657821]
18. Halloran ME, Piedra PA, Longini IM Jr, Gaglani MJ, Schmotzer B, Fewlass C, et al. Efficacy of trivalent, cold-adapted, influenza virus vaccine against influenza A (Fujian), a drift variant, during 2003–2004. Vaccine. 2007 May 16; 25(20):4038–45. [PubMed: 17395338]
19. Tam JS, Capeding MR, Lum LC, Chotpitayasunondh T, Jiang Z, Huang LM, et al. Efficacy and safety of a live attenuated, cold-adapted influenza vaccine, trivalent against culture-confirmed influenza in young children in Asia. Pediatr Infect Dis J. 2007 Jul; 26(7):619–28. [PubMed: 17596805]
20. Nichol KL, Mendelman PM, Mallon KP, Jackson LA, Gorse GJ, Belshe RB, et al. Effectiveness of live, attenuated intranasal influenza virus vaccine in healthy, working adults: a randomized controlled trial. JAMA. 1999 Jul 14; 282(2):137–44. [PubMed: 10411194]
21. Mendelman PM, Rappaport R, Cho I, Block S, Gruber W, August M, et al. Live attenuated influenza vaccine induces cross-reactive antibody responses in children against an A/Fujian/ 411/2002-like H3N2 antigenic variant strain. Pediatr Infect Dis J. 2004 Nov; 23(11):1053–5. [PubMed: 15545863]
22. Chen H, Matsuoka Y, Swayne D, Chen Q, Cox NJ, Murphy BR, et al. Generation and characterization of a cold-adapted influenza A H9N2 reassortant as a live pandemic influenza virus vaccine candidate. Vaccine. 2003 Oct 1; 21(27–30):4430–6. [PubMed: 14505926]
23. Suguitan AL Jr, McAuliffe J, Mills KL, Jin H, Duke G, Lu B, et al. Live, attenuated influenza A H5N1 candidate vaccines provide broad cross-protection in mice and ferrets. PLoS Med. 2006 Sep.3(9):e360. [PubMed: 16968127]
24. Joseph T, McAuliffe J, Lu B, Vogel L, Swayne D, Jin H, et al. A live attenuated cold-adapted influenza A H7N3 virus vaccine provides protection against homologous and heterologous H7 viruses in mice and ferrets. Virology. 2008 Jun 26.
25. Karron R, Callahan K, Luke C, Thumar B, McAuliffe J, Schappell E, et al. A live attenuated H9N2 influenza vaccine is well-tolerated and immunogenic in healthy adults. J Infect Dis. 2009 in press.
26. Abdel-Ghafar AN, Chotpitayasunondh T, Gao Z, Hayden FG, Nguyen DH, de Jong MD, et al. Update on avian influenza A (H5N1) virus infection in humans. N Engl J Med. 2008 Jan 17; 358(3):261–73. [PubMed: 18199865]
27. Stephenson I, Wood JM, Nicholson KG, Charlett A, Zambon MC. Detection of anti-H5 responses in human sera by HI using horse erythrocytes following MF59-adjuvanted influenza A/Duck/ Singapore/97 vaccine. Virus Res. 2004 Jul; 103(1–2):91–5. [PubMed: 15163495]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

28. Clements ML, O Donnel S, Levine MM, Chanock RM, Murphy BR. Dose response of A/Alaska/6/77 (H3N2) cold adapted reassortant vaccine virus in adult volunteers: role of local antibody in resistance to infection with vaccine virus. Infect Immun. 1983; 40:1044. [PubMed: 6852910]
29. Sasaki S, Jaimes MC, Holmes TH, Dekker CL, Mahmood K, Kemble GW, et al. Comparison of the influenza virus-specific effector and memory B-cell responses to immunization of children and adults with live attenuated or inactivated influenza virus vaccines. J Virol. 2007 Jan; 81(1):215–28. [PubMed: 17050593]
30. Kashyap AK, Steel J, Oner AF, Dillon MA, Swale RE, Wall KM, et al. Combinatorial antibody libraries from survivors of the Turkish H5N1 avian influenza outbreak reveal virus neutralization strategies. Proc Natl Acad Sci U S A. 2008 Apr 22; 105(16):5986–91. [PubMed: 18413603]
31. Shinya K, Ebina M, Yamada S, Ono M, Kasai N, Kawaoka Y. Avian flu: Influenza virus receptors in the human airway. Nature. 2006; 440(7083):435–6. [PubMed: 16554799]
32. Powers DC, Kilbourne ED, Johansson BE. Neuraminidase-specific antibody responses to inactivated influenza virus vaccine in young and elderly adults. Clin Diagn Lab Immunol. 1996 Sep; 3(5):511–6. [PubMed: 8877127]
33. Gillim-Ross L, Subbarao K. Can immunity induced by the human influenza virus N1 neuraminidase provide some protection from avian influenza H5N1 viruses? PLoS Med. 2007 Feb. 4(2):e91. [PubMed: 17298170]
34. Kilbourne ED, Laver WG, Schulman JL, Webster RG. Antiviral activity of antiserum specific for an influenza virus neuraminidase. J Virol. 1968 Apr; 2(4):281–8. [PubMed: 4911843]
35. Murphy BR, Kasel JA, Chanock RM. Association of serum anti-neuraminidase antibody with resistance to influenza in man. N Engl J Med. 1972; 286:1329. [PubMed: 5027388]
36. Sandbulte MR, Jimenez GS, Boon AC, Smith LR, Treanor JJ, Webby RJ. Cross-reactive neuraminidase antibodies afford partial protection against H5N1 in mice and are present in unexposed humans. PLoS Med. 2007 Feb.4(2):e59. [PubMed: 17298168]
37. Maines TR, Chen LM, Matsuoka Y, Chen H, Rowe T, Ortin J, et al. Lack of transmission of H5N1 avian-human reassortant influenza viruses in a ferret model. Proc Natl Acad Sci U S A. 2006 Aug 8; 103(32):12121–6. [PubMed: 16880383]
38. Karron RA, Belshe RB, Wright PF, Thumar B, Burns B, Newman F, et al. A live human parainfluenza type 3 virus vaccine is attenuated and immunogenic in young infants. Pediatr Infect Dis J. 2003 May; 22(5):394–405. [PubMed: 12792378]
39. Wright PF, Karron RA, Belshe RB, Thompson J, Crowe JE Jr, Boyce TG, et al. Evaluation of a live, cold-passaged, temperature-sensitive, respiratory syncytial virus vaccine candidate in infancy. J Infect Dis. 2000; 182(5):1331–42. [PubMed: 11010838]

