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Evaluation of the Safety and Immunogenicity of a Candidate Pandemic Live Attenuated Influenza Vaccine (pLAIV) Against Influenza A(H7N9)

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Background. We evaluated a candidate A/Anhui/2013(H7N9) pandemic live attenuated influenza vaccine (pLAIV) in healthy adults, and assessed the ability of 1 or 2 doses to induce immune memory.

Methods. Healthy subjects in 2 age groups (18–49 years and 50–70 years) with undetectable hemagglutination-inhibiting (HAI) antibody to H7N9 were enrolled. Younger subjects received either 1 or 2 intranasal doses of $10^{7.0}$ fluorescent focus units of A/Anhui/1/2013 pLAIV, while older subjects received a single dose. All subjects received a single 30-μg dose of unadjuvanted, antigenically matched A/Shanghai2/2013(H7N9) pandemic inactivated influenza vaccine (pIIV) 12 weeks after their first dose of pLAIV.

Results. Both vaccines were well tolerated. Serum HAI antibody responses were detected in 0 of 32 younger subjects and 1 of 17 older subjects after 1 dose of pLAIV and in 2 of 16 younger subjects after a second dose. Strong serum antibody responses were detected after a single subsequent dose of pIIV that was broadly reactive against H7 influenza viruses.

Conclusions. An A(H7N9) pLAIV candidate was safe in both age groups. Priming with pLAIV resulted in responses to subsequent pIIV that exceeded those seen in naive subjects in previous reports. The A(H7N9) pLAIV induces strong immune memory that can be demonstrated by exposure to subsequent antigenic challenge.

Clinical Trials Registration. NCT01995695 and NCT02274545.

Keywords. pandemic influenza; live vaccine; immune memory.

Severe human disease due to influenza A(H7N9) virus is continuing to occur in China [1, 2]. In contrast to the experience with influenza A(H5N1) virus in humans, severe disease and hospitalization due to influenza A(H7N9) virus have predominantly affected older adults and those with chronic illnesses [3]. New cases have been recognized each winter, and influenza A (H7N9) virus is considered to pose a pandemic threat.

Vaccines for control of influenza viruses with pandemic potential are under development, and results of clinical trials have suggested that these vaccines will require the use of adjuvants and multiple doses to induce substantial serum antibody responses [4, 5]. An alternative approach is the development of pandemic live attenuated influenza vaccines (pLAIVs). Since seasonal LAIVs appear to be immunogenic and highly effective in children [6–8], they would in theory be an excellent option for pandemic control. Evaluation of a number of pLAIV candidates have shown them to be well tolerated in healthy adults but infrequently associated with serum antibody responses [9–12].

Two studies have now demonstrated that pLAIV recipients respond to subsequent doses of antigenically matched pIIV with a rapid and vigorous antibody response that suggests that pLAIVs established immunologic memory [13, 14]. In a previous study involving H7 vaccines, recipients of 2 doses of an A/Netherlands/219/03(H7N7) pLAIV but not recipients of 1 dose of an antigenically distinct A/chicken/British Columbia/CN-6/04(H7N3) pLAIV had a vigorous serum antibody response to an unadjuvanted A(H7N7) pIIV given 18 months later [14]. It was unclear in that study whether the absence of a response to the pIIV by A (H7N3) pLAIV recipients was because of antigenic mismatch or because of the number of doses of pLAIV administered.

In the current study, we evaluated a candidate A(H7N9) pLAIV and directly compared the safety, viral shedding pattern, and immunogenicity in younger subjects who received either 1 or 2 doses of pLAIV. Because of the age distribution of individuals infected with influenza A(H7N9) virus, we also enrolled a second cohort of older subjects who received a single dose. All subjects received a single dose of antigenically matched unadjuvanted A(H7N9) pIIV 12 weeks after pLAIV receipt. We found that pLAIV primed both older and younger subjects to respond

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to a single subsequent dose of pIIV. These results suggest that A(H7N9) pLAIV can effectively prime the immune system to respond to subsequent doses of unadjuvanted A(H7N9) pIIV.

METHODS

Vaccines

The pLAIV used in this study was generated by plasmid rescue in Vero/primary chicken embryo kidney cell coculture as a 6–2 reassortant deriving the hemagglutinin (HA) and neuraminidase genes from human influenza A/Anhui/1/2013(H7N9) virus and all other gene segments from the cold-adapted influenza A/Ann Arbor/6/60 master donor virus and was produced in embryonated hen's eggs. This vaccine was shown to provide protection against challenge with wild-type influenza A(H7N9) and A(H7N7) viruses in the ferret model [15]. Sequence analysis of the neuraminidase gene predicted it to be sensitive to the antiviral drug oseltamivir.

