CONCISE COMMUNICATION

Comparison of the Safety, Vaccine Virus Shedding, and Immunogenicity of Influenza Virus Vaccine, Trivalent, Types A and B, Live Cold-Adapted, Administered to Human Immunodeficiency Virus (HIV)–Infected and Non–HIV-Infected Adults

James C. King, Jr.,[1] John Treanor,[4] Patricia E. Fast,[5] Mark Wolff,[2] Lihan Yan,[2] Dominic Iacuzio,[3,a] Bernard Readmond,[1] Diane O'Brien,[4] Kenneth Mallon,[5] William E. Highsmith,[1] John S. Lambert,[1] and Robert B. Belshe[6]

[1]University of Maryland Medical Center, Baltimore, [2]EMMES Corp., Potomac, and [3]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland; [4]University of Rochester Medical Center, Rochester, New York; [5]Aviron, Mountain View, California; [6]Saint Louis University School of Medicine, St. Louis, Missouri

Fifty-seven human immunodeficiency virus (HIV)–infected (CDC class A1-2) and 54 non–HIV-infected adults, not prescreened for influenza susceptibility, were randomized to receive trivalent live attenuated influenza vaccine (LAIV) or placebo intranasally. LAIV was safe and well tolerated with no serious adverse events attributable to vaccine. Reactogenicity rates were similar in LAIV and placebo recipients except that runny nose/nasal congestion was significantly more common in LAIV recipients regardless of HIV status. No prolonged shedding of LAIV was observed in HIV-infected participants. HIV RNA levels were not increased and CD4 counts were not decreased in HIV-infected LAIV recipients compared with placebo recipients after immunization. Shedding of LAIV and increases in antibody titers were infrequent, consistent with prior experience in unscreened adults. The data suggest that inadvertent vaccination with LAIV in relatively asymptomatic HIV-infected adults would not be associated with frequent significant adverse events.

Investigational trivalent, live attenuated, cold-adapted influenza vaccine (LAIV) is safe, well tolerated, and efficacious against influenza illness in children and adults [1–4]. Some undiagnosed asymptomatic or mildly symptomatic human immunodeficiency virus (HIV)–infected persons might be exposed to LAIV inadvertently if this vaccine were licensed and gained wide acceptance. Therefore, an assessment of the safety, duration of LAIV virus shedding, effect on HIV replication, and effect on CD4 cell counts in relatively asymptomatic HIV-infected persons would allow clinicians and public health officials to more accurately determine the risks and benefits of the use of this live vaccine in the general population. This study examined the overall safety of administering LAIV to adults with asymptomatic or mildly symptomatic HIV infection.

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Written informed consent was obtained from all study subjects. Human experimentation guidelines of the US Department of Health and Human Services and of each institution (University of Maryland at Baltimore, University of Rochester) were followed during this research study.

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[a] Present affiliation: Roche Laboratories, Nutley, New Jersey.

Reprints or correspondence: Dr. James King, Center for Vaccine Development, University of Maryland School of Medicine, 700 W. Lombard St., Baltimore, MD 21201 (jking@som.umaryland.edu).

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Methods

Vaccine. LAIV and placebo were supplied by Aviron (Mountain View, CA). LAIV consisted of 10^7 $TCID_{50}$ each of A/Shenzhen/227/95 (H1N1), A/Wuhan/359/95 (H3N2), and B/Harbin/7/94-like strains of attenuated cold-adapted influenza virus in egg allantoic fluid. Placebo consisted of normal egg allantoic fluid. LAIV and placebo were stored frozen at −20°C. Study vaccine was delivered as a 0.5-mL intranasal spray (0.25 mL in each nostril) as previously described [2]. The vaccine was reassayed for potency ~1 month after the study was completed and found to have ~10^7 $TCID_{50}$ of each of the 3 cold-adapted influenza virus strains.

Subjects and randomization. Approximately equal numbers of HIV-infected and non–HIV-infected participants were enrolled into the study from the University of Maryland Medical Center and the University of Rochester Medical Center. Participants were 18–58 years of age and in good general health and were not prescreened to have low serum influenza antibodies. Female participants agreed to use acceptable methods of birth control for 1 month after enrollment and vaccination. HIV-infected participants were to have a CDC class of A1-2 [5] and a plasma HIV RNA polymerase chain reaction (PCR) measurement of <10,000 copies/mL

and >200 CD4 cells/mm^3 within 3 months prior to vaccination and were to be on a stable antiretroviral regimen if they had ≤500 CD4 cells/mm^3. Exclusion criteria included any immunization received within 1 month prior to enrollment, influenza vaccination received within 6 months of enrollment, receipt of blood products within 3 months prior to enrollment, pregnancy or lactation, current upper respiratory or febrile illness, history of wheezing or bronchodilator use within 1 month before enrollment, severe allergy to chicken eggs, or any household member with severe immunosuppression. Study participants were retrospectively surveyed to assess whether they received parenteral, inactivated influenza vaccine in the year prior to enrollment. Participants were stratified according to HIV infection status and randomized 1 : 1 to receive either LAIV or placebo in a double-blind manner.