Table 1

Clinical and virologic responses to one or two doses of H5N1 recombinant live-attenuated A/Ann Arbor/6/60 *ca* influenza vaccines

			Virus detection in nasal wash					% with indicated illness or laboratory abnormality								
			Culture +			rRT-PCR+										
Participants	**N**	**% infected[a]**	**%**	**Duration[b] mean(±SD), days**	**Peak titer, mean(±SD), $\log_{10}$ $TCID_{50}$/ml[c]**	**%**	**Duration[b] mean (±SD), days**	**Fever**	**URI**	**LRI**	**Cough**	**OM**	**SI**	**HA**	**ALT Elevation[d]**	**Neutropenia[d]**
VN 2004 $10^{6.7}$ $TCID_{50}$																
Dose 1	21	5	0	0	0.6	10	2.0 (1.5)	0	5	5[d]	0	0	0	10	14	0
Dose 2	18	17	0	0	0.6	17	1.0 (0.0)	0	0	0	0	0	33	33	28	0
VN 2004 $10^{7.5}$ $TCID_{50}$																
Dose 1	21	43	0	0	0.6	10	1.3 (0.6)	0	0	0	0	0	0	24	14	0
Dose 2	19	75	0	0	0.6	8	2.5 (2.1)	5[d]	16	0	0	0	0	16	0	16
HK 2003 HK 2003 $10^{7.5}$ $TCID_{50}$																
Dose 1	17	29	6	1.0 (0.0)	1.0(0.0)	47	1.3 (0.5)	0	0	0	0	0	6	18	6	0
Dose 2	16	19	0	0	0.6	56	1.2 (0.4)	0	13	0	0	0	0	25	0	0

[a]Infection was defined as: 1) shedding of vaccine virus detected by culture and/or 2) shedding of vaccine virus detected by rRT-PCR any time after study day 1 and/or 3) a 4-fold rise in serum HI, neutralizing, or H5 IgG or serum IgA antibodies. Participants whose nasal washes were rRT-PCR+ on study day 1 only but were without other evidence of infection were not considered infected because this occurred frequently and we could not exclude the possibility that input virus was being detected.

[b]Duration calculated for those individuals with nasal washes that were culture positive or rRT-PCR positive for vaccine virus, respectively. Throat swabs from recipients of $10^{6.7}$ $TCID_{50}$ of VN 2004 AA *ca* were tested for vaccine virus by culture and rRT-PCR and none were positive.

[c]Culture-negative specimens were assigned a titer of $10^{0.6}$ $TCID_{50}$/mL.

[d]See text for additional details.

[e]URI, upper respiratory tract illness; LRI, lower respiratory tract illness; OM, otitis media; SI, systemic illness (defined as chills and/or myalgias in more than one muscle group); HA, headache.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 2

Antibody responses to one or two doses of H5N1 recombinant live-attenuated A/Ann Arbor/6/60 *ca* influenza vaccines