The inactivated vaccine used in this study was an unadjuvanted split-virion A(H7N9) vaccine derived from the antigenically identical influenza A/Shanghai/2/2013(H7N9) virus (Sanofi Pasteur, Swiftwater, Pennsylvania), provided by the Biomedical Advanced Research and Development Authority. The potency of the vaccine was determined to be 30 μg per 0.5-mL dose by reverse-phase high-pressure liquid chromatography [16].

Study Design

Evaluation of the A/Anhui/1/2013 pLAIV was performed using previously published methods [9,12,17]. The first stage enrolled subjects aged 18–49 years, and eligible subjects were assigned, based on their preference, to receive either a single dose of A(H7N9) pLAIV or 2 doses separated by 28 days. After we determined that the A(H7N9) pLAIV candidate was well tolerated in younger subjects, healthy older subjects aged 50–70 years were subsequently enrolled and received a single dose of A(H7N9) pLAIV.

For each dose of A(H7N9) pLAIV, subjects were admitted to an isolation facility and observed for 2 days, and then received $10^{7.0}$ fluorescent focus units of the vaccine virus in a volume of 0.5 mL by intranasal spray in open label fashion. Physical examination and assessment of reactogenicity events were performed daily after inoculation, until discharge. Among younger subjects, a nasal wash specimen was obtained daily for virus detection, while among older subjects, a nasal swab specimen was obtained daily. The presence of influenza virus was determined by inoculation of Madin-Darby canine kidney (MDCK) cells at 33°C and by real-time reverse-transcription polymerase chain reaction (RT-PCR) analysis as previously described [18]. Subjects were discharged from the facility on day 9 after inoculation if they had at least 2 consecutive real-time RT-PCR tests negative for vaccine virus.

Subjects returned approximately 12 weeks after their first dose of pLAIV to receive a single booster dose of 30 μg of the unadjuvanted A(H7N9) pIIV by intramuscular injection. The study was approved by the University of Rochester Institutional Review Board, and written informed consent was obtained from all participants.

Characterization of the RNA Sequence of Shed Virus

Viral RNA was extracted from nasal wash and swab samples, using the NucliSENS easyMag system (bioMérieux). The SuperScript III One-Step RT-PCR system and Platinum Taq DNA polymerase were used to reverse transcribe viral RNA and amplify complementary DNA (Invitrogen). RT-PCR products were amplified in nested PCR reactions using the Herculase II Fusion DNA polymerase enzyme with the dNTP Combo kit (Agilent). Nested PCR–amplified products were run on 1% agarose gels, and gels containing a visible band denoting the expected fragment size were purified and sent to Sequetech (Mountain View, California) for sequencing. The sequences were then assembled and aligned to the reference vaccine A(H7N9) sequence. The 3 temperature-sensitive/attenuated loci of the polymerase basic 1 (PB1) gene of the cold-adapted influenza A/Ann Arbor/6/60 master donor virus [19] were also sequenced with a similar strategy to ensure that the viral RNA originated from the A(H7N9) LAIV.

Serological Analysis

Sera were tested by hemagglutination-inhibition (HAI) and microneutralization (MN) assays against the vaccine virus and by MN assays against other wild-type H7 viruses. Wild-type influenza A/Anhui/1/2013(H7N9) and A/Hong Kong/734/2014(H7N9) viruses were kindly provided by Nancy Cox (Influenza Division, Centers for Disease Control and Prevention, Atlanta, Georgia). Wild-type influenza A/Netherlands/219/2003(H7N7) virus was kindly provided by David Swayne (Southeast Poultry Research Laboratory, US Department of Agriculture, Athens, Georgia), and wild-type influenza A/chicken/British Columbia/CN-7/2004(H7N3) virus was kindly provided by John Pasick (Canadian Food Inspection Agency, National Centre for Foreign Animal Diseases, Winnipeg, Canada).

HAI assays were performed on sera after treatment with receptor-destroying enzyme (Denka Seiken), using 0.75% or 1% horse erythrocytes [12] with 4 hemagglutination units of virus, beginning at a 1:4 dilution of serum. MN assays were performed on MDCK cells as previously described [20], except that assays using the pLAIV viruses were performed at 33°C. For serum HAI and MN antibody assays, subjects were defined as responders if they achieved a ≥4-fold increase in antibody titer, compared with the baseline value, at any time point after vaccination.