Safety evaluation. Study participants recorded daily on a diary card for 10 days after vaccination their evening temperature and the following reactogenicity events: runny nose, malaise, sore throat, cough, myalgia, nausea or vomiting, decreased appetite, abdominal pain, or headache. Participants were questioned and examined once during each of the following intervals: 3–5, 7–10, and 28–35 days after vaccination. All adverse events were noted.

Viral cultures. Nasal and throat swabs were collected once during each of 3 above-mentioned study visits in order to detect LAIV virus shedding by tissue culture. The first 2 culture intervals were chosen on the basis of previous LAIV shedding data that demonstrated maximum shedding in highly susceptible persons 2–10 days after vaccination [6]. In immunocompetent persons, LAIV shedding is highly unlikely to occur beyond 2–3 weeks; therefore, the day 28–35 interval was chosen to detect prolonged shedding. Immunofluorescent antibody was used to identify influenza A or B.

HIV RNA PCR. Quantitative plasma HIV RNA levels were measured by reverse-transcriptase PCR assay using the Amplicor Monitor kit (Roche Diagnostics, Branchburg, NJ) at the University of Maryland once during each of the following intervals: before vaccination and 7–10, 28–35, 90–100, and 170–190 days after vaccination. The lower limit of detection for this assay was 400 copies/mL. Undetectable levels were assigned a value of 200 copies/mL. Changes in HIV RNA levels of <0.5 log (~2.5-fold) are considered to be within the expected range of the laboratory assay and day-to-day variation within individuals [7]. Therefore, a rise of HIV RNA level of 1 log (10-fold) was considered clinically significant.

CD4 cell counts. Flow cytometry was performed at each clinical site to determine CD4 cell counts on blood obtained before vaccination and at 28–35, 90–100, and 170–190 days after vaccination.

Antibody assays. Influenza hemagglutination antibody inhibition (HAI) tests were performed on paired sera obtained before and 28–35 days after vaccination. HAI tests were performed at Saint Louis University with antigens derived from vaccine strains. Participants were considered serosusceptible if their prevaccination HAI titer was <1 : 8. Sera with a titer of <1 : 4 were assigned a titer of 1 : 2. Seroconversion was defined as a ≥4-fold rise in HAI titer.

Statistical methods. This study was designed to allow for a preliminary examination of LAIV in a relatively small number of asymptomatic HIV-infected subjects and to allow for the detection of large differences in rates of reactogenicity events and shedding of LAIV. The power to make statistically significant comparisons depends on both the sample size and response rates. For example, using a 1-sided test of proportions with a sample size of 28 in each treatment group of HIV-infected subjects, and assuming the observed rate of a reactogenicity event in placebo recipients is 10%–31%, there would be 80% power to detect from 28% to 33% higher reactogenicity rates in HIV-infected LAIV recipients.

Rates of fever (temperature >37.8°C) and reactogenicity events were compared within each HIV status group between LAIV and placebo recipients by χ^2 or Fisher's exact test. Linear regression was used to compare $\log_{10}$ changes in quantitative HIV RNA levels and CD4 cell counts between those who received LAIV and those who received placebo just before and at 7–10 days (HIV RNA only), at 28–35 days, at 90–100 days, and at 170–190 days after vaccination. Geometric mean HIV RNA levels and CD4 cell counts were computed at each time point with 95% confidence intervals. Seroresponse rates, defined by ≥4-fold increases in influenza HAI titers between baseline and 28–35 days after vaccination, were evaluated by logistic regression. The effect of immunization on the change in geometric mean HAI titers was evaluated on a log scale using the *t* test. All regression models included covariates adjusting for baseline demographic characteristics.

Results

In total, 57 HIV-infected (mean age, 40 years) and 54 non–HIV-infected (mean age, 34 years) study participants were enrolled. Forty-nine percent of the HIV-infected participants were male compared with 35% in the non–HIV-infected group. The racial distributions for the HIV-infected and non–HIV-infected participants, respectively, were 74% versus 22% African American; 23% versus 70% white; 4% versus 8% other. Twenty (45%) of 44 HIV-infected and 25 (56%) of 45 non–HIV-infected participants reported that they had received an influenza vaccine in the year prior to enrollment.