Vaccine, Dose	N	% with any Ab response	H5 HI titer, mean (±SD)			Neutralizing Ab titer, mean (±SD)			Serum IgG to rH5, mean (±SD)			Serum IgA to rH5, mean (±SD)			NW to rH9, mean (±SD)		
			Before	After	4-fold increase, %	Before	After	4-fold increase, %	Before	After	4-fold increase, %	Before	After	4-fold increase, %	Before	After	4-fold increase, %
VN 2004 $10^{6.7}$ $TCID_{50}$																	
Dose 1	21	19	2.6 (0.9)	2.8 (0.9)	0	2.4 (0.3)	2.4 (0.3)	0	5.5 (1.2)	5.4 (1.2)	0	8.0 (2.2)	8.0 (2.2)	0	5.7 (1.5)	6.2 (1.5)	19
Dose 2	18	11	2.7 (0.9)	3.1 (1.0)	6	2.3 (0.0)	2.3 (0.0)	0	5.5 (1.2)	5.6 (1.2)	0	8.0 (2.3)	8.3 (2.5)	11	6.2 (1.5)	5.9 (0.9)	6
Any dose	21	29	2.6 (0.9)	3.1 (1.0)	10	2.4 (0.3)	2.3 (0.0)	0	5.5 (1.2)	5.6 (1.2)	0	5.3 (2.9)	7.8 (0.8)	10	5.7 (1.5)	5.9 (0.9)	24
VN 2004 $10^{7.5}$ $TCID_{50}$																	
Dose 1	21	48	1.2 (0.6)	1.3 (0.7)	5	2.3 (0.0)	2.3 (0.0)	0	6.2 (1.2)	6.5 (1.4)	24	6.7 (1.8)	7.3 (2.3)	33	5.0 (2.0)	4.7 (1.7)	5
Dose 2	19	43	1.3 (0.7)	1.6 (1.1)	5	2.3 (0.0)	2.4 (0.5)	5	6.6 (1.5)	7.1 (1.3)	24	7.3 (2.2)	8.1 (2.4)	33	4.7 (1.7)	5.0 (1.5)	11
Any dose	21	66	1.2 (0.6)	1.6 (1.1)	10	2.3 (0.0)	2.4 (0.5)	5	6.2 (1.2)	7.1 (1.3)	38	6.7 (1.8)	8.1 (2.4)	52	5.0 (2.0)	5.0 (1.5)	19
HK 2003 $10^{7.5}$ $TCID_{50}$																	
Dose 1	17	18	1.0 (0.0)	1.0 (1.4)	0	2.3 (0.0)	2.3 (0.0)	0	6.3 (1.4)	6.4(1.4)	6	6.4 (1.6)	6.3(1.9)	6	5.0 (2.0)	4.7(1.7)	6
Dose 2	16	19	1.0 (0.0)	1.1 (0.3)	0	2.3 (0.0)	2.3 (0.0)	0	6.4 (1.4)	6.5(1.3)	0	6.3 (1.9)	6.7(1.9)	13	4.7 (1.7)	5.0(1.5)	13
Any dose	17	35	1.0 (0.0)	1.1 (0.3)	0	2.3 (0.0)	2.3 (0.0)	0	6.3 (1.4)	6.5(1.3)	6	6.4 (1.6)	6.7(1.9)	18	5.0 (2.0)	5.0(1.5)	18

All antibody titers are expressed as mean reciprocal $\log_2$ values. Specimens for measurement of serum and nasal wash (NW) antibody were obtained as indicated in Participants, Materials, and Methods; data shown here are from before and 4 to 6 weeks after each vaccination. HI and neutralizing antibodies were measured against the respective vaccine virus. Recombinant H5 VN 2004 was used to measure serum IgG and nasal wash IgA antibody by ELISA. Immulon 2 plates were coated with 30ng/well of recombinant baculovirus-expressed H5 G1 HA (influenza A/HK/1073/99 H9N2; Protein Sciences, Meriden, CT). “Percent of individuals with a response to either dose” includes some individuals with a two-fold response to each dose, so that the percent of participants with a response to both doses may be greater than the sum of the percent responding to each dose.

HI, hemagglutinaton inhibition; Ab; antibody; r, recombinant.

Table 3

Summary of Virus Shedding and Immunological Responses to $10^{7.5}$ $TCID_{50}$ of H5N1 VN 2004/AA *ca*

Subject No.	culture	rRT-PCR	HI Ab	Neut Ab	serum IgG	serum IgA	NW IgA
22	−	−	−	−	−	−	−
23	−	+	−	−	−	−	−
24	−	−	−	−	−	−	−
25	−	−	−	−	−	+	+
26	−	+	−	−	−	−	+
27	−	+	−	−	+	+	−
28	−	+	+	−	+	−	−
29	−	+	−	−	+	+	+
30	−	+	−	−	−	−	−
31[a]	−	−	−	−	−	−	−
32	−	+	−	−	+	+	−
33	−	+	−	−	−	−	−
34	−	+	−	−	−	+	−
35	−	+	−	−	−	−	−
36	+	+	−	−	+	−	−
37	−	+	−	−	−	+	−
38	+	+	+	+	+	+	+
39	−	+	−	−	−	+	−
40	−	−	−	−	−	+	−
41[a]	−	−	−	−	+	+	−
42	−	+	−	−	+	+	−
total	2/21	15/21	2/21	1/21	8/21	11/21	4/21

Abbreviations used are as follows: No.= number, HI= Hemagglutination inhibition assay, Neut= microneutralization assay, Ab=Antibody, NW= Nasal wash. Represents responses following either dose of vaccine.

[a]These subjects received only 1 dose of vaccine

Author Manuscript Author Manuscript Author Manuscript Author Manuscript