Sera in the younger cohort were also tested for HA-specific antibody by an enzyme-linked immunosorbent assay (ELISA). Ninety-six–well Nunc Maxisorb plates (Thermal Scientific) were coated with purified baculovirus-expressed H7 HA protein from influenza A/Anhui/1/2013 virus (BEI Resources) at

0.25 μg/well and blocked with 5% nonfat dry milk. Sera were tested at a starting dilution of 1:100, and binding was detected with alkaline phosphatase–conjugated isotype-specific goat anti-human immunoglobulin G (IgG), immunoglobulin M (IgM), or immunoglobulin A antibody (Invitrogen, Frederick, Maryland). The end point titer was the highest dilution giving an optical density at least twice that of background. A 4-fold increase in titer over baseline was considered a response.

Sera collected before and 28 days after pLAIV and pIIV boost in younger subjects were also tested for antibody that could mediate antibody-dependent cellular cytotoxicity (ADCC) using a modified flow-based assay as previously described [21, 22]. Briefly, wells of a 96-well ELISA plate (Maxisorp, Nunc) were coated with 600 ng/well of recombinant HA protein (Sino Biological) overnight at 4°C. Wells were washed with 1 × PBS and incubated with diluted human sera (1:20–1:81 920) for 2 hours at 37°C. Wells were washed with 1 × PBS and incubated with 100–500 000 NK-92 cells stably expressing human CD16 (Conkwest) for 5 hours at 37°C in 10% CO_2. Following incubation, cells were stained with CD107a APC-Cy7 (clone H4A3; BD) and fixed with 10% paraformaldehyde. Cells were analyzed via flow cytometry, and the end point titer was defined as the highest dilution of antibody inducing CD107a expression from natural killer (NK) cells at a level that was at least twice the background level in antigen-negative wells.

Statistical Analysis

Mann–Whitney rank sum tests were used to compare the duration of viral shedding between groups, and the Fisher exact test was used to compare rates. Because real-time RT-PCR positivity on the day after vaccination could represent input virus, infection was considered to be present on the basis of a positive result of the real-time RT-PCR assay on any day after day 1, isolation of vaccine virus in cell culture at any time after administration, or a 4-fold increase in serum HAI antibody titer between the specimen obtained before vaccination and the specimen obtained 28 days after vaccination [12].

RESULTS

Safety

Administration of 1 or 2 doses of A(H7N9) pLAIV to serosusceptible young adults was well tolerated (Table 1). The frequency of systemic complaints in the 9 days following pLAIV receipt was low, with mild headache being the most frequent complaint, and there were no differences in systemic reactogenicity between the first and second dose. Nasal symptoms were more frequent, but there were no complaints of greater than mild severity. The frequency of complaints of runny nose and stuffy nose was less after the second dose than after the first dose, but the differences were not statistically significant. All complaints had resolved by the time of discharge from the isolation facility.

Because of the age distribution of disease due to influenza A (H7N9) virus in humans, we also evaluated a single dose of A (H7N9) pLAIV in adults 50–70 years of age, an age group in which pLAIV candidates have not been tested previously. The reactogenicity profile in this age group was very similar to that in younger adults, consisting primarily of mild nasal symptoms and headache.

Two of the young adult subjects in the single-dose pLAIV group did not return for further visits, leaving 47 subjects across the 2 age cohorts who received the subsequent A(H7N9) pIIV boost. The inactivated vaccine was also well tolerated, with the most common adverse event being mild tenderness at the site of injection (Table 2). Local tenderness was slightly more common in the older age group. There were no severe complaints, and there was no difference in the reactogenicity of the pIIV between those who received 1 dose and those who received 2 doses of pLAIV.

Table 1. Frequency of Reactogenicity Events Following Administration of A(H7N9) Pandemic Live Attenuated Influenza Vaccine

	Subjects Experiencing Reactogenicity Event, by Cohort and Severity, No. (%)								
	Younger Cohort, Dose 1 (n = 32)			Younger Cohort, Dose 2 (n = 16)			Older Cohort, Dose 1 (n = 17)		
Complaint	Mild	Moderate	Severe	Mild	Moderate	Severe	Mild	Moderate	Severe
Body ache	6 (19)	1 (3)	0	0	0	0	1 (6)	0	0
Chills	4 (13)	0	0	0	0	0	1 (6)	0	0
Cough	3 (9)	0	0	1 (6)	0	0	3 (18)	0	0
Earache	2 (6)	0	0	0	0	0	1 (6)	0	0
Eye irritation	2 (6)	0	0	0	0	0	3 (18)	0	0
Headache	10 (31)	4 (13)	0	5 (31)	2 (13)	0	3 (18)	1 (6)	0
Nosebleed	4 (13)	0	0	1 (6)	0	0	3 (18)	0	0
Runny nose	12 (38)	0	0	0	0	0	3 (18)	1 (6)	0
Sore throat	3 (9)	0	0	1 (6)	0	0	0	0	0
Stuffy nose	11 (34)	0	0	3 (19)	0	0	5 (29)	0	0

For each subject, the most-severe event is reported.