Rates of reactogenicity events were similar between LAIV and placebo recipients regardless of HIV status, with the exception of a statistically significant higher rate of runny nose/nasal congestion in LAIV recipients (table 1). There were no significant differences in the rates of reactogenicity events between HIV-infected and non–HIV-infected LAIV recipients.

No serious adverse events were attributable to LAIV; however, 4 adverse events judged by the investigators as possibly vaccine related were encountered within 28–35 days after vaccination. Two of these adverse events occurred in HIV-infected LAIV recipients: One had clinical sinusitis and another wheezed. One HIV-infected placebo recipient wheezed and 1 non–HIV-infected placebo recipient had bronchitis. All adverse events resolved without apparent sequelae. One HIV-infected participant had a positive viral culture for vaccine virus (influenza B) 5 days after receiving LAIV. No other LAIV shedding was detected in study participants.

Plasma HIV RNA PCR levels were stable in both the LAIV and placebo HIV-infected participants (table 2). One LAIV recipient had a ≥10-fold rise in HIV RNA level with a pre-

Table 1. Postvaccination reactogenicity events (%) by human immunodeficiency virus (HIV) infection status and vaccine assignment (data are events occurring on >1 day during the 10-day follow-up period).

	HIV infected		Non–HIV infected	
Symptom	LAIV, $n = 28$	Placebo, $n = 29$	LAIV, $n = 27$	Placebo, $n = 27$
Fever[a]	2 (7)	3 (10)	0	3 (11)
Runny nose/nasal congestion	17 (61)[b]	9 (31)[b]	21 (78)[b]	12 (44)[b]
Malaise	7 (25)	7 (24)	7 (26)	7 (26)
Sore throat	7 (25)	2 (7)	6 (22)	6 (22)
Cough	11 (39)	8 (28)	3 (11)	7 (26)
Myalgia	10 (36)	6 (21)	4 (15)	6 (22)
Nausea/vomiting	4 (14)	5 (17)	1 (4)	3 (11)
Decreased appetite	3 (11)	3 (10)	3 (11)	5 (19)
Abdominal pain	2 (7)	1 (3)	1 (4)	3 (11)
Headache	11 (39)	9 (31)	12 (44)	11 (41)
Any of the above	22 (79)	18 (62)	22 (81)	19 (70)

NOTE. LAIV, live attenuated influenza vaccine.
[a] Fever was defined as a temperature >37.8°C.
[b] $P < .05$, Fisher's exact test, between respective LAIV and placebo groups for each HIV status category.

vaccination level of 200 copies/mL and the day 7–10 level of 135,664 copies/mL, which returned to near baseline at 407 copies/mL by the day 28–35 postvaccination visit. Two other participants had ⩾10-fold rises in HIV RNA levels, which occurred after the day 7–10 postvaccination visit: One LAIV recipient went from a prevaccination level of 1448 copies/mL to 19,550 copies/mL on the 28–35 day postvaccination visit, but the level returned to near baseline (1917 copies/mL) by the 90-day postvaccination visit. One placebo recipient had a prevaccination level of 200 copies/mL and a 28–35 day postvaccination level of 15,033 copies/mL; this level persisted to the 90-day postvaccination visit at 13,512 copies/mL.

Eight of the HIV-infected participants were not on antiretroviral therapy during this study; 5 received LAIV and 3 received placebo. There were no 10-fold rises in HIV RNA levels among the participants not on antiretrovirals. There was a slight decline in CD4 cells after vaccination in both LAIV and placebo HIV-infected recipients (table 2), but no significant differences were detected in the magnitude of these decreases between the 2 treatment groups. Importantly, none of the CD4 cell counts fell below 200 cells/mm^3 within 1 month of vaccination. There was no trend toward transient or prolonged decreases in CD4 cells in the HIV-infected participants not on antiretroviral therapy.

Few participants had a seroresponse to LAIV as defined by a ⩾4-fold rise in HAI titer (table 2). Of note, the proportion of LAIV recipients who were considered serosusceptible (titer <1 : 8) before vaccination was low. For the HIV-infected participants, 4%, 4%, and 31% were serosusceptible to H1N1, H3N2, and B, respectively, prior to vaccination. Among the non–HIV-infected participants, 11%, 4%, and 11% were serosusceptible to H1N1, H3N2, and B, respectively, before receiving LAIV. The geometric mean reciprocal HAI titers before and 28–35 days after LAIV did not differ significantly (table 2).

Discussion

LAIV was generally safe and well tolerated in HIV-infected adults with asymptomatic or mildly symptomatic HIV infection

Table 2. Summary of seroresponses (⩾4-fold hemagglutination inhibition [HAI] to titer rise) to live attenuated influenza vaccine (LAIV) antigens in all participants, geometric mean CD4 cell counts, and human immunodeficiency virus (HIV) RNA copies/mL (as determined by polymerase chain reaction) in HIV-infected LAIV and placebo recipients.

	HIV-infected participants		Non–HIV-infected participants	
Seroresponse	LAIV	Placebo	LAIV	Placebo
Influenza HAI seroresponse				
H1N1	1/26 (4%)	2/25 (8%)	1/27 (4%)	0/26 (0%)
H3N2	2/26 (8%)	2/25 (8%)	0/27 (0%)	0/26 (0%)
B	0/26 (0%)	1/25 (4%)	1/27 (4%)	0/26 (0%)
Geometric mean HAI titers (before, after vaccination)				
H1N1	55, 56	36, 36	67, 75	98, 103
H3N2	32, 35	35, 45	41, 44	48, 52
B	10, 11	18, 18	41, 48	44, 44
Geometric mean HIV RNA copies/mL of blood (95% CI), no. tested				
Before vaccination	544 (295–1002), 28	681 (366–1267), 29	NA	NA
After vaccination				
7–10 days	602 (310–1168), 27	517 (331–806), 27	NA	NA
28–35 days	467 (283–771), 27	507 (289–891), 27	NA	NA
3 months	617 (353–1078), 27	552 (302–1009), 26	NA	NA
6 months	574 (296–1115), 21	1035 (461–2327), 23	NA	NA
Geometric mean CD4 cells/mm^3 (95% CI), no. tested				
Before vaccination	598 (525–682), 28	498 (429–579), 29	NA	NA
After vaccination				
28–35 days	550 (480–630), 25	490 (426–564), 26	NA	NA
3 months	567 (493–651), 27	454 (376–548), 26	NA	NA
6 months	603 (524–693), 20	571 (493–662), 23	NA	NA

NOTE. CI, confidence interval; NA, not applicable.

and in non–HIV-infected adults. This information is important in that, although the study participants were not highly susceptible to influenza, they are likely to be similar to the population that may inadvertently receive LAIV if it becomes widely used. The excess of runny nose/nasal congestion in both the HIV-infected and non–HIV-infected LAIV recipients is an expected minor reactogenicity event [2] and was not significantly different between the 2 HIV status groups.

Prolonged LAIV shedding was not observed in the HIV-infected participants. HIV-infected subjects with advanced HIV disease have been reported to shed respiratory viruses, including influenza for prolonged periods [8, 9]. The lack of LAIV shedding in the present study was likely due to the attenuated phenotype of the vaccine and to the high degree of prevaccination influenza immunity and the relatively mild degree of HIV-related immunosuppression of the HIV-infected participants.

Finally, there was no evidence of significantly increased HIV replication as measured by plasma HIV RNA levels nor was there any evidence of significantly greater decreases in peripheral blood CD4 cell counts following LAIV compared to placebo in HIV-infected participants. Some previous studies in persons who received parenteral inactivated influenza vaccine showed increased HIV replication [10, 11], but others found no significant changes in HIV replication in HIV-infected persons after vaccination [12, 13].

This study had limitations. First, the sample size was small. Therefore, we cannot conclude that serious reactions would not occur if large numbers of HIV-infected persons are exposed to LAIV. However, it is unlikely that a large number of these persons would be exposed to LAIV since inactivated influenza vaccine is recommended for use in immunocompromised persons [14] and secondary transmission of LAIV to susceptible contacts has not been reported [15].

The lack of adverse events attributable to LAIV could have been due to lack of LAIV infectivity in this unscreened group. In adults who are not selected to have low preimmunization influenza antibodies, few serum antibody rises and little viral shedding are expected, as observed in the present study. However, even with infrequent LAIV shedding and seroresponses in our study participants, an immunologic effect was anticipated as this vaccine has been effective in reducing influenza illnesses in adults not screened to be influenza susceptible despite poor antibody responses [3, 4]. The higher proportion of runny nose/nasal congestion events in our LAIV recipients compared with the placebo recipients may indicate that there was limited local replication of vaccine virus in their nasal mucosa, despite the lack of evidence of vaccine virus infection. It is reassuring that no increase in infectivity of LAIV occurred in these HIV-infected participants despite the likelihood of at least some degree of HIV-related immunosuppression.

Study results indicate that serious adverse events would not be expected to occur frequently if relatively asymptomatic HIV-infected persons become exposed to LAIV inadvertently. However, it must be stressed that the safety of LAIV administration to highly influenza-susceptible HIV-infected persons was not assessed. Administration of LAIV to a more influenza-susceptible population, such as HIV-infected young children, could better address this issue. Finally, this study was not intended to suggest that LAIV should be recommended for HIV-infected adults: Parenteral inactivated influenza vaccine is already recommended for such persons.

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