Table 2. Frequency of Complaints Reported Within 7 Days After Receipt of Split-Virion A(H7N9) Pandemic Inactivated Influenza Vaccine

	Subjects Reporting Complaint, by Cohort and Severity, No. (%)								
	Younger Cohort, 1-Dose Prime (n = 14)			Younger Cohort, 2-Dose Prime (n = 16)			Older Cohort, 1-Dose Prime (n = 17)		
Site, Complaint	Mild	Moderate	Severe	Mild	Moderate	Severe	Mild	Moderate	Severe
Injection site									
Pain	1 (7)	0	0	2 (13)	0	0	2 (12)	0	0
Redness	0	0	0	1 (6)	0	0	4 (24)	0	0
Swelling	0	0	0	1 (6)	0	0	3 (18)	0	0
Tenderness	5 (36)	0	0	4 (25)	1 (6)	0	5 (29)	0	0
Systemic									0
Body ache	2 (14)	0	0	1 (6)	1 (6)	0	1 (6)	0	0
Chills	1 (7)	0	0	0	0	0	1 (6)	0	0
Cough	1 (7)	1 (7)	0	1 (6)	0	0	0	0	0
Headache	1 (7)	0	0	0	3 (19)	0	0	0	0

For each subject, the most-severe complaint is reported.

Vaccine Virus Shedding

Recovery of the pLAIV virus from the nasopharynx of study subjects was infrequent. (Table 3). Virus was primarily detected only by real-time RT-PCR, mostly on the day immediately following vaccine administration. Virus was recovered in cell culture after 8 of 65 vaccine doses (12.3%), with roughly equal frequency in young adults after dose 1 and dose 2 and slightly higher frequency in older subjects. If vaccine virus infection is defined as detection of the virus in culture or by real-time RT-PCR at any time after day 1 or as development of a ≥4-fold increased HAI antibody response, then approximately 20%–30% of subjects were infected after each dose of pLAIV. There did not appear to be significant differences in the infectivity of the vaccine between the first and second dose in the 2-dose group. Among those who received 2 doses, infection occurred in 3 of 16 (18%) after the first dose. Among the 3 subjects with evidence of infection after dose 1, 1 subject (33%) was also infected after dose 2, while of the 13 subjects without evidence of infection after dose 1, 2 (15%) were infected after dose 2.

Detection of vaccine virus was more frequent and of longer duration in the older subjects. One older subject in particular had shed virus detected by real-time RT-PCR daily for 8 days following inoculation and was also culture positive on days 3–8 after inoculation. To facilitate discharge from the isolation facility, the subject was treated with 75 mg of oseltamivir twice daily beginning in the afternoon of day 8 and was real-time RT-PCR and culture negative on days 9, 10, and 11.

Sufficient signal for sequencing was only obtained from 1 sample from the younger group and during the very prolonged period shedding in the older subject. A sample from day 5 after inoculation of dose 2 in a younger subject showed 100% identity to the open reading frame of the N9 vaccine reference sequence. The 3 temperature-sensitive and attenuated loci in the PB1 gene were present, indicating that the sequenced viral RNA originated from the administered vaccine. Sequencing of samples from day 4 and from day 8 of shedding from the older subject with prolonged shedding also determined that the HA and neuraminidase genes on both days were identical to the original vaccine sequences and that the

Table 3. Shedding of A(H7N9) Live Attenuated Influenza Vaccine (LAIV), Determined by Real-time Reverse-Transcription Polymerase Chain Reaction Analysis (PCR) and Culture, Among Subjects After Receipt of the First or Second Vaccine Dose

	Subjects, No. (%)				PCR Findings Among Shedders	
Cohort, Dose	Overall	PCR Positive[a]	Culture Positive	Infected[b]	Duration, d, Mean ± SE	CT AUC, Mean ± SE[c]
Younger						
Dose 1	32	18 (56)	2 (6)	6 (19)	1.7 ± 0.4	5.0 ± 0.9
Dose 2	16	11 (68)	2 (13)	5 (31)	1.6 ± 0.4	4.4 ± 0.9
Older						
Dose 1	17	13 (76)	4 (24)	6 (35)	2.4 ± 0.6	9.0 ± 3.4

None of the differences between groups reached statistical significance.

Abbreviation: SE, standard error.

[a] Subjects with any PCR-positive result on any day after administration of LAIV.

[b] Subjects were identified as infected if they shed vaccine virus beyond day 1 or if they manifested a ≥4-fold increased hemagglutination-inhibiting antibody response from baseline to day 28 (dose 1) or 56 (dose 2).

[c] The area under the curve (AUC) was calculated as the sum of the difference between the cycle time (CT) and 40 (the cutoff point) for all positive samples.

Table 4. Serum Antibody Response to A(H7N9) Pandemic Live Attenuated Influenza Vaccine (pLAIV) and Pandemic Inactivated Influenza Vaccine (pIIV)

		Subjects Responding[a] to pLAIV Prime or pIIV Boost, by Assay, No. (%)							
		Following pLAIV Prime				Following pIIV Boost			
Cohort, Dose	Overall	HAI	MN	IgG	IgA	HAI	MN	IgG	IgA
Young									
1 dose	14	0	0	0	0	8 (57)	9 (64)	9 (64)	6 (43)
2 dose	16	2 (13)	2 (13)	0	0	13 (81)	15 (93)	14 (88)	10 (63)
Older									
1 dose	17	1 (6)	1 (6)	NT	NT	10 (59)	8 (47)	NT	NT

Abbreviations: HAI, hemagglutination-inhibition assay; IgA, immunoglobulin A–specific enzyme-linked immunosorbent assay; IgG, immunoglobulin G–specific enzyme-linked immunosorbent assay; MN, microneutralization assay; NT, not tested.

[a] Defined as a ≥4-fold increase in titer between baseline and day 56, for pLAIV, or between the time before pIIV receipt to day 28 after pIIV receipt.

cold-adapted and temperature-sensitive loci on the PB1, PB2, and nucleoprotein genes were unchanged from the vaccine.

Immune Response

Two younger subjects, both in the 2-dose group, had a 4-fold increase in HAI antibody (from <1:4 to 1:32) and MN antibody (from <1:10 to 1:20) titers between day 28 and day 56 after receipt of pLAIV (Table 4). One of these subjects shed virus beyond day 1 after dose 1 but not dose 2, while the other subject did not shed virus beyond day 1 after either dose. In the older group, the subject who exhibited prolonged shedding of vaccine virus after inoculation also had an increase in both HAI and MN antibody to A(H7N9) pLAIV. No other subjects had measurable serum antibody HAI or MN responses to administration of either dose of pLAIV.

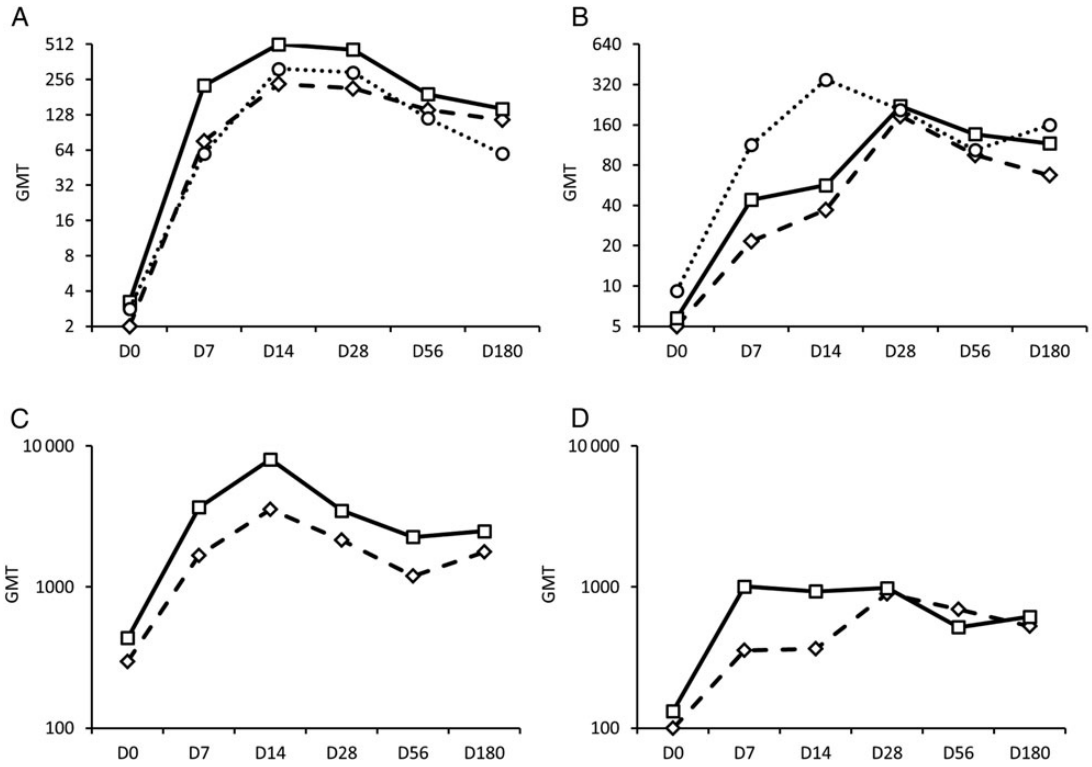

Figure 1. Kinetics of the serum antibody response to a single dose of unadjuvanted A(H7N9) inactivated influenza vaccine (IIV) in younger subjects who previously received 1 dose (diamonds, dashed lines) or 2 doses (squares, solid lines), or older subjects (circles, dotted lines) who received 1 dose of A(H7N9) live attenuated influenza vaccine (LAIV). Data are for subjects considered responders by that specific test. *A*, Results of the hemagglutination-inhibition assay, using the A(H7N9) LAIV and horse red blood cells. *B*, Results of the microneutralization assay, using the A(H7N9) LAIV. *C*, Results of the immunoglobulin G–specific enzyme-linked immunosorbent assay (ELISA), using baculovirus-expressed A/Anhui/1/13(H7N9) hemagglutinin. *D*, Results of the immunoglobulin A–specific ELISA, using baculovirus-expressed A/Anhui/1/13(H7N9) hemagglutinin. ELISA was only performed on sera obtained from younger subjects. Abbreviation: GMT, geometric mean titer.

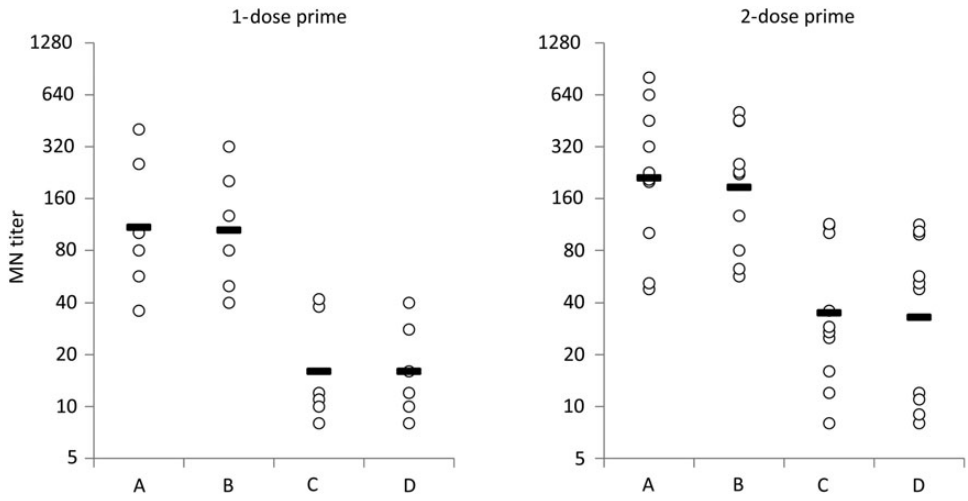

Figure 2. Neutralizing antibody titers against wild-type influenza A(H7N9) viruses. Sera collected 28 days after inactivated influenza vaccine boost in younger recipients of 1 (left) or 2 (right) doses of live attenuated influenza vaccine priming were tested by microneutralization (MN) assay against the following wild-type H7 influenza viruses under biosafety level 3 conditions: A/Anhui/1/2013 (H7N9, human; *A*), A/Hong Kong/734/2014 (H7N9, human; *B*), A/Netherlands/219/2003 (H7N7, human; *C*), A/ck/British Columbia/CN-7/2004 (H7N3, avian; *D*). Data are shown for sera that had a titer of ≥1:32 against the homologous influenza A/Anhui/1/2013 virus. The solid bar represents the mean.

In contrast, 57% of younger subjects in the 1-dose pLAIV group and 64% in the 2-dose group had ≥4-fold increased serum HAI responses, and 81% and 93% had serum MN responses to a subsequent dose of unadjuvanted A(H7N9) pIIV. Similarly, 59% of older subjects had a HAI response to a subsequent pIIV boost after a single dose of pLAIV, and 47% had a MN response. All of the subjects who responded achieved HAI titers of >1:40 against the A/Anhui/1/2013(H7N9) pLAIV virus. The frequency of MN and HAI responses was similar in younger and older subjects who received a single priming dose of pLAIV and somewhat higher in those who received 2 doses of the antigenically matching A(H7N9) pLAIV, but the differences were not statistically significant. Responses detected by ELISA to the H7 HA were primarily IgG, and no IgM responses were detected (data not shown).

The kinetics of the antibody response among those subjects who had a ≥4-fold increased response are shown in Figure 1. Increases in the antibody titer to A(H7N9) could be detected as early as 7 days after pIIV in both younger and older subjects. Antibody was still detectable in these subjects 180 days after receipt of the inactivated vaccine. There were no significant differences when the titers of antibody achieved by responders were compared between the 1-dose and 2-dose primed groups or between the 1-dose younger and older groups.

Sera obtained from the younger subjects after pIIV receipt were also tested for neutralization activity against the wild-type influenza A/Anhui/1/2013(H7N9) virus, as well as a more recent influenza A/Hong Kong/734/2014(H7N9) virus, the wild-type human influenza A/Netherlands/219/2003 (H7N7) virus, and the wild-type North American lineage avian influenza A/ck/British Columbia/CN-7/2004(H7N3) virus (Figure 2). Inactivated vaccine boosting resulted in neutralizing antibody that recognized the 2014 human influenza A(H7N9) virus isolate at approximately equal titers to the homologous 2013 influenza A(H7N9) virus. In addition, responding subjects generated antibody to the previous Eurasian lineage human influenza A(H7N7) virus, as well as the North American lineage avian influenza A(H7N3) virus, although neutralizing titers against these viruses were lower.

We also used a modified NK cell activation assay to measure ADCC responses to the H7 HA in the younger cohort. In subjects that received either a single dose or 2 doses of A(H7N9) pLAIV, there was no detectable rise in ADCC titer (Figure 3*A* and 3*B*). However, all subjects had a significant rise in H7-specific ADCC following pIIV receipt, with geometric mean titers increasing from 93 to 2128 (Figure 3*C*). Further, there was a significant difference in the post-IIV ADCC titer in subjects who had been primed with 1 versus 2 doses of pLAIV (Figure 3*D*). The ADCC titer strongly correlated with the HAI titer following pIIV receipt, suggesting that A(H7N9)-specific HAI antibodies may also have ADCC activity ($R = 0.7850$; $P < .001$; data not shown). Thus, the pLAIV-IIV regimen induced robust levels of H7-specific ADCC–mediating antibodies in healthy adults.

DISCUSSION

Consistent with the findings of other studies of pandemic formulations of LAIV based on this master donor virus, the A(H7N9) pLAIV was well tolerated in younger subjects, with a minority of subjects experiencing mild, self-limited nasal symptoms following vaccination. The safety of pLAIV candidates has not been previously evaluated in older subjects, but because the primary age group affected by influenza A(H7N9)

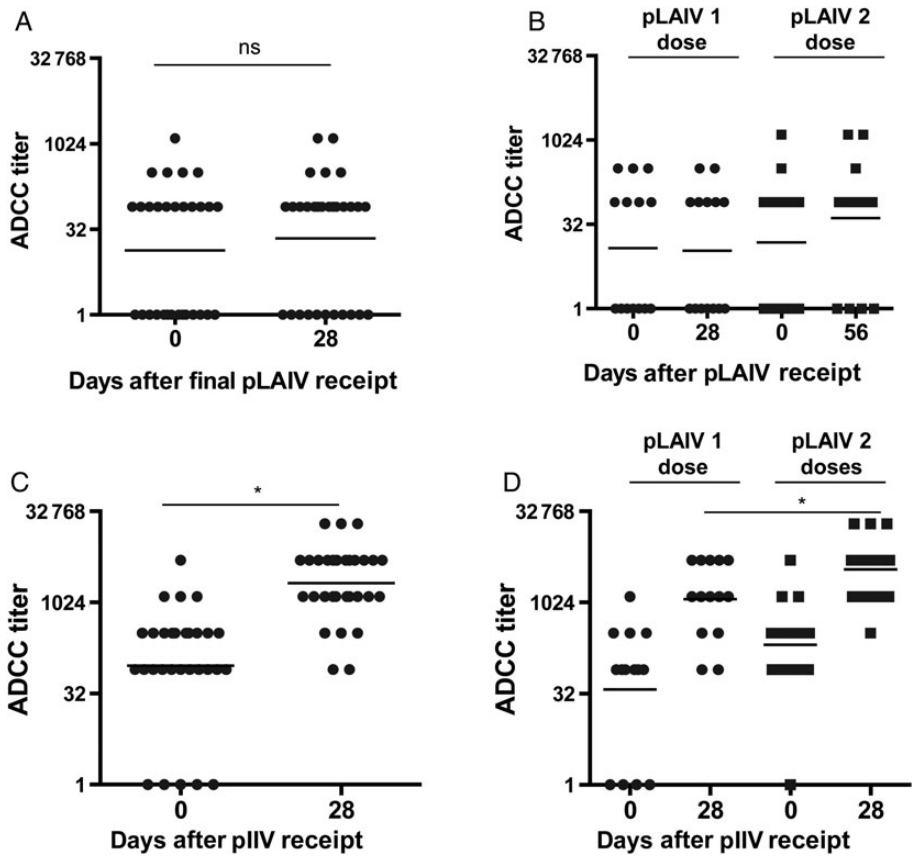

Figure 3. Induction of A(H7N9)-specific antibody-dependent cellular cytotoxicity (ADCC) following a pandemic live attenuated influenza vaccine (pLAIV) and pandemic inactivated influenza vaccine (pIIV) regimen. ADCC titer to rH7 hemagglutinin (A/Anhui/01/2013) in serum obtained from younger subjects at day 0 and day 28 following receipt of either 1 dose (n = 14) or 2 doses (n = 16) of A(H7N9) pLAIV (*A* and *B*) and following receipt of an A(H7N9) pIIV boost (*C* and *D*). The line indicates the geometric mean value for the group. $^*P < .05$, by the Student *t* test.

virus infection has been older, with a mean age of 63 years [3], the results of the current study, which suggest that pLAIV is well tolerated in this age group, are reassuring.

The nasopharyngeal replication of the A(H7N9) pLAIV was extremely limited in both younger and older subjects. The reasons for the highly restricted replication of the vaccine virus in most subjects are unclear, since previous studies have demonstrated that similar vaccine viruses replicate well in animal models [15]. However, some subjects demonstrated prolonged replication of the vaccine virus, which, in 1 subject, did not terminate until oseltamivir was administered.

Serum antibody responses to pLAIV were only detected in 3 subjects. Although the amount of virus detected after the second dose in younger subjects was somewhat reduced as compared to the amount detected after the first dose, there was no evidence that individuals with detectable virus after dose 1 were more or less likely to have detectable virus after dose 2. Detection of virus shedding by PCR or culture did not predict antibody response to pLAIV or boosting, although the single subject with prolonged viral replication manifested a detectable serum HAI and MN response.

Emerging data suggest that, although cold-adapted pLAIV candidates do not induce easily detectable primary immune responses, they prime individuals for vigorous serum antibody responses to subsequent doses of poorly immunogenic inactivated subvirion vaccines [13, 14]. These studies have evaluated subjects who received 2 doses of pLAIV and were given inactivated vaccine ≥2 years later. Therefore, in this study we directly compared the priming effect of a single dose of pLAIV to that of 2 doses administered 28 days apart and whether boosting could be detected at approximately 12 weeks after priming, as suggested by studies evaluating inactivated vaccine boosting after DNA vaccination [23, 24].

Both 1 and 2 doses of pLAIV primed subjects for a response to inactivated A/Shanghai/2/2013(H7N9) vaccine. Although we did not have a concurrent control group of naive subjects who received the inactivated vaccine, in a previous study of the same A(H7N9) pIIV performed in naive subjects [4], serum HAI antibody responses were detected in only 2 of 99 subjects (2%) after a single dose of 45 μg of pIIV, and MN responses were detected in 1 of 99 recipients (1%). Even 2 doses of 45 μg of unadjuvanted A(H7N9) generated HAI and MN responses in only 5% and 21% of subjects, respectively.

The induction of antibodies that have nonneutralizing effector functions, such as ADCC, may provide some enhanced protection from severe influenza virus infection. We have previously shown moderate to low H7-specific ADCC in the general population [22]. In the current study, we found that approximately half of the younger subjects (16 of 30) had low, but detectable cross-reactive ADCC toward recombinant H7 HA before they received the initial dose of pLAIV. While the A (H7N9) pLAIV did not induce substantial increases in ADCC toward H7 HA, a single subsequent dose of A(H7N9) pIIV induced significant levels of H7-specific ADCC in all subjects. Passive transfer studies in mice suggest that the protective ability of anti-HA stem antibodies is mediated by ADCC [25, 26]. Assessment of ADCC responses to pandemic vaccines could be helpful in defining their protective potential.

Other differences between the 1-dose and 2-dose groups were not statistically significant. However, there was a trend toward more-frequent HAI and MN responses to inactivated vaccine in those who received 2 priming doses of pLAIV. The titers of antibody achieved by those who responded were not different in the 2 groups, suggesting that the major contribution of the second dose was to prime additional subjects who, for unknown reasons, were not primed by the first dose.

In summary, with 3 different pLAIV-pIIV combinations (A [H5N1], A[H7N7], and A[H7N9]), we have demonstrated rapid, robust, high-quality, cross-reactive antibody responses after the boost with antigenically matched pIIV. The observation of priming for subsequent responses to pIIV in the absence of an antibody response to the priming immunization is similar in some ways to the response to A(H5N1) pIIV in recipients of DNA vaccine [23, 24]. Although the specific immune mechanisms responsible remain to be elucidated, these observations expand the options potentially available for eliciting robust responses to avian H5 and H7 HAs through vaccination.

Notes

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